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St George's, University of London

Invasive Meningococcal Disease in Preterm
Infants

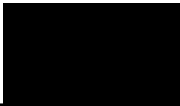
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Thesis for the degree of Doctor of Philosophy

4th June 2023

I declare that this thesis is an original report of my research, has been written by me and has not been submitted for any previous degree. All collaborative contributions have been indicated clearly and acknowledged. References have been provided for all supporting literature and resources.

Signed _____  _____

Date _____ 4th June 2023 _____

Abstract

Background

Invasive meningococcal disease (IMD) causes significant morbidity and mortality.

Preterm infants may be at greater risk of IMD and may have reduced responses to the current 2+1 4CMenB vaccine schedule. Serum bactericidal antibody assays, using adult complement with infant serum, may not accurately assess infant immunity against *N. meningitidis*.

Methods

The literature was systematically reviewed for outcomes of immunogenicity, efficacy, effectiveness, reactogenicity and safety of routine vaccines in preterm infants. An epidemiological study compared incidence of IMD in preterm and term infants. A clinical trial compared the immunogenicity of 4CMenB vaccine when given to preterm infants according to a 2+1 and 3+1 schedule. Cord blood samples were collected and pooled to create a neonatal complement source for SBA assays to allow comparison of titres between assays using adult and neonatal complement.

Results

Although antibody and cellular responses were reduced following some vaccines, where a protective threshold exists preterm infants usually achieved this, and vaccine effectiveness and efficacy is similar between term and preterm infants. Vaccination is well tolerated in preterm infants and there is no evidence that vaccines should be delayed in this group. The incidence of IMD is higher in preterm than term infants, and the incidence is higher in infants born at <32 weeks of gestation (WG) compared to those born at 32-37 WG. The immunogenicity of 4CMenB in preterm infants

Abstract

vaccinated according to a 2+1 or 3+1 schedule is similar for strains 5/99 and 44/76-SL, although somewhat reduced for strain NZ98/254 in those infants vaccinated with a 2+1 schedule. There is no evidence of differences in SBA titres when using adult compared with neonatal complement.

Conclusions

IMD has significant consequences for infants. Preterm infants have a higher incidence of IMD than term infants; whilst the current 4CMenB vaccine schedule is acceptable ongoing surveillance must be prioritised.

Words= 300

Table of contents

Abstract	2
Table of contents	4
Table of tables	8
Table of figures	11
Table of images	13
Attribution of work	14
Covid-19 statement	15
Acknowledgements	16
Abbreviations	18
1 Introduction	20
1.1 Invasive meningococcal disease (IMD)	20
1.1.1 Historical perspective	20
1.1.2 Microbiology	20
1.1.3 Immune responses	24
1.1.4 Epidemiology	26
1.1.5 Clinical features	28
1.2 Vaccination	30
1.3 Preterm birth	37
1.3.1 Epidemiology of preterm birth	37
1.3.2 Immunity in preterm infants	39
1.3.3 Infectious diseases in preterm infants	43
1.3.4 Vaccination in preterm infants	44
1.4 Assessing meningococcal vaccine immunogenicity	44
1.5 Summary and aims	47
1.5.1 Summary	47
1.5.2 Aims	47
2 Methods	48
2.1 Systematic review: Immunogenicity, efficacy, effectiveness, reactogenicity and safety of routine vaccines given in the first eighteen months of life to preterm infants. ..	48
2.1.1 Registration	48
2.1.2 Search strategy	48
2.1.3 Eligibility criteria	49
2.1.4 Study design	51
2.1.5 Data management	53
2.1.6 Selection process	53
2.1.7 Meta-analysis	54
2.1.8 Risk of bias assessment	54
2.1.9 GRADE assessment	55

Table of contents

2.2	Invasive Meningococcal disease in Preterm infants	55
2.2.1	Identification of cases.....	55
2.2.2	Data collection.....	55
2.2.3	Ethical approval	56
2.2.4	Population	56
2.2.5	Definitions.....	57
2.2.6	Risk factors.....	57
2.2.7	Vaccination status.....	57
2.2.8	Meningococcal antigen typing system (MATS).....	58
2.2.9	Sequelae	58
2.2.10	Analysis	58
2.3	Babies born Early Antibody Responses to MenB vaccination (BEAR Men B): a comparison of two MenB vaccine schedules in preterm infants.....	59
2.3.1	Ethical approval and registration.....	60
2.3.2	Study design.....	60
2.3.3	Statistical analysis	65
2.4	Sources of complement in SBA assays.....	67
2.4.1	Ethical approval and registration.....	68
2.4.2	Clinical study methods.....	68
2.4.3	Laboratory study methods.....	70
3	Systematic review: immunogenicity, efficacy, and effectiveness	81
3.1	Background.....	81
3.1.1	Vaccine schedules	82
3.1.2	Objectives	82
3.1.3	Methods.....	83
3.2	Results	83
3.2.1	Immunogenicity.....	84
3.2.2	Efficacy and effectiveness.....	129
3.2.3	GRADE assessments.....	133
3.3	Discussion	133
3.4	Conclusion	136
4	Systematic review: reactogenicity and safety.....	137
4.1	Background.....	137
4.1.1	Objectives	138
4.1.2	Methods.....	138
4.2	Results	138
4.2.1	Reactogenicity.....	138
4.2.2	Safety	156
4.2.3	GRADE assessments	158
4.3	Discussion	158
4.4	Conclusion	162
5	Invasive Meningococcal disease in Preterm infants.....	163
5.1	Background.....	163
5.1.1	Objectives	164
5.1.2	Methods.....	164
5.2	Results	164
5.2.1	Participants	164

Table of contents

5.2.2	Gestational age	165
5.2.3	Incidence rates and ratios.....	165
5.2.4	Clinical Presentation	167
5.2.5	Vaccination	169
5.2.6	Outcome	172
5.2.7	Sensitivity analysis reclassifying all infants with missing data as term.....	174
5.3	Discussion.....	175
5.4	Conclusion	181
6	Babies born Early Antibody Response to MenB vaccination- immunogenicity results.....	182
6.1	Background	182
6.1.1	Objectives	183
6.1.2	Methods.....	183
6.2	Results	184
6.2.1	Recruitment	184
6.2.2	Data analysis	190
6.3	Discussion	201
6.4	Conclusion	205
7	BEAR Men B reactogenicity and safety results.....	207
7.1	Background.....	207
7.1.1	Objectives	208
7.1.2	Methods.....	208
7.2	Results	208
7.2.1	Reactogenicity	209
7.2.2	Safety	231
7.3	Discussion.....	233
7.4	Conclusions.....	239
8	Sources of complement in serum bactericidal antibody assays.....	241
8.1	Background.....	241
8.1.1	Objectives	243
8.1.2	Methods.....	243
8.2	Results	243
8.2.1	Recruitment	243
8.2.2	Demographics	244
8.2.3	Antibiotic use.....	244
8.2.4	Laboratory analysis.....	245
8.3	Discussion	264
8.4	Conclusions.....	269
9	Conclusions and further work	270
9.1	Conclusions.....	270
9.2	Further work.....	273
9.3	Final comments.....	277
10	References	278

Table of contents

Appendix A.....	301
Appendix B.....	304
Appendix C.....	311
Appendix D.....	389
Appendix E	390
Appendix F	408
Appendix G.....	416
Appendix H.....	449
Appendix I	477
Appendix J.....	479

Table of tables

Table 1.1. Virulence factors of <i>N. meningitidis</i>	21
Table 1.2. Currently licensed meningococcal vaccines.	32
Table 1.3. Techniques to assess Men B vaccine coverage.	36
Table 2.1. Classification of studies (105, 106).	52
Table 2.2. Eligibility criteria for the BEAR Men B study.	60
Table 2.3. Routine vaccines during BEAR Men B study period.....	61
Table 2.4. Timing of vaccinations and blood sampling.....	61
Table 2.5. Details of the classification used for solicited adverse events following vaccination.	63
Table 2.6. Descriptions of terms used to describe changes in cardiorespiratory events between the period before and after vaccination.	65
Table 2.7. Eligibility criteria for the COMPARE study.	68
Table 2.8. Instructions for sample incubation for radial immunodiffusion assays.	70
Table 2.9. Details of the complement preparations used in the disc diffusion assay.	80
Table 3.1. Details of the vaccine schedules received in studies of Hib vaccine in preterm infants with no term comparison.	86
Table 3.2. GMC/T and percentage of preterm infants reaching protective thresholds following primary Hib vaccination with no term comparison.....	87
Table 3.3. Details of the vaccine schedules received in studies of Hib vaccine in preterm infants with term comparison.	89
Table 3.4. GMC/T and percentage of infants reaching protective thresholds following primary Hib vaccine series with comparison of term and preterm infants.	89
Table 3.5. Details of studies comparing term and preterm infants after administration of a booster dose.	92
Table 3.6. GMC/T of infants following Hib booster vaccination in studies comparing term and pre-term infants.....	92
Table 3.7. Percentage of infants reaching protective thresholds following booster Hib vaccination in studies comparing term and preterm infants.....	93
Table 3.8. GMC/T and percentage of infants reaching a protective threshold in studies comparing term and preterm infants after primary tetanus vaccine series.....	97
Table 3.9. GMC and proportions reaching a protective threshold in studies comparing term and preterm infants after primary series of diphtheria vaccine.	101
Table 3.10. GMC and proportions reaching a protective threshold in studies comparing term and preterm infants receiving booster diphtheria vaccine.	102
Table 3.11. GMC and percentages of infants reaching a protective threshold in studies comparing long term immunity following diphtheria vaccination.	103
Table 3.12. GMC and percentages of infants reaching a protective threshold in studies comparing long term immunity following hepatitis B vaccination.	105
Table 3.13. Comparison of IgG GMC against pertussis antigens following primary vaccination in term and preterm infants.....	108
Table 3.14. Comparison of IgG GMC against pertussis antigens following booster vaccination in term and preterm infants.....	109
Table 3.15. Comparison of persistence of IgG GMC and percentage reaching a 'protective threshold' against pertussis antigens in term and preterm infants.....	110
Table 3.16. SBA GMT following primary MenC vaccination in studies comparing term and preterm infants.....	115
Table 3.17. SBA GMT following booster MenC dose in studies comparing term and preterm infants. ...	116
Table 3.18. Percentages of preterm infants who reached the protective threshold of $\geq 0.35 \mu\text{g/ml}$ for serotypes covered in PCV7 following the primary vaccination series.	118
Table 3.19. Percentages of preterm infants who reached the protective threshold of $\geq 0.35 \mu\text{g/ml}$ for additional serotypes covered in PCV13 following primary vaccine series.	119
Table 3.20. Percentages of preterm infants who reached the protective threshold of $\geq 0.35 \mu\text{g/ml}$ for serotypes covered in PCV7 following a booster dose.	119

Table of tables

Table 3.21. Percentages of term and preterm infants with titres ≥ 0.35 $\mu\text{g/ml}$ for serotypes covered in PCV7 following primary vaccination series.	120
Table 3.22. Percentages of term and preterm infants with concentrations ≥ 0.35 $\mu\text{g/ml}$ for serotypes covered in PCV10/13 following primary vaccination series.	121
Table 3.23. Percentages of term and preterm infants with concentrations ≥ 0.35 $\mu\text{g/ml}$ for serotypes covered in PCV7 following booster dose.	124
Table 3.24. Percentages of term and preterm infants with concentrations ≥ 0.35 $\mu\text{g/ml}$ for serotypes covered in PCV10 following booster dose.	124
Table 3.25. Details of the vaccine effectiveness trials for pertussis vaccine.	130
Table 4.1. Details of the studies reporting cardiorespiratory events in preterm infants before and after vaccine administration.	140
Table 4.2. Details of studies reporting cardiorespiratory events with no comparison between period before and after vaccination.	141
Table 4.3. Details of the studies investigating cardiorespiratory events following vaccination.	147
Table 4.4. Definitions of fever used in the included papers.	152
Table 5.1. Incidence rates and incidence rate ratios according to gestation in infants aged <1 year with confirmed invasive meningococcal disease September 2015 to August 2020 in England.	166
Table 5.2. Age of onset of disease overall and according to gestational group.	168
Table 5.3. Presenting syndrome overall and according to gestational group.	168
Table 5.4. Fever as a presenting symptom overall and according to gestational group.	169
Table 5.5. Signs of shock at presentation overall and according to gestational group.	169
Table 5.6. Summary of vaccines received in infants infected with serogroups B and C.	170
Table 5.7. A breakdown of the number of doses of 4CMenB received according to the number of doses a participant was eligible for.	170
Table 5.8. Breakdown of MATS coverage according to vaccination status.	171
Table 5.9. Length of hospital admission by gestational age group.	172
Table 5.10. PICU admission by gestational age group.	172
Table 5.11. Case fatality rate by capsular group.	173
Table 5.12. Summary of sequelae reported according to gestational age category.	173
Table 5.13. Summary of sequelae classified as 'other'.	174
Table 5.14. Breakdown of results separated according to category- gestational age and missing.	174
Table 6.1. Number of participants completing each study visit (including all recruited infants, n=136).	185
Table 6.2. Baseline characteristics according to study group.	187
Table 6.3. Summary of non-study vaccinations received.	188
Table 6.4. Age at study visits.	188
Table 6.5. Time between study visits.	189
Table 6.6. Protocol deviations.	189
Table 6.7. GMT and 95% CI according to study group at five months.	190
Table 6.8. Proportions of participants with titres $\geq 1:4$ at 5 months.	191
Table 6.9. GMTs (95% CI) at 12 months (pre-booster) according to schedule.	192
Table 6.10. Proportions of participants with titres $\geq 1:4$ at 12 months.	193
Table 6.11. GMTs (95% CI) at 13 months (post-booster) according to schedule.	194
Table 6.12. Comparison of proportions in each group with SBA $\geq 1:4$ for all participants at 13 months.	195
Table 6.13. Results of ordered logistic regression investigating interaction between gestational age and study group.	196
Table 6.14. Fold decrease between post-primary and pre-booster for the three tested strains.	197
Table 6.15. Fold change between post-primary and post-booster for the three tested antigens.	198
Table 6.16. Calculated half-life of antibody between post-primary and pre-booster sampling points for all three tested antigens (days).	198
Table 6.17. Percentage of infants with SBA GMT $\geq 1:4$ following primary and booster vaccination, at both time points and at neither time point.	199
Table 6.18. Summary of variables of interest for GMTs following investigation using linear regression.	200
Table 7.1. Baseline characteristics of infants receiving vaccination in hospital or in the community.	210
Table 7.2. Percentage of infants experiencing apnoea, bradycardia and desaturation before and after vaccine administration at eight weeks showing median and range of episodes.	212

Table of tables

<i>Table 7.3. Percentage of infants experiencing apnoea, bradycardia and desaturation before and after vaccine administration at 12 weeks showing median and range of episodes.</i>	<i>214</i>
<i>Table 7.4. Percentage of infants experiencing apnoea, bradycardia, and desaturation before and after vaccine administration at 16 weeks showing median and range of episodes.</i>	<i>215</i>
<i>Table 7.5. Summary of cardiorespiratory events following vaccines at 8, 12 and 16 weeks.</i>	<i>215</i>
<i>Table 7.6. Overall clinical status following vaccines at 8, 12 and 16 weeks.</i>	<i>216</i>
<i>Table 7.7. Comparison of characteristics of infants who experienced deterioration or not following vaccination with results of logistic regression.</i>	<i>218</i>
<i>Table 7.8. Proportion of infants experiencing local reactions following 4CMenB administration.</i>	<i>220</i>
<i>Table 7.9. Proportions of infants experiencing local reactions following PCV13 administration.</i>	<i>221</i>
<i>Table 7.10. Proportion of infants experiencing local reactions following DTaP/IPV/Hib/HepB vaccination.</i>	<i>222</i>
<i>Table 7.11. Proportion of infants experiencing local reactions following MMR vaccination.</i>	<i>223</i>
<i>Table 7.12. Proportion of infants experiencing local reactions following Hib/MenC vaccination.</i>	<i>224</i>
<i>Table 7.13. Proportion of infants experiencing systemic reactions occurring following vaccination at each time point.</i>	<i>225</i>
<i>Table 7.14. Summary of the results for systemic reactions shown to have a significant relationship to gestational age (30-34⁺⁶ WG vs. <30+0 WG).</i>	<i>227</i>
<i>Table 7.15. Proportion of infants with fever up to seven days following vaccination.</i>	<i>228</i>
<i>Table 7.16. Paracetamol received following vaccination.</i>	<i>230</i>
<i>Table 7.17. Percentage of participants investigated for sepsis within 7 and 28 days.</i>	<i>231</i>
<i>Table 7.18. Recorded SAEs according to group.</i>	<i>232</i>
<i>Table 8.1. Participant demographics.</i>	<i>244</i>
<i>Table 8.2. Percentage of infants receiving antibiotics according to study group and mode of delivery.</i>	<i>245</i>
<i>Table 8.3. Results from RIDs showing absolute values and values as a percentage of the adult value, along with published term and preterm results presented as a percentage of adult values.</i>	<i>245</i>
<i>Table 8.4. SBA assay results using strain 5/99 (NadA) and adult serum with adult and neonatal complement.</i>	<i>254</i>
<i>Table 8.5. SBA assay results using strain 5/99 (NadA) and baby serum with adult and neonatal complement.</i>	<i>255</i>
<i>Table 8.6. SBA assay results using strain NZ98/254 (PorA) and adult serum with adult and neonatal complement.</i>	<i>255</i>
<i>Table 8.7. SBA assay results using strain NZ98/254 (PorA) and baby serum with adult and neonatal complement.</i>	<i>256</i>
<i>Table 8.8. SBA assay results using strain 44/76-SL and adult serum with adult and neonatal complement.</i>	<i>256</i>
<i>Table 8.9. SBA assay results using strain 44/76-SL and baby serum with adult and neonatal complement.</i>	<i>257</i>
<i>Table 8.10. Results from the SBA assays using adult and neonatal complement and a set of preterm infant sera and one adult serum samples.</i>	<i>258</i>

Table of figures

Figure 1.1. Diagram of <i>N. meningitidis</i> showing important virulence factors.	21
Figure 1.2. Diagram of the complement cascade.	25
Figure 1.3. Map showing the incidence and predominant serogroups by region (5, 22). Created with mapchart.net.	26
Figure 1.4. Incidence of IMD in England per 100,000 population from 2010/11 to 2019/20 (25).	27
Figure 1.5. Invasive meningococcal disease in England by capsular group: 2011/2012 to 2020/2021 (25).	28
Figure 1.6. Summary of presenting symptoms and signs of invasive meningococcal disease.	29
Figure 1.7. Preterm birth rate across countries in 2010, image taken from 'Born too Soon' (47).	37
Figure 1.8. The percentage of babies born at less than 37 weeks of gestation in England from 2006-2016 with breakdown according to gestational age (data from the Office of National Statistics) (49).	38
Figure 1.9. Serum bactericidal antibody assay.	45
Figure 2.1. PICO criteria for the systematic review.	51
Figure 2.2. Flow chart for the classification of studies (105, 106).	53
Figure 2.3. Dilution plate for SBA assay.	77
Figure 3.1. Summary of searching and selection process according to PRISMA flow chart.	84
Figure 3.2. Percentage of preterm infants reaching titres $\geq 0.15\mu\text{g/ml}$ and $\geq 1.0\mu\text{g/ml}$ after primary Hib vaccination.	87
Figure 3.3. Forest plot showing proportions of infants reaching a threshold of $\geq 0.15\mu\text{g/ml}$ following primary vaccination.	91
Figure 3.4. Forest plot showing proportions of infants reaching a threshold of $\geq 1.0\mu\text{g/ml}$ following primary vaccination.	91
Figure 3.5. Forest plot showing proportions of infants reaching a protective MenC SBA GMT in preterm and term infants.	116
Figure 3.6. Forest plot of the proportions of infants achieving protective threshold for serotype 4.	121
Figure 3.7. Forest plot of the proportions of infants achieving protective threshold for serotype 6B.	122
Figure 3.8. Forest plot of the proportions of infants achieving protective threshold for serotype 9V.	122
Figure 3.9. Forest plot of the proportions of infants achieving protective threshold for serotype 14.	122
Figure 3.10. Forest plot of the proportions of infants achieving protective threshold for serotype 18C.	123
Figure 3.11. Forest plot of the proportions of infants achieving protective threshold for serotype 19F.	123
Figure 3.12. Forest plot of the proportions of infants achieving protective threshold for serotype 23F.	123
Figure 4.1. Visual representation of the reports which include an investigation of factors associated with cardiorespiratory events.	144
Figure 4.2. Percentage of infants reported to have experienced fever following receipt of vaccines in different studies including term comparisons where available.	153
Figure 5.1. Percentage of cases of IMD caused by different serogroups of <i>N. meningitidis</i>	165
Figure 5.2. Number of cases/100,000 infants separated according to study year.	167
Figure 6.1. CONSORT flow diagram for BEAR Men B study including all recruited participants (n=136).	184
Figure 6.2. Graph showing the number of participants recruited at each site stratified according to gestational age category.	186
Figure 6.3. SBA GMTs at five months against <i>N. meningitidis</i> strains 44/76-SL (fHbp), 5/99 (NadA) and NZ98/254 (PorA).	191
Figure 6.4. Proportion of infants reaching a putative protective SBA GMT against reference strains 44/76-SL (fHbp), 5/99 (NadA) and NZ98/254 (PorA) at five months.	192
Figure 6.5. SBA GMTs at twelve months against <i>N. meningitidis</i> strains 44/76-SL (fHbp), 5/99 (NadA) and NZ98/254 (PorA).	193
Figure 6.6. Proportion of infants reaching a putative protective SBA GMT against reference strain 44/76-SL (fHbp), 5/99 (NadA) and NZ98/254 (PorA) at twelve months.	194
Figure 6.7. SBA geometric mean titres at thirteen months against <i>N. meningitidis</i> strains 44/76-SL (fHbp), 5/99 (NadA) and NZ98/254 (PorA).	195
Figure 6.8. Proportion of infants reaching a putative protective SBA GMT against reference strain 44/76-SL (fHbp), 5/99 (NadA) and NZ98/254 (PorA) at 13 months.	196

Table of images

Figure 6.9. GMT against 44/76-SL (fHBp), 5/99 (NadA) and NZ98/254 (PorA) at 5, 12 and 13 months shown according to group and gestational category.	197
Figure 7.1. Progress of participants vaccinated as inpatients through vaccine visits.	209
Figure 7.2. Ventilatory support following first vaccines displayed according to ventilatory support required before vaccination.	211
Figure 7.3. Percentage of infants experiencing apnoea (A), bradycardia (B) and desaturation (C) before and after vaccination at 8 weeks.	212
Figure 7.4. Ventilatory support following second vaccines displayed according to ventilatory support required before vaccination.	213
Figure 7.5. Percentage of infants experiencing apnoea (A), bradycardia (B) and desaturation (C) before and after vaccination at 12 weeks.	214
Figure 7.6. Clinical course of infants vaccinated as inpatients.	217
Figure 7.7. Classification of local reactions following 4CMenB administration at 2, 3, 4 and 12 months.	220
Figure 7.8. Classification of local reactions following PCV13 administration at 2, 3, 4 and 12 months.	221
Figure 7.9. Classification of local reactions following DTaP/IPV/Hib/HepB administration at 2, 3, 4 and 12 months.	222
Figure 7.10. Classification of local reactions following MMR administration at 12 months.	223
Figure 7.11. Classification of local reactions following vaccination with Hib/MenC vaccine.	224
Figure 7.12. Classification of systemic reactions following vaccination.	227
Figure 7.13. Highest recorded temperature in 7 days following vaccination.	229
Figure 8.1. Summary of published CH50 and AP50 results (59, 265).	242
Figure 8.2. CONSORT flow diagram for the COMPARE study.	244
Figure 8.3. Complement components in term and preterm samples presented as a percentage of the adult reference value.	246
Figure 8.4. SBA curves for SBA assays performed using serial dilutions of term neonatal and adult complement and strain 5-99 (NadA).	247
Figure 8.5. SBA results for SBA assays performed using serial dilutions of term neonatal complement and adult complement and strain NZ98/254 (PorA).	248
Figure 8.6. SBA results for SBA assays performed using serial dilutions of term neonatal complement and adult complement and N. meningitidis strain 44/76-SL (fHBp).	249
Figure 8.7. SBA assay results using adult complement neat, with added PBS or added β -lactamase and strain 5-99 (NadA).	253
Figure 8.8. SBA assay results using neonatal complement neat, with added PBS or added β -lactamase and strain 5-99 (NadA).	253
Figure 8.9. GMTs and 95% confidence intervals of MFI-C for C3b(A) and C5b9(B) comparing adult, term neonatal and preterm neonatal complement using strain 5/99.	259
Figure 8.10. GMTs and 95% confidence intervals of MFI-C for C3b(A) and C5b9(B) comparing samples known to have low, mid and high SBA titres.	260
Figure 8.11. GMTs and 95% confidence intervals of MFI-C for C3b(A) and C5b9(B) comparing adult, term neonatal and preterm neonatal complement using strain 44/76-SL.	261
Figure 8.12. GMTs and 95% confidence intervals of MFI-C for strain 44/76-SL for C3b(A) and C5b9(B) comparing samples known to have low, mid and high SBA titres.	262
Figure 8.13. GMTs and 95% confidence intervals of MFI-C for C3b(A) and C5b9(B) comparing adult, term neonatal and preterm neonatal complement using strain NZ98/254.	263
Figure 8.14. GMTs and 95% confidence intervals for MFI-C for strain NZ98/254 for C3b(A) and C5b9(B) comparing samples known to have low, mid and high SBA titres.	264

Table of images

<i>Image 2.1. Cord blood sampling.</i>	69
<i>Image 2.2. Examples of precipitin rings.</i>	71
<i>Image 2.3. Sterile purification of cord blood plasma, applied to chromatography system with attached IgG and IgM depletion columns.</i>	73
<i>Image 2.4. Flexible film isolator in a category three laboratory (left).</i>	76
<i>Image 2.5. Flexible film isolator in a category three laboratory (right).</i>	76
<i>Image 2.6. Discs on bacterial lawn.</i>	79
<i>Image 8.1. Ampicillin infused disc on meningococcal lawn.</i>	249
<i>Image 8.2. Disc diffusion assay using N. meningitidis strain 5/99 (NadA) and neonatal complement from infants in the vaginal delivery group (no antibiotics reported).</i>	250
<i>Image 8.3. a and b: Disc diffusion assay using N. meningitidis strain 5/99 (NadA) and neonatal complement from infants in term (A) and preterm (B) infants delivered by caesarean section (antibiotics reported).</i>	251
<i>Image 8.4. Disc diffusion assay using N. meningitidis strain 44/76-SL (fHbp) and neonatal complement from infants delivered by vaginal delivery (no antibiotics reported).</i>	251
<i>Image 8.5. Disc diffusion assay using N. meningitidis strain NZ98/254 (PorA) and neonatal complement from infants delivered by vaginal delivery (no antibiotics reported).</i>	252

Attribution of work

	Declaration
Chapter 1	Nil
Chapter 2	Nil
Chapter 3	Emily Shaw was the second reviewer for the selection process in the systematic review, which included joint screening all of the titles and abstracts identified in the literature searches, and then joint reviewing all potential full texts for inclusion. She was also a second reviewer for the risk of bias assessments and took part in the discussion of the GRADE assessment for each of the identified outcomes.
Chapter 4	
Chapter 5	Enhanced surveillance is routinely conducted by UKHSA, and the surveillance questionnaires were sent out and the data extracted and recorded in the database by a member of the immunisation team at UKHSA Colindale.
Chapter 6	• The BEAR Men B study was a multi-centre clinical trial at six UK sites. Recruitment and follow up at the five sites other than St George's, University of London was performed by the local research teams • SBA assays were performed at the meningococcal reference unit (MRU) in Manchester • The study statistician was Professor Nick Andrews who provided extensive advice during my analysis
Chapter 7	
Chapter 8	• The recruitment and sample collection for this work was assisted by the clinical research midwives at St George's University Hospitals NHS Foundation Trust • Immunoglobulin depletion of the pooled complement was performed by Mrs Frances Alexander at UKHSA Porton Down • Many of the ADCD and SBA assays were performed by Mrs Lauren Allen and Mr Steven Thomas, members of the Pathogen Immunology group at UKHSA Porton Down, along with repeats for the disc diffusion assays to confirm the results of these.
Chapter 9	Nil

Covid-19 statement

The Covid-19 pandemic had a significant impact on the COMPAre (Sources of **CO**mplement in **M**eningococcal and **P**ertussis serum bactericidal antibody **A**ssays) study (chapter 8). Because of the significant demands on the UKHSA laboratory teams, I had to delay and shorten the time I spent at UKHSA Porton Down. This meant that I was unable to personally complete all of the planned assays and I am very grateful to the Pathogen Immunology Group, particularly Mrs Lauren Allen and Mr Steven Thomas for their help in completing these. Although other aspects of the work contained within this thesis were delayed or disrupted by the Covid-19 pandemic, it is only this element which I was unable to complete in the way which was planned.

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Finally, many, many thanks are owed to my family for their extraordinary patience and encouragement. Thank you.

Abbreviations

ADCD	Antibody dependent complement deposition
AE	Adverse event
aHR	Adjusted hazard ratio
APC	Antigen presenting cell
APPs	Antimicrobial proteins and peptides
AP50	Alternative Pathway haemolysis 50% assay
BEAR Men B	Babies born Early Antibody Response to Men B vaccine
BPI	Bacterial/permeability increasing protein
BW	Birthweight
CFR	Case fatality rate
CH50	Complement Haemolysis 50% assay
CLD	Chronic lung disease
COMPAre	Sources of CO mplement in ME ningococcal and PE rtussis serum bactericidal antibody AS says
CPAP	Continuous positive airway pressure
CRM	Cross reacting material of diphtheria toxin
dOMV	Detergent extracted outer membrane vesicle
ED	Emergency Department
ELISA	Enzyme linked immunosorbent assay
FDA	Food and Drug Administration
FHA	Filamentous haemagglutinin
fHbp	Factor H binding protein
GMT	Geometric mean titre
GSK	GlaxoSmithKline
IMD	Invasive meningococcal disease
IR	Incidence rate
IRR	Incidence rate ratio
JCVI	Joint Committee on Vaccination and Immunisation
LBW	Low birthweight
LOS	Lipooligosaccharide
LPS	Lipopolysaccharide
MAC	Membrane attack complex
MASP	Mannan-binding lectin associated serine protease-1
MATS	Meningococcal antigen typing system
MBL	Mannose binding lectin
miTT	Modified intention to treat
MLST	Multilocus sequence typing
NadA	Neisserial adhesinA
NHBA	Neisserial heparin binding antigen
NHS	National Health Service
NK cells	Natural killer cells
Opa/Opc	Opacity proteins

Abbreviations

OMP	Outer membrane protein
PBMCs	Peripheral blood mononuclear cells
PorA/B	Porin A/B
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
PRN	Pertactin
PRP	Polyribosyl ribitol phosphate
PT	Pertussis toxin
RP	Relative potency
SAE	Serious Adverse Event
SBA	Serum bactericidal antibody
SGA	Small for gestational age
SmPC	Summary of product characteristics
ST	Sequence type
SUSAR	Sudden unexpected serious adverse event
TLR	Toll like receptor
VLBW	Very low birthweight
WG	Weeks of gestation

1 Introduction

1.1 Invasive meningococcal disease (IMD)

1.1.1 Historical perspective

Meningococcal disease, or cerebrospinal fever as it was known, was first clearly described in 1805 following an epidemic in Geneva. In 1887 Weichselbaum isolated the pathogen from meningeal fluid in post-mortem samples, which he described as *diplococcus intracellularis meningitidis* and in 1893 the first lumbar puncture was performed allowing identification of the organism from a living patient (1). Following these discoveries, immunotherapy using anti-meningococcal serum was developed, and this remained the established treatment until the advent of the antibiotic era. The requirement to notify cases of cerebrospinal fever was implemented in 1912 which allowed more accurate assessment of outbreaks throughout the 20th century (1).

1.1.2 Microbiology

Neisseria meningitidis (*N. meningitidis*; meningococcus) is a Gram-negative diplococcus which is genetically dynamic, allowing it to frequently adapt, and evade human immunity (figure 1.1) (2).

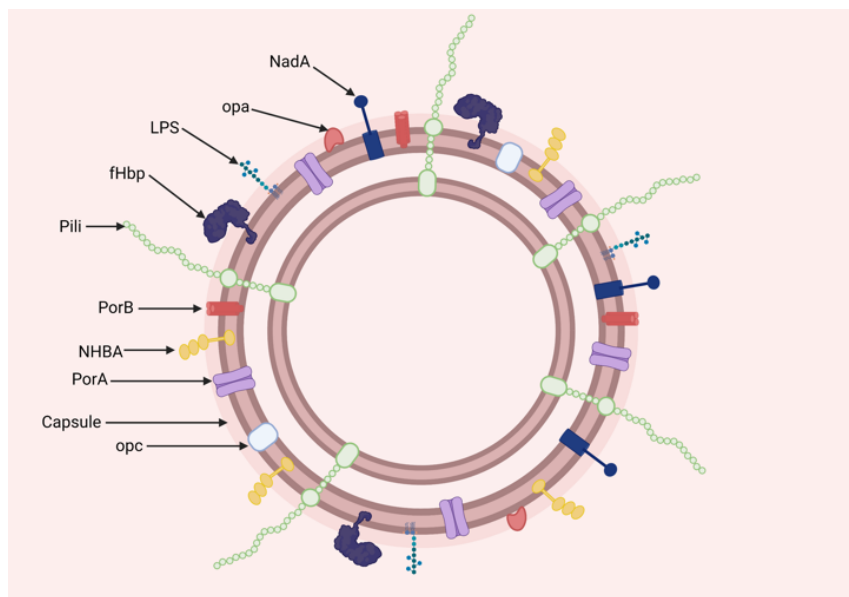


Figure 1.1. Diagram of *N. meningitidis* showing important virulence factors.

The virulence of *N. meningitidis* is determined by multiple factors, of which the major ones are shown in this diagram. Strains of *N. meningitidis* do not always have a capsule, but invasive strains are almost always encapsulated. The subcapsular cell envelope is made up of an outer membrane, a peptidoglycan layer and an inner membrane. Nad A= neisserial adhesin A; Opa/Opc= opacity proteins; LPS= lipopolysaccharide; fHbp= factor H binding protein; Por A/B= Porin A/B; NHBA= neisserial heparin binding antigen. Diagram created with biorender.com.

There are several virulence factors in the meningococcal repertoire which facilitate effective adhesion to human epithelial cells and immune evasion (table 1.1).

Table 1.1. Virulence factors of *N. meningitidis*.

Virulence factor		Role
Capsule		Not all meningococci are encapsulated and whether a strain is encapsulated or not impacts on whether it is likely to be a carriage or invasive strain. Loss of capsule expression favours carriage, as the presence of the capsule inhibits attachment by masking adhesion structures however, once invasion is achieved, the capsule allows the meningococcus to evade phagocytic activity through evasion of the complement system protecting it against destruction by the host (3).
Adhesins	Pili	Important in adhesion and invasion. The genes which code for the pilin subunit undergo frequent recombinational events which leads to a high degree of antigenic variation allowing the meningococcus to escape immune surveillance.
	Opacity proteins (Opa, Opc)	Facilitate the intimate adhesion of meningococci and invasion (2).

	Lipopolysaccharides (LPS)/Lipooligosaccharides (LOS)	Play an important role in adherence to host tissue and evading complement activity. They also play a role in activating the innate immune system and cause an inflammatory cascade (2).
	Porins (PorA and PorB)	Involved in host cell interaction and constitute a target for bactericidal antibodies (2).
	factor H binding protein	Downregulates the alternative complement system through binding to the human complement regulator factor H. It degrades complement component C3 on the surface of meningococci which enhances its capacity to resist complement mediated lysis.

Meningococcal strains can be classified in several ways: according to their polysaccharide capsule (serogroup), their class one outer membrane protein (OMP) (Por A) (serosubtype), their class two or three OMP (Por B) (serotype) and their lipopolysaccharide structure (immunotype) (4). According to the most commonly used of these, the serogroup classification, it is possible to identify 12 serogroups, of which six (A, B, C, W, X, Y) cause the vast majority of disease. Partly in response to the multiplicity of classification systems in use, a genetic typing system, multilocus sequence typing (MLST), was developed and is now used for the molecular typing of strains (4). This technique is performed by sequencing the DNA of portions of seven housekeeping genes (5) and allows the generation of sequence types (STs) which can be grouped together into clonal complexes (6) and represent closely related isolates. In the case of the meningococcus, STs which are grouped together share virulence characteristics and so specific clonal complexes can be identified as being 'hypervirulent'.

Meningococcal transmission is by inhalation of respiratory droplets, usually requiring close or prolonged contact. Acquisition of these meningococci can be transient, lead to colonisation or, in some cases, lead to development of invasive disease. Overall, in

non-epidemic settings about 10% of the population are asymptomatic carriers of *N. meningitidis* and only a fraction of these individuals develop invasive disease (7). Rates are different in different age groups and are highest in teenagers and young adults (8), although a recent cross-sectional study demonstrated that carriage rates in young people aged 15-19 have decreased significantly as a result of widespread vaccination and changes in risk behaviours for carriage (9).

In some people, colonisation results in the generation of protective mucosal and systemic antibody (10), functioning as an immunising event, whilst in a much smaller group, nasopharyngeal colonisation will be followed by invasion of the mucosa and IMD (4). This is usually within 14 days of colonisation being established (4). The factors which determine the development of invasive disease are complex and include pathogen and host factors. The virulence of the infecting strain is an important determinant of whether invasive disease occurs, with most cases of disease being caused by a limited number of clonal complexes (5, 11), in contrast to colonising strains which show more heterogeneity in the clonal complexes observed (6). The hypervirulent strains can, however, be found in the nasopharynx of asymptomatic carriers without progressing to disease (12). IMD is almost always caused by encapsulated organisms as this allows the meningococcus to evade complement mediated killing and provides a defence against phagocytosis (12). In contrast to this, only around 50% of colonising strains are encapsulated (7). Host factors are also important in the development of invasive disease – disruption of the mucosal barrier through smoking or exposure to cooking fires, or from recent viral upper respiratory tract infection leads to an increased risk of invasive disease (13). Because of the importance of the complement system, particularly the role played by the membrane

attack complex (MAC) in bacterial cell lysis, and the activity of bactericidal antibodies in defence against invasive meningococcal disease, individuals with hypogammaglobulinaemia and complement deficiencies, particularly deficiencies in components of the membrane attack complex (MAC), are also at increased risk of invasive disease (14). These aspects of immune defence will be discussed further in the next section.

1.1.3 Immune responses

The complement system plays an integral role in protection against meningococcal disease through a number of mechanisms, including opsonisation, chemotaxis, induction of a bactericidal response, enhancing antibody responses and immunological memory (15). In those cases where *N. meningitidis* penetrates the mucosa and enters the circulation, the complement system is rapidly activated. The complement system is a complex network of over 35 proteins which, in health, exist as inactive precursors (16). When complement proteins encounter meningococci, they are activated using one of three possible pathways: through the classical pathway mediated by antibody, through the alternative pathway mediated by binding of complement components to the meningococcal surface or through the mannose binding lectin (MBL) pathway in which MBL binds to mannose on the meningococcal surface (13). All these activation pathways involve a series of cleavage reactions and all result in the creation of convertase enzymes on the surface of the meningococcus (16). C3 convertases cleave C3 resulting in the production of C3b which binds to *N. meningitidis* and marks it for destruction by phagocytes, and becomes a component of the convertases which cleave C5 into C5a and b. The chemoattractant C5a, plays an important role in bringing phagocytes to the area, causes an oxidative burst and stimulates basophils

and mast cells (16), and C5b which initiates the terminal complement pathway (17).

The terminal complement pathway consists of a series of reactions which result in the creation of a MAC which assembles on the surface of *N. meningitidis* and creates a cytotoxic pore, causing bacterial death (18) (figure 1.2).

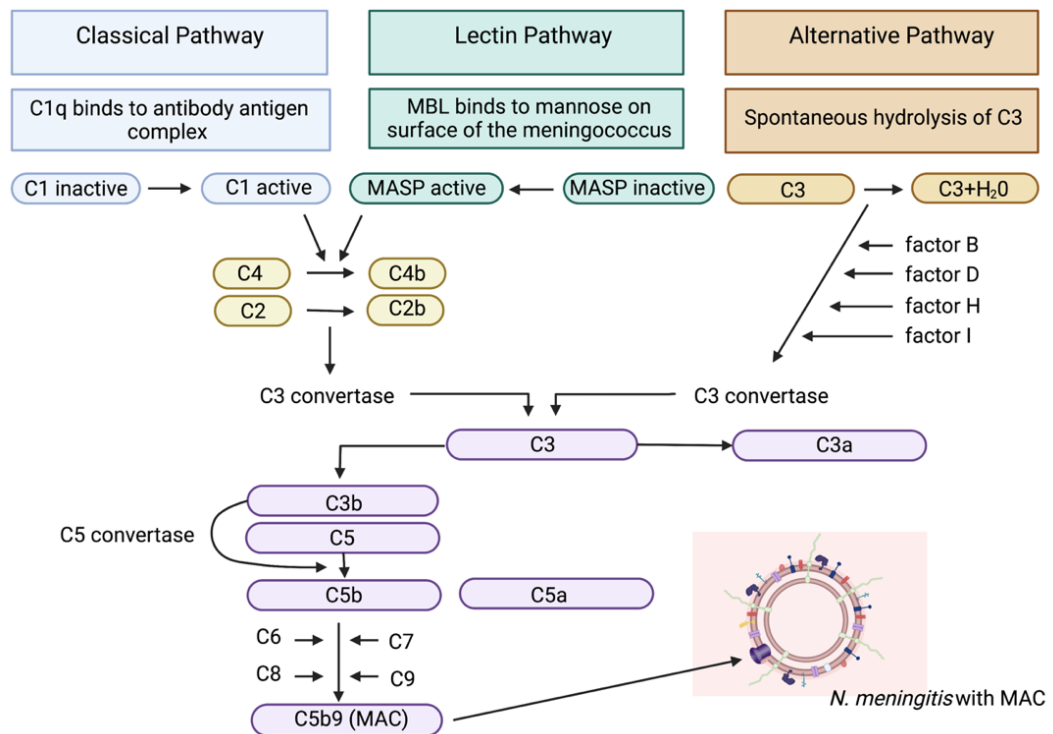


Figure 1.2. Diagram of the complement cascade.

All three pathways converge on the terminal pathway resulting in the creation of the membrane attack complex (MAC). MBL= mannose binding lectin; H₂O= water; MAC= membrane attack complex; MASP= mannan-binding lectin-associated serine protease-1. Diagram created with biorender.com.

The action of the MAC alongside the proinflammatory activity of C3a and C5a and the phagocytosis of opsonised cells play an important role in the innate immunity to *N. meningitidis* (19). As well as innate immunity, humoral immunity plays a vital role in defence against *N. meningitidis* and, in most cases, sufficient bactericidal antibodies are required for efficient immune defence. These antibodies can be maternally derived through placental transfer in young infants, through antibody production in response to carriage of either *N. meningitidis* or *N. lactamica*, or through vaccination. Although a

serum bactericidal antibody titre of $>1:4$ is usually thought to be protective, there is evidence that in some situations the mechanism of protection may be through alternative routes, particularly opsonophagocytosis (20).

1.1.4 Epidemiology

Information about the epidemiology of meningococcal disease is dependent on the nature and quality of the surveillance performed in a particular setting and wide variation in practice globally means that true comparisons of incidence can be extremely difficult (5, 21). From the available data, the epidemiology of meningococcal disease shows considerable global variation, both in terms of the number and pattern of cases and in the prevailing serogroups identified (figure 1.3).

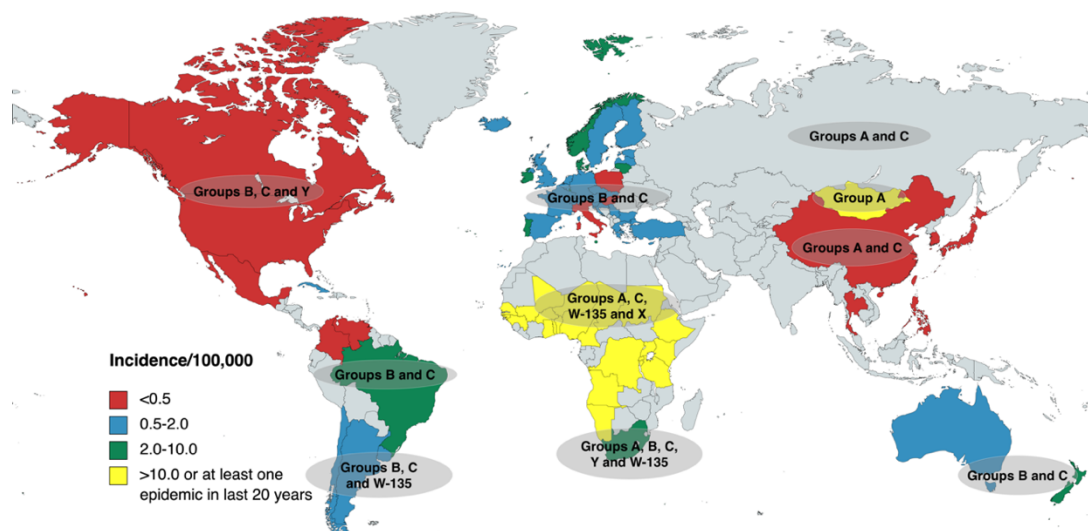


Figure 1.3. Map showing the incidence and predominant serogroups by region (5, 22). Created with mapchart.net

In North America there are fewer than 0.5 cases per 100,000 population, overall, in Europe just under 1 per 100,000 population and in most of Africa there are more than 10 cases per 100,000 population each year (23) with considerable variation between years depending on whether an epidemic, defined as a weekly incidence of 15 cases per 100,000 population, averaged over 2 consecutive weeks in an area with a

population of 30,000-100,000 (24), has taken place. In high income settings, serogroup B tends to predominate, although there has been an increase in cases caused by serogroup Y and serogroup W (12).

The incidence of IMD is usually highest in infants aged less than one year, half of all cases are in children under five years and there is a second peak in older teenagers and young adults (12). In 2019-2020 the overall incidence of IMD in England was 1 per 100,000 population: 10 per 100,000 population in infants and 2 per 100,000 population in those aged 1-4 years (25) (figure 1.4).

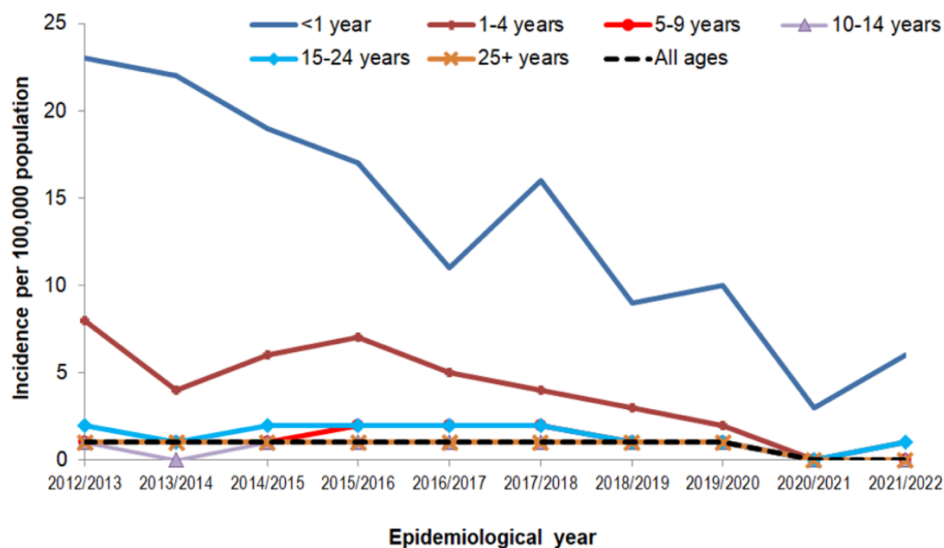


Figure 1.4. Incidence of IMD in England per 100,000 population from 2010/11 to 2019/20 (25).

Overall, the incidence decreased from 2010/11 to 2019/20 with the highest rates being seen in those less than one year followed by those aged 1-4 years. The very low rates reported in 2020/21 can be explained by the COVID-19 pandemic and the associated social distancing, following the removal of these restrictions case have increased again.

Serogroup B dominates in younger individuals, representing 88% of cases in infants (aged less than one year) and 90% in young children aged 1-4 years (26).

In England there has been a decline in confirmed cases of IMD since the peak of 2,595 cases in 1999-2000 (26). Initially this was driven by the introduction of the serogroup C

vaccination programme, and, in the last 10 years, this has mainly resulted from secular changes in serogroup B incidence (25) (figure 1.5).

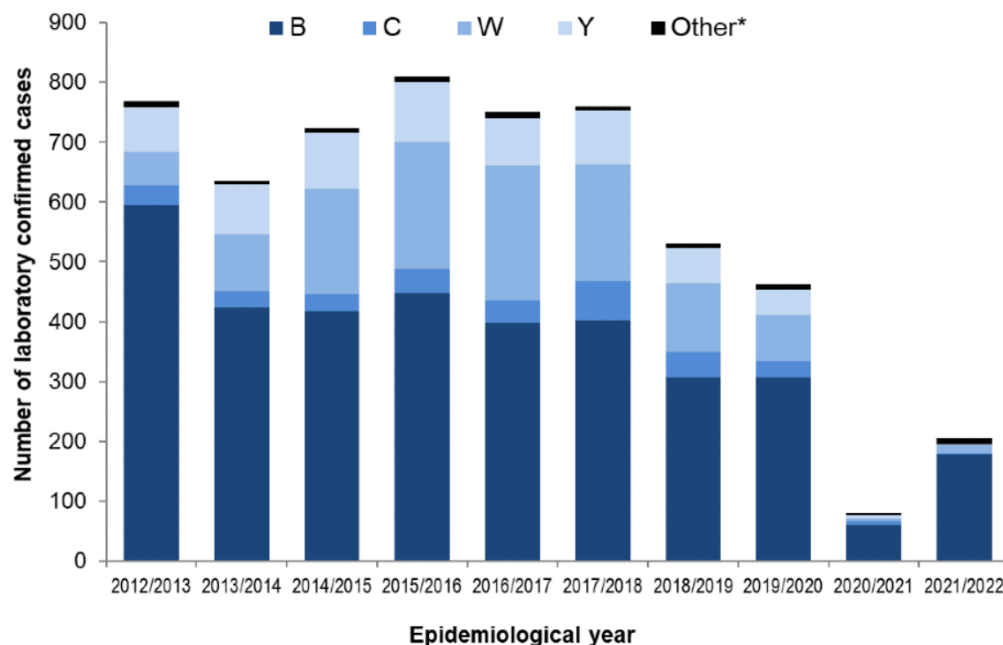


Figure 1.5. Invasive meningococcal disease in England by capsular group: 2011/2012 to 2020/2021 (25).

*Other refers to serogroups X, E, ungrouped (culture negative but PCR screen positive and negative for the four groups [B, C, W, Y] which are routinely tested for), and ungroupable.

There has also been a significant impact from the COVID-19 pandemic and the resulting social distancing and lockdown measures have led to a dramatic reduction in reported cases. For the epidemiological year (beginning of July to the end of June) 2020- 2021 there were 80 cases of IMD which is 83% lower than that seen in the epidemiological year 2019-2020 (25).

1.1.5 Clinical features

IMD most commonly presents as septicaemia or meningitis, but less common presentations such as septic arthritis and pneumonia are well recognised (figure 1.6).

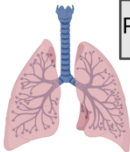
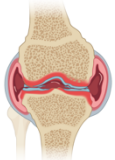
Presenting symptoms and signs		Differences in younger children
 Meningitis	•Fever •Nausea and vomiting •Photophobia •Headache •Agitation/reduced conscious level •Neck stiffness	•Most children <2 years do not develop specific features of meningitis •Neck stiffness less common in those aged less than 2 years •Bulging anterior fontanelle <18 months •Unconsolable crying
 Sepsis	•Fever •Drowsiness •Nausea and vomiting •Haemorrhagic rash •Leg pain •Cold peripheries	• Younger children sometimes present with increased work of breathing and diarrhoea
 Pneumonia	• Fever • Increased work of breathing • Chest pain • Cough	• Less common as a presentation in children
 Septic arthritis	• Hot, painful joint • Reduced range of movement • Usually monoarticular • Knee most commonly affected	• Less common as a presentation in children

Figure 1.6. Summary of presenting symptoms and signs of invasive meningococcal disease.

Clinical presentation differs somewhat according to the infecting serogroup (27), and as the epidemiology of meningococcal infection changes, some previously ‘atypical’ presentations, for example gastrointestinal symptoms associated with serogroup W, have become more common (28). In young children the clinical presentation may be less specific, which can make diagnosis difficult and may lead to delays. In children less than two years, irritability and lethargy are frequent presenting symptoms, and typical signs of meningeal irritation are rarely present (29). The case fatality rate (CFR) is lower in children than in adults; in a UK study for the period 2010-2015, children under 15 years had a CFR of 4.5% compared with a CFR of 6.6% in adults and 18.4% in older adults (30), in a Canadian study the CFR in children was 4.1% compared with a CFR of 12.5% in adults (31) and in a meta-analysis including 40 studies the CFR was lower in infants (CFR 9%) and young children (CFR 7%) than in young adults (CFR 15%), or older

adults (CFR 30%) (32). CFRs also vary according to the infecting serogroup, with highest CFR reported in groups W and C and the lowest CFR in group B (32).

1.2 Vaccination

Vaccines have had a transformative effect on the landscape of infectious diseases, including meningococcal disease. They induce an acute immune response, of which the most significant element is antibody production primarily through the activity of B-lymphocytes, although the importance of T-lymphocytes should not be underestimated, and also generate immune memory. Following vaccination B-lymphocytes are activated, engage with T-lymphocytes and proliferate, becoming plasma cells which produce antibody, or memory B-lymphocytes (33). After initial exposure there is a small, but rapid, increase in antibody followed by a greater, but slower, increase which is followed in turn by gradual waning (33). If there is repeated exposure, there is a much faster increase in antibody and the antibody produced decays at a slower rate to that produced after the initial exposure (33). This theory underpins the principle of priming and boosting in which one or more priming doses of vaccine are given fairly quickly, usually with a minimum of three weeks between doses, followed by a booster dose after a period of at least four months (33).

Long-term protection from vaccines depends on persistence of antibodies or memory B-lymphocytes which are able to respond to repeat exposure to the pathogen. The relative importance of these two elements of protection depends in part on the incubation period of the specific pathogen as, if disease onset is very rapid as seen in meningococcal disease, it is possible to become unwell before sufficient memory response has been achieved (34). Persistence of antibody response depends on the

nature of the antigen used in the vaccine, the schedule used, any adjuvants employed and the age at vaccination (34).

The first vaccines produced against IMD were polysaccharide vaccines, which although safe and immunogenic in adults, led to a weak, non-bactericidal response in infants.

The success of conjugate vaccines for *Haemophilus influenzae* type b (Hib) raised the possibility that such an approach could be used for meningococcal vaccines. In a conjugate vaccine the polysaccharide component of the bacterial target is bound to a protein and this combination is presented and processed in a T cell-dependent way, leading to long term memory even in infants aged less than two years (35, 36).

Conjugate vaccines for meningococcal disease were developed using tetanus toxoid, diphtheria toxoid or an inactive form of diphtheria toxin (CRM¹⁹⁷) as the protein for conjugation (37).

Attempts to produce a serogroup B vaccine using a capsular polysaccharide were unsuccessful because of the structural homology between group B polysaccharide and the poly-sialylated form of the neural cell adhesion molecule in the human foetal brain which resulted in poorly immunogenic vaccines because of immune tolerance (38). As a result of this, protein-based vaccines were developed based on detergent-extracted outer membrane vesicles (dOMV) (39). The antibody responses generated by dOMV vaccines depend on the PorA, which is highly variable, (39) and these consequently often provide only very narrow protection. They are therefore effective in the context of an outbreak when a single sero-subtype is implicated but are less useful as a vaccine in general use.

Following the development of dOMV vaccines, efforts were made to identify antigens which could generate broader responses (39) using a technique known as reverse vaccinology. In this process, the whole genome sequence of *N. meningitidis* was analysed to identify proteins which were likely to exist on the outer membrane and thus be able to generate an immunological response. These proteins were then amplified and used to vaccinate mice and the antisera analysed. A bactericidal response was elicited from 28 of these proteins and following assessment of the likely coverage and cross protective abilities, three were selected for use in the 4CMenB vaccine (40). As it was seen that the response was greater when these were combined with a dOMV, 4CMenB is made up of four components: Neisserial adhesin A (NadA), Neisseria heparin binding antigen (NHBA), factor H binding protein (fHbp), along with a dOMV (39).

Vaccines are routinely available against serogroup A, B, C, a combination vaccine for serogroups ACWY, and studies investigating serogroup X vaccines have been undertaken which show that it is immunogenic when combined with ACWY (41) (table 1.2).

Table 1.2. Currently licensed meningococcal vaccines.

	Manufacturer	Serogroup contained	Approved ages	Year licensed	Composition
Polysaccharide vaccines					
MenAfriVac®	Serum Institute of India	A	From 1 year	2010	<i>N. men</i> group A polysaccharide-not less than 10µg; Tetanus toxoid carrier protein-not less than 10-33µg
Conjugate vaccines					
Menactra®	Sanofi Pasteur	ACWY	9 months-55 years	2005	<i>N. men</i> group A polysaccharide-4µg

					<i>N. men</i> group C polysaccharide-4µg <i>N. men</i> group W-135 polysaccharide-4µg <i>N. men</i> group Y polysaccharide-4µg Diphtheria toxoid protein- 48µg
Menveo®*	GSK	ACWY	2 months to 55 years	2010	<i>N. men</i> group A oligosaccharide-10µg <i>N. men</i> group C oligosaccharide-5µg <i>N. men</i> group W-135 oligosaccharide-5µg <i>N. men</i> group Y oligosaccharide-5µg CRM ₁₉₇ protein
Nimenrix®*	Pfizer	ACWY	From 6 weeks	2012	<i>N. men</i> group A polysaccharide-5µg <i>N. men</i> group C polysaccharide-5µg <i>N. men</i> group W-135 polysaccharide-5µg <i>N. men</i> group Y polysaccharide-5µg Tetanus toxoid carrier protein-44µg
MedQuadfi®*	Sanofi Pasteur	ACWY	From 12 months	2020	<i>N. men</i> group A polysaccharide-10µg <i>N. men</i> group C polysaccharide-10µg <i>N. men</i> group W-135

					polysaccharide-10µg <i>N. men</i> group Y polysaccharide-10µg Tetanus toxoid carrier protein-55µg
Menitorix®*	GSK	C (and Hib)	From 2 months	2005	<i>Haemophilus</i> type b polysaccharide-5 µg Tetanus toxoid carrier protein-12.5µg <i>N. men</i> group C polysaccharide Tetanus toxoid carrier protein-5µg
Menjugate®*	GSK	C	From 2 months	2000	<i>N. men</i> group C oligosaccharide-10µg CRM ₁₉₇ protein-12.5-25µg
NeisVac-C®*	Pfizer	C	From 2 months	2001	<i>N. men</i> group C polysaccharide-10µg Tetanus toxoid-10-20µg
Protein vaccines					
Bexsero®*	GSK	B	From 2 months	2015	Recombinant <i>N. men</i> group B NHBA fusion protein- 50 µg Recombinant <i>N. men</i> group B NadA protein- 50 µg Recombinant <i>N. men</i> group B fHbp fusion protein- 50 µg OMV from <i>N. men</i> group B strain NZ98/254- 25 µg
Trumenba®*	Pfizer	B	From 10 years	2014	<i>N. men</i> serogroup B fHbp subfamily A- 60µg

Chapter 1- Introduction

					<i>N. men</i> serogroup B fHbp subfamily B- 60µg
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N. men= *Neisseria meningitidis*; GSK=GlaxoSmithKline; fHbp= factor H binding protein; OMV= Outer Membrane Vesicle; NadA=Neisserial adhesin A; CRM= cross reacting material of diphtheria toxin; NHBA, Neisserial heparin binding antigen

*Licensed in UK

4CMenB was introduced to the UK schedule in 2015 and uses a reduced two-dose priming schedule (8 and 16 weeks with a booster at 12 months), which has been shown to be immunogenic and effective in preventing serogroup B disease in term infants (42, 43). In addition, in the UK schedule, a serogroup C vaccine is offered in combination with Hib at 12 months and an ACWY vaccine is offered at 14 years (44). Because of the highly conserved structure of capsular polysaccharide, when adequate immune responses to reference strains are demonstrated for polysaccharide vaccines this can usually be assumed to extend to all strains belonging to the same serogroup (45). The situation is more complicated for protein vaccines such as 4CMenB because the target proteins show considerable variation both in their sequence and their expression. Protection against a strain depends on the infecting strain expressing an antigen which cross-reacts with one of those included in the vaccine. For this reason, several techniques have been developed which allow an assessment to be made of vaccine coverage within a population based on knowledge of the infecting serogroup B strains. A summary of these techniques is shown in table 1.3, but a slightly more detailed description of the meningococcal antigen typing system (MATS) is provided here as this is the system referred to most within this thesis. MATS is specific to the 4CmenB vaccine and employs three antigen specific enzyme linked immunosorbent assays (ELISAs) (factor H binding protein [fHbp], neisserial adhesinA [NadA] and neisserial heparin binding antigen [NHBA]) and genotyping and phenotyping

information for Porin A (PorA) with the aim of assessing whether a particular meningococcal strain expresses antigens which cross react with those in the 4CmenB vaccine (46). In the MATS ELISA, both the quantity of antigen expressed by the bacteria and immunologic cross-reactivity with the antigen in the vaccine is assessed and the reactivity of each strain is compared to a reference strain. The difference between the reactivities is known as the relative potency (RP). If the RP is higher than a previously established minimum RP predictive of killing in the serum bactericidal antibody (SBA) assay the strain is considered to be covered (46). For Por A, a strain is covered if it expresses the variable region 2 P1.4 that matches that seen in the vaccine's outer membrane vesicle (OMV) component (45).

Table 1.3. Techniques to assess Men B vaccine coverage.

Methods for prediction of coverage	Method	Limitations
Meningococcal antigen typing system (MATS)	ELISAs for fHbp, NadA and NHBA and genotyping for PorA. Coverage is considered to be achieved when relative potency is higher than the bactericidal threshold for at least one of fHbp, NadA or NHBA, or for Por A when variable region 2 P1.4 is expressed in the bacterial strain.	May underestimate the coverage as does not consider any synergistic effects between vaccine components or between different OMV components Requires cultured isolates
Genetic MATS (gMATS)	Assessment of the level of correlation between MATS coverage estimates and antigen genotyping for each of the four vaccine antigens in 4CmenB. Coverage is considered to be achieved if the sequence of the antigen-encoding gene corresponds to peptides predicted as covered on the basis of MATS.	Underestimates strain coverage as for MATS Does not predict coverage for new alleles where MATS data are not available
Bexsero antigen sequence typing (BAST)	Genotypic assessment of the antigen on a strain compared with the genetic profile of the 4CmenB antigens. Coverage is considered to be achieved when the genetic profile of the strain antigen matches that of the vaccine.	Based on limited phenotypic data May underestimate coverage as does not take into account any synergistic effects

		Cannot predict coverage for new alleles
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1.3 Preterm birth

1.3.1 Epidemiology of preterm birth

Preterm birth is defined as delivery of a liveborn infant before 37 weeks of gestation (WG), and this can be divided into extremely preterm (<28 WG), very preterm (28-31⁺⁶ WG), and moderate to late preterm (32-36⁺⁶ WG) (47). In 2014, the rate of preterm birth was estimated globally to be 10.6%, although there is considerable variability between different countries, with rates ranging from 5% in some northern European countries to 18.1% in Malawi (figure 1.7) (47). There are significant limitations in estimates of preterm birth, particularly those from low-income settings where there may be less established systems of data collection, less robust methods of gestational age assessment and misclassification of some important outcomes (48).

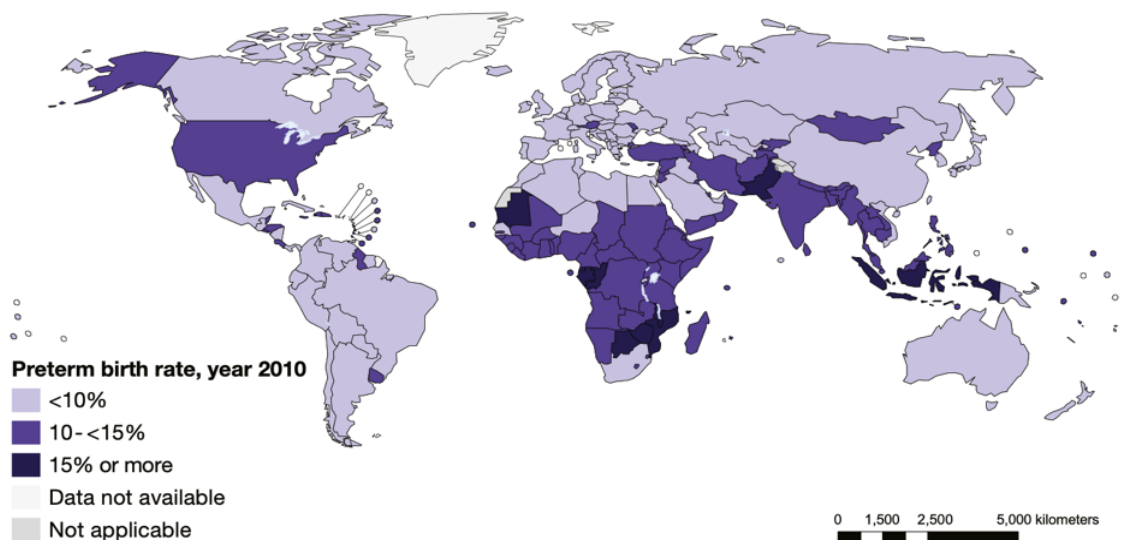


Figure 1.7. Preterm birth rate across countries in 2010, image taken from ‘Born too Soon’ (47).

Although the quality of the data is variable, there is a marked difference in estimates of preterm birth between countries globally. High income countries tend to have lower reported rates of preterm birth, with the United States being an interesting exception.

In England the rate of preterm birth is around 7-8% and has remained stable over the last 10 years. The vast majority of preterm infants are moderate to late preterm (figure 1.8).

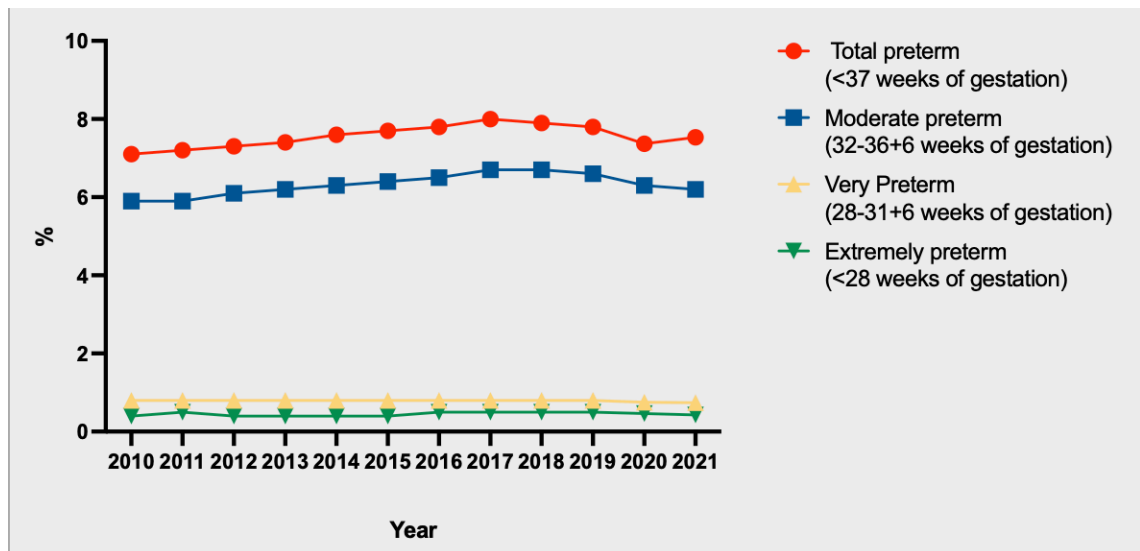


Figure 1.8. The percentage of babies born at less than 37 weeks of gestation in England from 2006-2016 with breakdown according to gestational age (data from the Office of National Statistics) (49).

Risk factors for preterm birth include maternal history of preterm delivery, maternal smoking, multiple pregnancy, increased or lower maternal age, shorter periods between pregnancy, low or high maternal body mass index, infection, maternal smoking, and socioeconomic deprivation (47, 50). The morbidity and mortality of preterm infants is strongly associated with gestational age: a systematic review reported survival rates (survival to discharge or follow up periods of 1-6 years) of babies born alive at 22 WG as 7.3%, compared with 90.1% of those born alive at 27 WG (51). Infection is an important cause of mortality in preterm infants (52, 53) and preterm infants have a continued increased risk of infection which extends throughout their first year of life (54).

1.3.2 Immunity in preterm infants

Immune development *in utero* is a delicate balance between developing foetal immunity in preparation for the transition to *ex utero* life and maintaining immune tolerance between the mother and foetus. Whilst there is increasing evidence that more immune development takes place in the foetus than was previously thought (55), infants at birth have an immune system which reflects this balance and can be seen in many respects to still be developing when compared with older children and adults. This situation is seen more dramatically in infants who are born preterm.

Optimal defence against pathogens requires an effective adaptive and innate immune system, and adequate communication between the two.

Newborn infants are dependent to a large extent on their innate immunity: the uterine environment provides little antigen exposure and adaptive immunity takes several years to develop fully (56).

Innate immunity

- **Antimicrobial proteins and peptides (APPs)**

Neonatal levels of APPs including alpha- and beta-defensins, bacterial/permeability increasing protein (BPI), cathelicidin, calprotectin and secretory phospholipase A₂ are variable in term infants, showing reductions for some APPs, for example calprotectin, whilst for others, for example cathelicidin and secretory phospholipase A₂, levels are similar to that seen in maternal blood (57). In preterm infants the pattern is of a general reduction in levels across APPs compared with older children and adults (57, 58).

- **Complement**

Most complement components are synthesised in the foetal liver and all components are detectable by 18-20 WG (59). Despite this development *in utero*, most complement components are found in lower levels in neonates compared with older children and adults, with even lower levels being seen in preterm infants. A notable exception to this is C7, which is predominantly produced in neutrophils rather than the liver and which is found in similar levels to the adult in term and preterm infants (60). In term infants, levels of C5, C6 and C8 are half or less than those found in adults and are around only 10% for C9 (59). Complement proteins C3, C4 and C5 are lower in preterm than term infants and the total functional activity of the classical pathway is also reduced in this population (59). Regulatory factors H and I are also reduced in neonates, with greater reduction seen in preterm infants (59). Factor H is an important regulator of the alternative complement pathway, and this factor is especially important in meningococcal infection as it binds to the factor H binding protein on the surface of meningococci.

- **Granulocytes**

Preterm infants have lower absolute counts of basophils, neutrophils and eosinophils compared to term infants (61). All neonates have impaired neutrophil function compared with older children and adults and this is more marked in those born preterm. Preterm infants have a reduced pool of neutrophils, leading to rapid depletion of stores at times of infection (62, 63) and their neutrophils show reduced functional capabilities: they are less efficient at chemotaxis, have reduced capacity for slow rolling and adhesion and are slower at phagocytosis, all of which mean that

neutrophils are slower to travel to sites of infection and less effective at killing when they arrive (63).

- **Monocytes and macrophages**

Whilst neonates have similar numbers of monocytes and macrophages as adults, in preterm infants these have a reduced ability to be recruited by chemotaxis to sites of inflammation, reduced efficiency of antigen presentation and less efficient phagocytosis (64).

- **Dendritic cells**

Dendritic cells play a vital role in linking the innate and adaptive immune systems through their role as the main antigen presenting cells (APC) for naïve T cells (64). In neonates, dendritic cells are less able to activate antigen specific T and B responses even though toll like receptor (TLR) expression is similar across adults, term and preterm infants. This can adversely affect the neonatal response to vaccination (64). When dendritic cells of preterm infants are stimulated, they produce anti-inflammatory cytokines rather than the pro-inflammatory cytokine response which is seen in term infants. This may leave preterm infants more vulnerable to infection (64).

- **Natural Killer (NK) cells**

Preterm infants have significantly reduced populations of NK cells; the absolute counts of these cells are correlated with gestational age (61, 65) and a reduction in these cells is associated with an increase in infection in this population (65, 66).

Adaptive immunity

- **Cell mediated responses**

Populations of both CD4⁺ and CD8⁺ cells are reduced in preterm infants; there is a greater reduction in CD8⁺ cells which leads to an increased CD4/CD8 ratio and may

contribute to the increased risk of infections in these infants (64, 67). Following antigen stimulation, CD4⁺ cells in preterm infants show a tendency towards Th2 and Th17 rather than Th1 polarisation, although this is probably not because of an intrinsic deficiency of the cells, but rather that they lack the stimulatory environment to mount a strong Th1 response (68). The reduction of T cells and the limited Th1 response renders infants vulnerable to persistent viral and disseminated infections and contributes to a reduced response to vaccines administered in early life.

- **Humoral responses**

Neonates are largely dependent on maternally derived immunoglobulin G (IgG), the only immunoglobulin able to cross the placenta. Placental transfer of IgG occurs at very low levels early in pregnancy, with a gradual increase as gestation progresses and an exponential increase in the last few weeks of pregnancy (69). Preterm infants have a shorter overall period for antibody transfer, and they miss the period of most effective transfer (>35 WG) rendering them deficient in maternal antibody compared with their term counterparts. Whilst the previous perception that the uterine environment is sterile has been challenged (64), it is undoubtedly the case that the foetus is antigenically naïve compared with older children and adults and this, combined with the neonate's reduced ability to class switch antibodies, leads to a reduction in immunoglobulin production and a dominance of IgM (56). This situation is exacerbated in preterm infants who are less able to class switch than their term counterparts (70), resulting in a situation in which preterm infants who have least maternal antibody because of their shortened gestation are also less able to produce endogenous IgG and are consequently doubly antibody deficient. This reduction in available antibody renders preterm infants more vulnerable to infection.

- **Immunoregulatory functions**

Regulatory T cells (Tregs) play a vital role in immune regulation during pregnancy and levels are increased during this period (61). Absolute counts of Tregs are similar in preterm and term infants, but the percentage of these populations are significantly higher in preterm infants with an inverse correlation being observed between gestation and percentage of Tregs (61, 71). This may partly explain the reduction in other immune cell populations in preterm infants, as Tregs are involved in the suppression of T cells, NK cells, B cells, macrophages and dendritic cells (61).

1.3.3 Infectious diseases in preterm infants

The combination of the immature immunity of preterm infants and the medical interventions they require render them vulnerable to infection and these infections are an important source of morbidity and mortality in this population. Preterm infants have been shown to be at more risk of early onset (72) and late onset sepsis (73) and are also at more risk of some vaccine preventable diseases. Despite a well-established pneumococcal vaccination programme, preterm infants remain at increased risk of invasive pneumococcal disease (Incidence Rate Ratio (IRR) 2.87 [95% CI 2.28-3.59]), with those infants born before 28 WG being at greatest risk (IRR 8.8 [95% CI 4.63-15.16]) (74). There has also been shown to be a higher incidence of pertussis infection in preterm infants (IRR 1.65 [95% CI 1.32-2.07]), greatest in those born before 28 WG (IRR 4.49 [95% CI 2.33-8.67]) (75), and an increased rate of hospitalisation in these infants (75, 76). This increased risk of hospitalisation is also seen for influenza (adjusted hazard ratio (aHR) 2.33 [95% CI 1.85-2.93]), with the greatest risk being for infants born extremely preterm (aHR 4.07 [95% CI 2.63-6.31]) (77).

1.3.4 Vaccination in preterm infants

It is recommended that babies born preterm are vaccinated according to their chronological age, with no adjustment made in the timing of routine vaccines according to prematurity, although there is evidence that preterm infants are often vaccinated later than their term counterparts (78-87). This delay may result from concerns that vaccines are less effective in preterm infants, or that the administration of vaccines might be associated with clinical deterioration. There are studies suggesting that antibody responses are reduced in preterm infants for some vaccines (88, 89). For meningococcal vaccines, there is evidence that vaccines against serogroup C are equally as immunogenic in preterm and term infants when given according to a multiple dose primary schedule (90-93), and there have not previously been any studies investigating the immunogenicity of vaccination against serogroup B disease using the reduced schedule in a preterm population.

1.4 Assessing meningococcal vaccine immunogenicity

IMD is relatively rare, which makes efficacy studies of meningococcal vaccines challenging. The work of Goldschneider and colleagues in the 1960s (94) showed that serum bactericidal antibody activity is highly correlated with immunity to IMD and in 1976 the WHO recommended using serum bactericidal antibody (SBA) assays for the evaluation of meningococcal vaccines (95). This is now established as the surrogate of protection for vaccine licensure (96). The SBA assay is a functional assay which measures the ability of antibody to kill bacteria, in this case meningococci, in the presence of complement (97). In assessments of immunity following 4CMenB vaccination, SBA assays are performed using three or four indicator strains (NZ98/254

[Por A], 44/76-SL [fHbp], 5/99 [NadA] +/- M10173 [NHBA]). These are strains which are matched to one of the main vaccine antigens (98). Heat inactivated sera is serially diluted in a 96 well plate and bacteria and complement are added to the relevant wells. A fixed volume from those wells containing complement and bacteria is plated out using the tilt method and incubated overnight (these are the time 0 (T_0) plates). The 96 well plates are incubated for 60 minutes, and a fixed volume then taken from the serial dilution wells and plated out using the same method (these are the time 60 (T_{60}) plates). These plates are then incubated overnight and the colony forming units counted. The bactericidal titre is expressed as the dilution of test sera yielding $\geq 50\%$ killing compared with the number of bacteria at the start of the test (99) (figure 1.9).

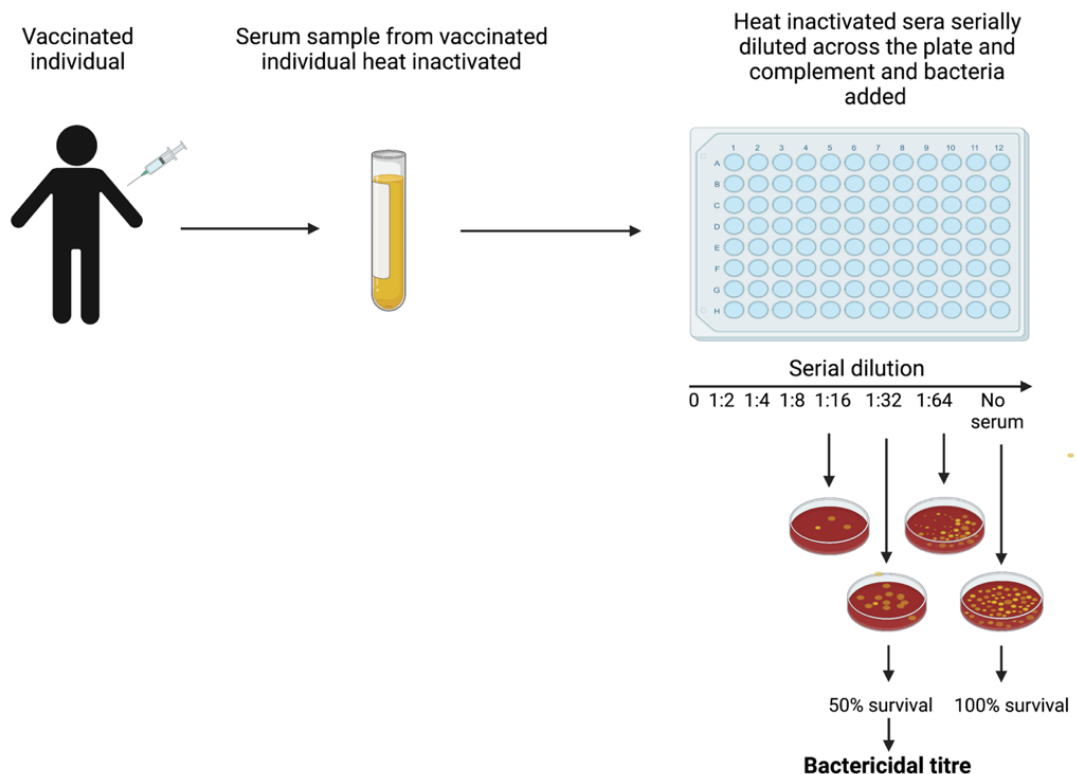


Figure 1.9. Serum bactericidal antibody assay.

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The source of complement used in SBA assays is important: for serogroups A, C, W and Y, both rabbit and human complement have been used (97). Rabbit complement tends to be preferred because of its wider availability, although the titres from SBA assays using rabbit complement are higher than those using human complement (100). This can be explained by the species specificity of some regulatory complement proteins, for example factor H, such that in the presence of rabbit complement the bacterial killing is enhanced because of the increased complement activity (101). For serogroup B, human complement must be used as rabbit complement leads to unacceptably high titres (102, 103). Neonatal complement differs in its composition from adult complement and these differences are more pronounced in neonates born preterm ([1.3.2](#)). Despite this, in assays evaluating the immunogenicity of vaccines in infants, complement from adults is used which may give a false estimation of the antibody titre in the infant.

Although SBA assays are the gold standard, reflecting the critical importance of bactericidal antibodies in protection against IMD, they do have some negative features such as the requirement for relatively large amounts of sera and their labour intensity, and other assays can also be helpful in exploring the immunogenicity of meningococcal vaccines. Antibody dependent complement deposition (ADCD) assays can allow investigation of specific parts of the complement pathway, for example an ADCD assay which uses the complement components C3b and C5b9 allows an exploration of both opsonophagocytosis and bactericidal activity.

1.5 Summary and aims

1.5.1 Summary

IMD continues to cause significant morbidity and mortality, although vaccination programmes have had a considerable impact on disease incidence. Preterm infants are vulnerable to infection and may be at increased risk of IMD. Despite this there are significant gaps in knowledge about the epidemiology of infection in preterm infants, the immunogenicity of vaccines against serogroup B, which is the most common serogroup in UK infants, in this population and the ways in which immunity is assessed following meningococcal vaccination in preterm infants.

1.5.2 Aims

1. To systematically review the evidence for the immunogenicity, efficacy and effectiveness of routine vaccines in preterm infants ([chapter three](#))
2. To systematically review the evidence for the reactogenicity and safety of routine vaccines in preterm infants ([chapter four](#))
3. To investigate the epidemiology of invasive meningococcal disease in preterm infants in England ([chapter five](#))
4. To compare the immunogenicity of two vaccine schedules of meningococcal group B vaccine in a clinical trial of UK preterm infants ([chapter six](#))
5. To describe the safety of routine vaccines (including 4CMenB) in preterm infants ([chapter seven](#))
6. To assess the use of human complement from age matched sources in assays measuring meningococcal immunity ([chapter eight](#)).

2 Methods

This chapter describes the methods used to address the specific research questions of the thesis.

2.1 Systematic review: Immunogenicity, efficacy, effectiveness, reactogenicity and safety of routine vaccines given in the first eighteen months of life to preterm infants.

2.1.1 Registration

This study has been registered on the International Prospective Register of Systematic Reviews (PROSPERO), registration number: CRD42021225937.

2.1.2 Search strategy

The systematic review was undertaken according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (104). The search strategy included medical subject headings (MeSH) and free text terms ([appendix A](#)).

MEDLINE, EMBASE, Web of Science, Cochrane CENTRAL and Latin American and Caribbean Health Sciences Literature database (LILACS) were searched. The search was restricted to English language and human participants only and included papers from the year 2000. The final search was performed on 6th November 2022. Snowball searching was performed in which the references of all papers were searched for further relevant references.

2.1.3 Eligibility criteria (figure 2.1)

-Population: Reports were included if they involved preterm infants (born at less than 37 weeks of gestation [WG]). If both term and preterm infants were included, only reports which allowed separation of the data relating to each group were included. Reports were not included if 'preterm birth' was specified, but included infants born after 37 WG, without separation of data for the two different populations.

-Intervention: Studies were included if there was administration of any of the vaccines currently given as part of the routine vaccination schedule in the UK in the first eighteen months of life (diphtheria, tetanus, pertussis, *Haemophilus influenzae* type B, polio, hepatitis B, pneumococcus, meningococcus B, meningococcus C, rotavirus, measles, mumps, and rubella). Studies were included irrespective of the schedule used, provided that vaccines were given after the neonatal period (i.e., at greater than 28 days). Vaccines which are given only to "at-risk" groups (e.g., Bacillus Calmette-Guérin [BCG], birth doses of hepatitis B vaccine, influenza) were not included.

-Comparison and outcome:

Immunogenicity:

Outcomes- Antibody concentrations or titres and/or proportions putatively protected (where a correlate of protection is known).

Comparisons- Preterm and term infants, vaccinated and unvaccinated preterm infants, as well as where no comparison between populations was made.

Reactogenicity:

Outcomes- Reactogenicity outcomes that refer to physical manifestations of the inflammatory response to vaccination which occur soon after vaccination.

Comparison- Local and systemic adverse events compared between preterm and term infants, vaccinated and unvaccinated preterm infants, as well as where no comparison between populations was made. Cardiorespiratory events following vaccination compared between vaccinated and unvaccinated preterm infants, as well as where no comparison between populations was made.

Safety:

Outcomes- Safety outcomes that refer to adverse events that could potentially have been caused by vaccination or worsened at any time after vaccination and include serious adverse events, hospitalisation and death.

Comparison- Term and preterm infants, vaccinated and unvaccinated preterm infants, no comparison between populations.

Efficacy/effectiveness:

Outcomes- Vaccine efficacy and vaccine effectiveness.

Comparison- Efficacy or effectiveness compared between preterm and term infants and vaccinated and unvaccinated preterm populations.

Population	Preterm infants born at <37 weeks			
Intervention	Routine vaccinations given to infants within the first 18 months of life			
Comparison	Vaccinated compared with unvaccinated preterm infants	Preterm compared to term	No comparison between populations was made.	
Outcomes	Immunogenicity	Local and systemic adverse events; cardiorespiratory events	Efficacy and/or effectiveness	Unsolicited adverse events or serious adverse events

Figure 2.1. PICO criteria for the systematic review.

2.1.4 Study design

Randomised controlled trials (RCTs), surveillance studies and observational studies including case series, cohort studies, case control studies, before and after studies, retrospective reviews and cross-sectional studies were included. Any study type other than these, including non-human studies, in vitro studies, single case reports, opinion pieces, conference proceedings and review articles were excluded, although the reference lists of review articles were checked for any relevant publications. Included studies were categorised based on the study descriptions in table 2.1 and using the flow chart in figure 2.2.

Table 2.1. Classification of studies (105, 106).

	Classification of study	Description
Experimental	Randomised controlled trial	Infants randomly allocated to intervention or control groups and followed up over time
	Quasi randomised trial	Infants allocated to intervention or control groups by the investigator, but the method of allocation falls short of true randomisation (e.g. by date of birth)
	Non randomised	Infants allocated to a group by investigator, but allocation entirely non-random (e.g. allocated according to clinician's preference)
Observational	Concurrent cohort study	A follow up study that compares outcomes between two groups. Participants are studied during the same period – this can either be prospective or retrospective
	Historical cohort study	The outcome from a new intervention is established for participants studied in one period and compared with those who did not receive the intervention in a previous period
	Case-control study	Participants with and without a given outcome are identified and exposure to vaccination is compared
	Cross-sectional study	Snapshot observation of a population at a single time point
	Case series	Report of a number of patients who have received vaccination and been followed up regardless of the duration of follow up. There is no control group
	Retrospective review	A study using existing data collected for clinical non-research related purposes
	Surveillance study	Report from national surveillance data

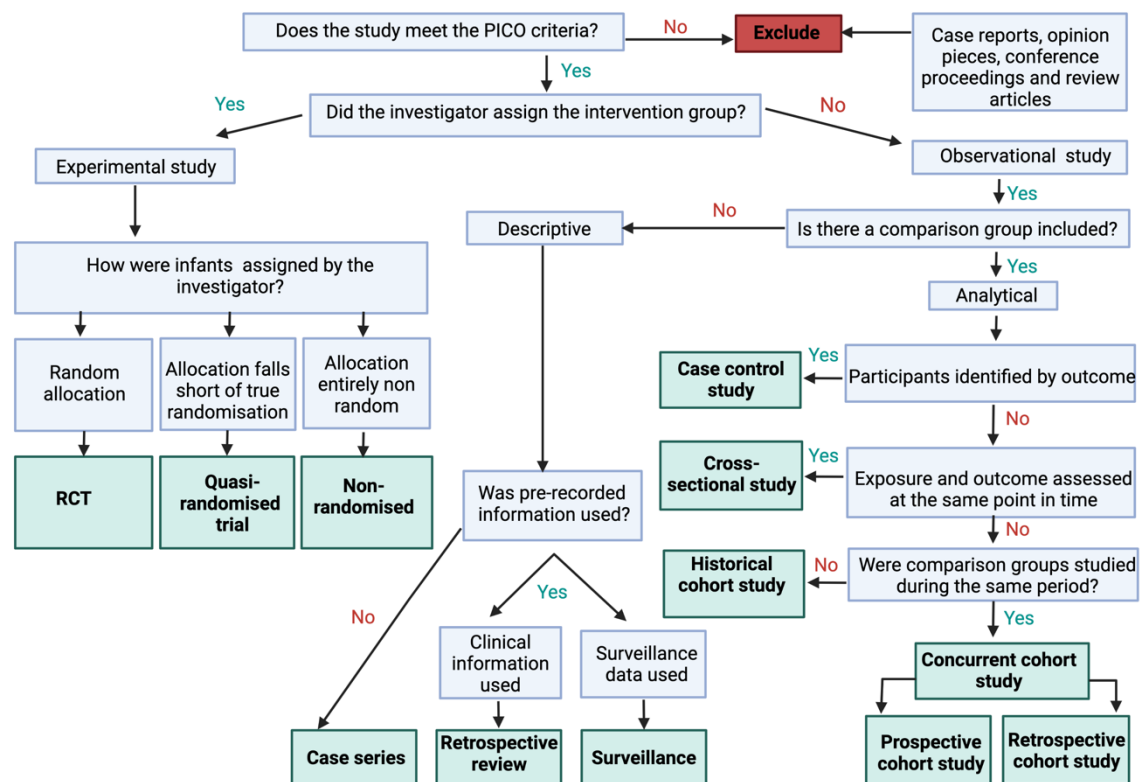


Figure 2.2. Flow chart for the classification of studies (105, 106).

-Timing of outcome data: There were no restrictions on reports based on the timing between vaccination and collection of samples for immunogenicity assessment. Reactogenicity studies were restricted to the recording of local and systemic adverse events within seven days of vaccine administration. Safety outcomes were included if they were collected at any point during the study.

2.1.5 Data management

Results from the literature search were uploaded onto Rayyan software to facilitate blinded reviews and de-duplication.

2.1.6 Selection process

Titles and abstracts were reviewed by two reviewers, Anna Calvert and Emily Shaw (neonatology GRID trainee), against the eligibility criteria using Rayyan software to

record decisions. Any disagreements were discussed with a third reviewer (Paul Heath, Professor of Paediatric Infectious Diseases). The full text of all the papers which appeared to meet the inclusion criteria were obtained and screened by both reviewers. For those papers where there was any uncertainty the paper was reviewed by a third reviewer (PH) and a decision made through discussion.

When multiple reports containing overlapping data were produced from the same study, these were treated as a single study with reference made to results from all available reports. When multiple reports were produced from the same study, containing entirely the same data, only one report was included in the systematic review.

2.1.7 Meta-analysis

Where there were sufficient studies with comparable data for a particular outcome, these were included in a meta-analysis using a random effects model. This was performed using Stata version 17 (StataCorp, College Station, Texas).

2.1.8 Risk of bias assessment

Risk of bias assessments were performed by the same two reviewers for all of the included studies using a tool selected specifically for the type of study. For randomised controlled trials the Cochrane Risk of Bias (ROB) tool was used, for non-randomised trials which included a comparison group the Risk of Bias in non-randomized studies of interventions (ROBINS-I) was used, for retrospective reviews the NIH quality assessment tool for before-after (pre-post) studies with no control group was used and for case series the NIH quality assessment tool for case series studies was used.

2.1.9 GRADE assessment

GRADE assessments were performed for each of the identified outcomes considering the domains of risk of bias, inconsistency of results, indirectness of evidence and publication bias. Using these domains, an overall assessment of quality of the evidence was produced. This was performed collaboratively by both reviewers through discussion.

2.2 Invasive Meningococcal disease in Preterm infants

This epidemiological study was a retrospective analysis of all cases of IMD in infants aged less than one year in England for the time-period 1st September 2015 to 31st August 2020, to allow comparison of cases of IMD in term (≥ 37 WG) and preterm (< 37 WG) infants.

2.2.1 Identification of cases

The UK Health Security Agency (UKHSA) collects information about all cases of IMD as part of their enhanced surveillance programme. The UKHSA are automatically informed about all invasive meningococcal isolates by NHS laboratories through the second-generation surveillance system (SGSS) as well as being informed about clinically suspected cases by clinicians. Invasive clinical isolates are sent to the UKHSA Meningococcal Reference Unit (MRU) for confirmation and strain characterisation, including serogrouping, typing and whole genome sequencing (107).

2.2.2 Data collection

For confirmed cases, clinical details including demographics, medical history, vaccination status, clinical course, outcome and sequelae were collected from the

patient's general practitioner (GP) using standard surveillance questionnaires ([appendix B](#)) as part of the enhanced UKHSA surveillance programme. Additional information was obtained in some cases from HPzone, an online public health platform supporting case and outbreak management (108), and from hospital clinicians and post-mortem reports where necessary. Fatalities were identified through the surveillance questionnaires, the personal demographics service (an online system which matches patients and their health records (109)) and electronic death registrations provided by the office of national statistics (ONS) to UKHSA for public health surveillance purposes. This data collection was performed by members of the UKHSA team as part of the routine surveillance process.

Gestational age information was missing for a significant number of infants in this study, as this data point had been added to the surveillance questionnaire quite recently. To obtain the missing gestational age information for surviving infants I contacted GPs and for those who had died and for whom this information was missing I contacted the treating hospitals. All information was stored on a password protected UKHSA laptop.

2.2.3 Ethical approval

UKHSA has legal permission, provided by Regulation 3 of The Health Service (Control of Patient Information) Regulations 2002, to process confidential patient information for national surveillance of communicable diseases and no additional ethical approval was needed for this study.

2.2.4 Population

All infants in England with IMD at less than 365 days of age between 1st September 2015 and 31st August 2020.

2.2.5 Definitions

Diagnosis

IMD was defined as detection of *N. meningitidis* from a normally sterile site, using culture and/or polymerase chain reaction (PCR). Meningitis was defined as presence of *N. meningitidis* in cerebrospinal fluid (CSF) or clinical features consistent with meningitis, alongside evidence of *N. meningitidis* in a blood sample. Septic arthritis was defined as the presence of *N. meningitidis* in joint fluid or clinical features consistent with septic arthritis, alongside evidence of *N. meningitidis* in a blood sample. Septicaemia was defined as evidence of *N. meningitidis* in a blood sample, without a clinical focus of infection, irrespective of the clinical condition of the patient.

2.2.6 Risk factors

Risk factors for IMD were defined as asplenia, complement deficiencies and receiving complement inhibitor medications, for example eculizumab.

2.2.7 Vaccination status

Information was collected about receipt of vaccines against *N. meningitidis* serogroups B and C. In England, vaccination against serogroup B using 4CMenB was implemented on 01 September 2015 as a two-dose schedule at 8 and 16 weeks with a booster at 1 year (42). There was a limited catch-up programme for infants born between 1st May 2015 and 31st August 2015 (42). For serogroup B, infants were considered to be ineligible if they were born before 1st May 2015, or were less than 56 days at disease onset, eligible for one dose of vaccine if born after 1st May 2015 and were ≥ 56 days at disease onset, and eligible for two doses of vaccine if they were born after 1st September 2015 and were ≥ 112 days at disease onset. Those infants who were eligible for one or two doses, but had received none or one respectively, were further

classified as having become eligible for vaccination less than or more than 14 days before disease onset. For serogroup C, infants were considered eligible if they were born before 1st June 2016 and were ≥ 84 days at disease onset.

2.2.8 Meningococcal antigen typing system (MATS)

In the UK, all isolates associated with IMD are analysed using the meningococcal antigen typing system (MATS), to assess the susceptibility of the isolate to 4CMenB. A MenB isolate was considered to be covered by 4CMenB if MATS positive for any antigens. If fewer than four antigens were characterised and none of the characterised antigens were MATS positive, 4CMenB coverage was defined as uncertain. If all four antigens were characterised and none were MATS covered, the isolate was defined as not covered.

2.2.9 Sequelae

Sequelae were recorded according to the categories used in the surveillance questionnaires (any, epilepsy, other neurological, amputation, hearing loss, other); where categorised as 'other', details were recorded.

2.2.10 Analysis

Data were analysed using Stata version 17 (StataCorp, College Station, Texas). All culture-confirmed and PCR-confirmed IMD cases in infants (age less than one year) during 1st September 2015 to 31st August 2020 in England were included. Population details including number of live births for each included year, and the breakdown of gestations of preterm births were obtained from the Office of National Statistics (ONS) (110)). Incidence rates per 100,000 infants along with 95% confidence intervals (95% CI) were calculated for the whole study population, for infants born at term and

preterm, and, for those born preterm, for those born at <32 WG and 32-36⁺⁶ WG.

Incidence rate ratios (IRR) with 95% CIs were used to compare rates. To adjust for cases where the gestational age was not known, analysis was first performed including only cases where gestation was known, then with all cases where gestational age was unknown reclassified assuming the distribution of cases with unknown gestation to be in the same proportion as those cases where gestational age was known. As it is possible that preterm birth is more likely to be recorded than term birth in primary care records and surveillance questionnaires a further analysis was performed in which all cases where gestational age was unknown were reclassified as being of term gestation. Data are mainly descriptive. Non-normally distributed data are described as medians with the interquartile range (IQR) and compared using the Mann-Whitney *U* test, while proportions were compared using the chi-squared or Fisher's exact test as appropriate.

2.3 Babies born Early Antibody Responses to MenB vaccination (BEAR Men B): a comparison of two MenB vaccine schedules in preterm infants

This was a phase IV multicentre study comparing 4CMenB vaccination given according to two different vaccination schedules (2+1 vs 3+1) in preterm infants born at <35WG (50% <30WG), investigating the immunogenicity of the two schedules and the reactogenicity of 4CMenB in a UK preterm population. Study documents including the protocol, information sheet, consent form, diary and case report form are included in [appendix C](#).

2.3.1 Ethical approval and registration

Ethical approval for BEAR Men B was given by Yorkshire and the Humber- Sheffield Research Ethics Committee (17/YH/0150) and by the Health Research Authority (HRA). Clinical trials.gov identification: NCT03125616.

2.3.2 Study design

Recruitment

Preterm infants aged <35WG (50% <30WG) were recruited from six sites in the UK. The eligibility criteria were designed to be broad to allow recruitment of a representative preterm population (table 2.2).

Table 2.2. Eligibility criteria for the BEAR Men B study.

Inclusion Criteria	Exclusion criteria
Preterm infant born at <35WG No contraindications to vaccination according to the Green Book Willing and able to comply with study procedures Written informed consent	Contraindication to vaccination according to the Green Book Life-limiting congenital anomaly or condition Prior diagnosis of an immunodeficiency syndrome Considered unlikely to complete expected follow up until the end of the study Child in care

WG= weeks of gestation

Randomisation

Two computerised block randomisation lists (one for gestational age <30 WG and one for 30-34⁺⁶ WG) were produced by the study statistician for each site. These were placed inside opaque envelopes bearing the corresponding participant number by an individual independent of the study. Each centre was provided with the necessary envelopes and on recruitment to the study, each participant was allocated, in order of inclusion, the next available participant number from the appropriate list depending on gestation. Participants randomised to group one received their vaccinations

according to the usual schedule, those randomised to group two received an additional dose at 12 weeks. Routine vaccines during the BEAR Men B study period are shown in table 2.3.

Table 2.3. Routine vaccines during BEAR Men B study period.

Vaccine	Product names	Age (months)			
		2	3	4	12
DTaP/IPV/Hib/HepB	Infanrix Hexa®	✓	✓	✓	
Rotavirus	Rotarix®	✓	✓		
MenB	Bexsero®	✓		✓	✓
PCV13	Prevenar13®	✓		✓	✓
MCC-TT/Hib-TT	Menitorix®				✓
MMR	Priorix®				✓

Study visits took place according to the timing shown in table 2.4.

Table 2.4. Timing of vaccinations and blood sampling.

Visit Number	1	2	3	4	5	6
Age	2 months/ 49-84 days	3 months; 28-42 days after V1	4 months; 28-42 days after V2	5 months; 28-42 days after V3	12 months; 364- 393 days	13 months; 28-42 days after V5
Group 1	4CMenB RV	RV	4CMenB RV	*	4CMenB RV *	*
Group 2	4CMenB RV	4CMenB RV	4CMenB RV	*	4CMenB RV *	*

RV=routine vaccines; * blood sampling; ages of scheduled vaccination refer to chronological ages.

Immunogenicity assessment

Blood samples were obtained from participants at 5 months of age (post-primary sample), 12 months (persistence sample) and 13 months (post-booster sample). These were sent by courier at ambient temperature to St George's, University of London where they were spun at 500g for 20 minutes and frozen at -80°C. These were then despatched to the UKHSA laboratories in Manchester where the analysis was

performed using SBA assays (111). The serum starting dilution was 1:2 and commercially sourced human complement was used, which is validated against each strain. The complement used for *N. meningitidis* strains 5/99 (NadA) and NZ98/254 (PorA) analysis was untreated and from a single individual and that used for *N. meningitidis* strain 44/76-SL (fHbp) was antibody depleted complement from a pooled human complement source. SBA results were reported against the three indicator strains 44/76-SL (fHbp), 5/99 (NadA) and NZ98/254 (PorA).

Reactogenicity assessment

Reactogenicity of the vaccine was assessed by caregiver completion of a seven-day diary after each vaccine dose, including both local and systemic adverse events. For those patients who were vaccinated in hospital, information was also collected about cardiorespiratory events including apnoea, bradycardia and desaturation (defined below) in the 24 hours before and 72 hours after vaccination, as well as details of any ventilatory support being received in this period.

Local reactions

Caregivers were asked to complete details of any erythema, swelling or pain. For erythema and swelling the area was measured with a ruler and recorded in the diary. 'Any' was defined as the presence of any local reaction and 'severe' if the erythema or swelling was ≥ 30 mm. For pain, caregivers were asked to assign a value using a scale of 0-3: 0=none, 1=mild, child whimpers when the site is touched, 2= moderate, child cries when the site is touched, 3= severe, child cries when the limb is moved. The site for vaccine administration was specified in the protocol so that local reactions could be differentiated. For most of the vaccines, local reactions from all participants were analysed together, however only those in group two received 4CMenB at 12 weeks

and at this point therefore local reactions to 4CMenB could only be analysed in group two, and at 16 weeks the local reactions to 4CMenB were compared between those in group one who were receiving a second dose of this vaccine and those in group two who were receiving a third dose.

Systemic reactions

Information about feeding, activity, irritability, crying, vomiting, diarrhoea, and fever were collected for the seven days following vaccination. Fever was classified according to the US Food and Drug Administration (FDA) toxicity grading scale (grade 1- 38.0-38.4°C, grade 2- 38.5-38.9°C, grade 3- 39.0-40°C, grade 4- >40.0°C). Non-febrile systemic adverse events were classified according to a four-point scale (0- none, 1- mild, 2- moderate, 3- severe). The details for each of the solicited adverse events are shown in table 2.5.

Table 2.5. Details of the classification used for solicited adverse events following vaccination.

Adverse event	Scale		Details
Has your child been feeding less than usual?	0	None	
	1	Mild	Feeding less than normal for 1-2 feeds
	2	Moderate	Missed 1-2 feeds completely
	3	Severe	Refused most or all feeds
Has your child been more irritable than usual?	0	None	
	1	Mild	Continuously irritable for less than 1 hour
	2	Moderate	Continuously irritable for 1 to less than 3 hours
	3	Severe	Continuously irritable for more than 3 hours
Has your child been less active than usual?	0	None	
	1	Mild	Less interested in surroundings, toys etc
	2	Moderate	No interest in surroundings, toys etc and sleeping through feeds
	3	Severe	Sleeping most of the time
Has your child cried persistently?	0	None	
	1	Mild	Cried continuously for less than 1 hour

	2	Moderate	Cried continuously for 1- less than 3 hours
	3	Severe	Cried continuously for 3 or more hours
Has your child been sick?	0	None	
	1	Mild	1-2 episodes without interfering with routine
	2	Moderate	Several episodes and cannot keep any food down
	3	Severe	Frequent episodes and taking nothing by mouth
Has your child had diarrhoea?	0	None	
	1	Mild	More loose stools than usual
	2	Moderate	Frequent runny stools without much solid material
	3	Severe	Multiple liquid stools without much solid material

Cardiorespiratory events

Details of inpatient monitoring for cardiorespiratory events including apnoea, bradycardia and desaturation were collected, along with details of any ventilatory support received, for 24 hours before and 72 hours after vaccine administration. This information was collected by a member of the research team using the clinical records maintained by nursing staff caring for the child. Apnoea was defined as a cessation in breathing of 20 seconds or longer, bradycardia as a heart rate of 100 beats per minute or less for 20 seconds or longer, and desaturation as oxygen saturations less than 88%. When comparing the ventilatory support in the period before and after vaccination, the most intensive support received in each time period was used. Cardiorespiratory events and ventilatory support before and after vaccination were compared (table 2.6). To allow an overall comparison to be made of the periods before and after vaccination, cardiorespiratory events and ventilatory support were combined and the clinical condition of each infant defined as 'deteriorated', 'stable' or 'improved'. If there was a discrepancy between the outcome for cardiorespiratory events and

ventilatory support (e.g., number of cardiorespiratory events decreased, but ventilatory support increased) ventilatory support was considered to be the more objective measure.

Table 2.6. Descriptions of terms used to describe changes in cardiorespiratory events between the period before and after vaccination.

Classification	Description
Deteriorated	Any episodes of desaturation, bradycardia or apnoea after vaccination if there had been none prior to vaccination
	An increase of $\geq 50\%$ in desaturation, bradycardia or apnoea after vaccination compared to prior to vaccination
	Increase in oxygen requirement of $\geq 10\%$
Improved	No episodes of desaturation, bradycardia or apnoea after vaccination if there had been any prior to vaccination
	A decrease of $\geq 50\%$ in desaturation, bradycardia or apnoea after vaccination compared to prior to vaccination
	Decrease in oxygen requirement of $\geq 10\%$
Stable	Any change in apnoea, bradycardia or desaturation less than that in description of deterioration or improvement
	Any change in oxygen requirement $< 10\%$

2.3.3 Statistical analysis

Definitions

Chronic lung disease was defined as supplementary oxygen +/- mechanical ventilation at >28 days of life and at a corrected gestational age of >36 WG. Small for gestational age (SGA) was defined as <10th percentile for gestational age at birth.

Sample size

Sample size calculations are complicated because of the multiple components in 4CMenB. The immune responses to three antigens were assessed. It was calculated that a comparison of two schedules of 4CMenB would require 60 infants in each arm. The standard deviation of the GMT responses to the three antigens was predicted to be around 1.0 loge units. Therefore, a sample size of 60 per group would allow a 1.7-fold difference between groups to be detectable at 80% power at the 5% significance

level. Based on experience from previous similar studies, a drop-out rate of around 10% was expected so the recruitment target was 132 infants.

Assessment of the distribution of the data

To allow selection of the appropriate test for comparison of the two study groups, the distribution was assessed for normality, firstly by visually representing the results using histograms and secondly using the Shapiro Wilks test ([appendix D](#)). Non-parametric tests were used as data was predominantly non-normally distributed.

Primary objective: For each schedule, geometric mean titres (GMTs) of the hSBA assay against three *N. meningitidis* strains (44/76-SL [fHbp], 5/99 [NadA] and NZ98/254 [PorA]) were calculated with 95% confidence intervals one month post primary vaccination. Schedules were compared using Kruskal-Wallis tests on log-transformed titres. Proportions with titres $\geq 1:4$ were also calculated with exact binomial 95% confidence intervals and compared between groups by Fisher's exact test. Significance was at a 5% level. Analysis was by modified intention to treat (mITT). This means that participants were analysed according to the group to which they were randomised, but only those with at least one blood sample taken and antibody result were included. Missing data (e.g., from withdrawals, non-compliers) was assumed to be missing at random. The mITT analysis included blood samples taken outside the recommended timing. Any queried data was checked with the site and investigated. The main subgroup analysis was stratification by level of prematurity (<30 WG/ 30+0-34⁺⁶ WG). A multivariable normal errors regression on logged titres was used to investigate the influence of receipt of blood products, postnatal steroids, gestational age, gender, prior BCG administration, chronic lung disease, early post vaccination paracetamol, ethnicity and birthweight.

Secondary objectives: Analysis of titres before and after the booster vaccination was performed in the same way as the analysis after the primary vaccine series. In addition, the geometric mean fold change between time points was calculated with 95% confidence intervals.

For the safety end-points analysis data was analysed “As Treated” (according to vaccine received) with proportions having each safety endpoint calculated with 95% exact confidence intervals. Proportions are according to the most severe level of the adverse event reached across the follow up period after each dose.

To investigate factors which may have affected the response to vaccination, regression models were constructed using covariates of receipt of blood products, postnatal steroids, gestational age, gender, prior BCG administration, chronic lung disease, early post vaccination paracetamol, ethnicity and birthweight all of which were identified as possible factors which might affect the antibody response. Logged SBA values were investigated using linear regression: univariable linear regression was performed to identify factors suitable for inclusion in a multivariable model using a qualifying p-value of 0.10 and always including the pre-identified factors of particular importance of study group and gestational category (<30 GW and 30-34⁺⁶ GW).

2.4 Sources of complement in SBA assays

In this study, cord blood was collected to allow the production of three pooled neonatal complement sources: preterm complement, term complement from infants born by caesarean section and term complement from infants born vaginally. This complement was then used in SBA and antibody dependent complement deposition (ADCD) assays to investigate if results obtained when using adult derived complement

are comparable to that obtained when using neonatal derived complement. Study materials including the protocol, participant information sheet and case report form is in [appendix E](#).

2.4.1 Ethical approval and registration

Ethical approval for the COMPAre study was given by the West of Scotland Research Ethics Service (19/WS/0105) and the Health Research Authority (HRA). Clinical trials.gov identification: NCT04023929.

2.4.2 Clinical study methods

Recruitment

Participants were recruited from St George's University Hospitals NHS Foundation Trust prior to delivery. The eligibility criteria for participation were broad (table 2.7)

Table 2.7. Eligibility criteria for the COMPAre study.

Inclusion criteria	Exclusion criteria
Pregnant and planning to deliver at St George's University Hospitals NHS Foundation Trust	Aged less than 16 years Known complement deficiency

Data collection

Information was collected about mode of delivery, gestational age, whether the participant had received antibiotics in the past seven days and, if so, details of the antibiotics, sex, and ethnicity of the infant.

Sample collection

After the placenta was delivered, it was received by a member of the research team and cord blood sampling was performed as soon as possible after delivery (image 2.1).



Image 2.1. Cord blood sampling.

Cord blood sampling was performed as quickly as possible after delivery using an aseptic technique and plastic syringes of a standardised type

A cord blood sample, consisting of as large a volume as possible, was obtained from each participant following delivery of the placenta. The sample was collected aseptically using a plastic syringe of a standardised make and the blood was transferred immediately into a hirudin containing falcon tube (recombinant Hirudin Creative Biomart, 2mg/50ml). The sample was transferred in a cool bag to the laboratories at St George's, University of London where it was placed into a polystyrene box containing wet ice as quickly as possible.

Sample processing and storage

The sample was decanted into falcon tubes ensuring that the volume in each tube was no greater than 2/3 of the total tube volume and placed in the refrigerated centrifuge. The samples were centrifuged at 1000g for 10 minutes at 4°C. After centrifugation, plasma was removed using polypropylene pastettes and put in labelled polypropylene cryovials. These cryovials were placed in a box of dry ice and left for 10 minutes to snap freeze and then stored in a -80°C freezer in the Vaccine Institute at St George's,

University of London. The samples were couriered on dry ice to UKHSA, Porton Down where the analysis took place.

2.4.3 Laboratory study methods

2.4.3.1 Development of a pooled complement source for term and preterm infants

To ensure that the pooled complement source for each gestational group contained samples with normally functioning complement, the functional performance of complement for each individual sample was tested. Functional activity of the classical and alternative pathways and quantity of individual complement proteins was assessed using radial immunodiffusion (RID) assays (Bindarid™, The Binding Site, Birmingham). The Complement Haemolysis 50% assay (CH50) and Alternative Pathway haemolysis 50% assay (AP50) provide functional assessments of the classical and alternative pathways respectively and single radial immunodiffusion assays were used to quantify individual complement components. These assays were performed according to the manufacturer's instructions. In summary, this method involved adding samples to the centre of cylindrical wells in pre-prepared agarose gel plates which were then incubated according to specific instructions (table 2.8).

Table 2.8. Instructions for sample incubation for radial immunodiffusion assays.

Component	Length of run	Temperature of run	Sample	Vol µl/well
AP50	18 hrs/ 6 hrs	4°C/ 37°C	Neat	5
CH50	18 hrs/0.5hrs	4°C/ 37°C	Neat	5
C1q	96 hrs	20-24°C	½ concentration	10
C1ina	72 hrs	20-24°C	Neat	5
C2	120 hrs	20-24°C	Neat	10
C3	48 hrs	20-24°C	Neat	5
C4	48 hrs	20-24°C	Neat	5
C4bp	120 hrs	20-24°C	Neat	10
C5	72 hrs	20-24°C	Neat	5
C6	96 hrs	20-24°C	Neat	10
C7	72 hrs	20-24°C	Neat	20
C8	72 hrs	20-24°C	Neat	10
C9	72 hrs	20-24°C	Neat	10
FB	72 hrs	20-24°C	Neat	5

Chapter 2- Methods

FH	72 hrs	20-24°C	Neat	5
FI	72 hrs	20-24°C	Neat	10

AP50= Alternative Pathway haemolysis 50% assay; CH50= Complement Haemolysis 50% assay; FB=factor B; FH=factor H; FI=factor I

For CH50, the agarose gel contains sheep red blood cells sensitised with a rabbit anti-sheep red blood cell antibody known as haemolysin. During the diffusion phase, C1 is fixed by the haemolysin. During the incubation stage the complement cascade activates, and the sheep red blood cells are lysed leading to a clear zone. For AP50 the agarose gel contains chicken red blood cells. During the diffusion phase C3b molecules bind to membrane surfaces resulting in the formation of C5 convertase. During incubation, the complement cascade activates, the chicken red blood cells are lysed, and a clear zone appears. For the individual components the agarose gel contains the monospecific antibody being tested. During incubation, antigen-antibody complexes formed creating a precipitin ring (image 2.2).

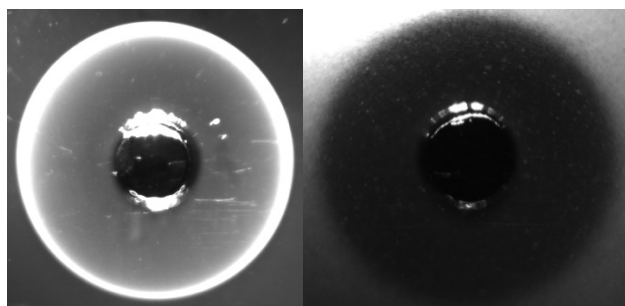


Image 2.2. Examples of precipitin rings.

The clear zones for CH50 and AP50 and the precipitin ring for the individual components were measured using a RID plate reader which allows measurement to the nearest 0.1mm. Through plotting the ring diameter of a number of samples of known concentrations, a calibration curve was produced which allows values for the control and the samples to be read. The values obtained from the RID analysis were reported, along with these results as a percentage of normal adult reference values.

Creating the pooled complement source

Once the individual complement components had been assessed, three pooled plasma sources were created: 1. Term (no antibiotics reported); 2. Term (antibiotics reported); 3. Preterm (antibiotics reported). Following this, the pooled plasma sources were sterilely depleted of IgG and IgM. This technique involves centrifuging the plasma at 1000g for 10 minutes at 4°C before applying this to linked sterile columns of 80ml Protein G Sepharose (GE), immediately followed by a 20ml CaptureSelect (Poros); the columns were connected to a sterile AKTA-Pure Chromatography system (GE) equilibrated with phosphate buffered saline (PBS) at 4°C (image 2.3). The plasma depleted of IgG and IgM was collected and C1q eluted from Protein G column using a salt gradient. IgG and IgM depleted plasma and C1q containing fractions were concentrated using 3,000k Da molecular weight cut off (mwco) dialysis membrane, laid upon a bed of 20,000 polyethylene glycol (PEG) at 4°C (112). Complement reconstitution was achieved through addition of C1q to the IgG and IgM depleted plasma and the pooled complement aliquoted into cryotubes and snap frozen at -80°C on dry ice and ethanol.



Image 2.3. Sterile purification of cord blood plasma, applied to chromatography system with attached IgG and IgM depletion columns.

2.4.3.2 Antibody dependent complement deposition (ADCD) assays

ADCD assays were used to measure the antibody mediated deposition of C3b and C5b-9 on meningococci. This allows an assessment of the opsonophagocytic (C3b) and bactericidal activity (C5b-9) in each complement-sera combination. The assays were performed using a pooled adult complement source, pooled term infant complement source from cord samples obtained following vaginal delivery and a pooled preterm complement source.

Preparing the bacteria

The three bacterial strains of interest (44/76-SL [fHbp], 5/99 [NadA] and NZ98/254 [PorA]), were streaked onto Columbia Agar + 5% horse blood plates (COH) (Biomérieux) in the flexible film isolator of a category three lab and incubated overnight in a flexible film isolator at 37°C with 5% CO₂. The following day, approximately 50 colonies were harvested from each plate and suspended in Frantz

medium (Media Services, UKHSA Porton Down). To kill the bacteria, 20% w/v sodium azide (Sigma-Aldrich) and 10mM Phenylmethylsulfonyl fluoride (PMSF) (Sigma-Aldrich) were made up and 100µl of both were added to 10mls of bacterial culture for each of the three strains of bacteria and incubated for 48 hours at 37°C. After 48 hours, the cultures were centrifuged and resuspended in 10ml Phosphate Buffered Saline (PBS), and 100µl of killed bacterial suspension was pipetted onto COH plates and incubated overnight at 37°C with 5% CO₂ to confirm that all bacteria had been killed. The killed culture was then centrifuged at 3060g for five minutes and resuspended in 1 ml of PBS.

Setting up the plates

5µl heat-inactivated sera was added to the relevant wells of a 96-well plate in triplicate for each sample. The complement sources were removed from the freezer immediately prior to use and defrosted at room temperature. 5µl of complement was added to the relevant wells. Inactivated bacteria were adjusted to an optical density (OD_{600nm}) of 0.1 in blocking buffer (2% bovine serum albumin [BSA] in PBS) and 90µl was added to all wells. The plates were incubated at 37°C for 45 minutes with shaking at 900rpm. Following this the plates were centrifuged for 5 minutes at 3060g, the supernatant was removed, 200µl of blocking buffer added to each well and the centrifugation and wash step was repeated. The bacterial pellet was then resuspended in 200µl of conjugate mix (1:500 dilution Rabbit polyclonal anti-human C3c-FITC (Abcam, UK); 1:4000 dilution Mouse anti-human mAb C5b-9 (Quidel, US) custom conjugated to Alexafluor 647 (Life Technologies, USA) and the plates incubated at 4°C for 20 minutes. Following this, the centrifugation and wash steps were repeated, and the bacterial pellet resuspended in 200µl live dead fixable violet stain (2µl/ml [Invitrogen, US]) in PBS. The plates were then incubated for 30 minutes at room

temperature in the dark. The centrifugation and wash steps were then repeated, and the bacterial pellet was resuspended in filter sterilised Hank's balanced salt solution (HBSS)(Gibco™). The plates were then transferred to the flow cytometer (Beckman Coulter CytoFLEX) for acquisition. Analysis was performed using the CytoFLEX acquisition and analysis software, CytExpert. For each sample, around 10,000 individual events were analysed for fluorescence and a horizontal gate was drawn to include 10% of the antibody independent control sample population (bacteria, complement and conjugate). A mean fluorescence index (MFI) was calculated through multiplication of the percent of events moving into the horizontal gate, by the average fluorescence of that population. The result was expressed as the average MFI of the test samples minus the average MFI of the antibody-independent control samples (MFI-C).

2.4.3.3 Serum bactericidal antibody (SBA) assays

SBA assays provide a functional assessment of the antibody and complement mediated killing of relevant bacteria, in this case meningococci, and are the gold standard to assess immunity to meningococcal disease. Serial dilutions of sera are incubated with target bacterial strains and complement. In the SBA assays performed as part of the COMPARE study, both infant and adult sera and neonatal and adult complement were used in different combinations to investigate any differences in the performance of the SBA assay. The adult serum was from an adult volunteer following 4CMenB vaccination and the infant sera were residual samples from the BEAR Men B study ([section 2.3](#)). All SBA assays involving meningococci are conducted in category three facilities (images 2.4 and 2.5).

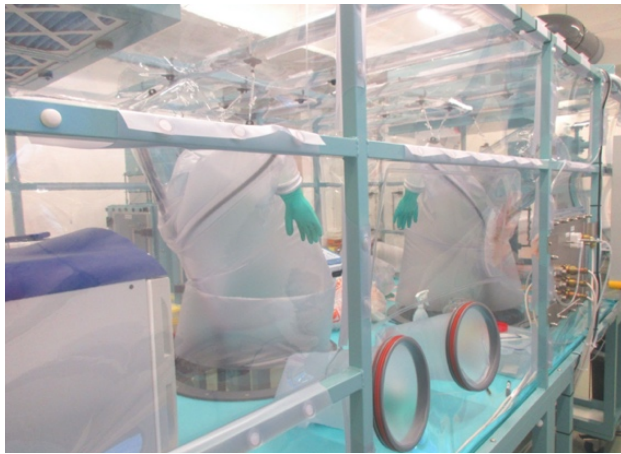


Image 2.4. Flexible film isolator in a category three laboratory (left).



Image 2.5. Flexible film isolator in a category three laboratory (right).

The three strains of bacteria (44/76-SL [fHbp], 5/99 [NadA] and NZ98/254 [PorA]) were prepared as in section [2.4.4.2](#). After the bacteria had been resuspended in the Frantz media (Media Services, UKHSA, Porton Down), the optical density (OD) of the suspensions were measured. For each suspension, this stock was used to inoculate 10mL of Frantz medium at an $OD_{600\ 0.1}$ and these were allowed to grow for three hours at 37°C with shaking.

Setting up the plates

Dilution plates were set up as shown in figure 2.3.

Dilution												
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	Bac + C'	Bac + HI C'
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	-	BB 20µl	BB 20µl	BB 20µl	BB 20µl	BB 20µl	BB 20µl	BB 20µl	BB 20µl	BB 20µl	BB 20µl	BB 20µl
C	Sera 20µl	Sera 20µl	Serial dilution from column 2 to column 10.								-	-
D	Bac 10µl	Bac 10µl	Bac 10µl	Bac 10µl	Bac 10µl	Bac 10µl	Bac 10µl	Bac 10µl	Bac 10µl	Bac 10µl	Bac 10µl	Bac 10µl
E	C' 10µl	C' 10µl	C' 10µl	C' 10µl	C' 10µl	C' 10µl	C' 10µl	C' 10µl	C' 10µl	C' 10µl	C' 10µl	C' 10µl
F												
G												
H												
I												

Figure 2.3. Dilution plate for SBA assay.

Bac= bacteria; BB= blocking buffer; C'= complement

20µl of blocking buffer was added to rows A-F in columns 2-12. For a starting dilution of 1:2, 20µl of heat inactivated serum was added to columns 1 and 2 and serial dilution performed from column 2 to 10 with 20µl being transferred from column to column and mixed six times with the final 20µl being discarded after column 10. 10µl of heat inactivated (HI) complement (C') was added to wells A-F in column 12, 10µl of adult complement was added to wells A-C and 10µl of cord complement was added to wells D-F in columns 1-11. The dilution plates were gently tapped and sealed with a plate sealer and transferred to the flexible film isolator in the category three lab.

In the isolator, the OD₆₀₀ of the three strains was measured and an appropriate strain-specific concentration was produced by performing a two-stage dilution in blocking buffer. Firstly a 1:100 solution was prepared, which was further diluted to create the final concentration. 10µl of the appropriate strain was added to wells A-F in all columns. A 10µl sample from wells A-F in column 12 were plated using the tilt method onto COH plates (Time 0 [T⁰] plates). The dilution plates were then sealed with a plate seal and incubated at 37°C for one hour shaking at 900 rpm. After one hour of incubation, 10µl from each well was plated out onto COH plates using the tilt method. These plates were left upright until all the liquid had dried or been absorbed before inverting and then placed in the incubator within the isolator overnight at 37°C with 5% CO₂ (Time 60 [T⁶⁰] plates).

The following day the plates were removed from the incubator and transferred using a sealed O-ring box into a Class I cabinet where colonies were counted using a colony counter (Scorcerer, Instem™, UK). Titres are calculated as the reciprocal dilution which shows 50% bacterial killing in T⁶⁰ plates in comparison to the number of colonies on the T⁰ plates from column 12 (bacteria and HI complement). For the assay to be valid, the number of colonies on the T⁰ plates must be between 50 and 250 per well and killing in the bacteria and complement wells must be below 30%.

2.4.3.4 SBA using serial complement dilutions

To investigate why some strains of bacteria appeared to grow less well in the presence of neonatal complement, SBA assays were performed using different concentrations of complement and using all three strains of bacteria according to the methods in section [2.4.4.3](#). The sera used was from an adult volunteer post vaccination with 4CMenB. In these assays, complement was prepared at a starting dilution of 1:2 and then serially

diluted using PBS. The final concentrations used in the assay were 25%, 12.5%, 6.25% and 3.125%.

2.4.3.5 Disc diffusion assay

To further investigate why some strains of bacteria appeared to grow less well in the presence of neonatal complement, disc diffusion assays were performed. The three strains of interest were prepared as described in section [2.4.4.3](#) but to a concentration equivalent to 0.5 on the MacFarland scale and then streaked onto COH plates to produce a bacterial lawn. Different preparations of complement (table 2.7) were pipetted onto paper discs and placed on the bacterial lawn along with a pre-prepared Oxoid™ Ampicillin Antimicrobial Susceptibility disc (10 µg/disc; Thermo Scientific™) (image 2.6).



Image 2.6. Discs on bacterial lawn.

Complement was used neat, with added β -lactamase to assess if there was any antibiotic present resulting in non-complement mediated bacterial killing and with added PBS as a control for the dilution effect of adding β -lactamase to the complement. Discs were prepared by pipetting 50µl of the complement preparation of interest onto the disc (Table 2.9). These complement preparations were neat complement, complement mixed with 100µl of β -lactamase (Sigma-Aldrich) to give a

concentration of approximately 50IU/ml and complement mixed with the same volume of PBS.

Table 2.9. Details of the complement preparations used in the disc diffusion assay.

Complement	Preparation
Adult	Neat
Term vaginal delivery	Neat
	Added β -lactamase
	Added PBS
Term C/S	Neat
	Added β -lactamase
	Added PBS
Preterm vaginal delivery	Neat
	Added β -lactamase
	Added PBS

C/S= caesarean section; PBS= phosphate buffered saline

These were placed in the incubator within the isolator overnight at 37°C with 5% CO₂.

The following day the plates were removed from the incubator and a visual inspection made of the area of bacterial killing.

2.4.3.6 SBA using complement with β -lactamase

To further investigate why *N. meningitidis* strain 5/99 (Nad A) appeared to grow less well in the presence of neonatal complement, an SBA assay was performed using this strain of bacteria according to the methods in section [2.4.4.3](#). In this assay, complement was used neat, or mixed with 100 μ l of either β -lactamase (final concentration of 50IU/ml) or PBS. Once added they were mixed thoroughly and incubated at 2-8°C for 10 minutes prior to use in the assay.

3 Systematic review: immunogenicity, efficacy, and effectiveness

3.1 Background

Preterm infants are at increased risk of infection and severe disease from a number of vaccine preventable pathogens, including *Bordetella pertussis* (76, 113, 114), pneumococcus (74, 115, 116) and rotavirus (117). It is recommended that preterm infants are vaccinated according to their chronological age, irrespective of their gestation at birth, but vaccination in this vulnerable group is often delayed (86, 87, 118). This delay in vaccination may, in part, result from concerns of parents and health care professionals about the safety and immunogenicity of vaccines in these infants (119). A fuller understanding of the available evidence relating to the benefits and risks of vaccination in this population is vital to ensure that the best possible information is disseminated to parents and health care professionals so that these delays can be addressed. This systematic review was undertaken to review the evidence about immunogenicity, efficacy, effectiveness, reactogenicity and safety of vaccines in preterm infants and is presented in two chapters. In this chapter the outcomes of immunogenicity, efficacy and effectiveness will be presented, and in [chapter four](#), the outcomes of reactogenicity and safety will be considered. Vaccines which are included in the current UK routine schedule for children under 18 months are considered in turn, with a brief introduction to the rationale for vaccination and then results presented including details of antibody concentrations/titres. For some vaccines a protective threshold is known, and where such a threshold exists, proportions of infants reaching this are often reported in the included papers.

The dominant protection elicited from vaccines comes from the production of antibodies and in keeping with this, the majority of included reports focus on the generation of humoral immunity, although some reports are concerned with cell mediated immunity. All the reports included in the review which studied the impact of corticosteroids used dexamethasone. A summary of the findings related to steroid treated infants are included in section [3.2.1.11](#).

3.1.1. Vaccine schedules

Most infant vaccines are given according to a primary and booster schedule. The total number of doses recommended, and the timing of these, depends on the vaccine and the local epidemiology of disease, but in general there is a minimum interval of three weeks between primary doses and at least four months between primary and booster doses to allow optimum responses to develop (33). Vaccine schedules are often described as X+Y with X being the number of primary doses given and Y being the number of booster doses given. For example, in the UK, the meningococcal serogroup B vaccine, 4CMenB, is currently given according to a 2+1 schedule in infants, with the first two doses given at 8 and 16 weeks, and the booster dose given at one year of age.

3.1.2 Objectives

1. To identify the evidence about immunogenicity, vaccine efficacy and vaccine effectiveness of routine vaccines given to preterm infants in the first 18 months of life
2. To perform a meta-analysis for those outcomes for which there are sufficient data from similar studies to allow meaningful statistical synthesis.

3.1.3 Methods

The full methods are described in [section 2.1](#). In summary, Medline, Embase, Web of Science, Latin American and Caribbean Health Sciences Literature (LILACS) and COCHRANE CENTRAL were searched on 25th November 2020 with updated searches performed on 15th December 2021 and 6th November 2022. The search strategy can be found in [appendix B](#). Data extraction was performed and is summarised in the sections below, with meta-analysis performed where possible. Quality assessments were performed using the GRADE framework and are discussed in [3.2.3](#). Full details of the GRADE assessments including risk of bias assessments are in [appendix H](#) and [appendix I](#).

3.2 Results

A total of 5105 titles were identified. After screening of titles and abstracts, 102 full texts were selected from the initial searches and a further three full texts from references of the original papers; therefore 105 full texts were reviewed, of which 80 papers, relating to 75 studies, were eligible for inclusion in this review (figure 3.1). Immunogenicity was the main outcome in 44 papers, reactogenicity in 23 papers, safety in three papers and efficacy or effectiveness in 10 papers. Vaccines received by participants included *Haemophilus influenzae* type B (Hib), diphtheria, tetanus, pertussis, polio, hepatitis B, group C meningococcus, pneumococcus, MMR and rotavirus.

A list of all full texts reviewed with reasons for exclusion is in [appendix F](#) and a full summary of all included papers is in [appendix G](#).

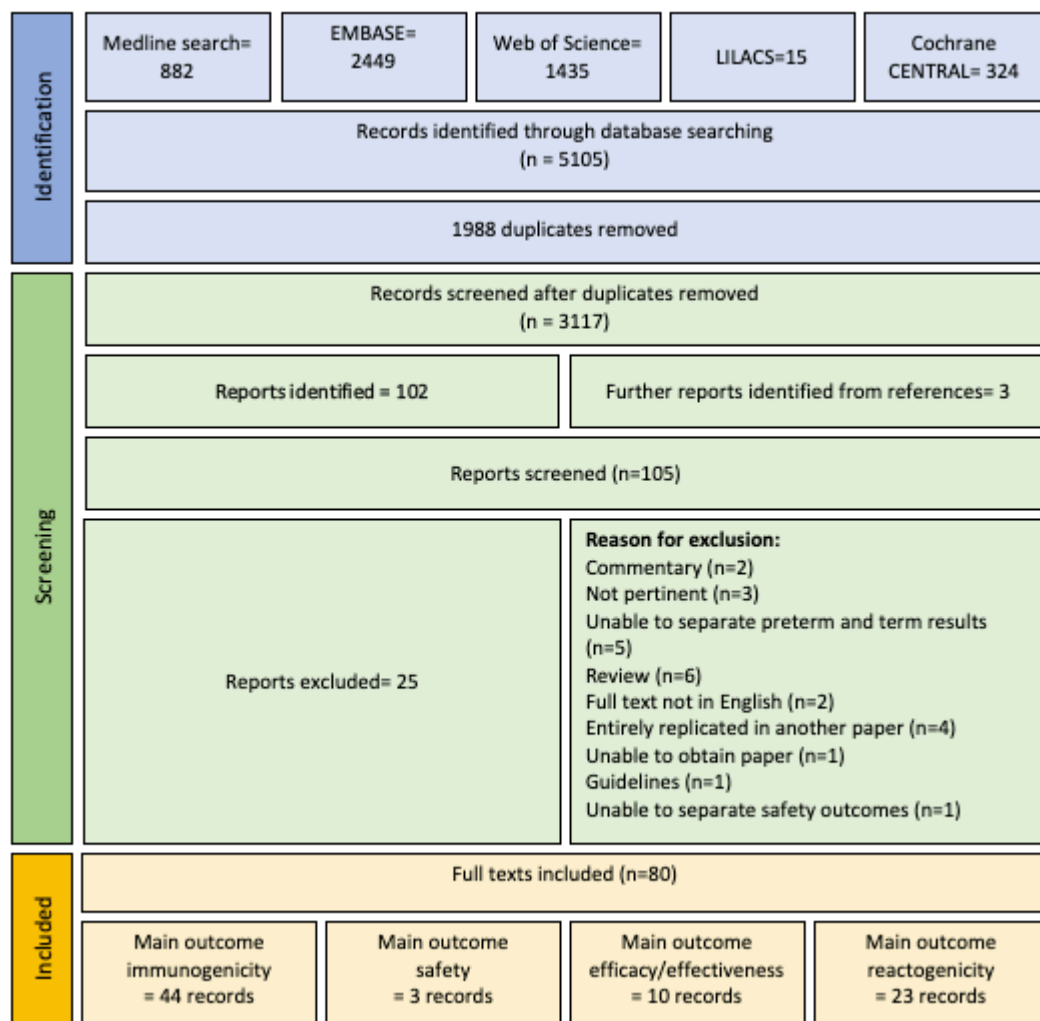


Figure 3.1. Summary of searching and selection process according to PRISMA flow chart.

3.2.1 Immunogenicity

3.2.1.1 *Haemophilus influenzae* type B (Hib)

Background

Hib vaccines were developed in the 1980s; the first of these were polysaccharide vaccines, but following the poor response in young children, conjugate vaccines were developed. These conjugate vaccines contain a length of polyribosylribitol phosphate (PRP) polysaccharide linked to a carrier protein and although all of these are immunogenic, there is some variation in immunogenicity between conjugate vaccines depending on the adjuvant and carrier protein used (120). Vaccination results in the

production of anti-PRP antibody, and antibody thresholds for short-term and long-term protection based on polysaccharide vaccine trials are established as $\geq 0.15\mu\text{g/ml}$ and $\geq 1.0\mu\text{g/ml}$ respectively (121). The antibody levels in the included trials are reported in the papers either as concentrations or titres and the same terms have been used in the review.

Description of studies

Immunogenicity of Hib vaccination in preterm infants was a widely studied outcome, being included in 19 reports (89-91, 122-137) relating to 17 studies, with three reports resulting from one study (123, 135, 137). All the reports came from observational studies: five case series (124, 125, 130, 134, 136), one cross-sectional study (122), ten prospective cohort studies (90, 91, 123, 126-128, 131-133, 135, 137) and two historical cohort studies (89, 129). The proportions of infants with antibody concentrations above the established thresholds for protection were reported in most studies.

Preterm infants with no term comparison

Six reports included results for preterm infants alone (124, 125, 130, 133, 134, 136), with no comparison to a term cohort. All the infants in the studies had received a three-dose primary schedule with similar immunogenicity sampling time points and the majority included infants born at lower gestational ages (table 3.1).

Table 3.1. Details of the vaccine schedules received in studies of Hib vaccine in preterm infants with no term comparison.

Author, year	GA of participants	Schedule	Timing of blood sampling	Hib component of vaccine used
Baxter, 2010 (136)	≤32 WG	2, 3 and 4 months	One month after 3 rd dose	PRP-T
Clarke, 2003 (124)	<30 WG	2, 3 and 4 months	5-8 weeks after 3 rd dose	PRP-T
D'Angio, 2013 (125)	<32 WG	3 dose series completed by 8 months of age	4-6 weeks after 3 rd dose	PRP-T, PRP-OMP, HbOc PRP
Slack, 2005 (133)	<32 WG	2, 3 and 4 months	4-6 weeks after 3 rd dose	PRP-T
Tsuda, 2012 (130)	<37 WG	3 vaccines starting at 2-6 months and given at 4-week intervals	One month after 3 rd dose	PRP-T
Vazquez, 2008 (134)	24-36 WG	2, 4 and 6 months	One month after 3 rd dose	PRP-T

GA= gestational age; WG= weeks of gestation; PRP-T =polysaccharide conjugated to tetanus toxoid; PRP-OMP=polysaccharide conjugated to group B meningococcal outer membrane protein; HbOc PRP=polysaccharide conjugated to mutant diphtheria toxoid

Following primary series

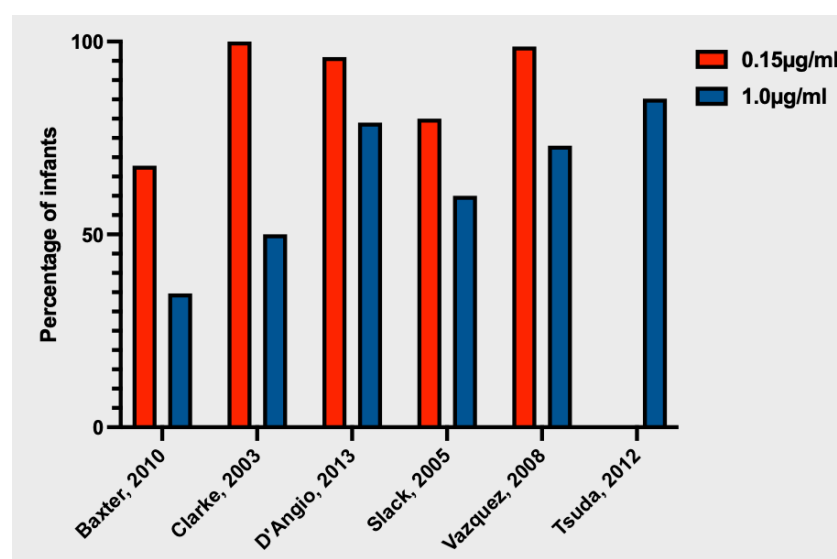
Five of the reports contained geometric mean concentration/titre (GMC/T) results. All the reports included information about the proportions of infants achieving protective thresholds after the primary series (table 3.1 and figure 3.2) and there was some variability in these results. The protective threshold of $\geq 0.15\mu\text{g/ml}$ was achieved in 67.8%-100% and $\geq 1.0\mu\text{g/ml}$ in 34.7%-85.2% of infants (table 3.2 and figure 3.2).

Table 3.2. GMC/T and percentage of preterm infants reaching protective thresholds following primary Hib vaccination with no term comparison.

Author, year	IgG GMC/T ($\mu\text{g/ml}$) (95% CI)	Percentage of infants reaching protective thresholds (%)	
		$\geq 0.15\mu\text{g/ml}$	$\geq 1.0\mu\text{g/ml}$
Baxter, 2010 (136)		67.8	34.7
Clarke, 2003 (124)	2.35 (0.77-7.17)	100	50
D'Angio, 2013 (125)	2.5 (1.7-3.4) [^]	96	79
	3.6 (2.7-4.8) ^{^^}		
Slack, 2005 (133)	1.21 (0.73-2.03)	80	60
Tsuda, 2012 (130)	2.89		85
Vazquez, 2008 (134)	2.10 (1.56-2.83)*	98.7 (93.1-100.0)	65.4 (53.8-75.8)
	2.50 (1.90-3.29)**		80.8 (70.3-88.8)

GMC/T=geometric mean concentration/titre; shaded boxes where data is not available

* $<1500\text{g}$ ** $1500\text{-}2000\text{g}$; [^] $<1000\text{g}$ ^{^^} $>1000\text{g}$

**Figure 3.2. Percentage of preterm infants reaching titres $\geq 0.15\mu\text{g/ml}$ and $\geq 1.0\mu\text{g/ml}$ after primary Hib vaccination.**

Clarke reported on infants who had received dexamethasone in the postnatal period and found the proportions protected within the range seen in other studies of preterm infants who had not received dexamethasone in the postnatal period, albeit at the

lower end of the range for the cut off of $\geq 1.0\mu\text{g/ml}$ (124). Vazquez compared the antibody response in low birthweight (LBW 1500-2000g) and very low birthweight (VLBW <1500g) infants and found that a lower proportion of VLBW infants reached the higher protective threshold of $1.0\mu\text{g/ml}$ compared with the LBW infants (134).

Following booster vaccine

Two reports also included results following the booster vaccine. In a population of infants who had received postnatal dexamethasone, Clarke reported a limited increase in anti-PRP GMT following the fourth dose (pre: $2.35\mu\text{g/ml}$ (95% CI 0.77-7.17); post $2.24\mu\text{g/ml}$ (95% CI 0.72-7.0), but a significant rise in avidity (124). Vazquez reported very high proportions reaching a protective threshold of $1.0\mu\text{g/ml}$ following the booster dose with no difference seen between low birthweight (LBW) and very low birthweight (VLBW) infants (LBW 100% [95% CI 94.2-100], VLBW 100% [95% CI 93.7-100]) (134).

Comparison of term and preterm infants following primary series

Seven reports included a comparison of results from term and preterm infants following the primary series. A summary of the schedules used is shown in table 3.3.

Table 3.3. Details of the vaccine schedules received in studies of Hib vaccine in preterm infants with term comparison.

Author, year	GA of participants	Schedule	Timing of blood sampling	Hib component of vaccine used
Berrington, 2006 (135)	<32 WG	2, 3 and 4 months	6-8 weeks after third dose	PRP-T
Heath, 2003 (89)	≤32 WG	2, 3 and 4 months	One month after third dose	PRP-T
Kulkarni-Munje, 2021 (127)	<37 WG	6, 10 and 14 weeks	One month after third dose	PRP-T
Omenaca, 2011 (91)	<31 WG	2, 4 and 6 months	One month after third dose	PRP-T
Omenaca, 2005 (128)	<37 WG	2, 4 and 6 months	One month after third dose	PRP-T
Rouers, 2020 (129)	<37 WG	2, 3 and 4 months	One month after third dose	PRP-T
Slack, 2001 (90)	<32 WG	2, 3 and 4 months	4-6 weeks after third dose	PRP-T

GA= gestational age; WG=weeks of gestation; PRP-T=polysaccharide conjugated to tetanus toxoid

There was variability seen in the GMC/T results (89-91, 127-129, 135) (table 3.4).

Table 3.4. GMC/T and percentage of infants reaching protective thresholds following primary Hib vaccine series with comparison of term and preterm infants.

Author, year	IgG GMC/T (µg/ml) (95% CI)			Percentage of infants reaching protective thresholds (%)					
				≥0.15µg/ml			≥1.0µg/ml		
	PT	T	P value	PT	T	P value	PT	T	P value
Berrington, 2006 (135)	0.9 (0.7-1.16)	1.45 (0.97-2.18)		86	95		51	67	
Heath, 2003 (89)	2.73 (1.63 - 4.58)	4.60 (3.51-6.04)	0.008	95	99	>0.05	67	88	<0.05
Kulkarni-Munje, 2021 (127)	1.58 *	7.71		95.8 3 (88.5-100)	98.14 (95.19-100)		54.16 (36.03-72.3)*	90.74 (84.38-97.09)	

				100) *					
	4.20 **			100 (100- 100) **			80 (70.49 - 89.5)* *		
Omenaca, 2011 (91)	16.0 8 (11.0 8- 23.3 3)^	10.47 (8.55- 12.83)		100 (92- 100) ^	99.3 (96.1- 100)		97.7 (88- 99.9) ^	94.4 (89.2- 97.5)	
	8.56 (6.59 - 11.1 3) ^^			99 (94.3 -100) ^^			93.8 (86.9- 97.7) ^^		
Omenaca, 2005 (128)	2.24 1 (1.65 5- 3.03 5)	4.24 (3.201 - 5.634)		92.5 (85.1 - 96.9)	97.8 (92.1- 99.7)		76.3 (66.4- 84.5)	86.5 (77.6- 92.8)	
Rouers, 2020 (129)	0.11 (0.10 - 0.14)	0.63 (0.41- 0.96)	<0.001	40.6	83.6				
Slack, 2001 (90)	0.27 (0.21 - 0.35)	0.81 (0.52- 1.25)	<0.001	55	80	0.003	21	46	0.001

GMC/T=geometric mean concentration/titre; T= term; PT= preterm; WG= weeks of gestation; shaded boxes where data is not available

*28-32 WG, **32-36 WG; ^<31 WG, ^^ 31-36 WG

Four reports detailed lower IgG GMC/Ts and proportions achieving both the lower and higher protective thresholds in preterm compared to term infants (90, 128, 129, 135), two found lower IgG GMCs and lower proportions reaching the higher threshold, but no difference in those reaching the lower threshold (89, 127), and one reported no differences in either IgG GMC or proportions reaching protective thresholds between term and preterm infants (91). All these studies used the same Hib component in the

vaccine and there was no pattern identified with respect to the timing of vaccination and antibody response (table 3.3).

A meta-analysis of the seven reports including thresholds $\geq 0.15\mu\text{g/ml}$ and the six reports including thresholds $\geq 1.0\mu\text{g/ml}$ following primary vaccination showed that the overall effect size favoured term infants (figures 3.3 and 3.4).

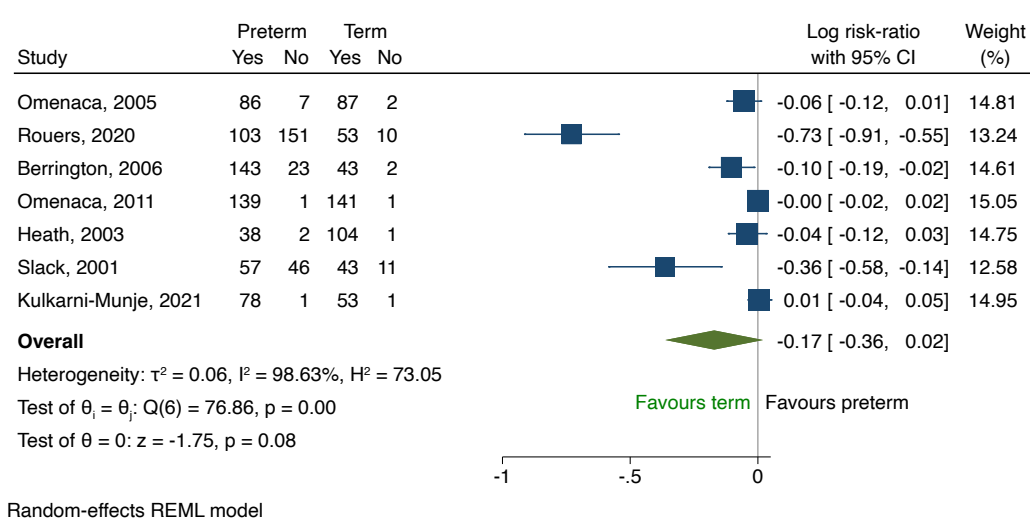


Figure 3.3. Forest plot showing proportions of infants reaching a threshold of $\geq 0.15\mu\text{g/ml}$ following primary vaccination.

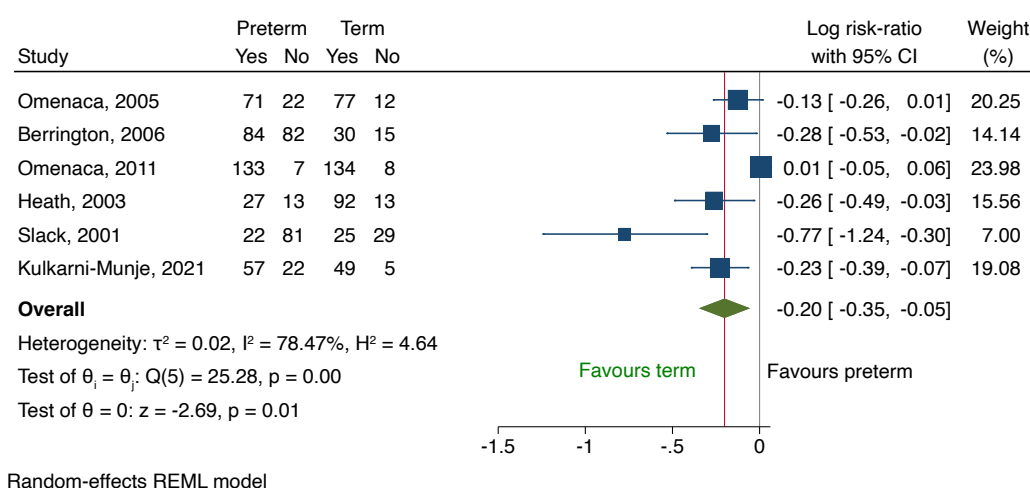


Figure 3.4. Forest plot showing proportions of infants reaching a threshold of $\geq 1.0\mu\text{g/ml}$ following primary vaccination.

Comparison of term and preterm infants following booster vaccination

Four studies investigated the impact of an additional dose following the primary vaccination series (91, 129, 131, 135) (tables 3.5 and 3.6). Details of the vaccines received are shown in table 3.5.

Table 3.5. Details of studies comparing term and preterm infants after administration of a booster dose.

Author, year	Primary schedule	Timing of fourth dose
Berrington, 2006 (135)	2, 3 and 4 months	Term: median 6.9; Preterm: median 9.2 months
Omenaca, 2007 (131)	2, 4 and 6 months	18-20 months
Omenaca, 2011 (91)	2, 4 and 6 months	16-18 months
Rouers, 2020 (129)	2, 3 and 4 months	11 months

Table 3.6. GMC/T of infants following Hib booster vaccination in studies comparing term and pre-term infants.

Author, year	IgG GMC/T (µg/ml) (95% CI)		
	PT	T	P value
Berrington, 2006 (135)	4.66 (3.12-6.42)	7.0 (2.3-21.0)	<0.0005
Omenaca, 2007 (131)	70.9 (56.0-89.8)	63.3 (45.7-87.8)	
Omenaca, 2011 (91)	58.71 (40.55-84.98) *	54.63 (45.33-65.83)	
	47.31 (37.80-59.21) **		
Rouers, 2020 (129)	2.95 (2.26-3.86)	17.0 (12.5-23.2)	<0.001

GMC/T= geometric mean concentration/titre; T= term; PT= preterm; shaded boxes show where data is not available; WG= weeks of gestation

*<31 WG **31-36 WG

Although there were differences reported in IgG GMC/T between studies, overall high proportions of both preterm and term infants reached protective thresholds following a booster dose (table 3.7).

Table 3.7. Percentage of infants reaching protective thresholds following booster Hib vaccination in studies comparing term and preterm infants.

Author, year	Percentage of infants reaching protective thresholds (%) (95% CI)					
	$\geq 0.15\mu\text{g/ml}$			$\geq 1.0\mu\text{g/ml}$		
	PT	T	P value	PT	T	P value
Berrington, 2006 (135)	98	100	0.001	93	100	<0.0005
Omenaca, 2007 (131)	100 (95.7-100)	100 (94.7-100)		100 (95.7-100)	98.5 (92.1-100)	
Omenaca, 2011 (91)	100 (90.7-100) *	100 (97.3-100)		100 (90.7-100) *	100 (97.3-100)	
	100 (96.2-100) **			100 (96.2-100) **		
Rouers, 2020(129)	88.1 (83.0- 92.0)	100 (93.0-100)	<0.01			

T= term; PT= preterm; WG= weeks of gestation; shaded boxes show where data is not available

* <31 WG **31-36 WG

Berrington reported results following administration of a fourth dose after completion of the primary series and found that anti-PRP GMTs were lower in preterm than in term infants, but a very high percentage of infants in both groups reached both the lower and higher thresholds, and in those infants who responded with an adequate antibody response, avidity was similar between term and preterm infants (123, 135).

Rouers found that anti-PRP GMCs were significantly lower in preterm infants compared to term infants following booster vaccination and that 88.1% of preterm infants overall achieved $\geq 0.15\mu\text{g/ml}$ compared with 100% in term infants. When the preterm infants were analysed separately according to gestational age, those born at less than 28 WG or 28-32 WG had significantly lower proportions reaching a protective threshold than those born at 32-36 WG or term infants (129). In contrast, Omenaca

reported similar anti-PRP GMC in term and preterm infants following a booster and very high proportions reaching a protective threshold in both groups (131). Similarly, in another report, Omenaca found no significant differences in antibody concentrations or proportions achieving either short-term or long-term protective thresholds between term and preterm infants. All infants in both the preterm and term groups had titres $\geq 1.0\mu\text{g/ml}$ (91). Infants in both of Omenaca's reports received an extended primary series at two, four and six months with a later booster compared with the other two studies which used an accelerated schedule at two, three and four months with an earlier booster.

Cellular immunity

One study investigated cell mediated immunity by stimulating infant peripheral blood mononuclear cells (PBMCs) with the antigens contained within the pentavalent vaccine. When the PBMCs were stimulated with Hib there was reduced response of CD4 and CD8 T cells in preterm compared with term infants (127).

Long-term immunogenicity

Three studies investigated longer term persistence of vaccine responses. Bednarek studied children at the age of 4.5-5.9 years, 2.5-3 years after receipt of last vaccinations, comparing the anti-Hib IgG in those who were term and preterm and found no significant differences between them, with high proportions of titres $\geq 1.0\mu\text{g/ml}$ being maintained in both term (90.2%) and preterm (84.8%) infants (122). At seven years of age Kirmani found significantly higher antibody concentrations in term than preterm infants (term $3.21\mu\text{g/ml}$, preterm $1.41\mu\text{g/ml}$; $p=0.03$), but no significant difference in the proportion of infants reaching either lower or higher protective thresholds ($\geq 0.15\mu\text{g/ml}$: term 100%, preterm 100%; $\geq 1.0\mu\text{g/ml}$: term 75%,

preterm 62%; $p=0.45$) (126). In contrast, Heath reported on infants at five years and found that preterm infants had significantly lower anti-Hib GMC (term 0.51 [95% CI 0.31-0.85], preterm 0.24 [95% CI 0.16-0.37]; $p<0.05$) and proportions with concentrations $\geq 0.15\mu\text{g/ml}$ (term 68%, preterm 59%) (89). The infants in this latter study had received an accelerated schedule at two, three and four months with no booster, in contrast to the earlier two in which vaccination had been given at two, four and six months with a booster between 12 and 18 months, and the immunogenicity samples for term and preterm populations were collected at different times: preterm sampling at 64 months, term sampling at 72 months.

Factors associated with reduced immunogenicity

Some reports included consideration of factors associated with reduced immunogenicity of Hib vaccine. Omenaca reported reduced antibody responses following the primary series in those babies $<10^{\text{th}}$ percentile for weight compared to those $>50^{\text{th}}$ percentile at six months and found no statistically significant difference in those infants who had received postnatal steroids (131). Berrington found that the probability of achieving a protective threshold increased with the number of whole cell pertussis doses received and, in a separate analysis, that use of an inactivated polio vaccination was significantly associated with reduced antibody concentrations (123, 137).

Summary

Whilst there is significant variability in the results seen for Hib vaccination, even when similar schedules are used, overall, there does seem to be a reduction in the anti-PRP GMC/T and proportions achieving protective thresholds reported for preterm infants following a primary vaccination series and this is supported by the results of the meta-

analysis. Whilst anti-PRP GMC/T remain lower in preterm infants in some reports following the booster vaccination, proportions of infants reaching both the lower and higher protective threshold are high and similar in both groups. The persistence of this equivalence in proportions protected between preterm and term infants may depend in part on the schedule used, as the one study investigating persistence of immune response following an accelerated schedule suggested that a lower proportion of preterm infants maintain a protective threshold of $\geq 0.15\mu\text{g/ml}$ (89).

3.2.1.2 Tetanus

Background

A tetanus vaccine has been available since the 1940s and is routinely given in a multivalent formulation, since 2017 this has been as part of a hexavalent vaccine in the UK. Vaccination results in the production of serum anti-toxoid antibody and there are several assays used to assess the response to vaccination. The thresholds for partial and optimal protection are established as ≥ 0.01 IU/ml and ≥ 0.1 IU/ml respectively.

Description of studies

Thirteen reports were identified which included response to tetanus vaccine as an outcome (122, 126-129, 132, 134-136, 138-141). All these studies were observational: three case series (134, 136, 140), one cross-sectional study (122), eight prospective cohort studies (126-128, 132, 135, 138, 139, 141) and one historical cohort study (129). The antibody levels in the included trials are reported in the papers either as concentrations or titres and the same terms have been used in the review.

Preterm infants with no term comparison

Two studies reported results for preterm infants only with no comparison made with term infants. Baxter found that after a three-dose schedule, 100% of infants achieved titres ≥ 0.01 IU/ml and 98.3% titres ≥ 0.1 IU/ml (136), and Vazquez that 100% of infants achieved titres ≥ 0.1 IU/ml after both the primary series and booster vaccine (134).

Comparison of term and preterm infants following primary series

Five studies compared term and preterm infants following the primary vaccine series (127-129, 135, 139) (table 3.8): in all of these, there were no significant differences in proportions reaching protective thresholds, but there was some variation in reported anti-tetanus IgG concentrations. Most studies described no difference between groups (127, 128, 135, 139), however one reported lower antibody concentrations in preterm infants (129) (table 3.8). In the two reports in which further analysis was performed according to gestational age, there were no significant differences in GMC or proportions above a protective threshold according to gestational age (127, 129).

Table 3.8. GMC/T and percentage of infants reaching a protective threshold in studies comparing term and preterm infants after primary tetanus vaccine series.

Author, year	IgG GMC/T (μ g/ml) (95% CI)			Percentage of infants reaching protective thresholds (%) (95% CI)			
				≥ 0.1 IU/ml		≥ 0.01 IU/ml	
	PT	T	P value	PT	T	PT	T
Berrington, 2006 (135)	0.59 (0.48-0.71)	0.48 (0.37-0.63)					
Kulkarni- Munje, 2021 (127)	1.44*	1.82		100 (100- 100)	100 (100- 100)	100 (100- 100)	100 (100- 100)
	1.47**			100 (100- 100)		100 (100- 100)	
Omenaca, 2005 (128)	2.46 (2.11-2.87)	2.30 (1.96-2.70)		100 (96.1- 100)	100 (95.9- 100)		

Rouers, 2020 (129)	1.25 (1.13-1.39)	2.1 (1.80-2.47)		100 (98-100)	100 (93-100)		
Slack, 2004 (139)	1.09 (0.94-1.26)	1.30 (1.03-1.63)	0.20				

GMT/C= geometric mean titre/concentration; PT= preterm; T= term; shaded boxes show where data is not available

*28-32 WG, **32-36 WG

Comparison of term and preterm infants following booster vaccination

Three studies compared responses following booster vaccination (129, 132, 138). Perin studied infants who had received a three-dose primary series prior to receiving a booster at 15 months and found that whilst preterm infants had lower anti-tetanus GMC prior to booster vaccination (term 0.205 [95% CI 0.012-1.080], preterm 0.147 [95% CI 0.015-0.969]; $p=0.025$), following the booster these were not significantly different (term 1.867 [95% CI 0.164-12.505], preterm 1.997 [95% CI 0.280-9.959]; $p=0.852$) and proportions with a titre ≥ 0.1 IU/ml after booster were 100% in both term and preterm infants (138). Omenaca followed up infants who had been included in a previous study at the time of booster vaccination at 18-20 months and found no differences in anti-tetanus GMC or proportions reaching a protective threshold either before (preterm: GMC- 0.198 [95% CI 0.160-0.244]; ≥ 0.1 IU/ml- 73.8% [95% CI 63.1-82.8]; term: GMC- 0.172 [95% CI 0.135-0.221]; ≥ 0.1 IU/ml- 68.7% [95% CI 56.2-79.4] or after booster vaccination (preterm: GMC 7.8 [95% CI 6.6-9.3]; ≥ 0.1 IU/ml 100% [95.7-100; term: GMC 9.1 [7.0-12.0] ≥ 0.1 IU/ml 98.5% [95% CI 92.1-100]) (132). Rouers reported significantly higher IgG GMC following booster vaccination in term infants (preterm 3.71 [3.42-4.03], term 5.55 [4.76-6.46]; $p<0.001$), but 100% of infants in both groups reached a protective threshold. In further analysis according to gestational age

there were no differences in IgG GMC between the different gestational age groups (129).

Cellular immunity following booster vaccination

In one study cellular immunity was assessed before and after booster vaccination following a three-dose primary series of tetanus vaccine. Following stimulation with tetanus toxoid, Perin found no significant difference in the percentages of CD4 and CD8 T cells, which express intracellular interferon-gamma, between preterm and term infants (138).

Long-term immunogenicity

Longer term persistence was studied by Kirmani, Bednarek and Omenaca, measuring the persistence of anti-tetanus antibody at 7, 4.5-5.9 and 4 years respectively (122, 126, 132). Kirmani studied infants who had received a three-dose primary series at two, four and six months and a booster at 12-18 months. Anti-tetanus GMTs were lower in preterm infants (term 4.22, preterm 1.99; $p=0.04$), but 100% of infants in both groups maintained titres ≥ 0.01 IU/ml (126). Infants studied by Bednarek received a three-dose primary series at two, four and six months followed by a booster at 16 months. There was no difference between term and preterm infants in the proportions who had maintained antibody concentrations ≥ 1.0 IU/ml, with high percentages in both groups (71.7% in preterm and 69.0% in term infants) (122). Omenaca included infants who had received a three-dose primary series at two, four and six months and a booster at 18-20 months and found no difference between term and preterm infants in anti-tetanus GMC (preterm 10.3 [95% CI 8.7-12.1], term 10.0 [95% CI 8.1-12.3]) or percentages with protective titres ≥ 0.1 IU/ml (100% in both groups) (132).

Summary

Overall, tetanus vaccine was highly immunogenic in preterm infants, with extremely high proportions of infants reaching a protective threshold and only one report detailing lower anti-tetanus antibody in preterm infants following primary vaccination. Following booster vaccination there were again extremely high proportions of infants with protective levels of antibody and no difference between term and preterm infants and this continued longer term. This was also seen in extremely preterm infants.

3.2.1.3 Diphtheria

Background

A vaccine against diphtheria has been available since 1921 and has been combined with tetanus and pertussis components since the 1940s. There is a well-established threshold for protection against disease, with ≥ 0.01 IU/ml providing partial protection and ≥ 0.1 IU/ml optimal protection.

Description of studies

Eleven papers studied the immunogenicity of diphtheria vaccination (122, 126-129, 132, 134, 136, 139-141). All the studies were observational: six prospective cohort studies (126-128, 132, 139, 141), one historical cohort study (129), three case series, (134, 136, 140) and one cross-sectional study (122).

Preterm infants following primary and booster vaccination with no term comparison

Two studies reported on results of preterm infants only with no comparison to term infants (134, 136). Baxter reported that 98.3% of infants achieved a minimum protective titre of ≥ 0.01 IU/mL and 76.5% an optimal titre of ≥ 0.1 IU/mL following a three-dose primary series (136). This study also showed a significant inverse relationship between gestational age and antibody response, with significantly more

infants born at an earlier gestation achieving a protective response than infants born at a later gestation. The authors speculated that this might have resulted from inhibition from maternal antibody (136). Vazquez reported that 99.3% of infants achieved a titre ≥ 0.1 IU/mL following primary vaccines and 99.2% following booster vaccines (134).

Comparison of term and preterm infants following primary series

Four studies compared responses in preterm and term infants following their primary vaccine series (table 3.9). Three of these studied proportions achieving protective titres and found that these were similar when preterm and term groups were compared overall and when Kulkarni-Munje and Rouers compared the preterm groups separately according to gestation there were no differences seen according to gestation (127-129). All four reports included a description of anti-diphtheria IgG concentrations and reported differing results (127-129, 139). Slack and Kulkarni-Munje found no significant differences between groups, whereas Omenaca and Rouers reported significantly lower concentrations in preterm infants (127-129, 139) (table 3.9)

Table 3.9. GMC and proportions reaching a protective threshold in studies comparing term and preterm infants after primary series of diphtheria vaccine.

Author, year	GMC (IU/ml) (95% CI)		Percentage of infants reaching protective thresholds (%) (95% CI)			
			≥ 0.01 IU/ml		≥ 0.1 IU/ml	
	PT	T	PT	T	PT	T
Kulkarni-Munje, 2021 (127)	0.53*	0.70	100 (100-100)*	100 (100-100)	91.66 (61.6-100)*	98.14 (95.19-100)
	0.65**		100 (100-100)**		98.18 (95-100)**	
Omenaca, 2005 (128)	3.661 (3.064-4.376)	5.441 (4.547-6.511)			100 (96.1-100)	100 (95.9-100)
Rouers, 2020 (129)	0.44 (0.35-0.55) †	0.64 (0.53-0.77)	100 (94-100) †	100 (93-100)		

	0.37 (0.31-0.43) ‡		99 (94-100) ‡			
	0.32 (0.26-0.40) ‡‡		100 (94-100) ‡‡			
Slack, 2004 (139)	1.50 (1.33- 1.68)	1.75 (1.36- 2.26)				

GMC=geometric mean concentration; IU=international units; PT= preterm; T= term; shaded boxes show where data is not available

*28-32 WG; **32-36 WG; †<28 WG; ‡≥28-<32 WG; ‡‡ ≥32-<=36 WG

Comparison of term and preterm infants following booster series

Omenaca followed up children at the time of their booster at 18-20 months and found no difference in antibody concentration or proportions protected following the booster (132). In contrast, Rouers reported significantly higher antibody concentrations in term infants compared to any of the preterm groups following booster vaccination, although 100% of both preterm and term infants reached protective titres (129) (table 3.10). Of note, infants in these two studies had received different priming schedules, with those in Omenaca receiving the vaccine at two, four and six months and those in Rouers receiving the vaccine at two, three and four months.

Table 3.10. GMC and proportions reaching a protective threshold in studies comparing term and preterm infants receiving booster diphtheria vaccine.

Author, year	GMC (IU/ml) (95% CI)		Percentage of infants reaching protective thresholds (%) (95% CI)			
			≥0.01 IU/ml		≥0.1 IU/ml	
	PT	T	PT	T	PT	T
Omenaca, 2007 (132)	13.5 (11.5-15.9)	17.7 (15.0-20.9)			100 (95.7-100)	100 (94.7-100)
Rouers, 2020 (129)	0.99 (0.80-1.23) *	1.80 (1.56- 2.08)	100 (94-100) *	100 (93-100)		
	1.17		100			

	(0.99-1.38) **		(95- 100) **			
	1.09 (0.90-1.31) ***		100 (94-100) ***			

GMC=geometric mean concentration; IU=international units; T= term; PT= preterm; shaded boxes show where data is not available

*<28 WG; **≥28-<32 WG; ***≥32-<=36 WG

Long-term immunogenicity

Omenaca, Bednarek and Kirmani measured the persistence of the antibody response at 4, 4.5-5.9 and 7 years respectively and found that there were no significant differences between proportions reaching a protective threshold, defined as ≥ 1.0 IU/mL for Bednarek and ≥ 0.1 IU/mL for Omenaca and Kirmani, although Kirmani reported significantly lower GMCs in preterm infants (122, 126, 132) (table 3.11).

Table 3.11. GMC and percentages of infants reaching a protective threshold in studies comparing long term immunity following diphtheria vaccination.

Author, year	GMC (IU/ml) (95% CI)			Percentage of infants reaching protective thresholds (%)(95% CI)					
				>0.1 IU/ml			>1.0 IU/ml		
	PT	T	P value	PT	T	P value	PT	T	P value
Bednarek, 2018 (122)				81	100	0.23	52.1	42.2	0.201
Kirmani, 2002 (126)	0.37	1.07	0.009	81	100	0.23			
Omenaca, 2007 (132)	28.6 (24.3- 33.7)	33.7 (27.3- 41.6)		100	100				

GMC=geometric mean concentration; IU=international units; T= term; PT= preterm; shaded boxes show where data is not available

Summary

Diphtheria vaccination was highly immunogenic, with high proportions of infants reaching the protective thresholds and no difference in these between term and preterm infants, although with some variation between studies in reported anti-diphtheria IgG GMC following primary vaccination despite similar schedules being

used. Following booster vaccination there were no differences in proportions reaching protective thresholds and this was sustained long term, although some studies continued to find lower anti-diphtheria IgG GMC in preterm infants.

3.2.1.4 Hepatitis B

Background

Routine hepatitis B vaccination was introduced in the UK infant schedule in 2017 in the form of the hexavalent vaccine Infanrix-hexa. The routine hepatitis B schedule is distinct from the schedule used in high-risk infants in which an additional monovalent hepatitis B vaccine is given at birth. There is a well-established correlate of protection for anti-HBs antibody concentrations of 10 mIU/mL.

Description of studies

Five reports included consideration of immune response to hepatitis B vaccination given outside the immediate neonatal period (91, 126, 128, 132, 134). All of the studies were observational: four prospective cohort studies (91, 126, 128, 132) and one case series (134).

Preterm infants with no term comparison

Vazquez reported on vaccine responses from preterm infants alone with no comparison made to a term cohort. Using a threshold of ≥ 10 mIU/mL, 94% of preterm infants reached a protective threshold following a three-dose primary vaccination series and 92% following a booster at 18-24 months (134).

Comparison of term and preterm infants following primary series

Two reports compared responses in term and preterm infants following primary vaccination, both of which reported high and comparable proportions of infants who reached a protective threshold between term and preterm groups, although one

report by Omenaca (2005) found no difference in anti-HBs antibody concentrations between groups (128), whereas Omenaca (2011) who separated preterm groups according to gestational age, found that preterm infants with earlier gestations had significantly lower antibody concentrations than those in the later gestation preterm group or term infants (91) (table 3.12). In contrast to these results, Omenaca (2005) found that all six of the preterm infants who did not reach the protective threshold were greater than 31 WG (128). Two of the six infants had severe growth restriction and poor weight gain after birth which may have contributed to the poor response, but the authors offered no suggestions about the other four infants.

Table 3.12. GMC and percentages of infants reaching a protective threshold in studies comparing long term immunity following hepatitis B vaccination.

Author, year	GMC (mIU/ml) (95% CI)		Percentage of infants reaching protective thresholds ≥ 10 mIU/ml (%) (95% CI)	
	PT	T	PT	T
Omenaca, 2005 (128)	634.1 (433.8-927.9)	867.1 (576.6-1303.9)	93.4 (86.2-92.7)	95.2 (88.3-98.7)
Omenaca, 2011 (91)	264.3 (180.2-387.8)*	586.6 (473.8-726.2)	97.5 (86.8-99.9)*	100 (97.2-100)
	434.3 (334.9-563.1)**		100 (95.9-100)**	

GMC=geometric mean concentration; T= term; PT= preterm

*<31 WG; **31-36 WG

Comparison of term and preterm infants following booster series

In one study, immunogenicity was assessed prior to and following booster vaccination at 18-20 months. The anti-HBs GMCs were comparable between term and preterm infants, but proportions with titres ≥ 10 mIU/mL were lower in preterm infants (prior to booster: percentage ≥ 10 mIU/mL- preterm 75.0 (95% CI 64.4-83.8), term 80.6 (95% CI 69.1-89.2), GMC- preterm 56.8 (95% CI 39.6-81.4), term 58.1 (95% CI 39.1-86.3); post booster: percentage ≥ 10 mIU/mL- preterm 91.6 (95% CI 83.4-96.5), term 98.5 (95% CI

92.1-100, GMC- preterm 1771.0 (95% CI 1060.3-2958.1), term 1965.0 (95% CI 1180.1-3272.0) (132).

Long-term immunogenicity

Kirmani studied the persistence of hepatitis B antibody at the age of seven years and found no significant difference in anti-HBs GMT or proportions with a concentration ≥ 10 mIU/mL between term and preterm infants (GMT: PT 186, FT 120, $p = 0.62$; proportions ≥ 10 mIU/mL PT 86%, FT 69%, $p = 0.27$) (126).

Summary

Hepatitis B vaccination given after the newborn period (first dose given after 28 days) was immunogenic after the primary series with high proportions reaching a protective threshold, although one report described lower antibody concentrations in the most preterm infants. Although the single study which compared term and preterm infant responses post-booster found that proportions reaching a protective threshold were lower in preterm infants, they were still high at 91.6% and there was no difference between groups seen in the persistence of immunogenicity.

3.2.1.5 Pertussis

Background

Pertussis vaccines have been part of the routine infant vaccination programme since the 1950s, first as a whole cell vaccine and then, since 2004, as an acellular vaccine.

The antigens included in acellular pertussis vaccines vary, but pertussis toxin (PT), filamentous haemagglutinin (FHA) and pertactin (PRN) are most commonly included.

Description of studies

Fifteen reports were included which investigated response to pertussis vaccine (122, 126-129, 132-134, 139-145). All were observational studies: seven were prospective

cohort studies (126-128, 132, 133, 139, 141), one was an historical cohort study (129), five were case series (134, 140, 142, 143, 145) and two were cross sectional studies (122, 144). The absence of an established correlate of protection for pertussis means that each study which described 'proportions protected' defined this putative protection differently which reduced the ability to compare outcomes from different studies.

Preterm infants with no term comparison following primary and booster vaccination

Two studies reported results for preterm infants only, with no comparison made to term infants (134, 142). One report by Sikora used a seroconversion threshold of Virotech units >11.0 and reported results for FHA and PT. In this, seroconversion occurred in 94.7% after primary vaccination and in 100% following a booster dose (142). Vazquez reported that protective titres (defined as ≥ 5 ELISA Units/mL) [EL.U/ml] were reached in very low birth weight infants in 92.4% (95% CI 84.2-97.2), 98.7% (95% CI 93-100) and 100% (95% CI 95.3-100) for PT, FHA and PRN respectively and in 96.3% (95% CI 89.7-99.2) for all pertussis antigens in low birthweight infants (134). Following the booster dose, the proportion putatively protected increased in very low birthweight babies to 98.4% (95% CI 96.3-100) for PT and 96.8% (95% CI 88.8-99.6) for FHA and PRN and in low birthweight babies to 98.2% (95% CI 90.4-100) for PT and 100% (95% CI 93.7-100) for FHA and PRN (134).

Comparison of term and preterm infants following primary series

Five studies compared responses in term and preterm infants with differing results (127-129, 133, 139). Slack (2005), Omenaca and Kulkarni-Munje found no differences in antibody concentrations for any of the tested pertussis antigens (127, 128, 133). In contrast, Rouers reported significantly lower IgG GMC in preterm infants for FHA and

PRN, but no difference for PT (129), and Slack (2004) found no differences in antibody concentrations against FHA and PRN, but these were significantly lower for PT for preterm infants (139). Rouers reported results according to gestational age and found that for PT there was a significant difference between those infants born at a gestational age <28 WG and those infants born at 32-36 WG (129) (table 3.13).

Table 3.13. Comparison of IgG GMC against pertussis antigens following primary vaccination in term and preterm infants.

Author, year	IgG GMC (95% CI)								
	FHA			PRN			PT		
	PT	T	P value	PT	T	P value	PT	T	P value
Kulkarni- Munje, 2021 (127)							29.23*	30.28	
							25.64**		
Omenaca, 2005 (128)	239.5 (205.8- 278.6)	252.8 (226.2- 282.5)		155.3 (131.3- 183.7)	200.3 (167.5- 239.5)		61.3 (53.1-70.8)	60.4 (53.8-67.8)	
Rouers, 2020 (129)	67.5 (61.1- 74.6)	98.7 (84.0- 116.1)		96.5 (86.2- 108.0)	148.6 (126.0- 175.3)		67.4 (61.1-74.4)	80.6 (68.8-94.4)	
Slack, 2004 (139)	54.7 (48.2- 62.1)	59.8 (48.7- 73.4)	0.46	82.6 (70.1- 97.3)	86.6 (64.9- 115.4)	0.77	21.0 (18.4-23.9)	33.4 (28.2-39.5)	<0.001
Slack, 2005 (133)	65.91 (55.31- 78.53)	48.1 (39.4- 50.3)		38.83 (26.43- 57.04)	34.1 (26-44.7)		76.82 (62.35- 94.64)	86.4 (68.5- 109.0)	

GMC=geometric mean concentration; FHA=filamentous haemagglutinin; PRN=pertactin; PT=pertussis toxin; T= term; PT= preterm; shaded boxes show where data is not available

*28-32 WG; **32-36 WG

Comparison of term and preterm infants following booster series

Omenaca studied the response to a booster vaccine at 18-20 months of age and found no significant difference between term and preterm infants prior to or following vaccination (132), whereas Rouers found significantly higher antibody concentrations for PRN in term infants following booster vaccination (129). Of note, the infants in

Omenaca's report had received a 3-dose primary series at two, four and six months compared with a three-dose primary series at two, three and four months used by Rouers. In a further analysis according to gestational age, Rouers found a significant difference between the gestational age groups for PT with increasing IgG GMC with increasing gestational age (<28 WG 95.9 [95% CI 81.2-113.3] vs 32-36 WG 147.9 [95% CI 121.8-179.6], $p<0.01$; 28-31⁺⁶ WG 109.5 [95% CI 94.3-127.1] vs 32-36 WG 147.9 [95% CI 121.8-179.6], $p<0.05$) (129).

Table 3.14. Comparison of IgG GMC against pertussis antigens following booster vaccination in term and preterm infants.

Author, year	FHA		PRN		PT	
	IgG GMC (95% CI)		IgG GMC (95% CI)		IgG GMC (95% CI)	
	PT	T	PT	T	PT	T
Omenaca, 2007 (132)	578.4 (491.0- 681.5)	583.6 (476.3- 715.0)	456.5 (360.4- 578.3)	544.4 (404.2- 733.4)	58.9 (49.8-69.7)	63.1 (51.0-78.1)
Rouers, 2020 (129)	158.8 (145.7- 173.2)	148 (126.3- 173.4)	193.1 (171.0- 218.1)	303.0 (247.6- 370.9)	115.4 (104.6- 127.3)	101.5 (87.1-118.2)

GMC=geometric mean concentration; FHA=filamentous haemagglutinin; PRN=pertactin; PT=pertussis toxin; T= term; PT= preterm

Long-term immunogenicity

Kirmani, Esposito, Bednarek, and Omenaca followed up a group of term and preterm infants 7, 5, 2.5-3.5, and 2-2.5 years after receiving vaccination respectively (122, 126, 132, 144). Kirmani, Bednarek and Omenaca found no significant differences between term and preterm groups (122, 126, 132), however Esposito found significantly lower IgG antibody levels and proportions of preterm infants maintaining a protective threshold for FHA and PRN, particularly in those infants born <31 WG (144). The infants in this study had received a 2+1 schedule in contrast to the 3+1 schedule administered to the infants in the other three studies.

Table 3.15. Comparison of persistence of IgG GMC and percentage reaching a 'protective threshold' against pertussis antigens in term and preterm infants.

Author, year	Antigen	IgG GMC (95% CI)		Percentage reaching 'protective' threshold (95% CI)	
		PT	T	PT	T
Bednarek, 2018 (15) †	FHA				
Esposito, 2002 (42) ^		9.53‡	28.97	53.8‡	93.3
		12.81‡‡		70.0‡‡	
Omenaca, 2007 (29) *		914.1 (776.4-1076.1)	1035.9 (832.8-1288.5)	100 (95.1-100)	100 (92.3-100)
Bednarek, 2018 (15) †	PRN				
Esposito, 2002 (42) ^		6.77‡	25.11	38.4‡	93.3
		2.59‡‡		60.0‡‡	
Omenaca, 2007 (29) *		596.7 (464.4-766.8)	686.5 (494.9-952.4)	100 (95.1-100)	100 (92.3-100)
Bednarek, 2018 (15) †	PTox			89.1	88.2
Esposito, 2002 (42) ^		1.17‡	1.77	0‡	20.0
		1.36‡‡		0‡‡	
Omenaca, 2007 (29) *		58.8 (48.6-71.1)	71.1 (53.0-95.2)	100 (95.1-100)	100 (92.1-100)

GMC=geometric mean concentration; FHA=filamentous haemagglutinin; PRN=pertactin; PT=pertussis toxin; T= term; PT= preterm; shaded boxes show where data is not available

* Threshold used >5EL U/ml; † threshold used >10 U/ml; ^threshold used post-vaccination titre at least four times that of pre-vaccination titre

‡ ≤31 WG; ‡‡32-37 WG

Cellular immunity

Vermeulen studied cellular immunity following pertussis vaccination in preterm infants and found that more than half of preterm infants were able to generate a specific T-cell response to PT or FHA (143). This persisted at one year of age and a booster did not significantly increase the cellular immune response (145). Kulkarni-Munje and Esposito compared the cellular immunity demonstrated by term and preterm infants. Kulkarni-Munje found an absence of proliferating memory T-cells in preterm infants compared with a good response in term infants (127). When following children up to

five years following vaccination, Esposito also found reduced lymphoproliferative responses in preterm compared to term infants, which was significant for those infants <31 WG (144).

Summary

There is considerable variation in humoral immunogenicity reported following both primary and booster vaccination, with some studies showing reduced immunogenicity particularly in infants born at the earliest gestations, and cellular immunity appears to be consistently reduced in preterm infants rendering those infants at increased risk of infection. Longer term immunogenicity between term and preterm infants was similar in those infants who had received a 3+1 schedule, although there were lower GMCs in preterm infants and a lower proportion of preterm infants maintaining a 'protective threshold' for two pertussis antigens in infants who had received a 2+1 schedule.

3.2.1.6 Polio

Background

The inactivated polio vaccine (IPV) was added to the routine infant schedule in the UK in 2004 following removal of the live vaccine. The IPV contains three poliovirus types and response to vaccination is measured using a poliovirus neutralising antibody assay. The results from these neutralising antibody assays can be reported in different ways: GMTs, the percentage of infants with neutralising antibodies above a 1:8 threshold (often referred to as the seroprotection rate) and the percentage of infants with a fourfold or greater rise in neutralising antibody (often referred to as the seroconversion rate) (146).

Description of studies

Eight reports were identified which investigated response to polio vaccination, all of the studies used inactivated polio vaccine (IPV) (122, 126, 128, 132-134, 147, 148). All of the studies were observational: two case series (134, 148), one cross sectional study (122) and five prospective cohort studies (126, 128, 132, 133, 147).

Preterm infants with no term comparison following primary and booster vaccination

Two studies reported results for preterm infants alone with no comparison made to term infants (134, 148). Sikora studied response to poliovirus type one only and reported that 95.5% of preterm infants achieved a protective titre, defined in this study as $>1:16$, following a three-dose primary schedule. After a booster dose at 16-18 months of age there was a significant increase in antibody concentration. There was no association between proportions reaching a protective threshold and gestational age or birthweight (148). Vazquez reported that 100% of low birthweight (LBW) and very low birthweight (VLBW) preterm infants achieved protective titres, defined as $\geq 1:8$, against all three poliovirus serotypes, both after the three-dose primary series and after booster vaccination at 18-24 months of age, although VLBW infants had significantly lower anti-poliovirus 3 GMT after the primary vaccine series (134).

Comparison of term and preterm infants following primary series

Three studies were identified which compared responses in term and preterm infants, all of which showed that there were no differences in proportions of term and preterm infants achieving protective titres, defined as $\geq 1:8$ (128, 133, 147). Some reports however described lower GMTs in preterm infants for some serotypes. Following a three-dose primary series Slack reported that titres were lower in preterm infants for serotypes two and three (133), Omenaca found that titres for serotype three were

significantly lower in preterm infants (128), and Klein identified lower titres for serotype one in preterm infants (147).

Comparison of term and preterm infants following booster series

Prior to and following booster vaccination at 18-20 months, Omenaca found that preterm and term infants were comparable in both their antibody titres and proportions achieving a protective threshold (132).

Long-term immunogenicity

Longer term persistence was studied by Kirmani and Bednarek, measuring the persistence of neutralising antibody against poliovirus at 7 and 4.5-5.9 years respectively. Neither reported a difference between term and preterm infants in the proportions of infants who achieved protective titres defined as $\geq 1:8$ and 12mIU/ml for Kirmani and Bednarek respectively, and, in addition, Kirmani showed comparable antibody titres (122, 126).

Cellular immunity

Klein also reported on cellular immunity following inactivated polio vaccination and found that term and preterm infants had comparable polio virus type three specific T cell responses and comparable circulating poliovirus specific responder T cells, but reduced poliovirus type three specific lymphoproliferation in preterm infants (147).

Summary

Overall, although GMTs were lower in preterm infants for some serotypes in some studies, there were no differences in term and preterm infants reaching protective titres either following primary or booster vaccination and immunogenicity was maintained longer term.

3.2.1.7 Meningococcal C

Background

Meningococcal serogroup C (MenC) vaccination was introduced in the UK in 1999 using a conjugate vaccine. Vaccination results in the production of bactericidal antibody which is measured using a serum bactericidal antibody (SBA) assay. An SBA titre of $\geq 1:8$ is established as the protective threshold (95).

Description of studies

Seven papers were identified which considered response to meningococcal group C (MenC) vaccination (90-93, 133, 136, 149). All of these were observational: five prospective cohort studies (90-93, 133), one case series (136) and one historical cohort study (149).

Preterm infants with no term comparison

Two studies reported results from preterm infants only with no comparison made to term infants. The first of these reported that 86.6% of infants developed a protective titre, with no association found between gestational age and SBA (136). Another study, in which a three-dose primary series had been used, reported 100% of preterm infants reached a protective SBA (133).

Comparison of term and preterm infants following primary series

Several studies compared responses between term and preterm infants and found no difference in antibody titres or proportions reaching protective titres following primary vaccinations (90-93). One study investigated the response following a single dose of MenC vaccine at 12-14 months of age and found that although the SBA GMT and Enzyme Linked Immunosorbent Assay (ELISA) derived antibody concentrations were comparable between the preterm population overall and an historical cohort of term

infants, the proportion with a protective SBA GMT in the preterm population overall was lower than that for term infants (149) (table 3.16).

Table 3.16. SBA GMT following primary MenC vaccination in studies comparing term and preterm infants.

Author, year	SBA GMT (95% CI)			Percentage of infants reaching protective thresholds (%) (95% CI)		
				SBA titre $\geq$ 1:8		
	PT	T	P value	PT %	T %	P value
Clarke, 2006 (149)	248.7 (110.8-558.1)	141.3 (89.2-224.1)	0.06	77	91	0.03
Collins, 2005 (92)	506 (30.6-836)	691 (467-1023)	0.33	95	98	0.3
Esposito, 2007 (93)	1243 (807-1915)	1389 (902-2139)		100 (86-100)	100 (86-100)	
Omenaca, 2011 (91)	1098.6 (790.2-1527.3)	1346.2 (1130.4-1603.1)		100 (92.1-100)	100 (97.4-100)	
	1036.8 (796.4-1349.8)			99 (94.4-100)		
Slack, 2001 (90)	398 (298-532)	380 (275-525)	0.44	99	98	1.0

SBA=serum bactericidal antibody; GMT=geometric mean titre; PT=preterm; T=Term; shaded boxes show where data is not available

*<31 WG; **31-36 WG

In a meta-analysis, there was equivalence seen between the proportions reaching a protective threshold in preterm and term infants (figure 3.5).

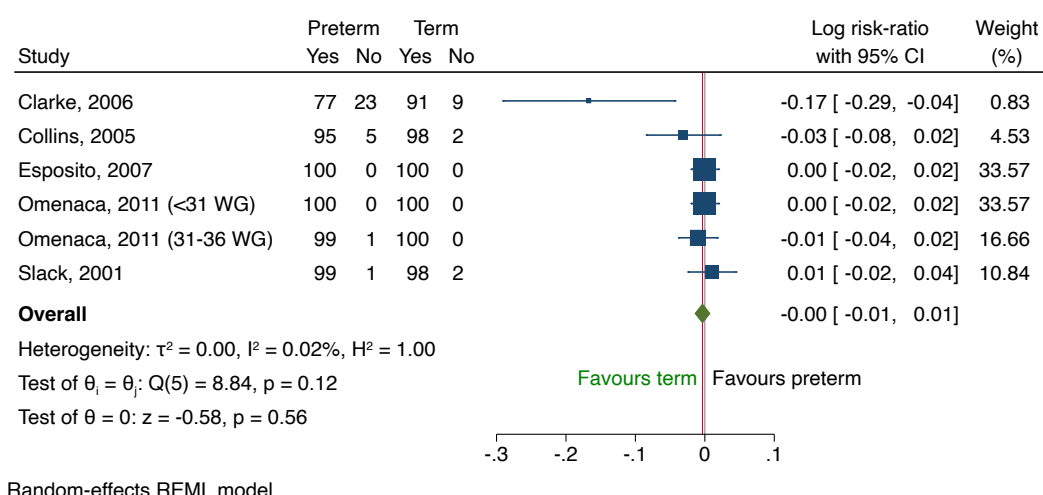


Figure 3.5. Forest plot showing proportions of infants reaching a protective MenC SBA GMT in preterm and term infants.

Comparison of term and preterm infants following booster vaccination

Following the booster dose, both Omenaca and Esposito found no differences

between term and preterm infants either in SBA GMT or proportions of infants with

protective titres between the two preterm or the term groups (91, 93). Collins also

found that the proportions with SBA titres $\geq 1:8$ were not significantly different

between term and preterm infants but reported significantly lower SBA GMT following

booster vaccine in preterm infants (92) (table 3.17).

Table 3.17. SBA GMT following booster MenC dose in studies comparing term and preterm infants.

Author, year	SBA GMT (95% CI)			Percentage of infants reaching protective thresholds (%) (95% CI)		
				SBA titre $\geq 1:8$		
	PT	T	P value	PT	T	P value
Collins, 2005 (92)	408 (232-717)	891 (568-1399)	0.03	90	98	0.08
Esposito, 2007 (93)	1992 (1339-2964)	2435 (1623-3654)		100 (86-100)	100 (86-100)	
Omenaca, 2011 (91)	4570.8 (2784.1-7504.3)*	5288.8 (4244.9-6589.4)		100 (90.5-100)*	99.3 (96.0-100)	

	5009.0 (3699.6- 6781.9)**			99 (94.3-100)**		
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SBA=serum bactericidal antibody; GMT=geometric mean titre; PT= preterm; T= term; WG= weeks of gestation; shaded boxes show where data is not available * <31 WG **31-26 WG

Summary

Overall MenC vaccine was very immunogenic in preterm infants when a multiple dose primary series was used, although some reduction in proportions with protective titres were seen when a single dose of MenC was used outside of infancy in children aged 12-14 months of age. The proportions of infants reaching a protective threshold were similar in term and preterm infants and this is supported by the results of a meta-analysis.

3.2.1.8 Pneumococcal

Background

Pneumococcal conjugate vaccines (PCV) were introduced in the UK in 2006 and the PCVs currently available include between 7 and 13 serotypes. An aggregate correlate of protection for all serotypes of ≥ 0.35 $\mu\text{g/ml}$ is used, although there is evidence that serotype specific correlates of protection may vary (150).

Description of studies

Twelve papers reported results on vaccine responses to pneumococcal vaccination (88, 129, 136, 151-159). These were mainly observational studies: three case series (136, 151, 154), six prospective cohort studies (88, 152, 155-157, 159), one historical cohort study (129) and two randomised controlled trials (153, 158).

Preterm infants with no term comparison

Five studies reported results from preterm infants only with no comparison made to term infants following the primary vaccine series (table 3.19 and 3.20). Both Baxter

and D'Angio reported variability in the antibody response between serotypes: the lowest response was seen for 6B and the highest for 19F (136, 151), although D'Angio found higher proportions with titres ≥ 0.35 $\mu\text{g/ml}$ for all serotypes (151) (table 3.19). D'Angio found that the proportion of infants achieving protective titres for serotypes 6B and 23F was reduced in those infants with a lower birthweight of 401-1000g and in those who had received postnatal steroid treatment. Szynczewska compared infants with birthweights of $<1000\text{g}$ and $\geq 1000\text{g}$ and identified no differences in the antibody concentrations or proportions with protective titres following a primary series. Serotypes 6B and 23F were the least immunogenic and serotype 14 the most immunogenic (154). Kent compared three pneumococcal vaccine schedules and found significant differences between schedules, with lack of seroprotection for more than 50% of PCV13 serotypes being seen in 25%, 12% and 3% for the reduced (2/4 month), accelerated (2/3/4 month) and extended (2/4/6 month) schedules respectively (153). Another study reported that proportions with protective titres were lowest for 6B and that pneumococcal antibody concentrations were higher following primary vaccination for serotypes 6B, 18C, 23F in those infants born at a later gestation (158).

Table 3.18. Percentages of preterm infants who reached the protective threshold of ≥ 0.35 $\mu\text{g/ml}$ for serotypes covered in PCV7 following the primary vaccination series.

Author, year	Percentage of infants reaching protective thresholds (%)					
	Serotypes					
	4	6B	9V	14	18C	19F
Baxter, 2010 (136)	77.5	67.5	87.5	87.5	87.5	92.5
D'Angio, 2010 (151)	96.7	84	95.5	97.1	98.4	99.2
Kent, 2016 (red) (153)	92.0	20.0	59.0	94.0	88.0	97.0
Kent, 2016 (acc) (153)	88.0	52.0	85.0	98.0	87.0	100
Kent, 2016 (ext) (153)	94.0	78.0	93.0	99.0	96.0	100

Szynczewska, 2013 (154)	93.3	71.6	95.0	96.7	96.7	98.3
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red=reduced; acc=accelerated; ext=extended

Table 3.19. Percentages of preterm infants who reached the protective threshold of ≥ 0.35 $\mu\text{g/ml}$ for additional serotypes covered in PCV13 following primary vaccine series.

Author, year	Percentage of infants reaching protective thresholds (%)					
	Serotypes					
	1	3	5	6A	7F	19A
Kent, 2016 (red) (153)	85.0	61.0	36.0	58.0	91.0	83.0
Kent, 2016 (acc) (153)	80.0	66.0	47.0	72.0	97.0	95.0
Kent, 2016 (ext) (153)	94.0	80.0	74.0	94.0	100	96.0

red=reduced; acc=accelerated; ext=extended

Following a booster dose, Szynczewska reported a significant increase in antibody concentration and high proportions achieving a protective threshold, with no difference seen between those infants weighing $<1000\text{g}$ and $\geq 1000\text{g}$. In a comparison of three schedules, Kent reported that following the booster dose, those infants who had received the extended schedule had significantly lower GMCs compared with those who had received the reduced (for 9/13 serotypes) or accelerated (for 4/13 serotypes) schedules, however almost all infants, irrespective of schedule, reached the protective threshold (153) (table 3.20). Van den Berg found that higher antibody concentrations were seen in those with higher gestations for 6B and 14 following booster vaccination (158).

Table 3.20. Percentages of preterm infants who reached the protective threshold of ≥ 0.35 $\mu\text{g/ml}$ for serotypes covered in PCV7 following a booster dose.

Author, year	Percentage of infants reaching protective thresholds (%)						
	Serotypes						
	4	6B	9V	14	18C	19F	23F
Kent, 2016 (red) (153)	100	98	98	100	100	100	98
Kent, 2016 (acc) (153)	98	97	98	100	97	100	100

Kent, 2016 (ext) (153)	99	99	99	100	94	100	97
Szynczewska, 2014 (154)	98	100	100	100	100	100	100

red=reduced; acc=accelerated; ext=extended

Comparison of term and preterm infants following primary series

Six studies compared term and preterm infants with variable results. Some authors reported reduced antibody concentrations and reduced proportions reaching a protective threshold to at least one serotype in preterm infants (88, 129, 155, 157) whereas others reported no difference between term and preterm infants in their response to PCVs (152, 159) (tables 3.21 and 3.22). In the two studies in which no difference was seen, the primary vaccination series had been given at two, four and six months (159) and three and five months (152), in contrast to a primary series given at two, three and four months in those studies where a difference was seen between term and preterm infants.

Table 3.21. Percentages of term and preterm infants with titres ≥ 0.35 $\mu\text{g/ml}$ for serotypes covered in PCV7 following primary vaccination series.

Author, year	Percentage of infants reaching protective thresholds (%)													
	Serotypes													
	4		6B		9V		14		18C		19F		23F	
	PT	T	PT	T	PT	T	PT	T	PT	T	PT	T	PT	T
Esposito, 2005 (152)	97.8	100	69.6	82.6	91.3	95.6	91.3	95.6	97.8	86.9	95.6	93.5	69.6	84.8
Martinon-Torres, 2015 (155)	97.0	99.0	73.0	87.6	97.0	97.0	100	98.0	97.0	95.0	99.0	99.0	85.9	93.0
Moss, 2010 (157)	88.0	96.0	39.0	38.0	86.0	90.0	96.0	98.0	91.0	96.0	96.0	100	71.0	96.0
Ruggeberg, 2007 (88)	86	100	41	79	92	100	93	97	95	98	97	98	81	92
Omenaca, 2011 (159)	98.3	100	94.2	93.9	99.2	100	100	100	100	98.5	100	100	95.9	95.4
Rouers, 2020 (129)	75.1	58.1	45.8	74.2	85.8	96.8	85.8	98.4	66.8	95.2	89.3	41.9	55.7	77.4

PT= preterm; T=term

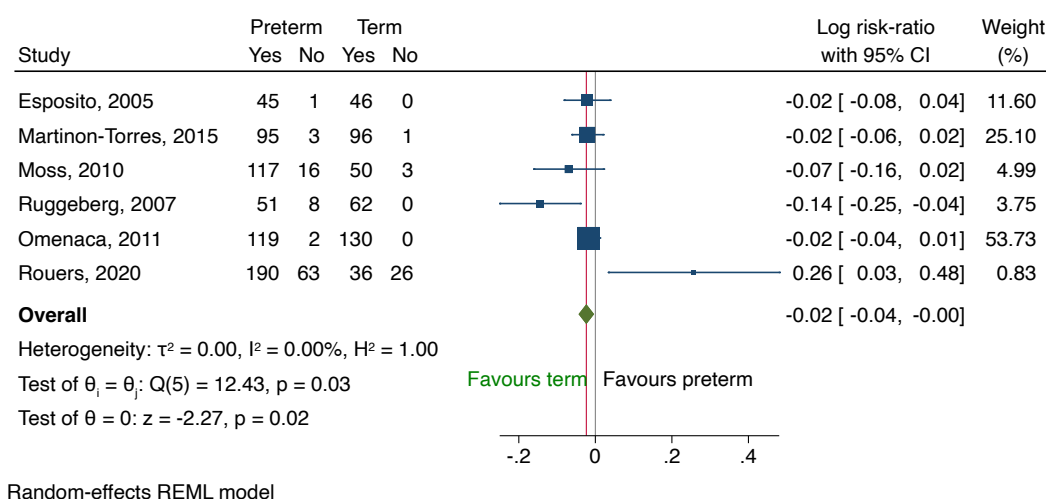
Table 3.22. Percentages of term and preterm infants with concentrations ≥ 0.35 $\mu\text{g/ml}$ for serotypes covered in PCV10/13 following primary vaccination series.

Author, year	Percentage of infants reaching protective thresholds (%)											
	Serotypes											
	1		3		5		6A		7F		19A	
	PT	T	PT	T	PT	T	PT	T	PT	T	PT	T
Martinon-Torres, 2015 (155)	94.0	95.9	86.0	0.7	72.0	90.7	83.0	94.9	99.0	99.0	99.0	99.0
Rouers, 2020 (129)	83.0	96.8			90.1	96.8			94.9	100		

PT= preterm; T=term; shaded boxes show where data is not available

Meta-analysis

In a meta-analysis of the proportions of infants reaching the threshold of ≥ 0.35 $\mu\text{g/ml}$ for each serotype included in PCV7 there was a tendency towards greater proportions of term infants reaching the threshold for serotype 4 (figure 3.6), 6b (figure 3.7), 9V (figure 3.8), 14 (figure 3.7), 18C (figure 3.9) and 23F (figure 3.11) and for preterm infants reaching the threshold for serotype 19F (figure 3.10).

**Figure 3.6. Forest plot of the proportions of infants achieving protective threshold for serotype 4.**

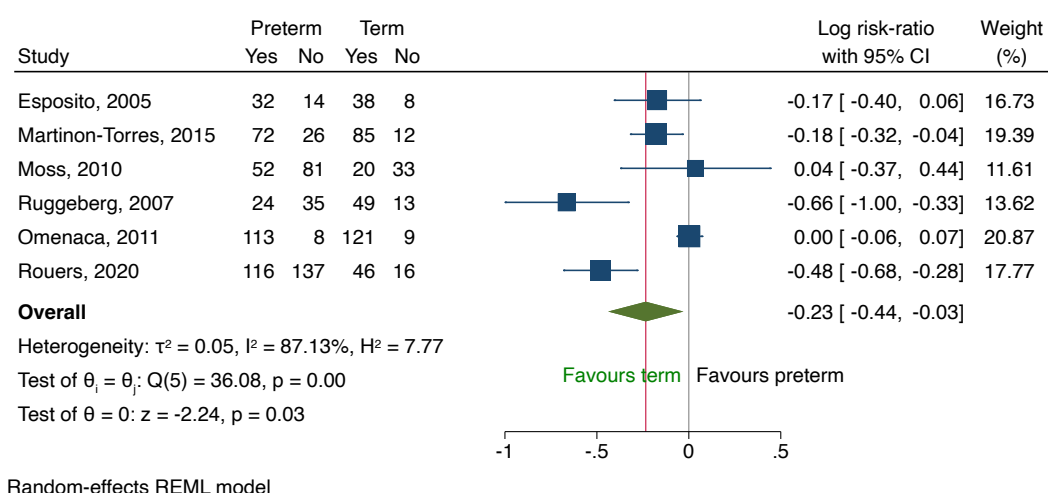


Figure 3.7. Forest plot of the proportions of infants achieving protective threshold for serotype 6B.

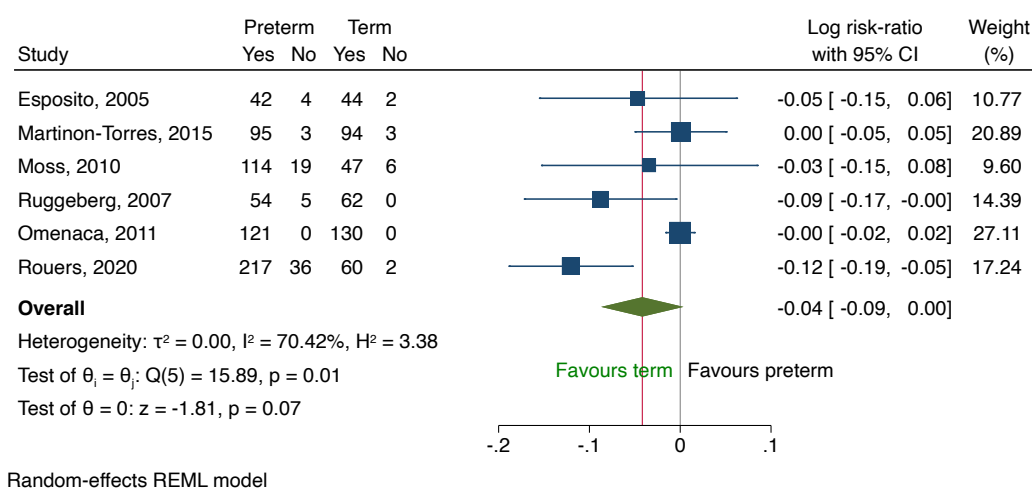


Figure 3.8. Forest plot of the proportions of infants achieving protective threshold for serotype 9V.

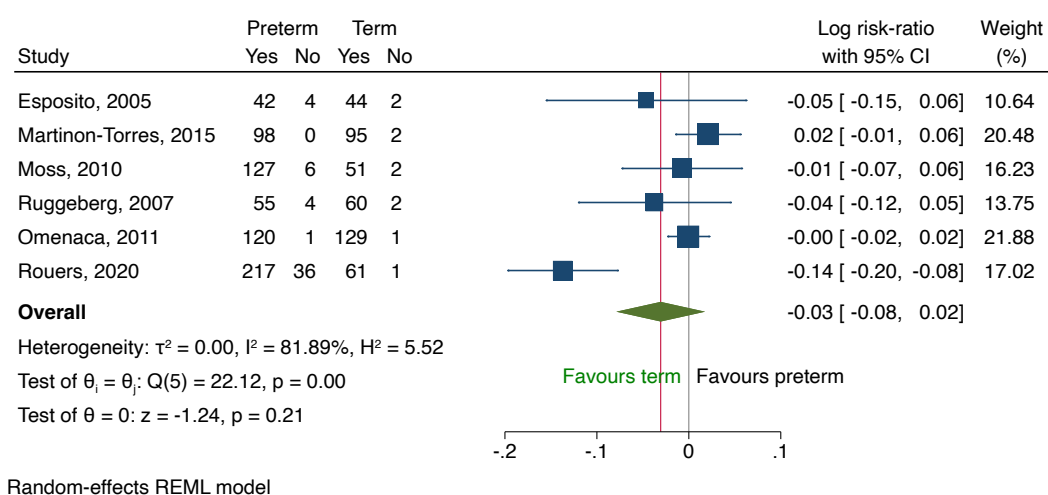


Figure 3.9. Forest plot of the proportions of infants achieving protective threshold for serotype 14.

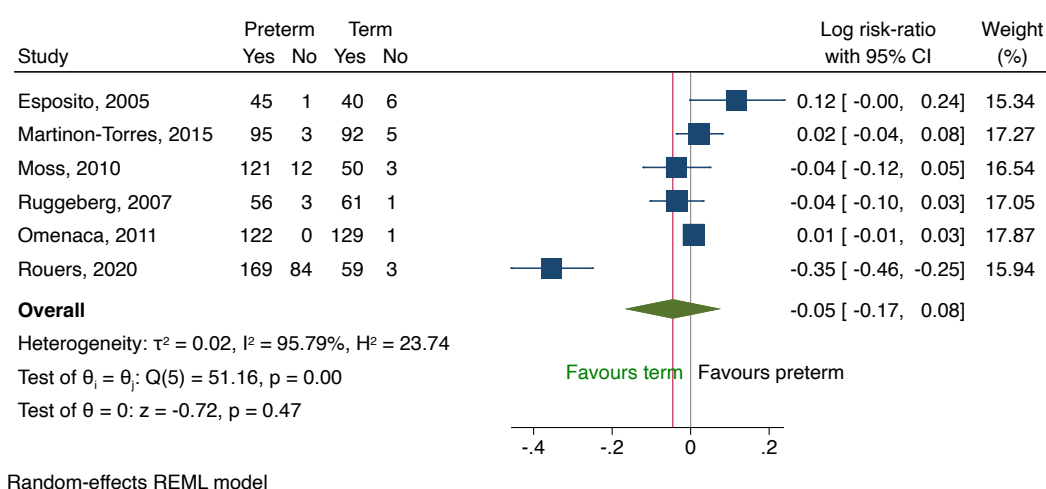


Figure 3.10. Forest plot of the proportions of infants achieving protective threshold for serotype 18C.

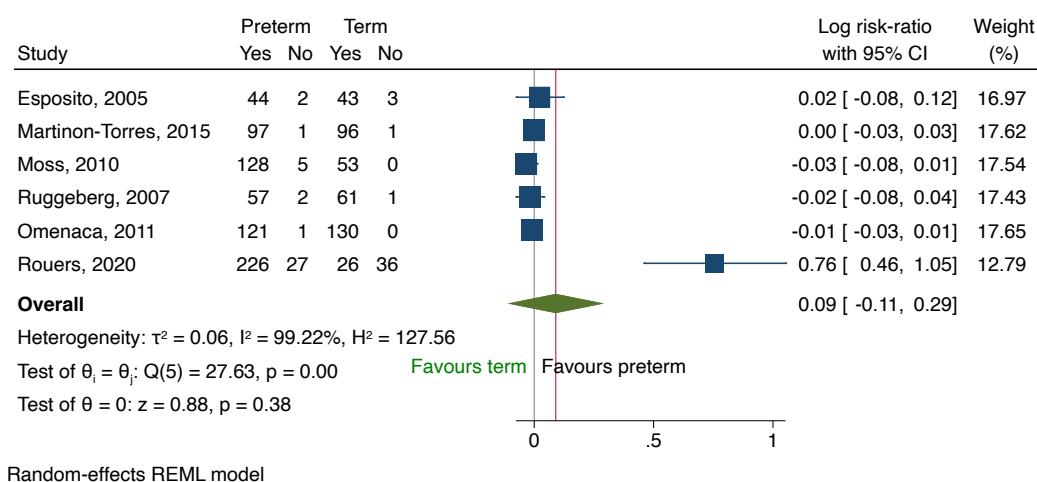


Figure 3.11. Forest plot of the proportions of infants achieving protective threshold for serotype 19F.

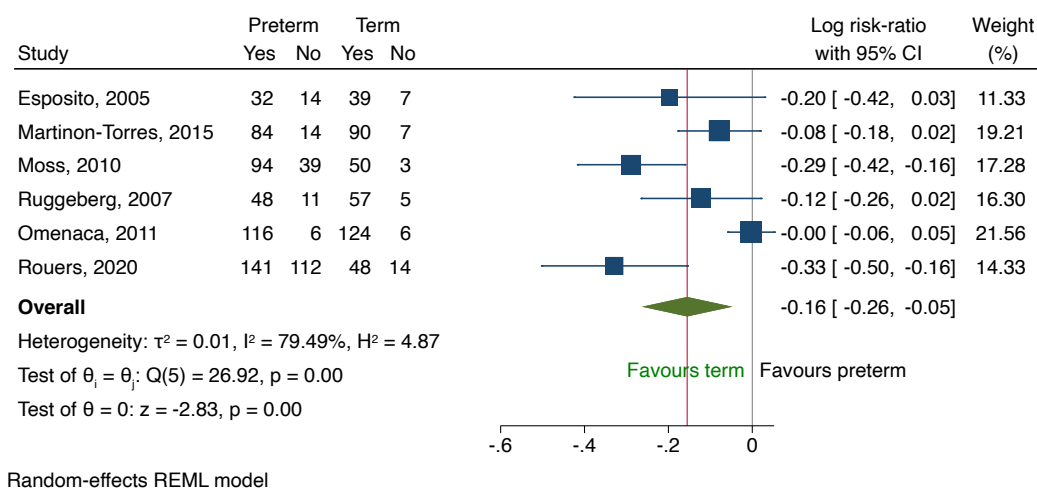


Figure 3.12. Forest plot of the proportions of infants achieving protective threshold for serotype 23F.

Comparison of term and preterm infants following booster vaccination

Six reports compared responses in term and preterm infants (88, 129, 152, 155, 157, 159). Of the studies which compared term and preterm infants, five reported proportions protected (table 3.23). Some reports identified a difference in antibody concentrations or proportion of infants reaching a protective threshold for at least one serotype (88, 129, 157) whereas others found no difference between groups following the booster (152, 155, 159).

Table 3.23. Percentages of term and preterm infants with concentrations ≥ 0.35 $\mu\text{g/ml}$ for serotypes covered in PCV7 following booster dose.

Author, year	Percentage of infants reaching protective thresholds (%)													
	Serotypes													
	4		6B		9V		14		18C		19F		23F	
	PT	T	PT	T	PT	T	PT	T	PT	T	PT	T	PT	T
Esposito , 2005 (152)	100	100	100	97.8	100	97.8	100	97.8	97.8	100	100	100	100	97.8
Moss, 2010 (157)	100	100	97	97	97	100	100	100	91	100	94	100	88	100
Ruggeb erg, 2007 (88)	98	98	94	95	98	93	100	100	98	98	94	100	96	93
Omenac a, 2011 (159)	100	100	99	100	100	100	100	100	100	99.2	100	100	99	100
Rouers, 2020 (129)	100	100	97	100	99	100	97	100	100	100	100	100	96	100

PT=preterm; T=term

Table 3.24. Percentages of term and preterm infants with concentrations ≥ 0.35 $\mu\text{g/ml}$ for serotypes covered in PCV10 following booster dose.

Author, year	Percentage of infants reaching protective thresholds (%)											
	Serotypes											
	1		3		5		6A		7F		19A	
	PT	T	PT	T	PT	T	PT	T	PT	T	PT	T
Rouers, 2020 (129)	100	98.8			100	98.4			100	100		

PT= preterm; T=term

Long-term immunogenicity

Martinon-Torres (2017) followed up infants one and two years after receiving the booster dose and found that antibody concentrations were significantly lower in preterm infants for the majority of serotypes, and proportions of infants with concentrations exceeding the protective threshold were significantly lower for 6/13 serotypes at one year and 3/13 at two years. The opsonophagocytosis assay (OPA) GMTs were lower for 4/13 serotypes at one year and 2/13 at two years, although the differences in OPA GMTs were less than that of IgG antibody concentrations suggesting that functional capacity of antibody in preterm infants may be more similar to their term counterparts (156).

Summary

There is considerable variation between reports about the immunogenicity of pneumococcal conjugate vaccine following the primary series. Overall, proportions reaching a protective threshold were lower in preterm than term infants following the primary series, but high proportions of infants in both groups reached protective concentrations following booster vaccination.

3.2.1.9 Measles, mumps and rubella (MMR)

Background

The MMR vaccine was introduced in the UK in 1988 and is an attenuated live viral vaccine containing measles, mumps and rubella viruses.

Description of studies

Four papers were identified which investigated immunogenicity of MMR vaccine (122, 160-162). Two investigated measles alone (160, 161) and the other two investigated measles, mumps and rubella (122, 162). All were observational studies: two

prospective studies (161, 162), one case series (160) and one cross-sectional study (122).

Preterm infants with no term comparison

One report included preterm infants only. In this, Ichikawa reported that all preterm infants <34 WG who received measles vaccine (AIK-C strain) at six months reached a titre of $\geq 1:4$, which is equivalent to 120 mIU/mL, and this was sustained for at least 12 months (160).

Comparison of term and preterm infants following primary series

Two reports compared term and preterm infants shortly after vaccination, both of which reported similar immunogenicity between populations. D'Angio found no statistically significant difference between term and preterm infants (<29 WG) in the percentage of infants with protective titres for measles (term: 100%, preterm: 100%), mumps (term: 84.6%, preterm: 92.9; $p=0.6$) or rubella (term: 100%, preterm: 92.9%; $p=1.0$) following vaccination at 15 months (162). Similarly, Ferreira reported no significant difference in anti-measles GMT in term and preterm infants (<37 WG) (term: 2.412 [95% CI 0.147-20.101, preterm 2.393 [95% CI 0.149-28.822; $p=0.97$] and 100% in both groups reached protective titres following a single dose of MMR at 12 months (161). Ferreira showed that increasing gestation was associated with a reduction in antibody titres which may relate to the higher levels of maternal antibody in babies born at later gestations.

Persistence of immunity

One report described the persistence of immunity from vaccination. Bednarek followed preterm infants ≥ 32 WG for 2.5-3 years after vaccination and found no significant differences in proportions protected against measles, mumps or rubella

between term and preterm infants after administration of MMR vaccination at 13-15 months (measles: term 100%, preterm 100%; mumps: term 81.4%, preterm 76.1%, rubella: term 81.4%, preterm 76.1%) (122).

Summary

MMR vaccine was highly immunogenic, including in those born before 29 WG, and this was sustained long-term. In all the studies in which a comparison was made between term and preterm infants, vaccination was given at 12 months or later by which time the impact of preterm birth may be reduced.

3.2.1.10 Rotavirus

Background

Rotavirus vaccine was introduced in the UK in 2013 and is a live, oral vaccine.

Description of studies

Only one study investigated immunogenicity of the rotavirus vaccine (163). This was a randomised controlled trial comparing infants who received rotavirus vaccine and those who received placebo. Rotavirus vaccination was immunogenic in preterm infants with anti-rotavirus seroconversion rates 30-83 days following dose 2 of 85.7% (95% CI 79.0-90.9) in the vaccine group and 16.0% (95% CI 8.8-25.9) in the placebo group. When the results were analysed according to gestational age, those 27-30 WG had a lower rate of seroconversion (75.9% [95% CI 56.5-89.7]) compared with those 31-36 WG (88.1% [95% 80.9-93.4]). This difference was not statistically significant, although this may have been because the group of the lower gestational age was small (163).

3.2.1.11 Steroid treated infants

Seven reports included results of infants receiving dexamethasone treatment, and these showed contrasting results (90, 124, 131, 140, 141, 149, 151). Previous dexamethasone treatment did not affect antibody response to MenC vaccine whether given as part of the primary series or at 13 months (90, 149). Results were varied for diphtheria, tetanus and pertussis. Broadly, Slack and Clarke reported that immunogenicity was maintained in children who had previously received dexamethasone (90, 124, 140), but Robinson reported significantly lower GMTs for diphtheria, tetanus, PT and FIM 2&3 in infants receiving dexamethasone at the time of vaccination, and a reduction in proportions reaching a threshold of ≥ 0.1 IU/ml for diphtheria and tetanus (141). The gestational ages of infants in these studies were similar and all were vaccinated according to the same schedule, but those reported by Slack and Clark had previously received dexamethasone whereas those reported by Robinson were currently receiving this treatment. Not only might this have an immunological effect, but there are also likely to be clinical differences between infants who currently require dexamethasone treatment and those who do not. It is also notable that Robinson found that the non-steroid treated comparator group had elevated titres for tetanus compared to other published studies which may have over-estimated the impact of dexamethasone for this antigen and the baseline titres for PT were elevated which may have confounded the results (141). Omenaca reported no significant decrease in anti-PRP GMC in dexamethasone treated infants (131). Only one report commented on the impact of dexamethasone on response to pneumococcal vaccination and found that antibody responses were reduced in those infants who had received postnatal dexamethasone (151).

3.2.2 Efficacy and effectiveness

3.2.2.1 Hib

One study was identified which compared the vaccine failures of Hib vaccine in term and preterm infants over an eight-year period and found no statistically significant difference in the vaccine failure rate between term and preterm infants (vaccine failure rate compared to term infants: <37 WG, relative risk (RR) 1.5 (95% CI 0.9-2.6, $p=0.14$); <35 WG, RR 1.7 (95% CI 0.8-3.5, $p=0.12$); ≤ 32 WG, RR 1.6 (95% CI 0.5-4.3, $p=0.2$) (89).

3.2.2.2 Rotavirus

Two papers reported the effectiveness or efficacy of the rotavirus vaccine in preterm infants (164, 165). Roué studied the effectiveness of the rotavirus vaccine in a population of French children who had been born preterm, through comparison of the rate of hospitalisation in children who had been born preterm in a period prior to the introduction of rotavirus vaccine with a period after its introduction. This study reported that introduction of the vaccine resulted in a risk reduction of 2.6 (95% CI 1.3-5.2) in hospitalisations during the first two epidemic seasons, followed by a factor of 11 (95% CI 3.5-34.8) in the third season (164). Goveia reported clinical efficacy results from preterm infants participating in a randomised controlled trial (RCT) of the rotavirus vaccine (REST study) and reported an efficacy against wild-type G1-G4 rotavirus occurring at least 14 days after the third dose of 73% (95% CI -2.2-95.2). The small sample size and wide confidence intervals mean that this was not statistically significant, but the point estimate was similar to that seen in the REST study population as a whole (74%) (165).

Summary

The evidence reviewed suggests that rotavirus vaccination protected preterm infants from rotavirus disease and that this protection is in keeping with that seen in a term population.

3.2.2.3 Pertussis

Four studies investigated the effectiveness of pertussis vaccination (75, 166-168) (table 3.25).

Table 3.25. Details of the vaccine effectiveness trials for pertussis vaccine.

Author	Vaccine details	Clinical endpoint	Time of assessment	VE preterm infants	VE term infants	
Riise (75)	2+1 schedule. Trivalent or pentavalent combination vaccines containing acellular pertussis component	Pertussis notification	Following 1 dose	73.0% (95% CI 44.1-87.0)	52.5% (95% CI 39.9-62.4)	
			Following 2 doses	80.1% (95% CI 63.7-89.1)	81.9% (95% CI 75.1-86.8)	
			Following 3 doses	93.0% (95% CI 85.8-96.5)	88.8% (95% CI 84.3-92.0)	
		Pertussis hospitalisation	Following 1 dose	71.2% (95% CI 29.9-88.1)	60.7% (95% CI 45.5-71.7)	
			Following 2 doses	93.8% (95% CI 72.2-98.6)	90.6% (95% CI 82.4-95.0)	
			Following 3 doses	88.7% (95% CI 54.3-97.2)	91.1% (95% CI 78.8-96.3)	
Hviid (166)	2+1 schedule. Either monovalent whole cell vaccine or quadrivalent or pentavalent combination vaccines containing an acellular pertussis component.	Pertussis hospitalisation	Acellular	Following 1 dose	45% (95% CI 12-65)	51% (95% CI 41-60)
				Following 2 doses	77% (95% CI 57-88)	85% (95% CI 81-89)
				Following 3 doses	90% (95% CI 69-97%)	96% (95% CI 93-98%)
		Whole cell	Following 1 dose	-23% (95% CI -90-21)	36% (95% CI 20-49)	
			Following 2 doses	47% (95% CI 14-67)	66% (95% CI 58-73)	
			Following 3 doses	95% (95% CI 62-99%)	87% (95% CI 80-91%)	

Van der Maas (167)	3+1 schedule. Pentavalent or hexavalent combination vaccines containing acellular pertussis component	Pertussis hospitalisation	Following 1 dose	73% (95% CI 20-91%)	95% (95% CI 92-97%)
			Following 2 doses	86% (95% CI 9-96%)	90% (95% CI 71-96%)
Sheridan (168)	3+ 1 schedule. Combination vaccine containing acellular pertussis component.	Pertussis notification	Following 1 dose	30.6 (-90.0-74.7)	Data not provided
				31.9 (21.6-40.9)	
			Following 2 doses	37.8 (-66.3-76.8)	
				68.8 (62.8-73.8)	
			Following 3 doses	68.0 (19.7-87.3)	
				73.3 (69.8-76.5)	
		Pertussis hospitalisation	Following 1 dose	-13.3 (95% CI -340.8-70.8)*	42.0 (95% CI 26.1-54.5)
				22.4 (-61.9-62.8) †	
			Following 2 doses	-11.2 (95% CI -362.2-73.2)*	83.2 (95% CI 73.9-89.2)
				82.8 (95% CI -23.6-96.1) †	
			Following 3 doses	71.6 (95% CI -34.6-94.0)*	87.6 (95% CI 81.6-91.6)
				54.9 (95% CI -63.8-87.6)†	

VE= vaccine effectiveness

*<32 WG †32-36+6 WG

Riise and Hviid reported similar vaccine effectiveness (VE) between term and preterm infants following all of the acellular pertussis vaccine doses. Hviid found that the first dose of whole cell vaccine did not provide any protection to preterm infants, but the VE were similar between term and preterm infants following the second and third doses of whole cell vaccine. Van der Maas described a significantly lower VE in preterm

infants following the first dose, but a similar VE after the second dose, and Sheridan reported a similar VE for pertussis hospitalisation between those born at <32 WG, 32-37 WG and at term following the third dose, but a lower VE for those infants born at <32 WG following the second dose. For the clinical endpoint of pertussis notification Sheridan compared those born before and after 32 WG and found a similar VE following the third dose of the vaccine, although again the VE was lower in those born at <32 WG following the second dose (168).

Summary

Overall, at the end of the primary course VE was similar in preterm and term infants for both acellular and whole cell pertussis vaccines, but there is some evidence that the protection provided by vaccination may be less following one or two vaccine doses for preterm infants. This reinforces the importance of preterm infants receiving appropriate vaccination in a timely manner.

3.2.2.4 Pneumococcus

Three studies reported on the vaccine effectiveness of pneumococcal vaccines, none of which found a difference between term and preterm populations (116, 169, 170). Nieminen reported on preterm infants who had received either two or three primary doses and a booster dose of a 10-valent pneumococcal vaccine and found similar point estimates of vaccine effectiveness for all of the clinical endpoints assessed, although there was insufficient power to give statistically significant results (169). Both Ruckinger and Riise compared the incidence of invasive pneumococcal disease (IPD) in infants before and after the introduction of PCV7 and estimated a similar reduction in cases of IPD in both term and preterm cohorts (116, 170).

Summary

Overall, there was no evidence that effectiveness differs between term and preterm populations for pneumococcal disease.

3.2.3 GRADE assessments

GRADE assessments were performed for all the outcomes according to the GRADE handbook (171) using risk of bias tools as described in [section 2.1](#). A summary of the risk of bias and GRADE ratings are in [appendix H](#) and [appendix I](#) respectively. Overall, the quality of the evidence relating to the immunogenicity and efficacy/effectiveness outcomes was low. Much of this uncertainty about the quality of the evidence came from the high risk of bias, which reflects the observational nature of many of the studies.

3.3 Discussion

There was variation seen in the immunogenicity of vaccines administered to preterm infants. This variation in part depended on the vaccine antigen. For example, for tetanus toxoid which is known to be strongly immunogenic, the antibody responses and proportions of infants reaching protective thresholds were similar for term and preterm infants. Similarly, MMR, the only live vaccine included for which a comparison was made between term and preterm infants, was equally immunogenic between term and preterm infants, although it is important to note that in these studies the vaccine was given after 12 months at which point the impact of preterm birth on vaccine responses may be waning.

For several vaccines, including Hib, diphtheria, pertussis and pneumococcus, the schedule employed appears to impact the antibody responses seen, with later

administration of the first dose and an extended primary series with longer gaps between doses reducing any differences between preterm and term responses. Longer intervals between vaccine doses are known to result in higher and more persistent antibody responses than schedules using closely spaced doses (33). It may be that the impact of this difference is greater in preterm infants. The schedule used for the primary series not only affected the primary response, but also impacted the response to booster doses, and longer-term persistence of antibody for some vaccines, for example Hib, pneumococcus and pertussis. Overall, preterm infants who had received a closely spaced primary series +/- an earlier booster dose had a lower response to the booster dose than their term counterparts, but this was not seen with an extended primary course and/or later administration of the booster dose. Whilst starting the primary series later and increasing the time between doses may result in an enhanced post-primary and booster response, it prolongs the duration of the period before infants have received any vaccines and therefore increases their vulnerability to infection in this period. Any decisions therefore about the best timing about vaccination in a particular setting needs to take account of local epidemiology.

The impact of degree of prematurity was investigated in some reports. Where this was found to be significant, earlier gestations were most commonly found to be associated with a lower antibody response after the primary series, including for hepatitis B (91), pertussis toxin (129) and Hib (129, 131), with the exception of one report of lower tetanus antibody titres in those infants who were born at a later gestation (136). One study investigating responses following booster doses of vaccine and persistence of responses at four years of age found that antibody levels were lowest in those born at less than 28 WG and that at four years this was also reflected in the proportions

reaching a protective threshold (132). Postnatal steroid administration was also considered. Although the timing of dexamethasone administration and the doses used are not always clear in the reports, where timing was known, previous dexamethasone administration did not seem to have an impact on antibody responses but dexamethasone administration at the time of vaccination was associated with lower antibody responses.

Overall, for most vaccines, the proportions of infants reaching a protective threshold where one was known was similar between term and preterm infants, even when in some cases the absolute antibody levels were lower in preterm infants, although there is variability in how long this protection persists. Although investigating the immunogenicity of vaccines is of paramount importance, the reason for vaccination is to protect the population at risk and the impact of immunogenicity on efficacy and effectiveness depends on the local epidemiology of disease.

Overall, only ten papers were identified which investigated the efficacy or effectiveness of vaccines in a preterm population. The results from these reports are reassuring about the protection provided by routine vaccination, even in the context of vaccines with lower immunogenicity observed in preterm infants, for example pertussis and pneumococcus. In the case of pertussis, analysis was performed following the administration of varying numbers of doses and there was some evidence that whilst protection between term and preterm infants is comparable at the conclusion of the primary course, infants may be more vulnerable before this time point. This reinforces the importance of preterm infants receiving the complete course of vaccinations in a timely manner, something which can be challenged by worries from both health care professionals and families about the reactogenicity and safety of

vaccination. These aspects will be reviewed in [chapter four](#). Overall, the quality of the evidence was assessed as low, which limits the confidence with which these findings can be interpreted.

3.4 Conclusion

Most vaccines which are administered to preterm infants result in similar proportions of infants with putative protective titres even if the antibody response is reduced. This translates into similar efficacy and effectiveness data at the conclusion of the vaccine series, although it is possible that for some pathogens preterm infants may be more vulnerable before all the recommended vaccines in a series have been administered and the persistence of their protection may be reduced. Overall, the quality of the data for the outcomes of immunogenicity, efficacy and effectiveness is low, which reflects the predominantly observational nature of the available studies

4 Systematic review: reactogenicity and safety

4.1 Background

Adverse events following vaccination can be related to a vaccine, or individual constituents of a vaccine, or result from an underlying medical condition in the recipient, or an idiosyncratic reaction (172). Although adverse events following vaccination are rare (173), they are often a significant concern for parents (174), and these concerns may be heightened in parents of preterm infants. Delayed vaccine administration in preterm infants has been widely reported (78-87) and this may result from concern on the part of parents and health care professionals about the impact of vaccinating this vulnerable group (175). Adverse events commonly include local reactions including erythema, swelling and pain, and systemic reactions, for example irritability, vomiting and fever. Fever is significantly more common following 4CMenB (176), and when this vaccine was introduced, there was concern that the increased incidence of fever in a population of young infants would result in an increase in evaluations for sepsis and antibiotic administration (177).

In a preterm population, a significant number of infants will have vaccines administered prior to discharge from hospital. Cardiorespiratory monitoring following vaccination is recommended in the UK for infants vaccinated as inpatients who were born at less than 28 WG for 48-72 hours following the first dose, but such monitoring may still be in place for infants born at later gestations, especially those with multiple comorbidities. If clinical instability is detected during a period of monitoring after the first vaccines are administered, a further period of inpatient monitoring is recommended after the second vaccines (178).

4.1.1 Objectives

To identify the evidence about reactogenicity and safety of routine vaccines given to preterm infants in the first eighteen months of life

4.1.2 Methods

The full methods are described in [section 2.1](#) and a short summary in [section 3.1.3](#).

4.2 Results

Details of the results of the search and paper selection is in [section 3.2](#). The outcome of reactogenicity was a primary focus of 23 records and the outcome of safety a primary focus of three records (figure 3.1, [section 3.2](#)). A list of all full texts reviewed with reasons for exclusion is in [appendix G](#) and a full summary of all included papers is in [appendix H](#).

The way in which reactogenicity and safety outcomes are presented varies between reports, with some outcomes being included under different headings. Whilst different approaches would have been possible, in this review reactogenicity outcomes will be discussed under the headings of cardiorespiratory instability in participants vaccinated as inpatients, solicited local and non-febrile adverse events, fever following vaccine administration, investigation for sepsis and administration of antibiotics. Safety outcomes are discussed under the headings of serious adverse events (SAEs), and hospitalisation and emergency department (ED) attendance.

4.2.1 Reactogenicity

4.2.1.1 Cardiorespiratory instability in participants vaccinated as inpatients.

There were 24 reports which included cardiorespiratory events as an outcome (128, 151, 179-200). Most studies investigating this outcome were observational: nine

retrospective reviews (179, 183-185, 187, 189, 194, 196, 197), one prospective cohort study (128), nine case series (151, 180, 184, 186, 188, 193, 195, 199, 200), two case control studies (181, 192), one historical cohort study (191) and two randomised controlled trials (182, 190). Because the outcome of interest is based on monitoring following receipt of vaccines in a hospitalised population, the studies predominantly report results from preterm infants alone, with only one paper including any term infants (183). There is considerable variation in the rates of cardiorespiratory events reported due to differences in study design, the included population, and the definitions used. Reported rates of apnoea following vaccine administration were between 2.5%-23% (151, 179, 182, 185, 188, 194, 195, 199), bradycardia between 10.8%-61% (182, 188, 194, 195) and desaturations between 2.2-72% (194, 195, 199). When rates of cardiorespiratory events were compared, this was most commonly between a period prior to and following vaccine administration, or between vaccinated and unvaccinated preterm infants. A further five reports discussed the recurrence of cardiorespiratory events with subsequent vaccine administration.

Comparison of periods before and after vaccination

There were 14 studies that compared rates of cardiorespiratory events before and after vaccination (128, 151, 181, 184-186, 189, 190, 193, 195-198, 200). Details of these studies are shown in table 4.1

Table 4.1. Details of the studies reporting cardiorespiratory events in preterm infants before and after vaccine administration.

Author, year	GA (WG)	Vaccines used	Timepoint for evaluation	Clinical details
DeMeo, 2015 (184)	<28	At least one of DTaP, IPV, HBV, Hib, PCV 7/13, combinations of these; given between 53-110 days	Before: three days After: three days	No exclusions. 42.7% of infants received caffeine in the pre- and post-immunisation periods
Mialet-Marty, 2013 (193)	<32	DTaP, Hib, HBV, PCV13; given after 56 days	Before: 24 hours After: 48 hours	Infants excluded if active infections, cardiac dysrhythmias, gastro-oesophageal reflux disease requiring treatment, severe intracerebral lesions, ventilatory support. No infants were receiving caffeine treatment.
Slack, 2003 (198)	<32	DTaP-Hib, MCC	Before: 24 hours After: 24 hours	No exclusions. Uncertain what proportion of infants had received caffeine.
Ben Jmaa, 2017 (190)	<32	DTaP, Hib, IPV;	Before: 24 hours After: 48 hours	Infants with cardiac dysrhythmias, congenital malformations, intraventricular haemorrhage grade 3 or 4 or peri-ventricular leukomalacia were excluded as were infants receiving ventilatory support or with unstable vital signs. Unknown whether any infants receiving caffeine treatment.
Ellison, 2005 (185)	<30	Hib, HBV, DTaP +/- IPV; mean age at vaccination 76 days (SD 20 days)	Before: 48 hours After: 48 hours	No exclusions. Unknown whether any infants receiving caffeine treatment.
Briggs-Steinberg, 2019 (181)	<36	Rotavirus; given 42-104 days	Before: one week After: one week	No exclusions. Unknown whether any infants receiving caffeine treatment.
Sen, 2001 (197)	<32	DTP +/-Hib	Before: three days After: three days	No exclusions. Unknown whether any infants receiving caffeine treatment.
Schulzke, 2005 (196)	<30	DTaP-IPV, Hib +/- HBV	Before: three days After: three days	All infants medically stable, none receiving ventilatory support or supplementary oxygen. No infants receiving caffeine treatment.
Faldella, 2007 (186)	<31	DTaP-IPV, Hib- HBV	Before: three days After: three days	No exclusions. Unknown whether any infants receiving caffeine treatment.

Pourcyrous, 2007(200)	<35	DTaP, IPV, Hib, HBV, PCV- single vaccine or combination	Before: Unknown After: three days	No exclusions. Unknown whether any infants receiving caffeine treatment.
Pfister, 2004(195)	<35	DTaP-IPV-Hib	Before: 24 hours After: 48 hours	No exclusions. Unknown whether any infants receiving caffeine treatment.
D'Angio, 2010(151)	<32	PCV7	Before: seven days After: three days	No exclusions. Unknown whether any infants receiving caffeine treatment.
Omenaca, 2005(128)	24-36	DTaP-IPV-Hib- HBV	Before: Unknown After: three days	Infants were excluded if any major congenital defects, serious chronic diseases, severe neurologic damage or non-treatable convulsions, acute disease, apnoea within seven days before vaccination. Unknown whether any infants receiving caffeine treatment.
Hacking, 2010(189)	Mean 27	DTaP-Hib, HBV, OPV/IPV +/-PCV7 +/- rotavirus	After: seven days	No exclusions. Unknown whether any infants receiving caffeine treatment.

DTaP= diphtheria, tetanus, acellular pertussis; IPV=inactivated polio vaccine; OPV=oral polio vaccine
HBV=hepatitis B virus; Hib=haemophilus influenzae type B; PCV=pneumococcal conjugate vaccine; GA= gestational age; WG= weeks of gestation

Eight reports included details of the change in cardiorespiratory events between the period prior to and following vaccine administration, but with no statistical comparison performed. All of these reported an increase in event frequency or severity, although the extent of this increase depended on the cardiorespiratory events of interest and the definitions used for these. A summary of these results is shown in table 4.2.

Table 4.2. Details of studies reporting cardiorespiratory events with no comparison between period before and after vaccination.

Author, year	Cardiorespiratory events of interest	Percentage of infants experiencing new or worsening cardiorespiratory events (%)
Sen, 2001 (197)	Apnoea, bradycardia, desaturation, increased oxygen requirement	29
Pfister, 2004 (195)	Apnoea, bradycardia, desaturation	47
Schulzke, 2005 (196)	Apnoea, bradycardia	13
Faldella, 2007 (186)	Apnoea, bradycardia, desaturation	11.1
Pourcyrous, 2007 (200)	Apnoea, bradycardia, desaturation	16
Omenaca, 2005 (128)	Bradycardia, desaturation	42
D'Angio, 2010 (151)	Apnoea	4
Hacking (189)	Apnoea requiring respiratory support	5.4

Where statistical analysis between the periods was performed, two studies using similar vaccines reported an increase in cardiorespiratory events following vaccine administration: Mialet-Marty reported a significant increase in bradycardia and desaturation in the period after vaccination compared to before (193) and DeMeo reported an increase in the respiratory support required following vaccination (adjusted rate ratio (ARR) 2.1, 95% CI 1.9-2.5) and an increase in the number of infants who required intubation (ARR 1.7 95% CI 1.3-2.2) (184).

In contrast, four studies found no significant increase in cardiorespiratory events (181, 185, 190, 198). Slack found no statistically significant increase in cardiorespiratory events following vaccination in a group of infants born at <32 WG (198). Ben Jmaa found no significant differences in the number of cardiorespiratory events occurring within a 24-hour period for the time following vaccine administration compared to that before, either in those infants who received ibuprofen or those who did not but reported significantly more variability in cardiorespiratory events in the placebo group (190). Ellison found no increase in apnoea requiring stimulation or the need for supplemental oxygen, although there was a small statistically significant increase in the maximum oxygen concentration used following vaccination (185). In a study of the rotavirus vaccine, Briggs-Steinberg reported no difference in proportion of infants experiencing an increase in apnoea or bradycardia in the period following rotavirus vaccination compared with the period before, although there was an increase in the number of infants with an increase in apnoea, apnoea requiring stimulation and bradycardia requiring stimulation following rotavirus vaccination compared with historical controls of the same chronological age (181).

Comparison between vaccinated and unvaccinated infants

Two studies compared the rates of cardiorespiratory events between vaccinated and unvaccinated infants. In a case control study comparing cardiorespiratory events in preterm infants ≤ 32 WG receiving DTP-IPV-Hib with unvaccinated controls, Lee found a significant increase in the cardiorespiratory events during the 72 hours following vaccination compared with unvaccinated controls, although the number of infants who required treatment was not increased in the vaccinated group (192). Carbone, in an RCT investigating cardiorespiratory events in preterm infants born at less than 37 WG following DTaP found no significant difference in prolonged bradycardia or prolonged apnoea between the vaccinated and unvaccinated populations (182).

Impact of cardiorespiratory events on clinical course

The significance of any cardiorespiratory events on clinical course is variably reported. Some reports suggested that cardiorespiratory events were associated with a need for increased respiratory support in 5.4% to 100% of infants (189, 196, 197, 200), whereas others found no increase in the need for respiratory support following cardiorespiratory events (185), and one study found that all infants with increased cardiorespiratory events returned to baseline within 48-72 hours (195).

Factors predicting cardiorespiratory events

Several authors attempted to investigate the impact of a variety of factors on the frequency of cardiorespiratory instability. A summary of the reports investigating these factors is shown in figure 4.1.

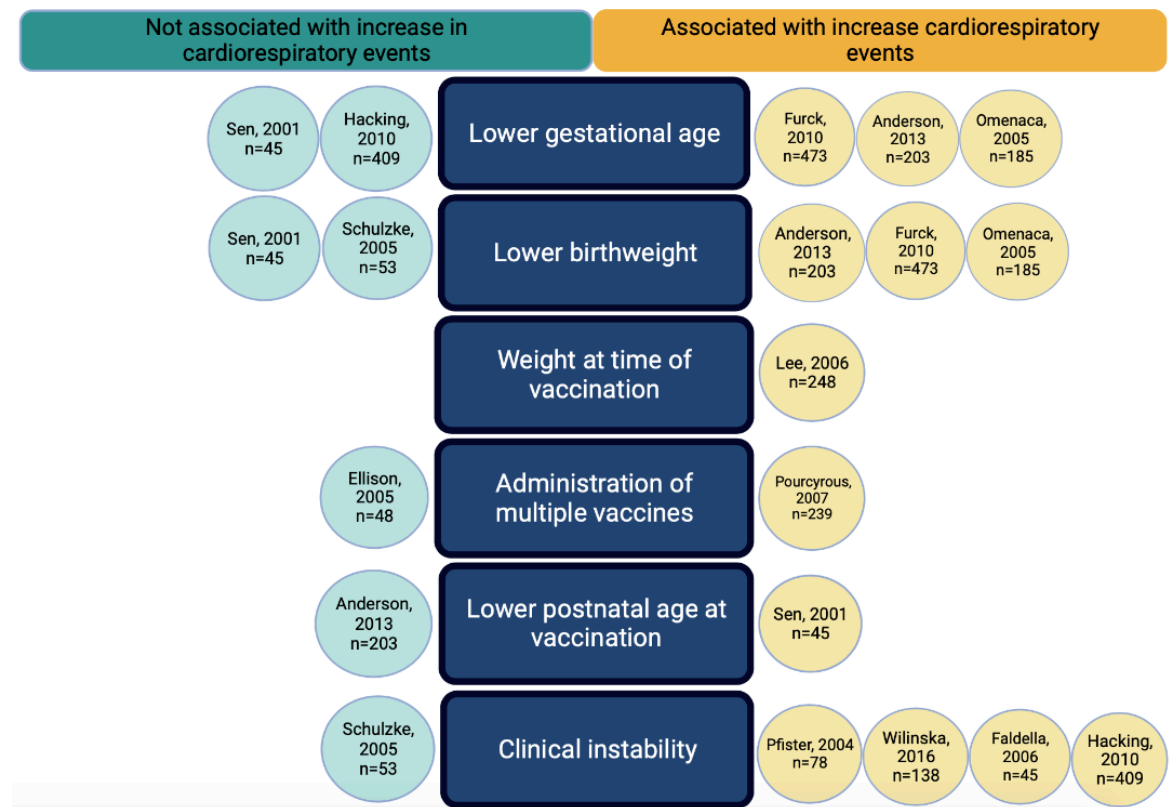


Figure 4.1. Visual representation of the reports which include an investigation of factors associated with cardiorespiratory events.

In a preterm population, advancing gestational age is usually associated with fewer cardiorespiratory events and the impact of gestational age is therefore of particular interest. Omenaca found that all the infants who experienced deterioration in cardiorespiratory events were less than 28 WG and had a birthweight of $\leq 1000\text{g}$ (128). Both Furck and Anderson reported that the risk of apnoea was greater in those of lower gestational age (179, 188), whereas Sen and Hacking found that gestational age was not a predictive factor in experiencing cardiorespiratory events (189, 197). Other predictive factors for cardiorespiratory events have also been considered. Some reports found that lower birthweight was associated with an increased risk of apnoea (179, 188), some that birthweight did not influence incidence of cardiorespiratory events (196, 197) and others that current lower weight was a risk factor for increased cardiorespiratory events (192). One study found that receiving multiple vaccines

simultaneously was associated with an increased risk of cardiorespiratory events (200), but another reported no significant association (185). Unstable clinical condition at the time of vaccination has been shown by some to be a significant risk factor for cardiorespiratory events. Pfister found that clinical instability, defined as severity of underlying clinical conditions or significant apnoea and bradycardia, at the time of vaccination was significantly associated with the incidence of cardiorespiratory events (195). Although Wilinska reported much lower rates of cardiorespiratory events, this study also supported an association between clinical condition at the time of vaccine administration with incidence of cardiorespiratory events: those infants experiencing cardiorespiratory events after vaccine administration had a significantly higher rate of late onset sepsis and a history of longer duration of continuous positive airway pressure (CPAP) (199). Hacking found that a longer duration of CPAP was associated with cardiorespiratory events requiring respiratory support along with incidence of previous septicaemia, and Faldella found that all the infants who had experienced a cardiorespiratory event related to vaccination had chronic disease (186). Schulzke in contrast, did not see any difference between those infants who had a recurrence of cardiorespiratory events and those who did not according to a history of chronic lung disease, intraventricular haemorrhage, or duration of respiratory support but of note all of the infants in this study were clinically well and not receiving any ventilatory or oxygen support at the time of vaccine administration (196). Sen found that lower age at vaccination rendered infants more vulnerable to cardiorespiratory events with those receiving vaccination at ≤ 70 days being significantly more likely to be affected (197), whereas Anderson did not find that age of vaccination was significant for apnoea (179).

Cardiorespiratory events following 4CMenB

There has been much interest in the reactogenicity of 4CMenB. Two studies focussed particularly on cardiorespiratory events following administration of this vaccine.

Mukherjee reported an increase in cardiorespiratory events including apnoea, bradycardia, and desaturations in preterm infants <28 WG following the addition of 4CMenB (194). In contrast, in a multicentre historical cohort study studying infants prior to and after introduction of 4CMenB, with and without prophylactic paracetamol, Kent found no difference in rates of apnoea, bradycardia or increase in respiratory support following vaccination between groups although there were more desaturations reported in infants who received 4CMenB without prophylactic paracetamol. Infants born at <28 WG in all groups were significantly more likely to experience desaturation and bradycardia following vaccination (191).

Recurrence of cardiorespiratory events after subsequent vaccination

Five studies focussed on cardiorespiratory events in infants after subsequent vaccines if they had experienced these after receipt of their first vaccines (179, 180, 183, 187, 188) with significant variability in reported results. A table with details of these studies are shown in table 4.3.

Table 4.3. Details of the studies investigating cardiorespiratory events following vaccination.

Author, year	GA (WG)	Timing of vaccine administration	Data collection	Outcome of interest
Anderson, 2013 (179)	<29	2, 4 months	Retrospective	Clinically important apnoea [^]
Furck, 2010 (188)	<35	Not provided	Prospective	Apnoea* Bradycardia ^Ψ Desaturation ^{ΨΨ}
Flatz-Jequier, 2008 (187)	<32	2, 3 and 4 months	Retrospective	Apnoea* bradycardia **
Clifford, 2011 (183)	<37	2, 4, 6 months	Retrospective	Apnoea* bradycardia**
Bohnhorst, 2021 (180)	<31	2, 3, 4 months	Prospective	Apnoea [^] , bradycardia ^{^^} . Desaturation ^{^^^}

GA= gestational age; WG= weeks of gestation

*Apnoea defined as cessation of respiration for ≥ 20 secs; **Bradycardia defined as a fall in heart rate to < 100 bpm

[^] Apnoea defined as cessation of respiration for ≥ 10 secs; ^{^^}Bradycardia defined as a fall in heart rate to < 80 bpm; ^{^^^}Oxygen saturation $< 80\%$

^Ψ Bradycardia defined as a fall in heart rate to < 90 bpm ≥ 20 s requiring intervention; ^{ΨΨ} Oxygen saturation $< 85\%$

Neither Anderson nor Furck identified any recurrence of cardiorespiratory events in infants who were monitored following receipt of their second vaccines, although it is important to note that only half of infants who experienced apnoea after the first set of vaccines in Anderson's report were monitored following the second vaccines (179). Whilst Anderson was concerned only with clinically significant apnoea and therefore the recurrence rate may have been predicted to be very low, Furck included new or worsening apnoea, bradycardia, or desaturation within the definition of cardiorespiratory events. In contrast to this absence of recurrence, both Flatz-Jequier and Clifford reported a recurrence of 18%. For Flatz-Jequier a cardiorespiratory event included new or worsening apnoea, bradycardia, and desaturation and for Clifford apnoea alone. Flatz-Jequier also studied infants who were monitored because they were still inpatients, but who had not had a cardiorespiratory event following their

first vaccinations and of these, none had a cardiorespiratory event following receipt of the second vaccines (187). Clifford studied the eight term infants who had experienced apnoea following their first vaccination and identified recurrence in none of the infants (183).

Bohnhorst reported a very high incidence of recurrence of cardiorespiratory events following administration of the second vaccines. In a comparison of the period six hours before and 24 hours after vaccine administration a significant increase in episodes of apnoea following vaccination was reported (26.5% vs. 62%) (180).

Although the incidence of cardiorespiratory events decreased in the second 24 hours of monitoring, there were four episodes of cardiorespiratory instability which were identified only in the second 24-hour period suggesting that a shorter period of monitoring may miss some events. Increasing age at the time of the second vaccination was associated with a reduction in the incidence of cardiorespiratory events requiring stimulation, with no events being reported in infants aged over 43.5 weeks (180).

There are considerable differences in study design and the definitions used between the studies, and some of the increase in events reported by Bohnhorst may have resulted from the prospective design of this study. Also, the second vaccines were given at three months in Bohnhorst's study, in comparison with later vaccination at four months in Clifford and Anderson's studies which may have contributed to their lower rates, although the second vaccines were also given at three months in Flatz-Jequier's study, and this study also reported rates of cardiorespiratory events far lower than that reported by Bohnhorst.

Two studies considered risk factors for recurrence. Clifford identified lower birthweight and the need for continued hospitalisation for complications related to prematurity to be important in predicting which infants would have recurrence following subsequent vaccines but found that mechanical ventilation or gestational age were not related (183). In contrast Bohnhorst found that lower gestational age was a risk factor for recurrence as was low birthweight, need for analeptic medication and a requirement for respiratory support at the time of the first vaccines (180).

Summary

Overall, results about cardiorespiratory stability following vaccination are extremely variable. Most studies are retrospective, there is significant potential for observer bias, and it is very difficult to separate out confounders in this vulnerable population.

Overall clinical instability prior to vaccination appears to be more associated with instability following vaccination, but it is difficult to separate the impact of the underlying causes from that of vaccine administration.

4.2.1.2 Solicited local and non-febrile systemic adverse events

Fifteen papers were identified which reported on local and systemic reactions (91, 93, 128, 132, 134, 152, 153, 155, 159, 163, 165, 181, 197, 199, 201): one retrospective review (197), two case series (134, 199), eight prospective cohort studies (91, 93, 128, 132, 152, 155, 159, 201), one case control study (181) and three RCTs (153, 163, 165).

Local adverse events of interest were quite consistently reported, usually including erythema, swelling and tenderness at the vaccination site, however there was much more variability in the range of systemic reactions reported and the definitions used for these.

Preterm infants only

Three reports included preterm populations only with no term comparison. Sen reported 'poor handling' and irritability in 13.3%, and feed intolerance in 11.1% (197), Wilinska identified no local reactions, but 'activity dysfunction' with no further description in 13% (199), and in one study which compared low birth weight (LBW) and very low birth weight (VLBW) infants, a higher rate of local pain at the injection site in LBW babies was seen, but there were no differences for any other local or systemic solicited symptom (134).

Term and preterm infants

Eight reports compared the incidence of local and systemic reactions between term and preterm infants with variable results. Some investigators identified differences between the populations for some of the recorded outcomes, but with no consistent pattern identified. Martinon-Torres found significantly more irritability, decreased appetite and decreased sleep in preterm infants, but similar rates for all other systemic effects. In terms of local reactions there was no difference between groups in the rates of reactions and these tended to decrease after each dose (155). Omenaca (2005) reported no difference in the incidence of local reactions, but an increased incidence of irritability in preterm infants and an increased incidence of drowsiness in term infants (128). Omenaca (2011) found no differences between term and preterm infants for systemic symptoms, although local reactions of redness and swelling were more frequently seen in term infants than preterm infants at all vaccine doses (159). Omenaca (2011) reported higher rates of local erythema in preterm infants, loss of appetite was significantly less frequently reported in preterm infants and there were no differences between groups in the rates of the other solicited systemic reactions of

irritability or drowsiness. Unsolicited reactions were reported significantly more frequently for term infants following the primary series. This was primarily due to the higher rate of injection site reactions in the term infants (91). Esposito (2007) reported variable differences between term and preterm infants in local reactions, and an increase in reported systemic reactions including decreased feeding after the third dose, sleepiness after the third dose and irritability after the second dose in preterm infants, but these were of minimal clinical significance (93). Other investigators found no significant differences in the frequency of local or systemic effects between preterm and term infants following the primary vaccine series (152, 153), or following booster vaccination (132).

Specific vaccines

Both Goveia and Omenaca reported on selected systemic reactions following rotavirus vaccination and found no difference between vaccine and placebo groups (163, 165). Briggs-Steinberg reported more episodes of non-bilious vomiting in the period following rotavirus vaccine administration than before, but no increase compared with historical controls receiving two-month vaccinations without rotavirus vaccine (181).

Summary

Overall, there was significant variability in the results from different studies, however most studies suggested that preterm infants are not at higher risk of either local or systemic reactions.

4.2.1.3 Fever following vaccine administration.

Sixteen papers reported on fever as an outcome (91, 128, 152, 155, 163, 165, 185, 188, 191, 194-197, 199, 201, 202). Most of the identified studies were observational: five retrospective reviews (185, 194, 196, 197, 202), five prospective cohort studies (91,

128, 152, 155, 201), three case series (188, 195, 199), one historical cohort study (191), and two RCTs (163, 165). There is considerable variation in the rates of fever reported in different studies; comparing rates of fever can be difficult as different definitions are used in different studies (table 4.4).

Study	Classification
Dubus, 2020 (202)	>37.5°C
Ellison, 2005 (185)	>37.5°C
Esposito, 2005 (152)	≥38.0°C
Furck, 2010 (188)	>38.5°C
Goveia, 2007 (165)	≥38.1°C
Kent, 2019 (191)	≥38.0°C
Martinon-Torres, 2015 (155)	>38.0°C
Mukherjee, 2018 (194)	>38.0°C
Omenaca, 2005 (128)	≥38.0°C
Omenaca, 2011 (91)	>38.0°C
Omenaca, 2012 (163)	>38.0°C
Pfister, 2004 (195)	≥38.0°C
Sadarangani, 2017 (201)	≥38.0°C
Schulzke, 2005 (196)	>38.0°C
Sen, 2001 (197)	>37.5°C
Wilinska, 2016 (199)	>38.0°C

Table 4.4.
Definitions of fever
used in the
included papers.

In the included studies, rates of fever following the first vaccination varied from 0.7-40.7% (Figure 4.2).

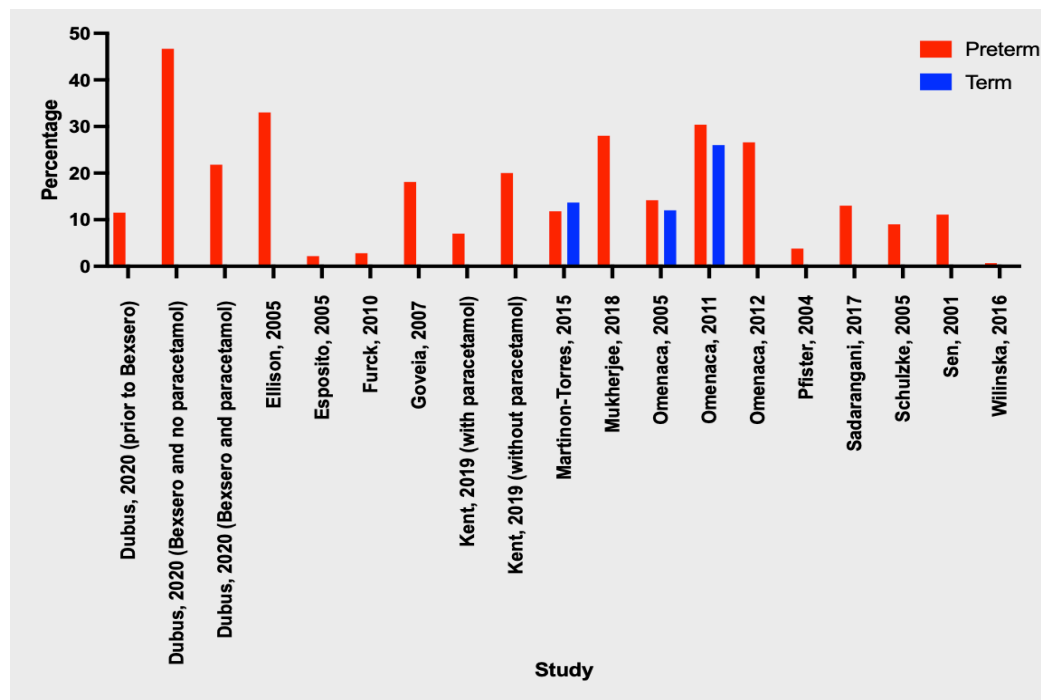


Figure 4.2. Percentage of infants reported to have experienced fever following receipt of vaccines in different studies including term comparisons where available.

Two studies using similar vaccines compared rates of fever pre- and post-vaccination in preterm infants: Ellison reported significantly higher rates of fever in the period following vaccination (185), whereas Klein found no difference between the period before and after vaccination (147).

Four studies compared incidence of fever in term and preterm infants (128, 152, 155, 159). None of these found any differences in rates of fever between term and preterm infants following any of the vaccine doses. In two studies of the rotavirus vaccine, Goveia and Omenaca compared rates of fever in infants who had received rotavirus vaccine and those who had received placebo and found no difference between groups (163).

4CMenB is associated with an increase in rates of fever following vaccination in all infants (203). Four studies, including preterm infants only, focussed on the period before and after introduction of 4CMenB (191, 194, 201, 202). Mukherjee reported an

increase in fever following the addition of 4CMenB to the vaccine schedule (0% before addition of 4CMenB, 33% following addition of 4CMenB) despite receiving three doses of prophylactic paracetamol (194). Kent compared reactions after 4CMenB had been added to the schedule with reactions reported in a historical cohort prior to its addition. In the historical cohort there was a rate of fever of 0%, in those who received 4CMenB without paracetamol there was a rate of 20%, and a rate of 7% in those who received 4CMenB with paracetamol (191). Sadarangani compared preterm infants <30 WG before and after introduction of 4CMenB to the UK vaccine schedule. Temperature instability, but not fever, was reported more commonly following introduction of 4CMenB (201). This study included eight infants who had received 4CMenB, of whom only three received paracetamol. Dubus compared the frequency of fever in preterm infants <37 WG in three groups: prior to addition of 4CMenB to the infant schedule, following the addition of 4CMenB given without paracetamol prophylaxis, and following the addition of 4CMenB given with paracetamol prophylaxis. More infants developed fever when 4CMenB was given alongside routine vaccines without paracetamol prophylaxis compared with routine vaccines without 4CMenB (RR 4.04; 95% CI 1.23-13.35), but this was much less marked when paracetamol prophylaxis was added (Pre 4CMenB vs 4CMenB with paracetamol RR 1.89, 95% CI 0.58-6.13; 4CMenB without paracetamol vs 4CMenB with paracetamol RR 0.47, 95% CI 0.22-0.98) (202).

Summary

The rates of fever following vaccination are very variable, but not more common in preterm compared with term infants. Fever is particularly associated with 4CMenB administration and giving paracetamol appears to reduce the rate of fever following this vaccine.

4.2.1.4 Investigation for sepsis and administration of antibiotics

Eight reports included the outcomes of investigation for sepsis and administration of antibiotics: seven including evaluation for sepsis and three antibiotic administration (181, 184, 185, 191, 194, 197, 200, 202).

The rates of evaluation for sepsis varied between 2.5% and 40% (181, 184, 185, 191, 197, 200, 202). DeMeo reported an adjusted rate ratio for the evaluation of sepsis of 3.7 (95% CI 3.2-4.4) in a population of infants who had received diphtheria, tetanus, acellular pertussis, inactivated polio, hepatitis B and 7- and 13- valent pneumococcal conjugate vaccines (184). In this study, DeMeo also found that the risk of sepsis evaluation was elevated in those infants born at 23-24 WG compared with those of later gestation (ARR 1.5 [95% CI 1.2-2.0] and those who had a history of episodes of sepsis (ARR 1.4 [95% CI 1.2-1.5) (184). In three further studies using similar vaccines, Pourcyrus, Sen and Ellison reported a rate of sepsis evaluation of 5%, 4% and 8% respectively (185, 197, 200). Briggs-Steinberg found no difference in the number of infants being investigated for sepsis in the period before and after rotavirus vaccination, in the period after rotavirus vaccination compared with the period following routine infant vaccination or for infants in this study in the period following rotavirus vaccination and an unvaccinated historical controls of the same age (181). Two reports focussed on the period after administration of 4CMenB. Dubus reported that 40% of infants who had received 4CMenB without paracetamol prophylaxis were investigated for sepsis. This was a 22-fold increase compared with the period prior to the addition of 4CMenB. After the addition of prophylactic paracetamol alongside 4CMenB administration, there was a 91% reduction in the number of infants being evaluated for sepsis (202). Kent reported a lower incidence of evaluations for sepsis

compared to Dubus, with 25% having screening bloods and 9% having a blood culture collected although of note this was for a population in which the majority (81%) of the infants had received prophylactic paracetamol (191). Three reports commented on administration of antibiotics following vaccination, all these studies were investigating 4CMenB (191, 194, 202). Mukherjee found that 12% of infants prior to the addition of 4CMenB received antibiotics and 33% in the period following addition of 4CMenB (194). Dubus found that 33% of infants received antibiotics following 4CMenB, when this was given without paracetamol, and this rate was significantly reduced following the addition of prophylactic paracetamol (202). Kent found a much lower rate of 5%, in common with a historic control population prior to addition of 4CMenB to the schedule (191). This lower rate of antibiotic administration may result from this population including infants who had been treated with prophylactic paracetamol as well as those who had not.

Summary

Vaccine administration was shown in some studies to be associated with an increased rate of evaluations for sepsis and antibiotic administration, particularly following 4CMenB which was markedly attenuated with the use of prophylactic paracetamol.

4.2.2 Safety

4.2.2.1 Serious Adverse events (SAEs)

Serious adverse events (SAEs) were included as a specific outcome in 12 papers (91, 93, 128, 134, 151-153, 155, 159, 163-165). In three of these there were comparisons made between term and preterm infants. In a study of rotavirus vaccine, Roué reported no statistically significant difference in the rate of SAEs between preterm and term infants and no cases of intussusception in either group (164). In a study of

pneumococcal conjugate vaccine alongside DTaP-HBV-IPV/Hib vaccine, Omenaca (2011) also found similar numbers in term and preterm infants, none of which were related to vaccination (159). In contrast, both Omenaca (2005) (128) and Martinon-Torres reported more SAEs in preterm compared with term infants (155).

In two reports, both relating to rotavirus vaccination, a comparison was made between vaccinated and unvaccinated preterm infants. Neither Goveia nor Omenaca found a difference in the rate of SAEs between preterm infants receiving the rotavirus vaccine and placebo or any cases of intussusception in either group (163, 165).

Summary

Overall, there are more SAEs reported for preterm infants, however these events were not related to vaccination and may reflect the vulnerability of this population, particularly their increased risk of admission with infections.

4.2.1.2 Hospital admission and ED attendance

Three papers reported emergency department (ED) attendance or hospital admission (147, 151, 204). All of these were observational studies: one was a case control study (204), one a case series (151) and another a prospective cohort study (147). In the case control study, Wilson reported a reduced incidence in events defined as ED presentation or hospitalisation following vaccination in all gestational ages with a trend toward lower rates of events in the more preterm infants. This trend became statistically significant when weight was adjusted for, suggesting that preterm infants may have a lower rate of hospitalisation and ED attendance following vaccination (204). D'Angio reported high rates of hospitalisation of participants over the course of the study, but only three of these were within seven days of vaccination (151). Klein

found no differences in ED attendances between term and preterm infants following poliovirus vaccine administration and no hospital admissions in either group (147).

Summary

Overall, there appears to be no increased risk of admission in the period soon after vaccination and the increased risk seen by D'Angio over a longer period is likely to be explained by the vulnerability of preterm infants and their increased risk of hospital admission with infections, particularly respiratory infections in their first year of life.

4.2.3 GRADE assessments

GRADE assessments were performed for all the outcomes according to the GRADE handbook using risk of bias tools as described in [section 2.1.8](#). A summary of the risk of bias and GRADE ratings are in [appendix H](#) and [appendix I](#) respectively. Overall, the quality of evidence for all the outcomes was low. Most of the studies were observational and many were retrospective with a considerable potential for confounding. The definitions used were variable and not always well reported.

4.3 Discussion

Vaccines are unusual pharmaceutical products in that they are given to otherwise healthy individuals to prevent future disease and it is therefore vital to adequately understand their adverse events and safety profile so that accurate information can be disseminated to health care professionals, individuals and, in the case of preterm infants, parents. Unfortunately, the quality of data collected for safety outcomes is often of poorer quality than that for immunogenicity or efficacy outcomes. This is partly because studies are rarely powered for safety outcomes, but also because case definitions are often not standardised, which makes it very hard to interpret the

results from different studies investigating similar outcomes (205). This lack of case definitions has been highlighted by the Brighton collaboration who emphasise the need for standardisation so that data from different studies can be combined and compared with greater confidence (206).

In the identified reports in this review there was considerable variation in the quality of the studies and of the reporting with most of the included studies being assessed as being of low quality. The quality assessment included consideration of risk of bias, inconsistency of results, indirectness of evidence and publication bias and in this review the main reasons for downgrading the evidence were the first three of these. The risk of bias was often high because studies were not blinded and, in many cases, there was considerable subjectivity in recording safety outcomes. There was substantial inconsistency in results because of poorly standardised definitions of the outcomes of interest and different methods used to monitor the infants. The evidence from the studies was indirect, with differences in the populations being studied, the interventions being received and the outcome measures, often with insufficient information provided to completely understand these differences.

As well as the general issues in drawing conclusions about safety outcomes, the position regarding preterm infants is further complicated by the wide clinical variation in this population, which can make it hard to separate some adverse events which are related to vaccination from the consequences of preterm birth.

Cardiorespiratory instability is common in preterm infants, particularly those born at the earliest gestations because of respiratory immaturity. There is often a perception that vaccine administration results in an increase in cardiorespiratory events, and an awareness of this perception is important when interpreting the results from

unblinded observational studies in which there is a significant degree of subjectivity in the reporting of events in the period following vaccination. This is exacerbated by the variety in how such events are monitored. In addition to this considerable source of possible bias, there are also some details which are not comprehensively included throughout the reports which limit the comparisons which can be made.

Pharmaceutical treatment with caffeine is widely prescribed to reduce the rate of apnoea and the need for respiratory support, but there is considerable variation in treatment protocols between neonatal units (207). It is a limitation in the papers discussing cardiorespiratory events that few of them provide any information about whether the included infants were receiving caffeine treatment, and none provide any details about this.

Although some of the observational studies reported an increase in cardiorespiratory events in the period after compared with before vaccination, this was not consistent and, of note, the only randomised controlled trial in this review which compared infants who had received vaccination and those who had not, did not show any difference in incidence of apnoea and bradycardia (182). In this study the infants had not received the more reactogenic 4CMenB vaccine and infants with 'unstable vital signs' as well as those with active infection or who were critically ill were excluded which may have restricted the study population to infants less likely to experience instability following vaccination. Although the results about the impact of various infant factors on the degree of cardiorespiratory instability experienced varies between studies, there does seem to be an increase in instability in the most preterm and those who are clinically unstable prior to vaccination. The healthy vaccinee effect may be operational in some of the observational studies in which the timing of vaccine

administration is selected according to the clinical stability of the infants and so the period prior to vaccination which is used for comparison may have a particularly low number of cardiorespiratory events.

The evidence in this review supports the current UK guidance about the monitoring of preterm infants at the time of vaccination, including inpatient monitoring following the second vaccines in those who were unstable at the time of their first vaccines, although the necessity for this may depend on the age at administration of the second vaccines.

Infants and young children usually experience fewer local adverse reactions following vaccination, compared to older children and adults (208) which may be because of differences in immune maturity. This pattern was not seen when comparing reactions in preterm and term infants with no indication that there are consistent differences between these groups in either local or non-febrile systemic reactions.

Fever was frequently observed, but importantly did not seem to be more frequent in preterm compared with term infants. Preterm infants are at increased risk of sepsis (209), and although fever is an unreliable sign of sepsis in infants, particularly those who are born preterm (210) the concern that increased temperature following vaccination will lead to extensive investigation and antibiotic administration is likely to be of even more relevance for this population. It is therefore reassuring that fever does not seem to be more common in preterm infants. In this review, there is evidence that 4CMenB is associated with an increase in fever and the effect of paracetamol in reducing the fever following 4CMenB is therefore reassuring. Although Mukherjee showed a high rate of fever following 4CMenB even with paracetamol, the numbers in this study were small (n=18). The reports in this review emphasise the

importance of paracetamol in preterm infants. When 4CMenB was first introduced there were concerns expressed about the routine administration of paracetamol to preterm infants (211). This has now become a routine part of clinical practice and these studies highlight the importance of providing education to families and health care professionals about the importance of this.

There were a large number of SAEs reported in studies involving preterm infants, most of which were not considered to be related to vaccination. Preterm infants are at increased risk of readmission to hospital following discharge compared with term infants and this risk is greatest for those born at the earliest gestations. The majority of these readmissions are for infections, particularly lower respiratory tract infections which are not related to the administration of vaccines (212).

4.4 Conclusion

Drawing conclusions about the reactogenicity and safety of vaccines is difficult because of the lack of standardised case definitions and the variation in the ways in which outcomes are recorded. Vaccine administration did not seem to be associated with more adverse events in preterm compared with term infants. For those infants who are still in hospital at the time of vaccination there are some studies which suggest that vaccination may be associated with cardiorespiratory instability particularly for those infants who were clinically less stable at the time of vaccine administration, but there was no increased incidence seen in the only randomised controlled trial investigating this. Overall, there is no evidence to support delaying vaccination in preterm infants.

5 Invasive Meningococcal disease in Preterm infants

5.1 Background

In the UK, invasive meningococcal disease (IMD) is most common in the first year of life, with a predominance of cases caused by serogroup B (213). Infants in the UK have been offered vaccination against serogroup B (MenB) since 2015, with doses given at 8 and 16 weeks and a booster at 12 months. Until 2016, infants in the UK received a vaccine against serogroup C (MenC) at 12 weeks and a booster at 12 months. This was withdrawn in 2016 following the success of the MenC vaccination programme resulting in good herd immunity and a very small number of cases of IMD caused by serogroup C (214).

Previous work has shown that preterm infants are more at risk of invasive pneumococcal disease (IPD) (74, 115, 116, 170). This increased risk results from their immature immunity, particularly the lower levels of antibody seen in preterm infants as a result of reduced transplacental transfer because of the shorter gestation and less efficient transplacental transfer earlier in gestation (215). In common with IPD, immunity to IMD is heavily dependent on bactericidal antibody, along with complement, most components of which are reduced in preterm infants (59), and therefore there may be an increased risk of IMD in preterm infants. The rarity of IMD, however, makes any significant association between IMD and preterm birth difficult to identify. A previous UK case-control study of adolescents hospitalised with IMD during 1999-2000 reported those with a history of preterm birth to have a 3.7-fold (95% CI, 1.0-13.5) increased risk of IMD, although this was no longer significant when only microbiologically confirmed cases were included (216).

There may also be differences in presentation and outcome of IMD in infants born preterm, compared with their term counterparts. A UK study investigating all-cause bacterial meningitis in infants less than 90 days found that clinical presentation differed between term and preterm infants and reported that the case fatality rate (CFR) was higher in preterm infants (17% in preterm infants, 4% in term infants) (217, 218). This latter finding is in contrast with the results for IPD from Kent, who investigated IPD in infants less than one year of age and found no difference in the CFR between term and preterm infants (74).

This epidemiological study was designed to explore the incidence, clinical presentation, and outcome of IMD in infants less than one year of age in the UK during a five-year period after the introduction of 4CMenB to the routine infant schedule.

5.1.1 Objectives

1. To investigate the incidence of IMD in preterm infants and compare this with term infants over the same period
2. To investigate the clinical presentation and outcome of IMD in preterm infants and compare this with term infants over the same period

5.1.2 Methods

A full description of the methods can be found in [section 2.2](#).

5.2 Results

5.2.1 Participants

During the five-year surveillance period of 1st September 2015-31st August 2020 there were 393 cases of IMD identified in infants less than one year: 49.1% (n=193) of the

infants were female. Information about ethnicity was available for 263 infants, of whom 90.1% (n=237) were white. Serogroup B was the most common infecting serogroup (69.7%, n=274) followed by W (17.6%, n=69), C (6.1%, n=24), Y (5.1%, n=20), ungrouped (1.5%, n=6) (Figure 5.1). Two infants (0.5%) were reported to have a risk factor of interest, both infants had complement component 2 (C2) deficiency.

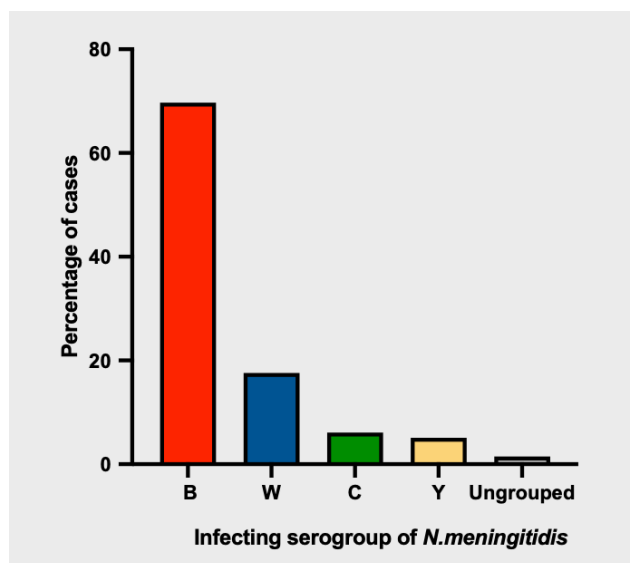


Figure 5.1. Percentage of cases of IMD caused by different serogroups of *N. meningitidis*.

5.2.2 Gestational age

Gestational age information was available for 92.4% (n=363) of the infants, of whom 87.6% (318/363) were term and 12.4% (n=45/363) preterm. Of the 45 preterm infants, 13 were born before 32 WG and 32 at 32-36⁺⁶ WG.

5.2.3 Incidence rates and ratios

Overall, the incidence of IMD in the infant population was 12.4/100,000 infants. After excluding the 30 infants for whom gestational age information was unknown, the incidence was significantly higher in preterm compared with term infants [18.3/100,000 vs 10.9/100,000; IRR 1.59 (95% CI 1.21-2.10), p=0.001], with the rate being significantly higher in those born at <32 WG [32.9/100,000 vs 10.9/100,000; IRR

2.93 (95% CI 1.72-4.99); $p < 0.001$) but not in those born at 32-36⁺⁶ WG (15.5/100,000 vs 10.9/100,000; IRR 1.38 (95% CI 0.99-1.93); $p = 0.06$). When adjustment was made reclassifying infants with unknown gestation according to the proportion of gestational ages in the infants where gestational age was known, the incidence rate ratios were slightly reduced, although these remained significant when comparing term vs preterm infants overall (IRR 1.49 [95% CI 1.13-1.96, $p = 0.005$]) and for a comparison of term vs preterm infants <32 WG (2.72 [95% CI 1.59-4.63, $p = 0.0002$]) (Table 5.1).

Table 5.1. Incidence rates and incidence rate ratios according to gestation in infants aged <1 year with confirmed invasive meningococcal disease September 2015 to August 2020 in England.

	Incidence rate (95% CI) (number of cases/infant population)		Incidence rate ratio (95% CI) compared to term/adjusted term	P value/ adjusted p value
Overall	12.4 (11.2-13.7) (393/3,156,963)			
Term ≥ 37 WG	10.9 (9.7-12.1) (318/2,911,479)	11.82* (10.6-13.1) 344/2,911,479		
Preterm <37 WG	18.3 (13.0-23.7) (45/245,484)		Unadjusted: 1.59 (1.21-2.10) Adjusted: 1.49 (1.13-1.96)	Unadjusted: 0.001 Adjusted: 0.005
<32 WG	32.9 (15.01-50.8) (13/39,551)		Unadjusted: 2.93 (1.72-4.99) Adjusted: 2.72 (1.59-4.63)	Unadjusted: <0.001 Adjusted: 0.0002
32-36 ⁺⁶ WG	15.5 (10.2-20.9) (32/205,933)		Unadjusted: 1.38 (0.99-1.93) Adjusted: 1.29 (0.92-1.79)	Unadjusted: 0.06 Adjusted: 0.14

WG= weeks of gestation

***Adjusted incidence/100,000:** Infants with missing gestational age assumed to be distributed in proportion to distribution of known gestational ages and reclassified accordingly

There was a trend towards reducing incidence through the years 2015-2020, although with an uptick seen in 2018 (figure 5.2).

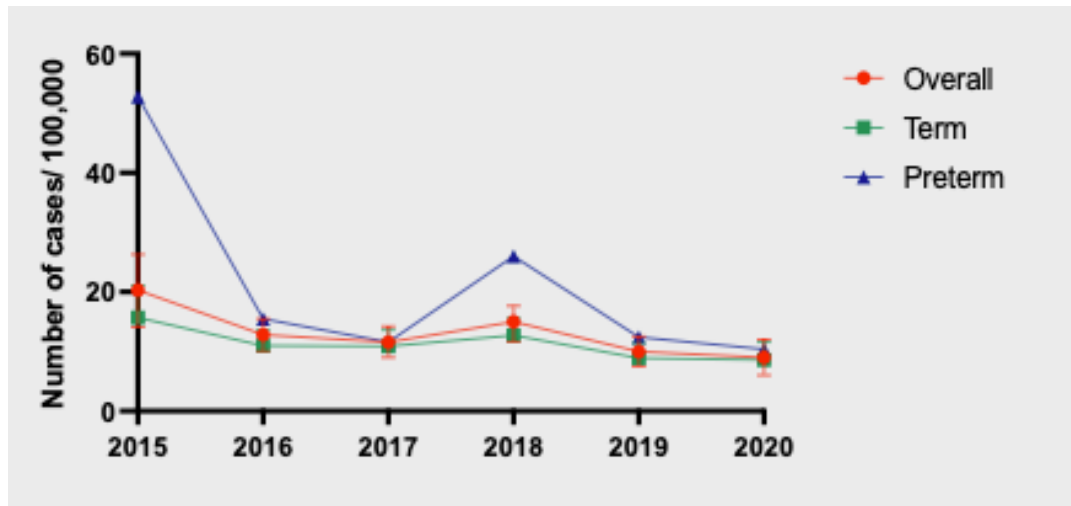


Figure 5.2. Number of cases/100,000 infants separated according to study year.

Term= ≥ 37 WG; preterm= <37 WG

5.2.4 Clinical Presentation

The age of onset was available for 388 infants. For the population overall, the median age of onset was 132.5 days (Interquartile range [IQR] 83-240.5). Gestational age information was available for 92.3% (n= 358) of these infants. For term infants, the median age of onset was 126 days (IQR 78-239) and for preterm infants, the median age of onset was 154 days (IQR 88-243). Where gestational age was known, there was no statistically significant difference in mean age of onset between term and preterm infants ($p=0.24$) and no correlation seen between age of onset and gestation in days in the preterm infants ($p=0.20$).

About half of all cases of IMD had disease onset prior to 126 days by which point the antibody response following the second dose of 4CMenB could have been seen, and around two thirds developed disease before six months of age (table 5.2).

Table 5.2. Age of onset of disease overall and according to gestational group.

Age of onset	Overall (n=388) % (n)	Term infants (n=313) % (n)	Preterm infants (n=45) % (n)
0-70 days	19.9 (77)	22.0 (69)	11.1 (5)
71-126 days	28.6 (111)	28.1 (88)	37.8 (17)
127-182 days	14.4 (56)	14.4 (45)	8.9 (4)
183-269 days	19.6 (76)	18.5 (58)	22.2 (10)
270- 364 days	17.5 (68)	16.9 (53)	20 (9)

Information about the presenting syndrome was available for 99% of infants (n=389): septicæmia was most common (49.6%, 193/389), followed by meningitis (33.9%, 132/389), meningitis and septicæmia (15.4%, 60/389) and septic arthritis (1%, 4/389). Gestational age information was available for 360 of these infants and there was no difference in presenting syndrome between term and preterm infants ($p=0.94$) (table 5.3).

Table 5.3. Presenting syndrome overall and according to gestational group.

		Overall (n=389)	Term (n=315)	Preterm (n=45)	P value*
Presentation % (n)	Septicæmia only	49.6 (193)	50.5 (159)	48.9 (22)	0.94
	Meningitis only	33.9 (132)	33.3 (105)	37.8 (17)	
	Meningitis and septicæmia	15.4 (60)	15.2 (48)	13.3 (6)	
	Septic arthritis	1.0 (4)	0.95 (3)	0 (0)	

*Fisher's exact test

Information about presence of fever at presentation was available for 80.2% (n=315) of the infants, of whom 81.3% (256/315) presented with fever. Gestational age information was available for 94.6% (298/315) of these infants and where this was

known, there was no statistically significant difference in the proportion of term and preterm infants presenting with fever ($p=0.46$) (table 5.4).

Table 5.4. Fever as a presenting symptom overall and according to gestational group.

		Overall (n=315)	Term (n=264)	Preterm (n=34)	P value*
Fever % (n)	Yes	81.2 (256)	67.3 (214)	60 (27)	0.46
	No	18.7 (59)	15.7 (50)	15.6 (7)	

*Chi squared test

Information about signs of shock at presentation was available for 74.8% of infants ($n=294$) of whom 38.1% (112/294) had signs of shock. Gestational age information was available for 95.2% (280/294) of these infants. There was no statistically significant difference between term and preterm infants ($p=0.57$) (table 5.5).

Table 5.5. Signs of shock at presentation overall and according to gestational group.

		Overall (n=294)	Term (n=246)	Preterm (n=34)	P value*
Signs of shock % (n)	Yes	38.1 (112)	37.8 (93)	47.1 (16)	0.57
	No	61.9 (182)	62.2 (153)	52.9 (18)	

*Chi squared test

5.2.5 Vaccination

There were 24 infants infected with serogroup C, none of whom had received MenC vaccination: one infant was too young to have received the vaccine before becoming infected, and 23 infants were born after the infant dose had been withdrawn from the routine schedule. Of those infants who were born after the infant dose had been withdrawn, onset of disease was between 11 and 358 days; 7/23 infants would have been too young to be protected from MenC vaccination at 12 weeks of age. Of the three infants who were infected with serogroup C and died, all were born after the

MenC vaccine dose had been removed from the schedule, and one was too young anyway to have received the dose.

There were 274 infants who were infected with serogroup B: 55 had received two vaccine doses, 103 had received a single dose, and 116 were unvaccinated. Gestational age information was available for 256 of these infants (table 5.6).

Table 5.6. Summary of vaccines received in infants infected with serogroups B and C.

		Number of infants	Term (n=229)	Preterm (n=27)
Serogroup B (n=274) % (n)	0 doses	42.3 (116)	41.5 (95)	40.7 (11)
	1 dose	37.6 (103)	38.9 (89)	40.7(11)
	2 doses	20.1 (55)	19.6 (45)	18.5 (5)
Serogroup C (n=24) % (n)	Unvaccinated	100 (24)	100 (18)	100 (4)

Of the 80 infants who were eligible to receive a single dose of vaccine, 50 received this dose prior to developing disease. Of the 30 who had not received a dose before developing disease, 15 had become eligible for the first dose within the 14 days prior to developing disease and the remaining 15 had become eligible more than 14 days previously. Of the 117 infants who were eligible for 2 doses, 55 had received both doses, 51 had received a single dose and 11 had received no doses. Of those who had received only one dose, 15 had become eligible within the 14 days before developing disease and 36 had become eligible more than 14 days before. For three infants the age of onset was unknown and therefore eligibility could not be determined (table 5.7).

Table 5.7. A breakdown of the number of doses of 4CMenB received according to the number of doses a participant was eligible for.

N (% of eligible infants who received the indicated number of doses)		Received		
		0	1	2
Eligible (n=274)	0	74 (100%)		
	1	30 (37.5%)	50 (62.5%)	

	2	11 (9.4%)	51 (43.6%)	55 (47%)
	Unknown	1	2	0

Of the 11 infants who died from serogroup B disease, nine were unvaccinated (three not eligible as prior to introduction of 4CMenB to the routine programme, five were too young to be vaccinated and one was eligible, but unvaccinated) and two were eligible for and had received one 4CMenB dose. There were no MenB fatalities among two-dose vaccinated infants.

Culture results were available for 106 (38.7%) with serogroup B disease (culture only n=49, Culture/PCR n=57) and 46/106 (43.4%) isolates were MATS-covered (33 one antigen, 9 two antigens and 4 three antigens), 27/106 (25.5%) were not covered and 33/106 (31.1%) had undetermined coverage. Among infants who had received at least one vaccine dose 40.6% (39/96) were infected with isolates which were MATS covered (24/63 [38.1%] after one dose, 15/33 [45.5%] after two doses), compared with 70% (7/10) of unvaccinated infants (table 5.8).

Table 5.8. Breakdown of MATS coverage according to vaccination status.

	Vaccines received		
	0 vaccines	1 vaccine	2 vaccines
Not covered	2	16	9
Unsure	1	23	9
Covered	7	24	15

There was no difference in admission to PICU according to MATS status (PICU admission: MATS covered- admitted 10/49 [20.4%]; MATS unsure- admitted 19/84 [22.6%]; MATS not covered- admitted 7/25 [28%], p=0.76) or CFR (MATS covered- died 1/54 [1.9%]; MATS unsure died 2/94 [2.1%]; MATS not covered died 0/27 [0%], p=1.0).

5.2.6 Outcome

Length of stay

Information about length of stay was available for 336 infants. Overall, the median length of hospital stay was six days (IQR 4-9 days). Gestational age information was available for 316 infants. There was no significant difference in the length of stay between term and preterm infants when these were compared ($p=0.48$) (table 5.9).

Table 5.9. Length of hospital admission by gestational age group.

	Overall (n=336)	Term (n=280)	Preterm (n=36)	P value*
Hospital length of stay (days) Median (IQR)	6 (4-9)	6 (4-9)	7 (4-10)	0.48

*Mann Whitney *U* test

PICU admission

Information about PICU admission was available for 86.8% ($n=341$) infants of whom 20.2% (69/341) had required PICU admission. Gestational age information was available for 319 infants. PICU admission occurred in 19.1% (54/282) of term infants and 32.4% (12/37) of preterm infants. There was not a statistically significant difference in the proportion of term and preterm infants admitted to PICU ($p=0.06$) (table 5.10).

Table 5.10. PICU admission by gestational age group.

		Overall (n=341)	Term (n=282)	Preterm (n=37)	P value*
PICU admission % (n)	Yes	20.2 (69)	19.1 (54)	32.4 (12)	0.06
	No	79.8 (272)	80.9 (228)	67.6 (25)	

*Chi squared test

Information about PICU length of stay was available for 43 of the infants: median length of stay four days (IQR 2-6 days). Where gestation was known, there was no

statistically significant difference in PICU length of stay between term and preterm infants ($p=0.89$).

Case Fatality Rate

Information about mortality was available for all infants and gestational age was identified for all but one of the infants who died. Overall, 4.1% ($n=16$) infants died.

Although the case fatality rate was highest with MenC infection, this was not statistically significant between the different capsular groups ($p=0.20$) (table 5.11) and there were also no significant differences seen between term and preterm infants (preterm: 1/45 [2.2%] vs. term: 14/318 [4.4%]; $p=0.49$).

Table 5.11. Case fatality rate by capsular group.

	Number of cases	Number of fatalities	Case Fatality Rate (%)	P value*
Overall	393	16	4.1	0.20
Capsular group B	274	11	4.0	
Capsular group W	69	1	1.4	
Capsular group C	24	3	12.5	
Capsular group Y	20	1	5	
Ungrouped	6	0	0	

*Fisher's exact test

Sequelae

At least one sequela was reported in 69 (21.2%) of 326 infants who survived their infection and for whom information was recorded, with a higher prevalence in preterm compared with term infants (14/39 [35.9%] vs. 52/268 [19.4%]; $p=0.04$); preterm infants had significantly more sequelae reported in the category of 'other' than term infants (preterm 6/33 [18.2%] vs. 15/253 [5.9%]; $p=0.02$) (table 5.12).

Table 5.12. Summary of sequelae reported according to gestational age category.

	Number of infants % (n)	Term % (n)	Preterm % (n)	P value*
Any sequelae	21.2 (69/326)	19.4 (52/268)	35.9 (14/39)	0.035
Epilepsy	4.8 (15/310)	5.1 (13/257)	5.9 (2/34)	0.63
Other neuro	4.5 (14/308)	5.1 (13/257)	3.1 (1/32)	0.30
Amputation	1.3 (4/312)	1.2 (3/259)	2.9 (1/34)	0.28

Other	7.2 (22/304)	5.9 (15/253)	18.2 (6/33)	0.02
Hearing loss	14.5 (33/227)	12.6 (23/183)	25.9 (7/27)	0.17

*Fisher's exact test

'Other' sequelae

Twenty-two infants had a reported sequelae classified as 'other' (table 5.13).

Table 5.13. Summary of sequelae classified as 'other'.

	Number of infants		
	Total (n=22)	Term (n=15)	Preterm (n=6)
Skin necrosis/scarring	7	5	2
Mild fatigue	1	0	1
Autism	1	1	0
Developmental delay	6	3	2
Squint	1	1	0
Renal pole scarring	1	1	0
Visual impairment	1	1	0
Mobility disturbance	2	1	1
No details	2	2	0

5.2.7 Sensitivity analysis reclassifying all infants with missing data as term

Repeating the analysis reclassifying all infants with missing gestation as term, resulted in slightly higher incidence in term infants (11.95/100,000), while the IRR remained significantly higher in preterm compared to term infants (IRR 1.47 [95% CI 1.12-1.94]; $p=0.007$). Infants born term, preterm and with missing gestational age were similar (table 5.14).

Table 5.14. Breakdown of results separated according to category- gestational age and missing.

		Term (n=318)	Preterm (n=45)	Missing (n=30)
Sex (female) (%)		49.4 (157/318)	46.7 (21/45)	50 (15/30)
Ethnicity (white) (%)		89.8 (194/216)	96.4 (27/28)	84.2 (16/19)
Serogroup (%)	B	72.0 (229/318)	60 (27/45)	60 (18/30)
	C	6.0 (19/318)	8.9 (4/45)	3.3 (1/30)
	W	15.1 (48/318)	22.2 (10/45)	36.7 (11/30)

	Y	5.7 (18/318)	4.4 (2/45)	0 (0/30)
	Ungrouped	1.3 (4/318)	4.4 (2/45)	0 (0/30)
Age at onset category (%)	1 (0-70 days)	22.0 (69/313)	11.1 (5/45)	10 (3/30)
	2 (71-126 days)	28.1 (88/313)	37.8 (17/45)	20 (6/30)
	3 (127-182 days)	14.4 (45/313)	8.9 (4/45)	23 (7/30)
	4 (183-269 days)	18.5 (58/313)	22.2 (10/45)	26.7 (8/30)
	5 (270- 364 days)	16.9 (53/313)	20 (9/45)	20 (6/30)
Presentation (%)	Septicaemia	50.5 (159/315)	48.9 (22/45)	4 (12/29)
	Meningitis	33.3 (105/315)	37.8 (17/45)	33.3 (10/29)
	Meningitis and septicaemia	15.2 (48/315)	13.3 (6/45)	20 (6/29)
	Septic arthritis	0.95 (3/315)	0 (0/45)	3.33 (1/29)
PICU (%)	Admission	19.1 (54/282)	32.4 (12/37)	13.6 (3/22)
	Not admitted	80.9 (228/282)	67.6 (25/37)	86.4 (19/22)
Survived (%)		95.6 (304/318)	97.8 (44/45)	96.7 (29/30)

PICU Paediatric Intensive Care Unit

5.3 Discussion

Preterm infants are at increased risk of infections, including vaccine preventable infections, for example IPD and pertussis (74-76). This study has demonstrated a significantly increased risk of IMD in UK infants born at less than 37 WG compared with term infants (IRR 1.59 (95% CI 1.21-2.10), $p=0.001$).

A higher IMD risk in preterm compared with term infants has been reported in three previous case-control studies, prior to 4CMenB licensure (216, 219, 220). In Denmark, the odds ratio (OR) of IMD was increased in preterm infants in their first year of life

(OR 1.3, 95% CI, 1.1-1.9) (220) while, in France, the odds ratio of hospitalisation with IMD was increased in any individual with a history of preterm birth (OR 2.7, 95% CI, 1.5-5.0) (219) and, in the UK, there was a 3.7 fold increase in IMD in adolescents who had been born preterm, although this was no longer significant when analysis was restricted to those microbiologically confirmed cases (216). Interestingly, two of these showed an increased risk in individuals beyond the first years of life, when the implications of preterm birth might be hypothesised to be less (216, 219). Increased incidence in preterm infants can be explained by the immature immunity of this population, particularly the lower bactericidal antibody and deficient complement profile, and there is evidence that preterm infants remain at an increased risk of hospitalisation for infections throughout childhood (221). The reasons for an increase in risk later in life are less clear. This work is the first to assess IMD risk in term and preterm infants after the introduction of a highly effective vaccine against MenB, which also provides some cross-protection against other meningococcal serogroups (222, 223).

In common with previous work investigating IPD which found that the incidence of IPD increased with lower gestations (74), this study found an increased incidence in infants born before 32 WG compared with those born at 32-36⁺⁶ WG with the difference between term and preterm infants being significant only for those born before 32 WG. This is likely to be explained by the increased capacity for transplacental transfer of antibody throughout the pregnancy as well as an increase in cumulative time for this transfer to occur (224, 225).

Routine vaccination with 4CMenB was introduced from 1st September 2015, therefore the majority of infants included in this study were born after the introduction of this

programme. Despite this, serogroup B remained the most prevalent infecting serogroup. 4CMenB vaccination is offered at 8 and 16 weeks with a booster at 12 months. A single dose of 4CMenB provides limited protection (223), and thus the period before administration of the second dose can be considered to be the most vulnerable period. Since the introduction of 4CMenB to the routine schedule, the peak age of onset has shifted to an earlier point of 1-3 months, compared with a previous peak of 5-6 months by which point infants have received their second dose of 4CMenB (226). The finding in this study that two thirds of infants had developed disease before six months is consistent with this. It is not known whether a two-dose priming schedule at 8 and 12 weeks is adequately immunogenic, and this is being investigated (EudraCT number 2021-001561-21), but this accelerated schedule has the potential to provide protection earlier in life. It has recently been reported that over half of the infants who develop disease in the window between the first and second 4CMenB doses at 8 and 16 weeks could be protected if the second dose was immunogenic when given at the earlier timepoint of 12 weeks (227).

There were 92 infants (33.9%) who were infected with group B meningococcus and under-vaccinated; 41 infants who had not received any doses of 4CMenB although they were eligible to have received at least one and 51 infants who had received a single dose although they were eligible to have received a second dose. That one third of all the infants infected with serogroup B had not received all of the vaccines to which they were eligible at an appropriate time is concerning and highlights the importance of infants receiving their vaccines in a timely manner.

Although vaccination with 4CMenB has been shown to be effective against serogroup B (223), the nature of the vaccine means that its protection is dependent on

compatibility between the surface proteins of the vaccine and the expressed antigens on the infecting strain. Estimates using the Meningococcal Antigen Typing System (MATS) suggest that around 66% (95% CI 52-80) of meningococcal isolates in 2014-15 in the UK would be covered by 4CMenB (228). In this study, of those who had received at least one vaccine and for whom MATS information was available, 40.6% had been infected with a strain which would have been predicted to be covered by the vaccine compared with 70% of those who were unvaccinated. This is likely a consequence of vaccine protection against MATS-positive MenB strains – a proxy for vaccine-preventable strains – in vaccinated infants (226). In most cases, this coverage was limited to one antigen (33/46, 71.7%). There are a number of possible explanations for these vaccine breakthrough cases, some of which are related to the nature of MATS testing and others related to individual immunity. MATS positivity refers to a $\geq 80\%$ chance of being killed by immune sera where one antigen is present or $\geq 96\%$ chance of being killed by immune sera where two or more antigens is present- it is therefore not designed to express certainty of killing. Additionally, as MATS was developed based on a 3+1 schedule in contrast to the 2+1 schedule used in the UK the use of pooled sera may overestimate MATS protection and the bactericidal activity of 4CMenB-induced antibodies relies on both the presence and expression of cross-reactive surface-exposed antigens which can be highly variable *in vivo* (229). A previous study has shown that there is some evidence of more severe disease in those infected with MATS positive isolates (228). This was not seen in this analysis when using PICU admission and case fatality rate as proxies for severity, with no difference being observed between the MATS status of isolates.

Routine vaccination against serogroup C at three months was removed from the UK schedule during the study period, in July 2016. We identified 24 children who had been infected with serogroup C, none of whom had been vaccinated: 23 of these infants were born after withdrawal of the infant vaccination, and although about one third of these infants would have been too young to receive the vaccine before presenting with disease, there are 16 infants who could potentially have been protected through vaccination if this were still offered at 12 weeks. An adolescent MenACWY conjugate vaccine implemented in August 2015 has been highly effective in providing both direct and indirect (herd) protection, such that cases due to the four meningococcal serogroups remain very low in England (230, 231), however continued surveillance is vital to ensure that cases of serogroup C do not increase in infants following the removal of this dose.

There was no difference in the age of onset or presenting syndrome between term and preterm infants. In contrast to the findings in a study of all cause bacterial meningitis that fever was more commonly seen in term than preterm infants (218), we found no differences between these groups. We also found that fever was a common presenting sign, being reported in 81.3% of infants, whereas Okike reported fever in just over half of infants (218). Okike found that infants with fever were older than those without and the increased number of febrile infants in our population may be because of their increased age (median age of 132.5 days in comparison with a median age of 15 days reported by Okike (218)).

In this study, the case fatality rate was 4.1% which is in keeping with previous UK based studies (232). There were no differences seen between CFR in term and preterm infants, which is consistent with previous findings for IPD (74). A systematic

review showed that CFR was lower for cases of serogroup B (32) compared with other serogroups. Although in this study the case fatality rate was higher in infants with MenC, case numbers due to non-MenB disease were too small to achieve statistical significance.

Where information about sequelae was available, at least one sequela was reported for 21.4% of infants, with preterm infants being significantly more likely to experience at least one sequela compared with their term counterparts (35.9% vs. 19.4%). The most common sequelae reported in all infants was hearing loss, which is consistent with other studies (233), however the only category of sequelae reported more commonly in preterm infants was that of 'other' which included a range of conditions including skin necrosis and scarring, visual disturbance and developmental delay. The significance of this is hard to determine as there is considerable overlap between the conditions included in the category of 'other', particularly developmental delay, with those which are often seen in preterm infants (234-238), making it very difficult to assess the increased risk of such sequelae in preterm infants who experienced IMD over that which they may have experienced as a consequence of preterm birth alone.

Strengths and limitations

The nature of surveillance in the UK means that very few cases of IMD should have been missed (239). Importantly, gestational age information was obtained for more than 90% of the infants which strengthens the main finding of a difference in the incidence rate between term and preterm infants, as does the fact that this relationship is still seen, albeit to a lesser extent, when the analysis is repeated reclassifying the infants of unknown gestational age according to the proportion seen

in infants with known gestational ages and reclassifying all infants with missing gestational ages as term.

Information about sequelae was poorly reported in the surveillance questionnaires with considerable missing data. It is conceivable that those completing the surveillance questionnaires were more likely to report the presence rather than the absence of sequelae which may have led to an over-estimation of sequelae. The surveillance period of 3-6 months following infection provided little opportunity to fully appreciate longer-term sequelae, particularly more subtle outcomes relating to neurodevelopment. When information about sequelae in the category of 'other' was provided, this was often quite brief, and the format of the surveillance survey did not allow this data to be further interrogated to provide a fuller picture.

5.4 Conclusion

This study has shown that in the five years after the implementation of 4CMenB, there was an increased risk of IMD in preterm infants with the greatest increase being in the most preterm infants and all efforts should be made to ensure that they benefit from preventative programmes, including vaccination. Continued efforts are needed to reduce the residual IMD burden in infants focussing on improved vaccine scheduling, encouraging timeliness of vaccination, and developing new MenB vaccines with greater breadth of coverage. As serogroup B is the most commonly infecting serogroup, it is important to understand the immunogenicity of 4CMenB vaccination in preterm infants, particularly as in the UK this is given according to a reduced 2-dose primary schedule.

6 Babies born Early Antibody Response to MenB vaccination- immunogenicity results

6.1 Background

The four-component meningococcal B vaccine, 4CMenB, was licensed in 2013 by the European Medicines Agency to be given according to a three-dose priming schedule followed by a booster in the second year of life (3+1 schedule) (223). In the UK, an initial review by the Joint Committee on Vaccination and Immunisation (JCVI) found that routine vaccination with 4CMenB was 'highly unlikely' to be cost effective, irrespective of the vaccine price, and on this basis the addition of 4CMenB to the routine schedule was not recommended (240). After further consideration of the evidence, particularly of some factors which had not been included in the initial analysis, for example litigation costs to the National Health Service (NHS) from MenB disease, the JCVI recommended inclusion of 4CMenB in March 2014 (241). 4CMenB was included in the schedule from 1st September 2015 and was administered according to a reduced schedule of two priming doses at 8 and 16 weeks and a booster dose at 12 months (2+1 schedule). At the time of the vaccine's introduction there was a short catch-up campaign offered to infants receiving their 12-and 16-week vaccinations. As well as studies investigating administration of a three-dose priming schedule, there have also been three studies specifically investigating the immunogenicity of a reduced two-dose priming schedule in term infants (43, 242, 243) and a further study which included an immunogenicity assessment following the second priming dose prior to administration of a third priming dose (244). All these

studies suggest that a two-dose priming schedule is adequately immunogenic in term infants.

Surveillance in the UK has shown that vaccine effectiveness against meningococcal serogroup B disease is 52.7% (95% CI -33.5-83.2%) when a two-dose priming schedule is used and 59.1% (95% CI -31.1-87.2%) when a two-dose priming schedule followed by a booster at 12 months is employed (223).

No studies have previously been performed investigating the immunogenicity of the reduced 2+ 1 schedule in preterm infants, and the BEAR Men B study was designed to compare a 2+1 and a 3+1 schedule in this population.

6.1.1 Objectives

Primary objective:

To compare the immunological responses in infants born preterm to 4CMenB after two primary doses given at 8- and 16-weeks chronological age with three doses given at 8-, 12- and 16-weeks chronological age

Secondary objectives:

1. To compare the persistence of immunological responses to 4CMenB at 12 months chronological age after two doses at 8- and 16-weeks chronological age with three doses given at 8-, 12- and 16-weeks chronological age
2. To compare the immunological responses following the 12-month 4CMenB booster in infants who received two doses at 8- and 16-weeks chronological age with three doses given at 8-, 12- and 16-weeks chronological age

6.1.2 Methods

A full description of the methods can be found in [section 2.3](#).

6.2 Results

6.2.1 Recruitment

The progress of participants in the study is shown in the CONSORT flow diagram (figure 6.1).

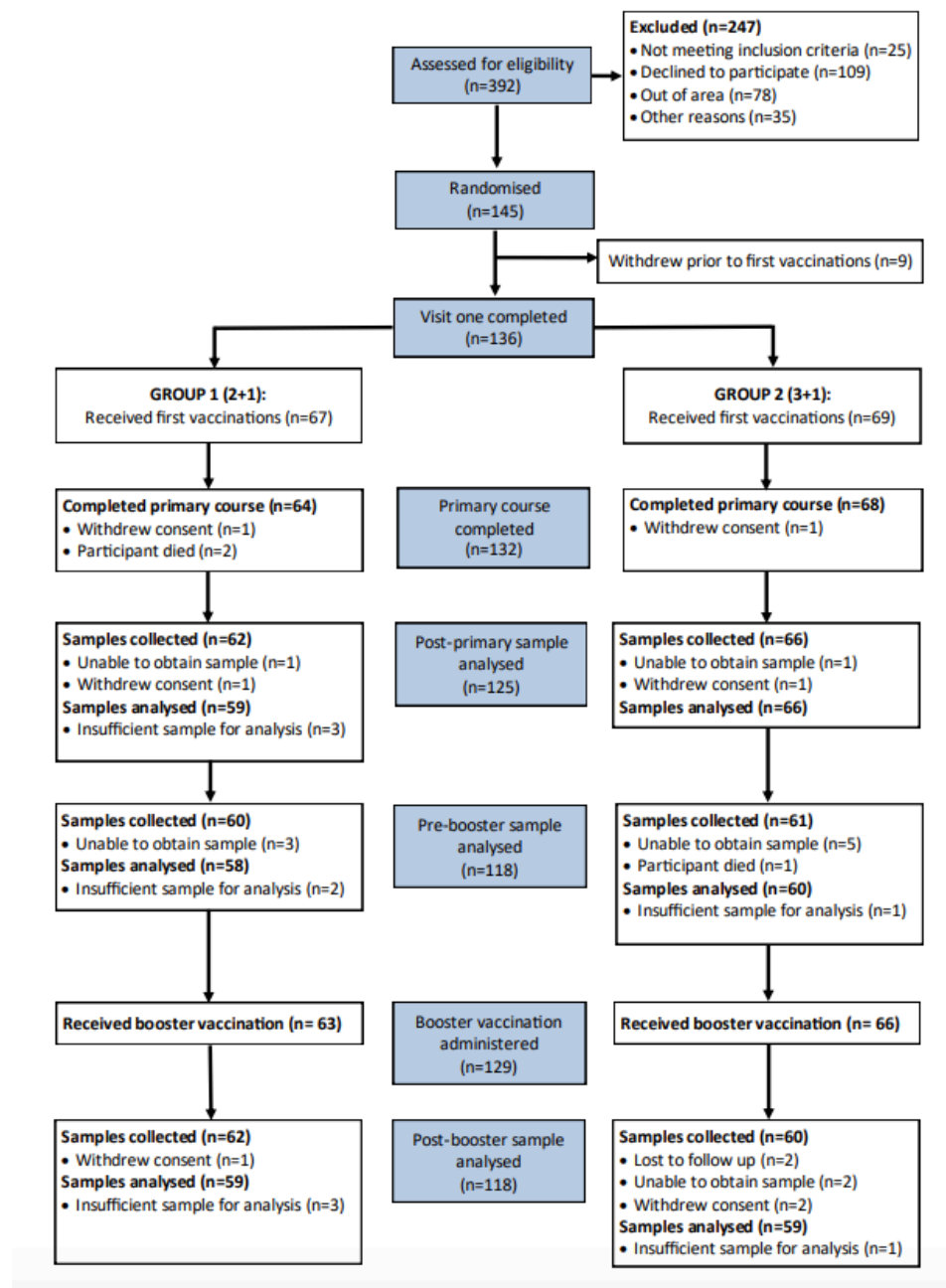


Figure 6.1. CONSORT flow diagram for BEAR Men B study including all recruited participants (n=136).

Screening identified 392 potentially eligible participants, of whom 247 were excluded.

The main reason for exclusion was parental unwillingness for their baby to participate (n=109), but a considerable number were excluded as they lived too far away from the hospital to allow follow up for the duration of the study (n=78). A smaller number of babies were ineligible (n=25) because they did not meet the eligibility criteria.

A total of 145 babies were recruited into the study; nine babies withdrew before the first vaccination visit as they had already received their first vaccines in primary care.

These infants were therefore not included in the total number of recruits. The first study visit was completed by 136 infants, and these are considered to be participants in the study. The study was completed by 124 infants: three participants died, seven were withdrawn and two lost to follow up. The number of participants completing each study visit shown in table 6.1.

Table 6.1. Number of participants completing each study visit (including all recruited infants, n=136).

	Group 1 (2+1 schedule)	Group 2 (3+1 schedule)	Total
V1 (Eight weeks; routine vaccines)	67	69	136
V2 (Twelve weeks; routine vaccines + 4CMenB if group 2)	64	68	132
V3 (Sixteen weeks; routine vaccine)	64	68	132
V4 (samples analysed) (4-6 weeks after primary series; post primary blood sample)	63 (59)	67 (66)	130 (125)
V5 (samples analysed) (12 months; routine vaccines; pre- booster blood sample)	63 (58)	66 (60)	129 (118)
V6 (samples analysed) (13 months; post-booster blood sample)	62 (59)	62 (59)	124 (118)

All infants received the recommended schedule apart from two infants who did not receive Rotarix (1 in each group). V1-6=visit 1-6.

For seven of the babies who did not complete the study there was no confirmation of consent from the parents that data could be used; these infants were not included in

the analysis. There are therefore 129 infants included in the analysis: 64 in group one (2+1 schedule), 65 in group two (3+1 schedule).

Serum bactericidal antibody assay control fail

There were 17 results for which non complement mediated lysis was observed in the control (seven for strain NZ98/254 [PorA], six for strain 5/99 [NadA] and four for strain 44/76-SL [fHbp]). The results which showed this failure in the control were not included in the analysis. For one of these results, antibiotics had been prescribed at the time of blood sampling, but for the others there was no obvious explanation for the failure of the control.

Recruitment by site

We recruited infants from six UK sites. The distribution of recruits by gestational age varied by site (figure 6.2).

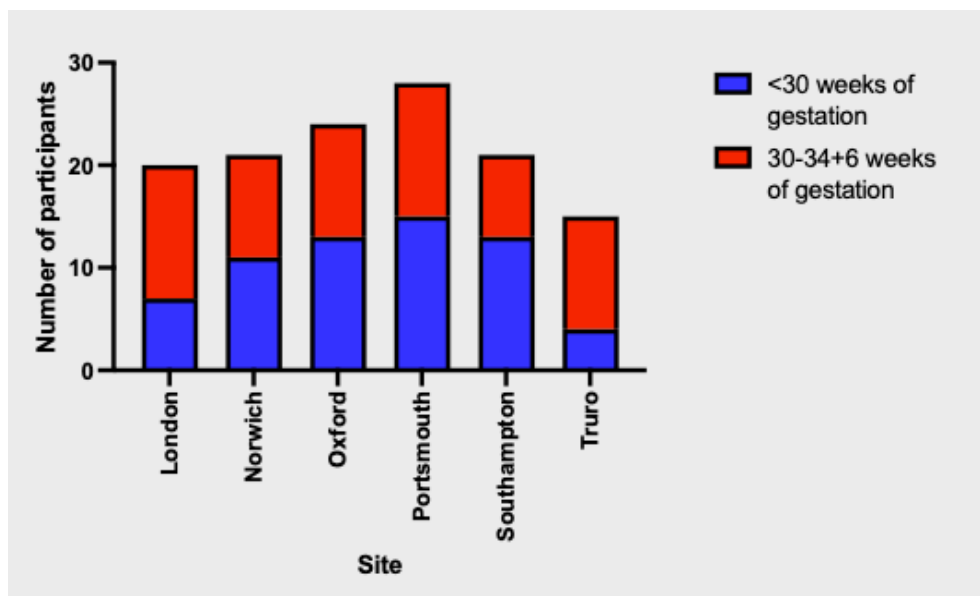


Figure 6.2.Graph showing the number of participants recruited at each site stratified according to gestational age category.

Baseline characteristics

The baseline characteristics of study participants are shown in table 6.2.

Table 6.2. Baseline characteristics according to study group.

Characteristic	N	Group 1 (2+1 schedule)	N	Group 2 (3+1 schedule)
Gestational age Median (range)	64	30 ⁺² (23 ⁺⁰ -34 ⁺³)	65	30 ⁺² (24 ⁺² -34 ⁺³)
Ethnicity (white) Number (%)	64	52 (81.3)	65	57 (87.7)
Sex (female) Number (%)	64	32 (50)	65	41 (63.1)
Birthweight (g) Median (range)	64	1262.5 (575-2790)	65	1350 (490-2610)
CLD Number (%)	64	17 (26.6)	65	15 (23.1)
SGA Number (%)	64	5 (7.8)	65	10 (15.4)
Blood transfusion Number (%)	64	25 (39.1)	65	27 (41.5)
Antenatal steroids Number (%)	64	59 (92.2)	65	63 (96.9)
Postnatal steroids Number (%)	64	5 (7.8)	65	2 (3.1)

CLD=chronic lung disease; SGA= small for gestational age; g grammes

Past medical history

There were a significant number of events of past medical history reported for participants, with a median of two episodes (range 0-12) being recorded for each participant. This is consistent with a preterm population. The most reported conditions were respiratory distress of the newborn (n=48), jaundice (n=46), suspected sepsis (n=32) and patent ductus arteriosus (PDA) (n=31).

Non-study vaccinations

Prior to their recruitment, nine participants had received the Bacillus-Calmette Guerin (BCG) vaccine, and none had received a hepatitis B vaccine. During the study, some participants received BCG (n=7), palivizumab (n=32), influenza (n=2) and yellow fever vaccination (n=1) (table 6.3).

Table 6.3. Summary of non-study vaccinations received.

Time period	Non-study vaccine		
	BCG	Palivizumab	Other
Prior to V1	9	0	
V1-V2	1	4	
V2-V3	3	6	
V3-V4	2	7	
V4-V5	1	13	Influenza (2); Yellow fever (1)
V5-V6	0	2	

V1-6= visit 1-6; BCG= Bacillus-Calmette Guerin

Maternal pertussis vaccination

The mothers of 71 of the participants had received pertussis vaccination in pregnancy, 57 had not and for one the information was unavailable. The median gestation for receiving this vaccination was 24⁺⁶ WG (range 16⁺⁴- 32⁺⁵ WG) and the median time for vaccination prior to delivery was 41 days (range 0-110 days).

Timing of study visits

The age at study visits and time between study visits are shown in tables 6.4 and 6.5.

Table 6.4. Age at study visits.

	Group 1 (2+1 schedule)			Group 2 (3+1 schedule)		
	n	Age at study visit (days) Median (range)	Visit taking place out of window Number (%)	n	Age at study visit (days) Median (range)	Visit taking place out of window Number (%)
V1	62	61 (52-94)	1 (1.6)	63	60 (51-84)	0 (0)
V2	61	95 (80-138)	2 (3.2)	63	93 (79-131)	2 (3.1)
V3	61	127 (109-175)	5 (7.9)	63	123 (110-164)	1 (1.5)
V4	60	163 (143-213)	4 (6.5)	62	157 (138-200)	2 (3.1)
V5	60	378 (345-397)	4 (6.7)	62	377 (366-415)	2 (3.2)
V6	60	412.5 (384-459)	5 (8.1)	59	415 (397-460)	6 (9.8)

V1-6= visit 1-6; 2+1 refers to a 2-dose priming schedule and 3+1 to a 3-dose priming schedule

Table 6.5. Time between study visits.

	Group 1 (2+1 schedule)			Group 2 (3+1 schedule)	
	n	Time between study visit (days) Median (range)	n	Time between study visit (days) Median (range)	
V1-V2	63	33 (25-68)	65	31 (28-49)	
V2-V3	63	32 (28-49)	65	30 (28-44)	
V3-V4	62	35 (27-49)	64	34 (28-50)	
V4-V5	62	216 (166-238)	62	218.5 (176-258)	
V5-V6	62	34 (27-90)	61	34 (28-85)	

V1-6= visit 1-6; 2+1 refers to a 2-dose priming schedule and 3+1 to a 3-dose priming schedule.

Protocol deviations

A summary of protocol deviations is shown in table 6.6. The most common of these were out of window visits (n=34) and no post-vaccination diary card being completed (n=25).

Table 6.6. Protocol deviations.

Protocol deviation	n	Impact of deviation on primary outcome
Vaccination of incorrect limb	4	None
No paracetamol given with inpatient vaccination	1	None
Out of window visit	34	Potentially significant depending on number of days out of window
Late reporting SAE	4	None
Late follow up of SAE	1	None
No study letter sent to GP	3	None
Freezer storing samples out of range	1	Minimal as quickly resolved. No thawing of samples.
No Rotarix given	4	None
Study vaccination took place without a member of research team being in attendance	1	None
Blood sample taken within 48 hours of antibiotics	2	Can interfere with SBA assay results leading to non-complement mediated lysis in the control.
No diary completed	25	None. Significant for secondary outcomes.
Incomplete diary	2	None. Significant for secondary outcomes.
Delegation log not signed by PI	1	None

No blood obtained	10	None. Minimal as sample size calculations included a 10% allowance for attrition.
Incomplete redaction of participant information sent to sponsor	1	None
Time observation completed not recorded	2	None

SAE= serious adverse event; SBA= serum bactericidal antibody

6.2.2 Data analysis

6.2.2.1 Post-primary results at 5 months of age

The geometric mean titres (GMTs) at five months are shown in table 6.7 and figure 6.3.

There were no significant differences in GMTs between the two groups for any of the tested strains.

Table 6.7. GMT and 95% CI according to study group at five months.

	Group One (2+1 schedule)		Group Two (3+1 schedule)		GMR	P value*
	N	GMT (95% CI)	N	GMT (95% CI)		
Strain 44/76-SL (fHbp)	56	40.5 (31.0-52.8)	63	27.7 (20.3-37.8)	0.69 (0.46-1.0)	0.13
Strain 5/99 (NadA)	56	481.3 (386.6-599.1)	63	603.9 (509.0- 716.4)	1.25 (0.96-1.64)	0.19
Strain NZ98/254 (PorA)	56	8.7 (5.8-13.0)	61	11.1 (8.3-14.8)	1.27 (0.79-2.05)	0.4

GMT= geometric mean titre; GMR= geometric mean ratio; fHbp= factor H binding protein; Nad A Neisseria adhesin A; PorA Porin A

*Kruskal Wallis test

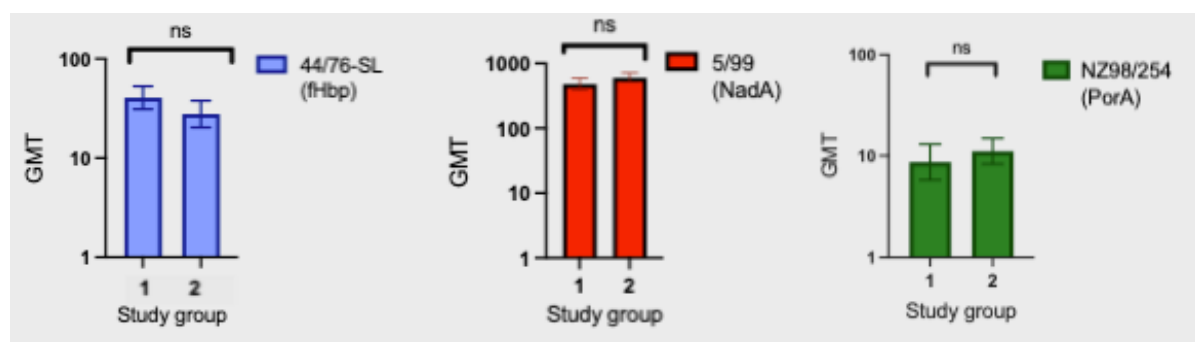


Figure 6.3. SBA GMTs at five months against *N. meningitidis* strains 44/76-SL (fHbp), 5/99 (NadA) and NZ98/254 (PorA).

Study group 1 refers to 2+1 schedule and study group 2 to 3+1 schedule. A: 44/76-SL; no significant difference between study groups, B: 5/99; no significant difference between groups, C: NZ98/254; no significant difference between groups. Error bars show 95% confidence intervals. Exact values are shown in table 6.7. SBA= serum bactericidal antibody, GMT=geometric mean titre, fHbp=factor H binding protein, Nad A=Neisseria adhesin A; PorA=Porin A; ns=not significant.

Proportions of infants with GMTs $\geq 1:4$

Proportions of infants with GMTs $\geq 1:4$ were compared between groups (table 6.8;

figure 6.4). There was a significant difference for strain NZ98/254 (PorA) with a higher proportion of infants achieving a titre $\geq 1:4$ in those who received a 3+1 schedule.

Table 6.8. Proportions of participants with titres $\geq 1:4$ at 5 months.

	N	Group One (2+1)	N	Group Two (3+1)	P value*
		Proportion (95% CI)		Proportion (95% CI)	
Strain 44/76-SL (fHbp)	56	0.98 (0.90-1.0)	63	0.94 (0.85-0.98)	0.37
Strain 5/99 (NadA)	56	1.0 (0.94-1.0)	63	1.0 (0.94-1.0)	-
Strain NZ98/254 (PorA)	56	0.70 (0.56-0.81)	61	0.87 (0.76-0.94)	0.03

Study group 1 refers to 2+1 schedule and study group 2 to 3+1 schedule. fHbp=factor H binding protein; Nad A=Neisseria adhesin A; PorA=Porin A

*Fisher's exact test

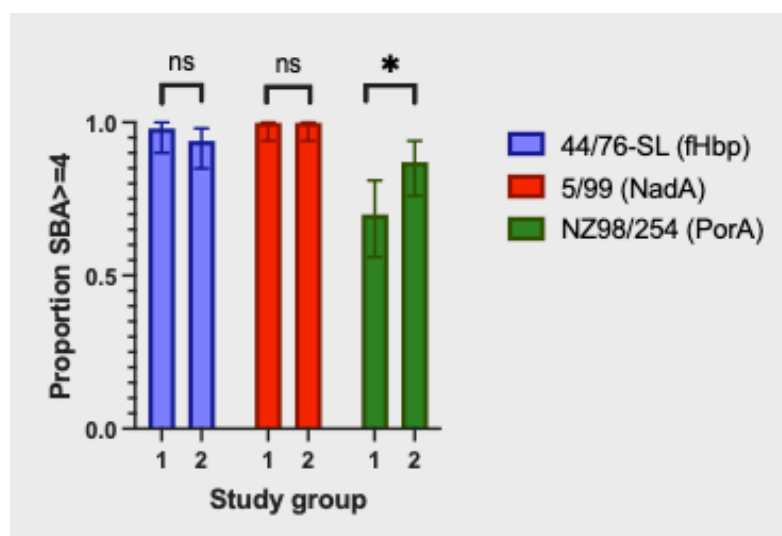


Figure 6.4. Proportion of infants reaching a putative protective SBA GMT against reference strains 44/76-SL (fHbp), 5/99 (NadA) and NZ98/254 (PorA) at five months.

Study group 1 refers to 2+1 schedule and study group 2 to 3+1 schedule. There is a significant difference between study groups for strain NZ98/254 ($p=0.03$); no significant difference between groups for strains 5/99 and 44/76-SL. Error bars show 95% confidence intervals. Exact values are shown in table 6.8. SBA serum bactericidal antibody; fHbp=factor H binding protein (44/76), Nad A=Neisseria adhesin A; PorA=Porin A, ns= not significant, * $p \leq 0.05$

6.2.2.2 Pre-booster results at 12 months of age

The GMTs at twelve months are shown in table 6.9 and figure 6.5. There is a significant difference between groups for strain NZ98/254 (PorA), with those receiving a 3+1 schedule having a significantly higher GMT.

Table 6.9. GMTs (95% CI) at 12 months (pre-booster) according to schedule.

12 months	N	Group 1 (2+1)	N	Group 2 (3+1)	GMR	P value*
Strain 44/76-SL (fHbp)	56	2.2 (1.9-2.6)	60	2.3 (1.7-3.0)	1.03 (0.73-1.45)	0.26
Strain 5/99 (NadA)	57	36.1 (22.5-58.0)	60	52.0 (34.5-78.4)	1.44 (0.78-2.65)	0.09
Strain NZ98/254 (PorA)	57	1.4 (1.2-1.6)	60	2.5 (1.8-3.4)	1.8 (1.3-2.5)	0.002

GMT= geometric mean titre; GMR= geometric mean ratio; fHbp=factor H binding protein; Nad A=Neisseria adhesin A; PorA= Porin A

*Kruskal Wallis test

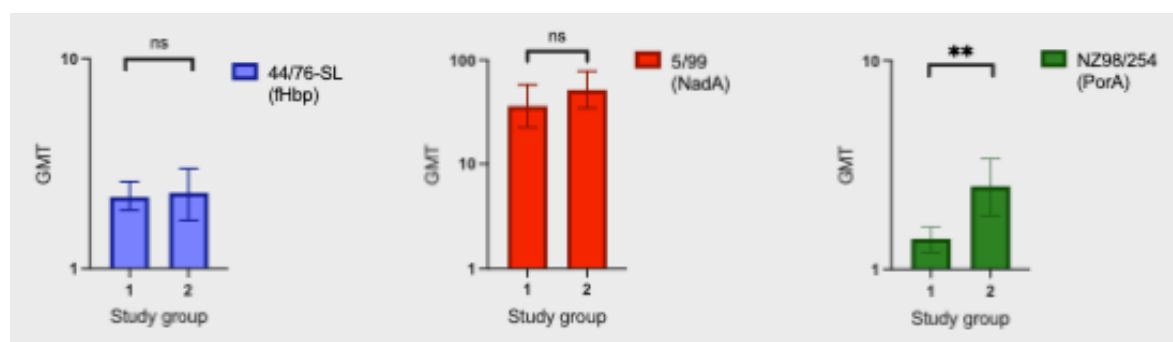


Figure 6.5. SBA GMTs at twelve months against *N. meningitidis* strains 44/76-SL (fHbp), 5/99 (NadA) and NZ98/254 (PorA).

Study group 1 refers to 2+1 schedule and study group 2 to 3+1 schedule. A: 44/76-SL; no significant difference between study groups, B: 5/99; no significant difference between groups, C: NZ98/254; significant difference between groups, $p=0.002$. Error bars show 95% confidence intervals. Exact values are shown in table 6.9. SBA= serum bactericidal antibody, GMT=geometric mean titre, fHbp=factor H binding protein, Nad A=Neisseria adhesin A; PorA=Porin A, ns= not significant, ** $p \leq 0.01$

Proportions of infants with GMTs $\geq 1:4$

Proportions of GMTs $\geq 1:4$ at 12 months were compared between groups (table 6.10; figure 6.6). There was a significantly higher proportion of infants with an SBA titre $\geq 1:4$ for strain NZ98/254 (PorA) in those who received a 3+1 schedule.

Table 6.10. Proportions of participants with titres $\geq 1:4$ at 12 months.

	N	Group One (2+1 schedule)	N	Group Two (3+1 schedule)	P value*
		Proportion (95% CI)		Proportion (95% CI)	
Strain 44/76-SL (fHbp)	56	0.30 (0.19-0.44)	60	0.33 (0.22-0.47)	0.84
Strain 5/99 (NadA)	57	0.86 (0.74-0.94)	60	0.88 (0.77-0.95)	0.79
Strain NZ98/254 (PorA)	57	0.11 (0.04-0.22)	60	0.33 (0.22-0.47)	0.004

Study group 1 refers to 2+1 schedule and study group 2 to 3+1 schedule. fHbp=factor H binding protein; Nad A=Neisseria adhesin A; PorA=Porin A

*Fisher's exact test

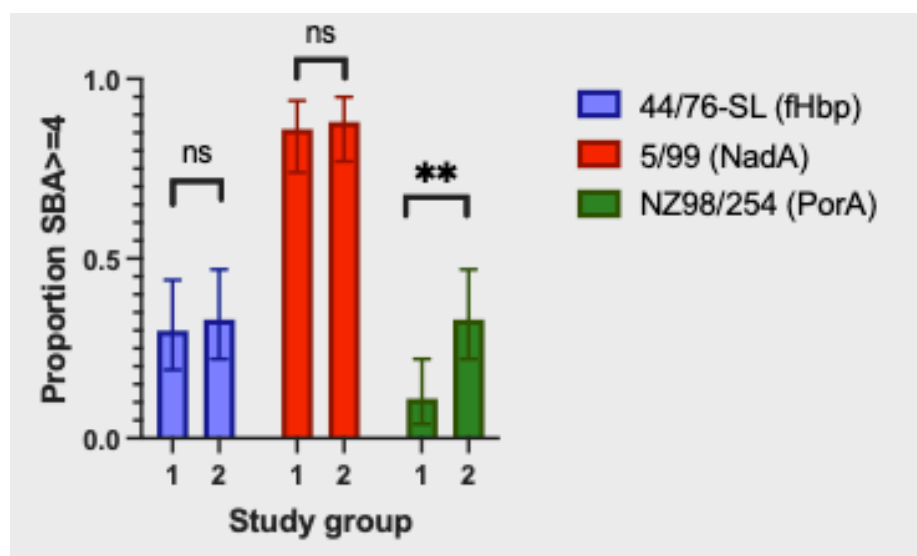


Figure 6.6. Proportion of infants reaching a putative protective SBA GMT against reference strain 44/76-SL (fHbp), 5/99 (NadA) and NZ98/254 (PorA) at twelve months.

Study group 1 refers to 2+1 schedule and study group 2 to 3+1 schedule. There is a significant difference between study groups for PorA ($p=0.004$); no significant difference between groups for NadA and fHbp. Error bars show 95% confidence intervals. Exact values are shown in table 6.10. SBA serum bactericidal antibody; fHbp=factor H binding protein (44/76), Nad A=Neisseria adhesin A (5/99); PorA=Porin A (NZ98/254), ns= not significant, ** $p \leq 0.01$

6.2.2.3 Post-booster results at 13 months of age

The GMTs at thirteen months of age are shown in table 6.11 and figure 6.7. There are no differences between study groups in GMTs.

Table 6.11. GMTs (95% CI) at 13 months (post-booster) according to schedule.

13 months	N	Group 1 (2+1)	N	Group 2 (3+1)	GMR	P value*
Strain 44/76-SL (fHbp)	57	26.3 (18.5-37.5)	55	34.5 (23.7- 50.4)	1.31 (0.79-2.17)	0.3
Strain 5/99 (NadA)	58	1906.3 (1405.5-2585.5)	57	2124.1 (1655.5-2725.3)	1.11 (0.76-1.64)	0.6
Strain NZ98/254 (PorA)	58	21.1 (14.2-31.2)	55	27.2 (18.9-39.0)	1.29 (0.76-2.18)	0.5

GMT Geometric Mean Titre; GMR Geometric Mean Ratio; fHbp factor H binding protein; Nad A Neisseria adhesin A; PorA Porin A

*Kruskal Wallis test

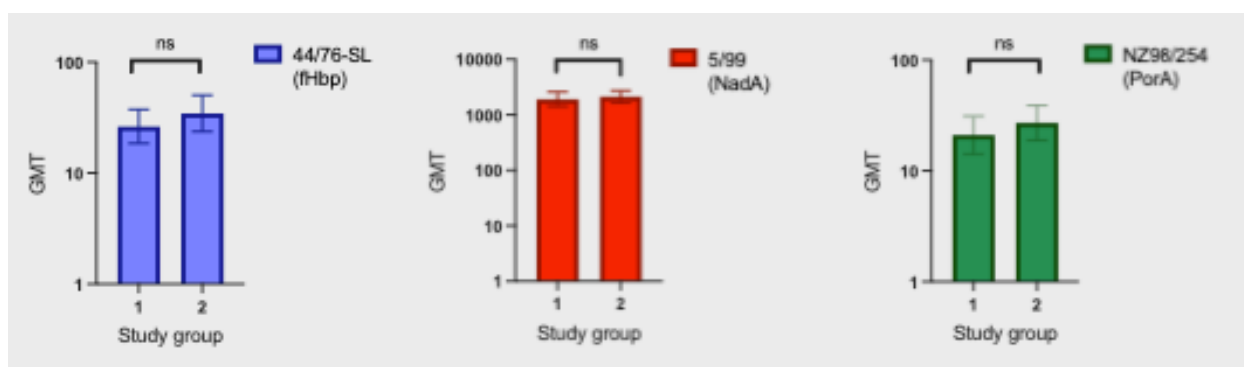


Figure 6.7. SBA geometric mean titres at thirteen months against *N. meningitidis* strains 44/76-SL (fHbp), 5/99 (NadA) and NZ98/254 (PorA).

Study group 1 refers to 2+1 schedule and study group 2 to 3+1 schedule. A: 44/76-SL; no significant difference between study groups, B: 5/99; no significant difference between groups, C: NZ98/254; no significant difference between study groups. Error bars show 95% confidence intervals. Exact values are shown in table 6.11. SBA= serum bactericidal antibody, GMT=geometric mean titre, fHbp=factor H binding protein, Nad A=Neisseria adhesin A; PorA=Porin A, ns= not significant

Proportions of infants with GMTs $\geq 1:4$

Proportions of GMTs $\geq 1:4$ at 13 months were compared between groups (table 6.12; figure 6.8). There were no differences between study groups for any of the tested strains.

Table 6.12. Comparison of proportions in each group with SBA $\geq 1:4$ for all participants at 13 months.

	N	Group One	N	Group Two	P value*
		Proportion (95% CI)		Proportion (95% CI)	
Strain 44/76-SL (fHbp)	57	0.98 (0.91-1.0)	55	0.95 (0.85-0.99)	0.36
Strain 5/99 (NadA)	58	1.0 (0.94-1.0)	57	1.0 (0.94-1.0)	-
Strain NZ98/254 (PorA)	58	0.88 (0.77-0.95)	55	0.95 (0.85-0.99)	0.32

Study group 1 refers to 2+1 schedule and study group 2 to 3+1 schedule. fHbp=factor H binding protein; Nad A=Neisseria adhesin A; PorA=Porin A

*Fisher's exact test

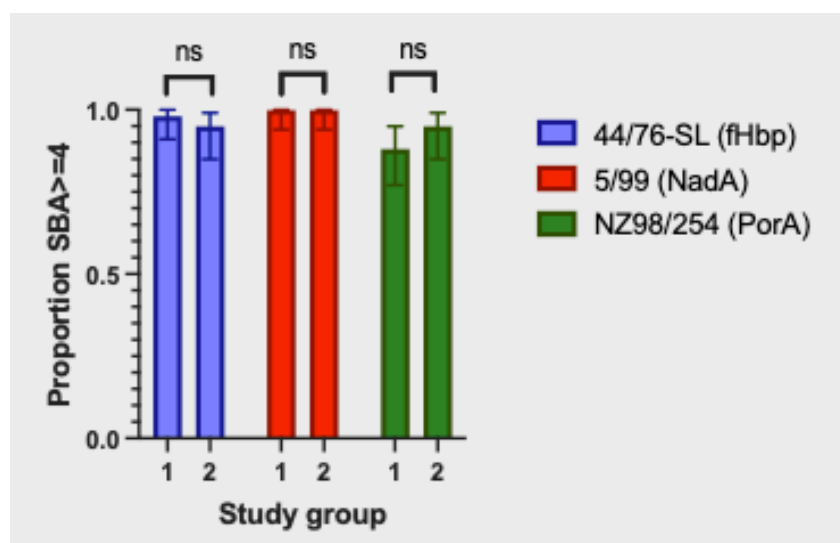


Figure 6.8. Proportion of infants reaching a putative protective SBA GMT against reference strain 44/76-SL (fHbp), 5/99 (NadA) and NZ98/254 (PorA) at 13 months

Study group 1 refers to 2+1 schedule and study group 2 to 3+1 schedule. There are no significant differences between study groups for any of the tested strains. Error bars show 95% confidence intervals. Exact values are shown in table 6.12. SBA= serum bactericidal antibody; fHbp=factor H binding protein; Nad A=Neisseria adhesin A; PorA=Porin A, ns= not significant

Interaction between schedule and gestational age

The interaction between study group and gestational category was investigated using ordered logistic regression. No significant interaction was seen for any of the strains at any of the time points, suggesting that the impact of group is not significantly affected by gestation (table 6.13).

Table 6.13. Results of ordered logistic regression investigating interaction between gestational age and study group.

Time point	Strain	Odds ratio	P value*
5 months	44/76-SL (fHbp)	1.0 (0.97-1.03)	0.97
	5/99 (NadA)	1.0 (1.0-1.06)	0.07
	NZ98/254 (PorA)	1.03 (0.99-1.06)	0.06
12 months	44/76-SL (fHbp)	1.0 (0.97-1.03)	0.86
	5/99 (NadA)	1.0 (0.98-1.0)	0.74
	NZ98/254 (PorA)	1.02 (0.99-1.06)	0.22
13 months	44/76-SL (fHbp)	0.98 (0.95-1.0)	0.13
	5/99 (NadA)	1.0 (0.97-1.03)	0.99
	NZ98/254 (PorA)	1.0 (0.99-1.1)	0.11

Study group 1 refers to 2+1 schedule and study group 2 to 3+1 schedule. fHbp factor H binding protein; Nad A Neisseria adhesin A; PorA Porin A

*Ordered logistic regression

6.2.2.4 Antibody kinetics over time

The variation in GMTs over time are shown for the three tested strains in figures 6.9.

There was a considerable reduction in GMTs for all tested strains between the post-primary and pre-booster timepoints (table 6.14 and figures 6.9). There was a low post booster response for 44/76-SL, particularly for those in group one in which the post booster response was lower than that seen following the primary vaccines (figure 6.9 and table 6.14).

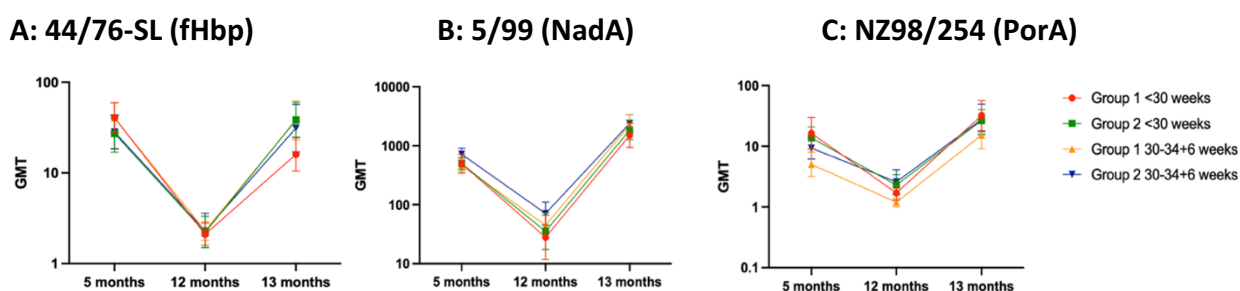


Figure 6.9. GMT against 44/76-SL (fHbp), 5/99 (NadA) and NZ98/254 (PorA) at 5, 12 and 13 months shown according to group and gestational category.

Study group 1 refers to 2+1 schedule and study group 2 to 3+1 schedule. fHbp= factor H binding protein; Nad A= Neisseria adhesin A; PorA= Porin A.

The greatest difference between groups in fold decrease between post-primary and pre-booster titres was for NZ98/254 (PorA), with a greater decrease in infants in group one (table 6.14). The greatest difference between groups in fold change between post-primary and post-booster titres was for fHbp, with a greater fold change seen for infants in group two compared with those in group one (table 6.15).

Table 6.14. Fold decrease between post-primary and pre-booster for the three tested strains.

	Fold decrease post-primary to pre-booster		
	Group 1 (2+1 schedule)	Group 2 (3+1 schedule)	Ratio of fold changes
Strain 44/76-SL (fHbp)	17.8 (13.3-23.8)	13.1 (9.2-18.6)	0.74 (0.47-1.2)
Strain 5/99 (NadA)	13.0 (8.7-19.4)	12.5 (8.7-18.0)	0.96 (0.57-1.6)

Strain NZ98/254 (PorA)	6.49 (4.4-9.5)	4.5 (3.3-6.1)	0.69 (0.43-1.1)
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Study group 1 refers to 2+1 schedule and study group 2 to 3+1 schedule. fHbp= factor H binding protein; Nad A=Neisseria adhesin A; PorA=Porin A.

Table 6.15. Fold change between post-primary and post-booster for the three tested antigens.

	Fold change post-primary to post-booster		
	Group 1 (2+1 schedule)	Group 2 (3+1 schedule)	Ratio of fold changes
Strain 44/76-SL (fHbp)	0.73 (0.5-1.0)	1.2 (0.8-1.8)	1.65 (1.0-2.74)
Strain 5/99 (NadA)	3.9 (2.8-5.3)	3.2 (2.5-4.1)	0.83 (0.57-1.2)
Strain NZ98/254 (PorA)	2.6 (1.7-3.9)	2.3 (1.6-3.4)	0.91 (0.53-1.6)

Study group 1 refers to 2+1 schedule and study group 2 to 3+1 schedule. fHbp= 198ator H binding protein; Nad A=Neisseria adhesin A; PorA=Porin A.

The half-life calculated between the post-primary and pre-booster samples was longest for strain NZ98/254 (PorA), but similar between those vaccinated with two or three priming doses of 4CMenB (table 6.16).

Table 6.16. Calculated half-life of antibody between post-primary and pre-booster sampling points for all three tested antigens (days).

	Overall	Group 1 (2+1 schedule)	Group 2 (3+1 schedule)
Strain 44/76-SL (fHbp)	58.3 (53.2-63.8)	54.9 (49.2-61.3)	61.6 (53.4-71.0)
Strain 5/99 (NadA)	67.5 (61.1-74.7)	68.1 (57.5-80.8)	67.0 (59.4-75.6)
Strain NZ98/254 (PorA)	84.2 (75.1-94.4)	76.8 (63.2-93.3)	90.9 (79.4-104.2)

6.2.2.5 Assessment of individual participant immunity

To investigate protection against individual indicator strains, analysis was also performed using paired post-primary and post-booster samples to assess protective thresholds (SBA titre $\geq 1:4$) in individual infants at these two time points. In those receiving a 2+1 schedule, 96.2% of infants achieved a protective titre against 44/76-SL following both primary and booster vaccinations while one (1.9%) achieved it after

primary vaccination only and another one (1.9%) after booster vaccination only; 100% of infants achieved a protective titre against 5/99 at both time points, while only 67.3% achieved a protective titre against NZ98/254 at both time points with 19.2% (n=10) achieving a protective titre after the booster dose only and 13.4% not achieving it at either time point. In those infants receiving a 3+1 schedule, 90.7% reached a protective titre against 44/76-SL at both time points while 5.6% (n=3) achieved this after the primary vaccines only and 3.7% (n=2) after the booster dose only; 100% of infants achieved a protective titre against 5/99 at both time points, while 87.0% achieved a protective titre against NZ98/254 at both time points with 7.4% (n=4) achieving this after the booster only and 5.8% (n=3) not achieving it at either time point (table 6.17)

Table 6.17. Percentage of infants with SBA GMT $\geq 1:4$ following primary and booster vaccination, at both time points and at neither time point.

	Strain								
	44/76- SL (fHbp)			5/99 (NadA)			NZ98/254 (PorA)		
	Overall (n=106)	Group 1 (n=52)	Group 2 (n=54)	Overall (n=108)	Group 1 (n=52)	Group 2 (n=54)	Overall (n=106)	Group 1 (n=52)	Group 2 (n=54)
Neither time point	0% (n=0)	0% (n=0)	0% (n=0)	0% (n=0)	0% (n=0)	0% (n=0)	9.4% (n=10)	13.4% (n=7)	5.8% (n=3)
Post primary only	3.8% (n=4)	1.9% (n=1)	5.6% (n=3)	0% (n=0)	0% (n=0)	0% (n=0)	0% (n=0)	0% (n=0)	0% (n=0)
Post booster only	2.8% (n=3)	1.9% (n=1)	3.7% (n=2)	0% (n=0)	0% (n=0)	0% (n=0)	12.3% (n=14)	19.2% (n=10)	7.4% (n=4)
Both time points	93.4% (n=99)	96.2% (n=50)	90.7% (n=49)	100% (n=108)	100% (n=52)	100% (n=56)	77.4% (n=82)	67.3% (n=35)	87.0% (n=47)

6.2.2.6 Determinants of immunogenicity

Linear regression for GMT

Multivariable regression of GMT against strain NZ98/254 (PorA) at five months, showed that sex and gestational category were significant determinants, with SBA titres being 1.6 times higher in female than male infants and titres in those born at 30-34⁺⁶ WG 0.49 of those born at less than 30 WG (table 6.18). When analysis was adjusted for these variables, the difference between study groups remained non-significant. For GMT against strain 5/99 (NadA) at five months, only ethnicity was significant, with white infants having SBA titres 0.59 of non-white infants (table 6.19). On adjustment for ethnicity, the difference between the two study groups went from borderline non-significant to borderline significant. At 12 months for GMT against strain 5/99 (NadA), female sex and gestation 30-34⁺⁶ WG were associated with higher GMTs, and white ethnicity and early paracetamol at third immunisations were associated with lower GMT (table 6.18). When analysis is adjusted for these variables, the difference between study groups remains non-significant. No factors were identified as significant for GMT against strain 44/76-SL (fHbp) at any time point.

Table 6.18. Summary of variables of interest for GMTs following investigation using linear regression.

Time point	Strain	Variable	GMT (95% CI)	P value*
5 months	NZ98/254	Group adjusted for significant variables (2 vs. 1)	1.33 (0.83-2.11)	0.24
		Sex (Female vs. male)	1.64 (1.04-2.58)	0.034
		Gestational category (30-34 ⁺⁶ vs. <30+0)	0.49 (0.31-0.76)	0.002
	5/99	Group adjusted for significant variables	1.30 (1.00-1.69)	0.049

12 months	5/99	(2 vs. 1)		
		Ethnicity (White vs. non-white)	0.59 (0.42-0.85)	0.004
		Group adjusted for significant variables (2 vs. 1)	1.35 (0.78-2.35)	0.29
		Gestational category (30-34 ⁺⁶ vs. <30+0)	1.74 (1.00-3.03)	0.049
		Sex (Female vs. male)	2.28 (1.30-4.00)	0.004
		Ethnicity (White vs. non-white)	0.24 (0.11-0.52)	<0.001
		Early paracetamol following third vaccines (Those who did receive paracetamol compared to those who did not)	0.66 (0.47-0.92)	0.013

*Linear regression

6.3 Discussion

This study is the first to investigate the immunogenicity of 4CMenB vaccination in preterm infants, comparing a 2+1 and 3+1 schedule. Overall, both schedules were immunogenic, although the GMT against strain NZ98/254 (PorA) at 12 months and the proportions of infants with titres $\geq 1:4$ at 5 and 12 months were significantly higher in the infants who received the 3+1 schedule. This strain is matched to the vaccine antigen Porin A, the immunodominant component of outer membrane vesicle (OMV) vaccines, which are known to be more immunogenic when a three rather than two dose priming schedule is used (245, 246). The improved response of the OMV component of 4CMenB following a three-dose priming schedule is consistent with this. The immunogenicity against strain 5/99 (NadA) was good at all time points, irrespective of the schedule used. All the participants had titres $\geq 1:4$, both post-primary and post-booster, and even immediately prior to the administration of the

booster vaccine the proportions protected were 0.86 and 0.88 in the two-dose and three-dose priming schedule respectively. These results are consistent with those reported in term infants vaccinated with a 2+1 schedule (242, 243), and GMTs were higher in these preterm infants than was seen in a population of term infants after a second dose in a study in which immunogenicity assessments were made following second and third doses (244). In this latter study, in which analysis was performed in the same lab as in this clinical trial, GMTs were also lower after the third dose in term infants compared with preterm infants vaccinated with three priming doses and remained lower following the booster dose (244). The impact of inter-laboratory variation can be considerable for SBA assays (247), which makes comparison with results from other laboratories harder, however the results from the three-dose priming schedule in the clinical trial were within the range seen from other studies involving term infants (203, 248-252).

The immunogenicity for strain 44/76-SL (fHbp) was also good overall, with 93.4% of the preterm infants having titres $\geq 1:4$ after both the primary series and the booster dose. Following a two-dose primary series, GMTs against strain 44/76-SL (fHbp) and proportions with titres $\geq 1:4$ were very similar in these preterm infants to those seen in previous term studies following the primary series (43, 242-244). Following the booster dose, proportions with titres $\geq 1:4$ in the preterm infants were similar to those reported in other studies (43, 242, 243), and GMTs were similar to those reported by Davis (243), but lower than those reported by Pinto (43). One month after a three-dose primary schedule the preterm GMTs against fHbp were very similar to the results seen in a study of term infants with analysis performed in the same lab (244), although somewhat lower than the results from other studies (203, 248-252) and after the

booster dose the preterm infants had lower GMTs than were seen in any of the published studies involving term infants. Although there was no significant difference in GMTs against 44/76-SL (fHbp) between study groups following the booster dose, the increase in GMT following the booster dose compared with the increase following the primary series was minimal, indeed for those infants vaccinated with two priming doses, the GMTs following the booster dose were lower than those seen following the primary dose. This absence of a high post-boost response against 44/76-SL (fHbp) when using a 2+1 schedule has also been observed for term infants in the studies by Pinto and Davis, perhaps suggesting that a three-dose primary schedule is necessary for optimal priming of this response. The same pattern was not seen in infants vaccinated according to a 2+1 schedule in Martinon-Torres' study, however of note, both the reduced schedules given by Martinon-Torres were slightly later (3.5 and 5 months or 6 and 8 months) than either those given in the BEAR Men B study, or in the infants studied by Pinto or Davis (all two and four months) which may explain this. The immunogenicity against strain NZ98/254 (PorA) was considerably lower than the other two studied strains and was lower in those infants who had received a two-dose priming schedule. This is consistent with what is known about OMV vaccines. It is of concern that in those preterm infants vaccinated according to a 2+1 schedule, 13.4% of infants did not achieve a titre of $\geq 1:4$ either post primary or post booster. Following the primary series and the booster dose, preterm infants vaccinated with a two-dose priming schedule had similar GMTs and proportions $\geq 1:4$ to those reported in previous studies of term infants with analysis performed in the same lab (243, 244), but lower proportions $\geq 1:4$ than term infants in another study where analysis was performed elsewhere (242). Preterm infants who had received three priming doses had GMTs

against NZ98/254 (PorA) which were within the range of results reported in term studies (244, 248-252).

As discussed in chapter one, the mechanism of action of 4CMenB is somewhat different to polysaccharide-based vaccines, in that it depends on the production of bactericidal antibodies directed against any one of the antigens included in the vaccine which are variably expressed on the surface of different meningococci. This means that the protection experienced by an individual is dependent on the presence of circulating bactericidal antibodies against the proteins expressed on the particular meningococci an individual is exposed to. Estimates of coverage from 4CMenB within a region are possible using the Meningococcal Antigen Typing System (MATS). In a study published in 2017 the MATS coverage in the UK was reported for two time periods of 2007-2008 and 2014-2015. This showed that overall, 66% of serogroup B isolates were estimated to be covered by 4CMenB in 2014-2015 compared with 73% in 2007-2008 (228). When looking at the results from the clinical trial in the context of the most recent MATS results it is reassuring that a high proportion of preterm infants in this study had titres $>1:4$ against strain 44/76-SL (fHbp) both post-primary and post-booster vaccination, as MATS coverage is often due to fHbp, although the non-significantly lower GMTs seen following the 2+1 schedule may be of concern if this results in earlier waning of protective antibody in infants who received this schedule. MATS coverage is less dependent on PorA (single matched antigen in $<1\%$ of isolates), which is reassuring given that up to 30% of infants in our cohort had not achieved a titre of $\geq 1:4$ following primary vaccination, potentially leaving them without protection against such a strain. It is also important to remember the dynamic nature of meningococcal epidemiology and the changes in expressed antigens seen between the

two time periods in Parikh's paper highlight the importance of the ongoing surveillance in the UK. It is important that this surveillance continues to consider the gestational age of any infants with invasive meningococcal disease to ensure that preterm infants are not over-represented among cases.

Strengths and limitations

A major strength of this study was the ability to recruit a large and broad sample of preterm infants, including those with a range of common comorbidities of prematurity making this population representative of UK preterm infants. One potential limitation of the study is that we did not collect baseline pre-vaccination antibody titres, but most infants are likely to have very low antibody titres (203), and, in any case, pre-vaccine antibody prevalence should be similar between the two study groups. We also did not include a term comparator group and, therefore, our comparisons with vaccine responses in term infants should be interpreted with caution, although at least one of the UK studies in term infants was conducted in the same reference laboratory using the same methodology and testing protocols. Finally, we did not follow up the participants beyond 13 months and, therefore, we cannot comment on long-term immune responses or impact on clinical protection against disease.

6.4 Conclusion

This study demonstrated that there were no significant differences for GMTs or proportions protected for strains 44/76-SL (fHbp) or 5/99 (NadA) between those vaccinated according to a two-dose or three-dose primary schedule. For strain NZ98/254 (PorA), a three-dose primary schedule was superior in preterm infants, which is consistent with what is known about OMV vaccines in term infants, and

4CMenB in a term population. For strain NZ98/254 (PorA), infants in the clinical trial had higher proportions with titres below the putative protective threshold, both post-primary and pre-booster which emphasises the need to capture gestational age as part of the surveillance programme. The most recent MATS results provide some reassurance that this will not result in excessive cases among preterm infants vaccinated according to this schedule, but meningococcal epidemiology is dynamic, and this should continue to be monitored. There is currently no evidence from this study that preterm infants should be vaccinated using a schedule different from that used in term infants.

7 BEAR Men B reactogenicity and safety results

7.1 Background

As discussed in chapter four, reactogenicity and safety outcomes are of interest to both parents and health care professionals when making decisions about the vaccination of infants, especially those born preterm. Timely vaccination is important in all infants, but despite recommendations to vaccinate preterm infants at their chronological age, vaccination is delayed more often in preterm than in term infants (118, 253). Reasons for delay in the administration of vaccines may include concerns about the vulnerability of this population and the perceived potential for vaccine administration to cause clinical deterioration, the range of comorbidities which are experienced by preterm infants, and the challenge in differentiating adverse events related to vaccination from the normal fluctuations in their clinical course. 4CMenB vaccination has been particularly associated with fever when given alongside routine vaccinations (203, 242, 248). This is of concern to parents (254), and also to health care professionals, as it is impossible to definitively distinguish between fever caused by recent vaccination and that caused by intercurrent infection, which in turn can lead to infants receiving additional invasive investigations or being admitted to hospital for investigation of possible sepsis. Several UK studies which took place shortly after the introduction of 4CMenB showed an increase in Emergency Department (ED) attendance and hospital admission following vaccination in term infants (255-257). In previously published studies of preterm infants receiving 4CMenB, the rate of fever was variable, ranging from 5% in a preterm population who received paracetamol prophylaxis when fever was defined as greater than 38°C, to 46.7% in preterm infants

not receiving paracetamol prophylaxis when fever was defined as greater than 37.5°C (191, 194, 202). Paracetamol administration at the time of 4CMenB administration has been shown to reduce the incidence of fever in term infants, with no reduction in immunogenicity (252). Paracetamol is therefore routinely recommended following 4CMenB vaccination at 8 and 16 weeks, with the first dose being given as close as possible to the time of vaccine administration, and two subsequent doses being given at four to six hourly intervals thereafter. This advice is the same for term and preterm infants, although the recommendation is that infants born at a gestational age of less than 32 WG should receive a dose calculated according to their weight (258).

In the BEAR Men B study information was collected about the reactogenicity of routine vaccines, including 4CMenB, incorporating the following outcomes: fever, local and non-febrile systemic reactions, investigation for sepsis, cardiorespiratory events in those infants monitored in hospital, and information about the safety of vaccination including serious adverse events (SAEs).

7.1.1 Objectives

To describe the safety of routine vaccines (including 4CMenB) in preterm infants.

7.1.2 Methods

The full methods are described in [section 2.3](#).

7.2 Results

Participant recruitment, baseline characteristics and progress through the trial is as described in [section 6.2.1](#).

7.2.1 Reactogenicity

Reactogenicity outcomes included cardiorespiratory events in those infants vaccinated as inpatients and solicited local and systemic adverse events including fever. Because of the importance of fever as an adverse event following 4CMenB, this is considered in a separate section from other systemic reactions.

7.2.1.1 Cardiorespiratory instability in participants vaccinated as inpatients

A proportion of infants were vaccinated in the neonatal unit (NNU): 84 vaccination episodes involving 56 participants.

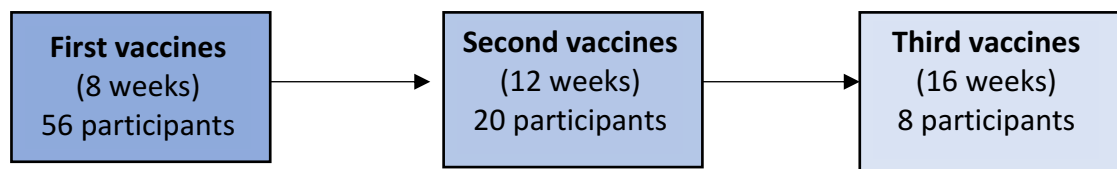


Figure 7.1. Progress of participants vaccinated as inpatients through vaccine visits.

Details of the participants vaccinated at each study visit are shown in table 7.1. These infants tended to be of lower gestational age and birthweight than those having vaccines administered in the community and had a higher rate of chronic lung disease (CLD).

Table 7.1. Baseline characteristics of infants receiving vaccination in hospital or in the community.

	8 weeks		12 weeks		16 weeks	
	Vaccinated in hospital (n=56)	Vaccinated in community (n=73)	Vaccinated in hospital (n=20)	Vaccinated in community (n=108)	Vaccinated in hospital (n=8)	Vaccinated in community (n=120)
Gestational age category (<30 WG) % (n)	96.4 (54)	12.3 (9)	95 (19)	39.8 (43)	87.5 (7)	45.8 (55)
Gestational age at birth (WG) Median (range)	27 ⁺² (23 ⁺⁰ -32 ⁺³)	32 ⁺² (28 ⁺⁰ -34 ⁺³)	25 ⁺⁵ (23 ⁺⁰ -30 ⁺⁶)	31 ⁺² (25 ⁺³ -34 ⁺³)	25 ⁺⁵ (23 ⁺⁰ -30 ⁺⁶)	30 ⁺⁴ (24 ⁺² -34 ⁺³)
Birthweight (g) Median (range)	925 (490-1605)	1700 (930-2790)	754 (490-1170)	1449.5 (672-2610)	669 (490-780)	1372 (580-2610)
Sex (female) % (n)	55.4 (31)	57.5 (42)	50 (10)	58.3 (63)	37.5 (3)	58.3 (70)
CLD % (n)	51.8 (29)	4.1 (3)	70 (14)	15.7 (17)	62.5 (5)	21.7 (26)
Age at vaccination (days) Median (range)	60.5 (52-84)	61 (51-94)	94 (82-138)	93 (80-131)	136 (110-175)	124 (110-164)

WG= weeks of gestation; CLD= chronic lung disease

Cardiorespiratory events before and after vaccination at eight weeks

In the 24 hours prior to administration of the first vaccines, 53.6% (n=30) of the inpatient infants were receiving ventilatory support (four continuous positive airway pressure [CPAP], 13 high flow oxygen, 13 low flow oxygen). In the 72 hours following vaccination 64.3% (n=36) of infants required ventilatory support (one biphasic positive

airway pressure [BiPAP], four CPAP, 16 high flow oxygen, 15 low flow oxygen). The details of the respiratory support required following vaccination according to that required before is shown in figure 7.2.

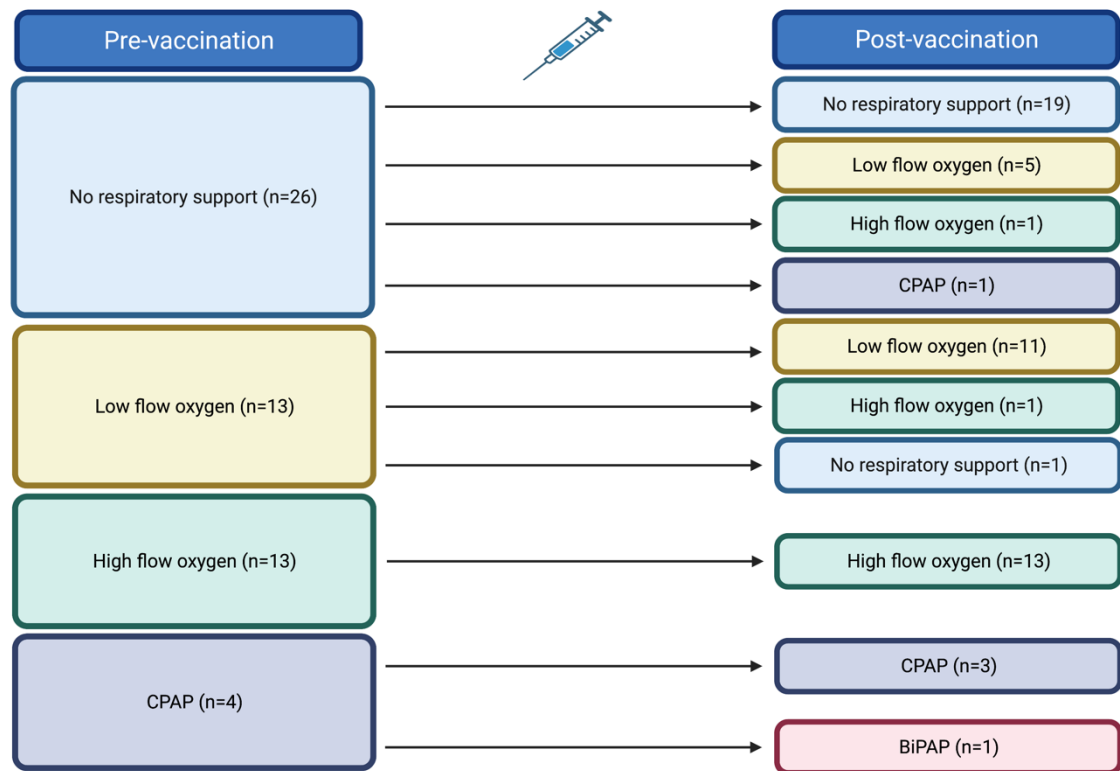


Figure 7.2. Ventilatory support following first vaccines displayed according to ventilatory support required before vaccination.

Nearly 30% (n=7) of infants who required no respiratory support prior to vaccination required some respiratory support in the 72 hours following vaccination. Of the infants who had required low flow oxygen, the majority continued on low flow oxygen, with one needing increased support to high flow oxygen, and one infant no longer needing support after vaccination. All the infants who needed high flow oxygen before vaccination remained on this after vaccination. One infant who had required CPAP before vaccination required an increase in support to BiPAP, and the remainder of these infants continued on CPAP.

CPAP= continuous positive airway pressure; BiPAP= biphasic positive airway pressure.

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The percentage of infants experiencing apnoea, bradycardia and desaturation was higher in the 72 hours after vaccination compared with the 24 hours before vaccination (table 7.2; figure 7.3). This difference was not statistically significant for any of the cardiorespiratory events.

Table 7.2. Percentage of infants experiencing apnoea, bradycardia and desaturation before and after vaccine administration at eight weeks showing median and range of episodes.

	Infants experiencing apnoea % (n)	Median (range)	Infants experiencing bradycardia % (n)	Median (range)	Infants experiencing desaturations % (n)	Median (range)
Pre-vaccine (n=55)	7.3 (4)	1 (1-4)	21.8 (12)	2.5 (1-13)	29.1 (16)	5.5 (1-14)
Post-vaccine (n=55)	20 (11)	2 (1-11)	30.9 (17)	3.5 (1-38)	41.8 (23)	4.5 (1-69)

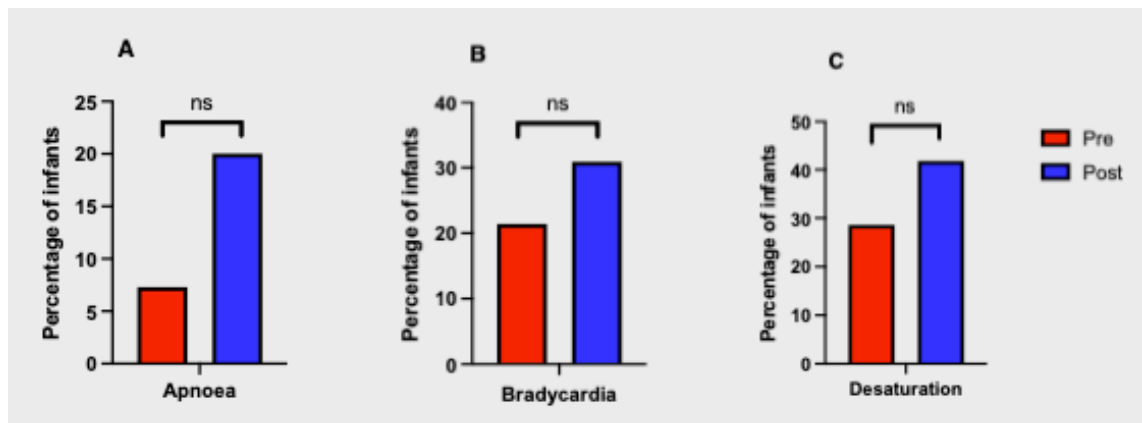


Figure 7.3. Percentage of infants experiencing apnoea (A), bradycardia (B) and desaturation (C) before and after vaccination at 8 weeks.

There was an increase in the percentage of infants experiencing apnoea, bradycardia, and desaturation in the period after vaccine administration compared to that before, but this was not statistically significant. ns= not significant

Cardiorespiratory events before and after vaccination at 12 weeks

In the 24 hours before administration of the second vaccines, 65% (n=13) of the inpatient infants required ventilatory support (one conventional ventilation, one CPAP, two high flow oxygen and nine low flow oxygen). Following these vaccines, the same percentage of infants required ventilatory support, with one infant who had been receiving low flow oxygen before vaccination needing high flow oxygen after vaccination, and the other infants receiving the same ventilatory support after vaccination (figure 7.4).

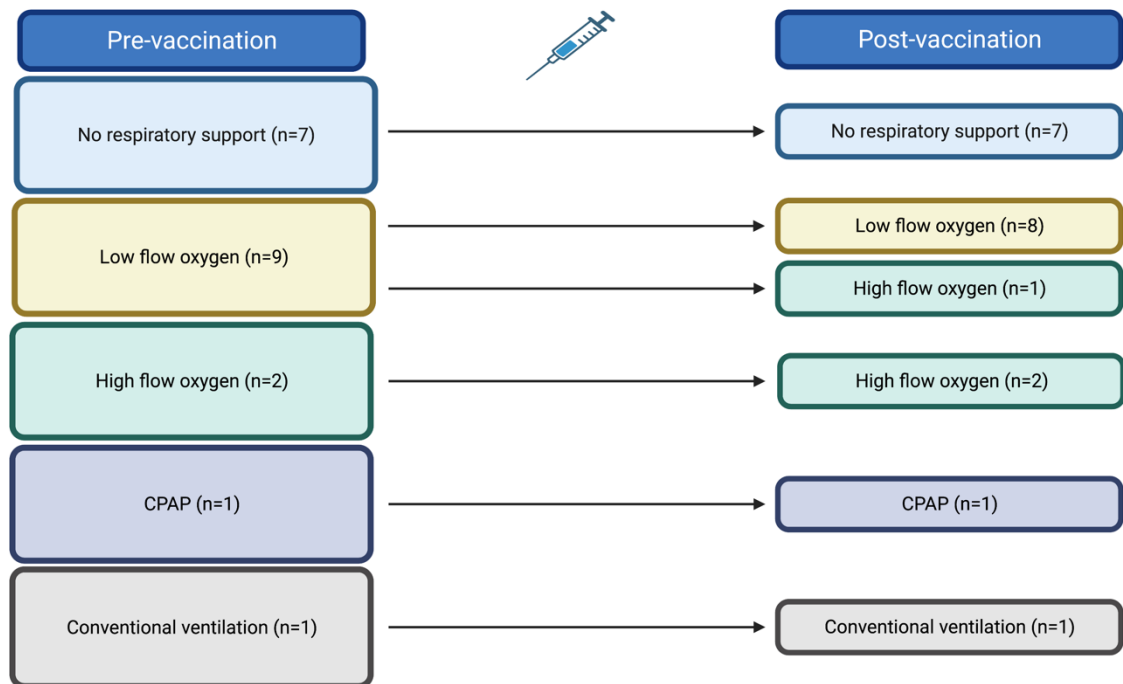


Figure 7.4. Ventilatory support following second vaccines displayed according to ventilatory support required before vaccination.

No infant required ventilatory support following vaccination who had not needed this prior to vaccination. Of the infants who had required low flow oxygen, the majority continued on low flow oxygen with one needing increased support to high flow oxygen. All the infants who needed high flow oxygen, CPAP or conventional ventilation before vaccination remained on this after vaccination.

CPAP= continuous positive airway pressure.

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The percentage of infants experiencing apnoea, bradycardia or desaturation was again higher in the 72 hours following vaccination compared with the 24 hours before at 12 weeks, but this was not significant for any of the recorded cardiorespiratory events (table 7.3; figure 7.5).

Table 7.3. Percentage of infants experiencing apnoea, bradycardia and desaturation before and after vaccine administration at 12 weeks showing median and range of episodes.

	Infants experiencing apnoea % (n)	Median (range)	Infants experiencing bradycardia % (n)	Median (range)	Infants experiencing desaturations % (n)	Median (range)
Pre-vaccine (n=20)	5 (1)	1 (1-1)	5 (1)	1 (1-1)	15 (3)	2.5 (1-4)
Post-vaccine (n=20)	15 (3)	1 (1-5)	15 (3)	8 (4-12)	35 (7)	2.5 (1-26)

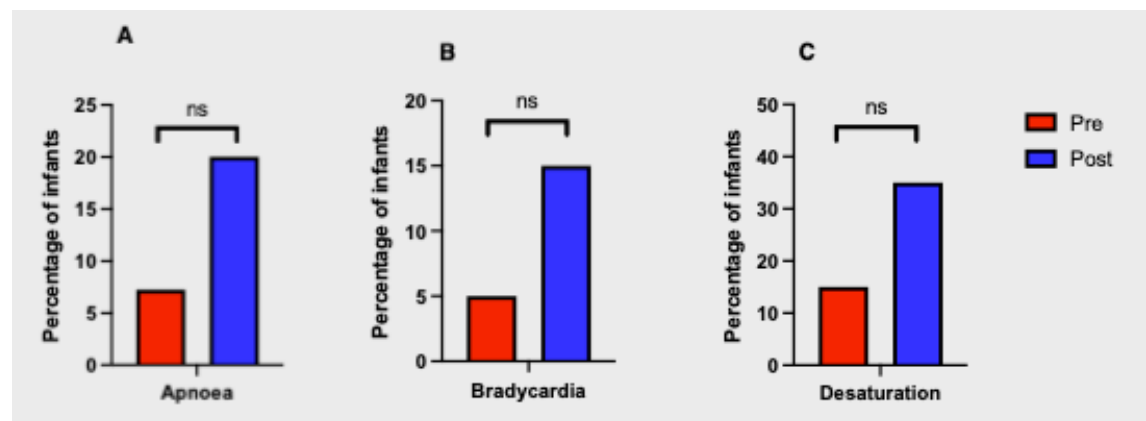


Figure 7.5. Percentage of infants experiencing apnoea (A), bradycardia (B) and desaturation (C) before and after vaccination at 12 weeks.

There was an increase in the percentage of infants experiencing apnoea, bradycardia, and desaturation in the 72 hours after vaccine administration compared with the 24 hours before at 12 weeks, although this was not statistically significant for any of these cardiorespiratory events.

ns= not significant

Cardiorespiratory events before and after vaccination at 16 weeks

In the 24 hours prior to administration of the third vaccines, 62.5% (n=5) infants

continued to require ventilatory support (one CPAP, two high flow oxygen, two low

flow oxygen). The same percentage of infants required ventilatory support in the 72

hours following vaccination, with one infant who had been receiving low flow oxygen

prior to vaccination needing an increase to high flow oxygen (one CPAP, three high

flow oxygen, one low flow oxygen). There were no episodes of apnoea or bradycardia

reported before or after receipt of the third vaccines and one infant experienced a single desaturation following vaccination (table 7.4).

Table 7.4. Percentage of infants experiencing apnoea, bradycardia, and desaturation before and after vaccine administration at 16 weeks showing median and range of episodes.

	Infants experiencing apnoea % (n)	Median (range)	Infants experiencing bradycardia % (n)	Median (range)	Infants experiencing desaturations % (n)	Median (range)
Pre-vaccine (n=8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Post-vaccine (n=7)	0 (0)	0 (0)	0 (0)	0 (0)	14.3 (1)	1 (1-1)

Overall cardiorespiratory events

Overall, there was an increase in cardiorespiratory events in 36.4% (n=20) of infants after the first vaccines, 20% (n=4) after the second vaccines and 14.3% (n=1) after the third vaccines (table 7.5).

Table 7.5. Summary of cardiorespiratory events following vaccines at 8, 12 and 16 weeks.

	Increase in CREs % (n)	No change % (n)	Decrease in CREs % (n)
First vaccines (n= 55)	36.4 (20)	56.4 (31)	7.3 (4)
Second vaccines (n=20)	20 (4)	65 (13)	15 (3)
Third vaccines (n=7)	14.3 (1)	85.7 (6)	0 (0)

CREs= cardiorespiratory events

When an overall assessment of stability was made, incorporating both cardiorespiratory events and ventilatory support, 41.8% (n=23) of infants can be considered to have deteriorated following first vaccines, 45% (n=9) after second and 28.6% (n=2) after third (table 7.6). There was no statistically significant difference between groups when the percentage of infants experiencing deterioration following

the second vaccines, at which point only those in group two received 4CMenB, was compared.

Table 7.6. Overall clinical status following vaccines at 8, 12 and 16 weeks.

Table 10: Overall clinical status following vaccines at 8, 12 and 16 weeks.					
Overall clinical condition	First vaccines (n=55) % (n)	Second vaccines (n=20) % (n)			Third vaccines (n=7) % (n)
Deterioration	41.8 (23)			P value*	28.6 (2)
		Gp. 1 (n=11)	36.4 (4)	0.65	
		Gp. 2 (n=9)	55.6 (5)		
Stable	49.1 (27)	Gp. 1 (n=11)	63.6 (7)		71.4 (5)
		Gp. 2 (n=9)	33.3 (3)		
Improvement	9.1 (5)	Gp. 1 (n=11)	0 (0)		0 (0)
		Gp. 2 (n=9)	11.1 (1)		

*Fisher's exact test

The clinical course of infants vaccinated as inpatients is displayed in figure 7.6.

Chapter 7- Babies born Early Antibody Response to MenB vaccination: reactogenicity and safety

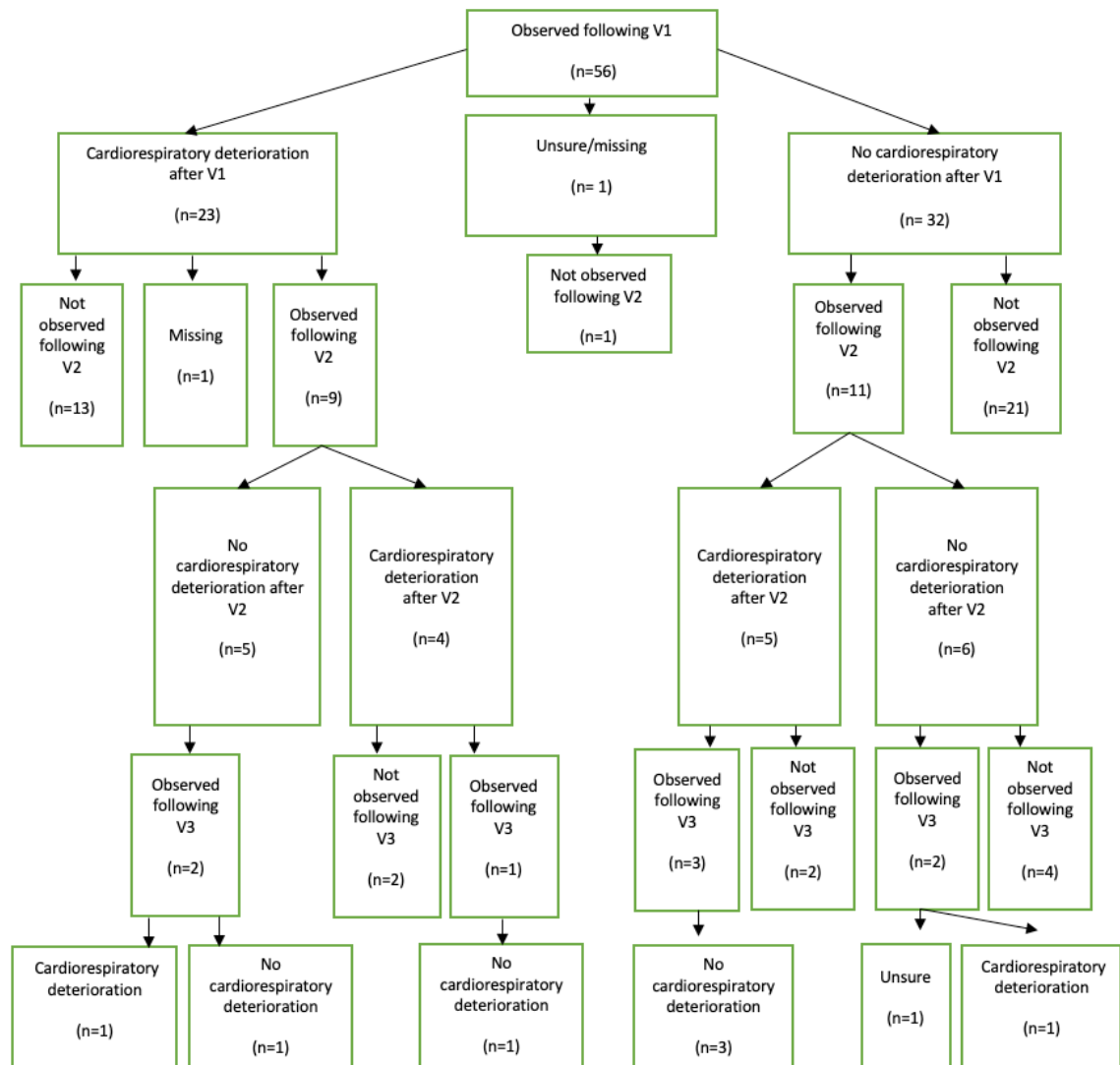


Figure 7.6. Clinical course of infants vaccinated as inpatients.

V1-V3= visit 1- visit 3

Overall, deterioration occurred in a similar percentage of infants following the first and second vaccines but was less frequent after the third (table 7.6). Of those infants who experienced deterioration following their first vaccines, nine were monitored following their second vaccines and of these, 44.4% (n=4) deteriorated. Monitoring took place for eleven infants who had not deteriorated after their first vaccines, of whom 45.5% (n=5) deteriorated.

Understanding which infants experience overall deterioration

Details of those infants who experienced overall deterioration and those who did not are summarised in table 7.7. Logistic regression was performed to identify whether gestational age, birthweight, CLD, paracetamol use, age at vaccination and, for the second and third vaccines, whether there had been any deterioration after first or second vaccines, could explain which infants experienced deterioration. No factors were identified as being significant in predicting those infants who would experience deterioration.

Table 7.7. Comparison of characteristics of infants who experienced deterioration or not following vaccination with results of logistic regression.

	Overall deterioration first vaccinations			Overall deterioration second vaccinations			Overall deterioration third vaccinations		
	Yes (n=23)	No (n=32)	p value*	Yes (n=9)	No (n=11)	p value*	Yes (n=2)	No (n=5)	p value*
Gestational age Median (range)	26 ⁺⁵ (25 ⁺⁰ - 30 ⁺⁶)	27 ⁺³ (23 ⁺⁰ - 32 ⁺³)	0.88	25 ⁺⁰ (23 ⁺⁰ - 28 ⁺⁶)	26 ⁺⁰ (25 ⁺⁰ - 30 ⁺⁶)	0.22	28 ⁺⁰ (25 ⁺¹ - 30 ⁺⁶)	25 ⁺³ (23 ⁺⁰ - 28 ⁺⁶)	0.29
Birthweight (g) Median (range)	839 (580- 1370)	967.5 (490- 1605)	0.30	660 (575- 955)	755 (490- 1170)	0.13	754 (753- 755)	660 (575- 780)	0.22
CLD % (n)	65.2 (15)	43.8 (14)	0.12	77.8 (7)	63.6 (7)	0.50	1 (50)	3 (60)	0.81
Paracetamol on day of vaccination % (n)	60.9 (14)	81.3 (26)	0.15	44.4 (4)	63.6 (7)	0.97	2 (100)	3 (60)	1.0
Age at vaccination Median (range)	61 (52- 77)	58 (52- 84)	0.37	91 (82- 110)	106 (82- 138)	0.05	136 (136- 136)	128 (110- 175)	1.0
Overall deterioration at V1 %(n)				44.4 (4)	55.6 (5)	0.96			
Overall deterioration at V2							0 (0)	80 (4)	1.0

%(n)									
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*Logistic regression

7.2.1.2 Local solicited adverse events

Local reactions were recorded according to the definitions in the methods chapter, [section 2.3.3](#). Local reactions were common following all vaccinations, with pain being the most commonly reported local reaction for each vaccine used (table 7.8-7.12; figure 7.7-7.10).

Severe reactions were unusual ($\leq 10\%$ of infants), apart from following 4CMenB vaccination at 12 months at which point 16% of infants were reported to have experienced severe pain. Following 4CMenB administration at four months, at which point those in group one had just received their second dose and those in group two had just received their third dose of 4CMenB, there were no differences identified between groups (any erythema $p=0.56$; any swelling $p=0.85$; any pain $p=0.32$; severe redness $p=1.0$; severe swelling $p=1.0$; severe pain $p=0.71$).

Overall, there was no consistent pattern in the reported local reactions according to gestational age category. The proportion of infants 30-34⁺⁶ WG experiencing swelling following the third dose of DTaP/IPV/Hib/HepB was higher than seen in infants less than 30 WG ($p=0.04$), and the proportion of infants 30-34⁺⁶ experiencing severe pain following PCV13 at 12 months of age was greater than seen in infants less than 30 WG ($p=0.02$).

4CMenB

Table 7.8. Proportion of infants experiencing local reactions following 4CMenB administration.

Age (months)	Group	N	Reaction	Any		Severe	
				n	Proportion (95% CI)	n	Proportion (95% CI)
2	Combined	109	Erythema	34	0.31 (0.23-0.41)	5	0.05 (0.02-0.11)
		111	Swelling	37	0.33 (0.25-0.43)	11	0.10 (0.05-0.17)
		115	Pain	59	0.51 (0.42-0.61)	7	0.06 (0.02-0.12)
3	Group 2	59	Erythema	19	0.32 (0.21-0.46)	2	0.03 (0.04-0.12)
		59	Swelling	17	0.29 (0.18-0.42)	5	0.08 (0.03-0.19)
		59	Pain	31	0.53 (0.40-0.66)	3	0.05 (0.01-0.14)
4	Combined	104	Erythema	50	0.48 (0.38-0.58)	1	0.01 (0.0002-0.05)
		107	Swelling	45	0.42 (0.33-0.52)	5	0.05 (0.02-0.11)
		105	Pain	61	0.58 (0.48-0.68)	7	0.07 (0.03-0.13)
12	Combined	95	Erythema	59	0.62 (0.52-0.72)	10	0.11 (0.05-0.19)
		94	Swelling	44	0.47 (0.36-0.57)	14	0.15 (0.08-0.24)
		96	Pain	64	0.67 (0.56-0.76)	15	0.16 (0.09-0.24)

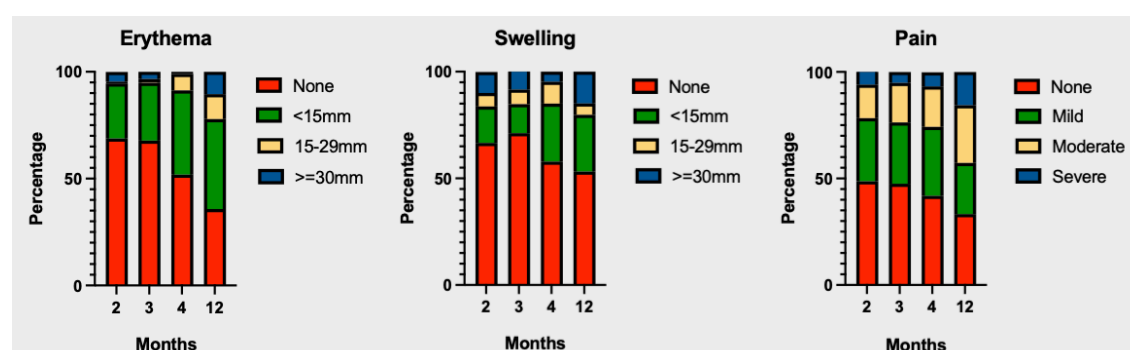


Figure 7.7. Classification of local reactions following 4CMenB administration at 2, 3, 4 and 12 months.

At three months, only infants in group two received 4CMenB vaccination.

PCV13

Table 7.9. Proportions of infants experiencing local reactions following PCV13 administration.

Age (months)	Group	N	Reaction	Any		Severe	
				n	Proportion (95% CI)	n	Proportion (95% CI)
2	Combined	108	Erythema	25	0.23 (0.16-0.32)	1	0.01 (0.0002-0.05)
		109	Swelling	13	0.12 (0.07-0.20)	1	0.01 (0.0002-0.05)
		110	Pain	41	0.37 (0.28-0.47)	5	0.05 (0.01-0.10)
4	Combined	94	Erythema	34	0.36 (0.27-0.47)	0	0 (0-0.04)
		95	Swelling	25	0.26 (0.18-0.36)	1	0.01 (0.003-0.06)
		93	Pain	43	0.46 (0.36-0.57)	3	0.03 (0.007-0.09)
12	Combined	92	Erythema	46	0.50 (0.39-0.61)	5	0.05 (0.02-0.12)
		92	Swelling	28	0.30 (0.21-0.41)	3	0.03 (0.007-0.09)
		94	Pain	55	0.59 (0.48-0.69)	7	0.07 (0.03-0.15)

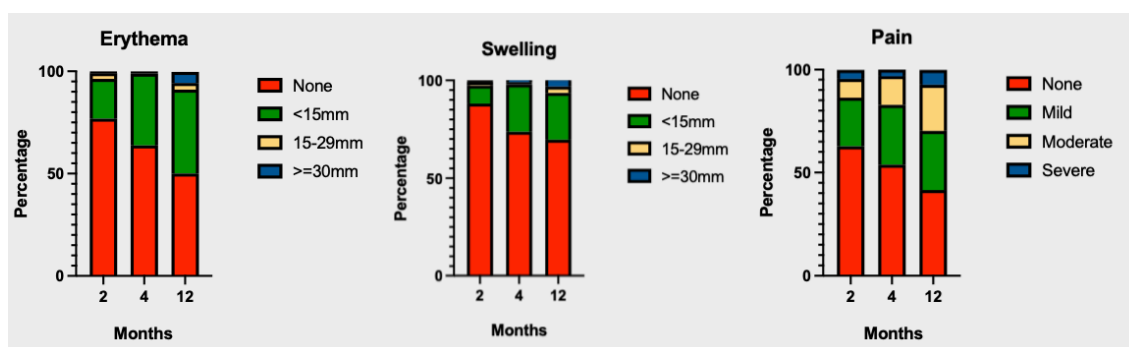


Figure 7.8. Classification of local reactions following PCV13 administration at 2, 3, 4 and 12 months.

DTaP/IPV/Hib/HepB

Table 7.10. Proportion of infants experiencing local reactions following DTaP/IPV/Hib/HepB vaccination.

Age (months)	Group	N	Reaction	Any		Severe	
				n	Proportion (95% CI)	n	Proportion (95% CI)
2	Combined	106	Erythema	26	0.25 (0.17-0.34)	1	0.009 (0.0002-0.05)
		106	Swelling	18	0.17 (0.10-0.26)	1	0.009 (0.0002-0.05)
		109	Pain	38	0.35 (0.26-0.45)	5	0.05 (0.02-0.10)
3	Combined	109	Erythema	26	0.24 (0.16-0.33)	2	0.02 (0.002-0.06)
		107	Swelling	27	0.25 (0.17-0.35)	6	0.06 (0.02-0.12)
		109	Pain	48	0.44 (0.35-0.54)	2	0.02 (0.002-0.06)
4	Combined	101	Erythema	45	0.45 (0.35-0.55)	1	0.01 (0.0003-0.05)
		103	Swelling	40	0.39 (0.29-0.49)	1	0.01 (0.002-0.05)
		104	Pain	54	0.52 (0.42-0.62)	5	0.05 (0.02-0.11)

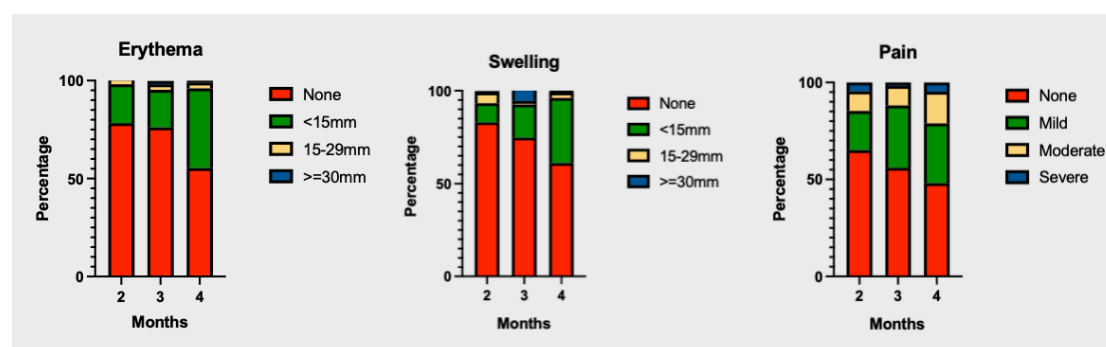


Figure 7.9. Classification of local reactions following DTaP/IPV/Hib/HepB administration at 2, 3, 4 and 12 months.

MMR

Table 7.11. Proportion of infants experiencing local reactions following MMR vaccination.

Age (months)	Group	N	Reaction	Any		Severe	
				n	Proportion (95% CI)	n	Proportion (95% CI)
12	Combined	93	Erythema	33	0.35 (0.26-0.46)	1	0.01 (0.0002-0.06)
		93	Swelling	25	0.27 (0.18-0.37)	1	0.01 (0.0003-0.06)
		92	Pain	47	0.51 (0.40-0.62)	6	0.07 (0.02-0.14)

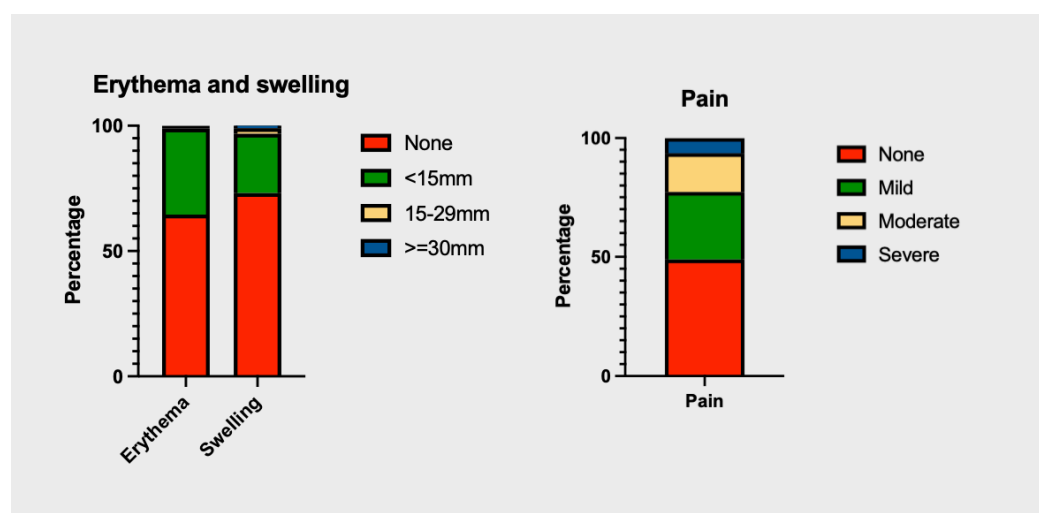


Figure 7.10. Classification of local reactions following MMR administration at 12 months.

Hib/MenC

Table 7.12. Proportion of infants experiencing local reactions following Hib/MenC vaccination.

Age (months)	Group	N	Reaction	Any		Severe	
				n	Proportion (95% CI)	n	Proportion (95% CI)
12	Combined	91	Erythema	27	0.30 (0.21-0.40)	1	0.01 (0.0003-0.06)
		92	Swelling	9	0.10 (0.05-0.18)	0	0 (0-0.04)
		94	Pain	41	0.44 (0.33-0.54)	5	0.05 (0.02-0.12)

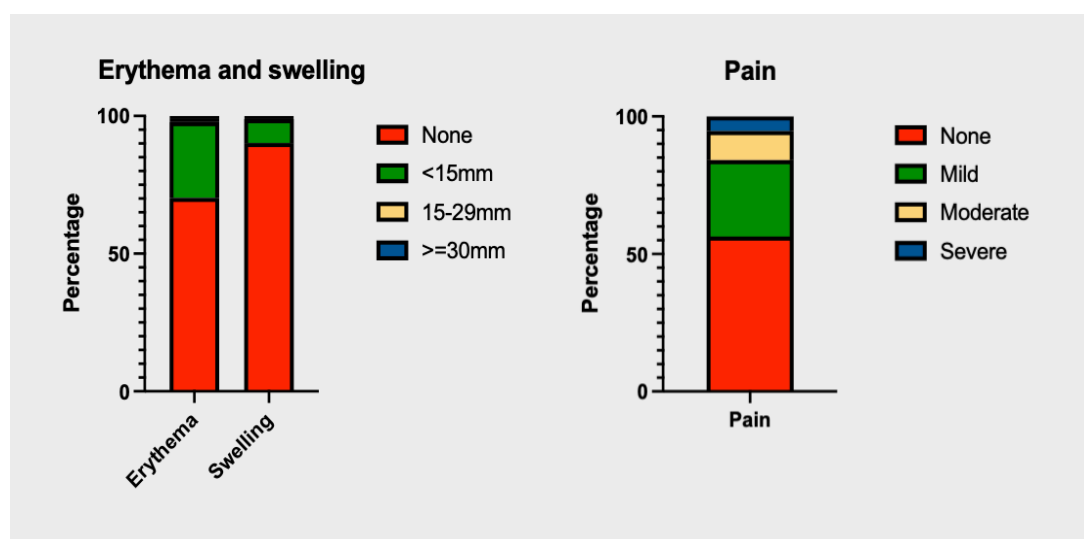


Figure 7.11. Classification of local reactions following vaccination with Hib/MenC vaccine.

7.2.1.3 Non-febrile solicited systemic adverse events

Non-febrile systemic reactions were common after receipt of all vaccines. The most commonly reported systemic reactions were irritability and reduced activity (table 7.13; figure 7.12).

Table 7.13. Proportion of infants experiencing systemic reactions occurring following vaccination at each time point.

Age (m)	n	Adverse event	Group	Any		Severe		P value	
				n	Proportion (95% CI)	n	Proportion (95% CI)	Any	Severe
2	107	Reduced feeding	All	56	0.52 (0.42-0.62)	4	0.04 (0.01-0.09)		
	108	Reduced activity		71	0.66 (0.56-0.75)	21	0.19 (0.12-0.28)		
	103	Irritability		64	0.62 (0.52-0.72)	4	0.04 (0.01-0.10)		
	104	Crying		48	0.46 (0.36-0.56)	3	0.03 (0.006-0.08)		
	102	Vomiting		45	0.44 (0.34-0.54)	0	0 (0-0.04)		
	103	Diarrhoea		36	0.35 (0.26-0.45)	1	0.01 (0.0002-0.05)		
3	106	Reduced feeding	Gp. 1 (n=51)	19	0.37 (0.24-0.52)	1	0.02 (0.0005-0.10)	0.12	0.48
			Gp. 2 (n=55)	29	0.53 (0.39-0.66)	0	0 (0-0.06)		
	107	Reduced activity	Gp. 1 (n=52)	24	0.46 (0.32-0.61)	1	0.02 (0.0005-0.10)	0.05	0.11
			Gp. 2 (n=55)	36	0.65 (0.51-0.78)	6	0.11 (0.04-0.22)		
	106	Irritability	Gp. 1 (n=51)	35	0.69 (0.54-0.81)	1	0.02 (0.0005-0.10)	1.0	0.37
			Gp. 2 (n=55)	38	0.69 (0.55-0.81)	4	0.07 (0.02-0.18)		
	106	Crying	Gp. 1 (n=51)	30	0.59 (0.44-0.72)	2	0.04 (0.005-0.13)	0.56	1.0
			Gp. 2 (n=55)	29	0.53 (0.39-0.66)	3	0.05 (-0.01-0.15)		
	104	Vomiting	Gp. 1 (n=50)	19	0.38 (0.25-0.53)	0		1.0	-
			Gp. 2 (n=54)	21	0.39 (0.26-0.53)	0			
	103	Diarrhoea	Gp. 1 (n=50)	20	0.40 (0.26-0.55)	2	0.04 (-0.005-0.14)	0.05	0.23

Chapter 7- Babies born Early Antibody Response to MenB vaccination: reactogenicity and safety

			Gp 2 (n=53)	11	0.21 (0.11-0.34)	0	0 (0-0.07)		
4	101	Reduced feeding	All	50	0.50 (0.39-0.60)	3	0.03 (0.006-0.08)		
	102	Reduced activity		65	0.64 (0.54-0.73)	6	0.06 (0.02-0.12)		
	102	Irritability		84	0.82 (0.74-0.89)	11	0.11 (0.06-0.18)		
	102	Crying		68	0.67 (0.57-0.76)	3	0.03 (0.006-0.08)		
	100	Vomiting		40	0.4 (0.30-0.50)	2	0.02 (0.002-0.07)		
	101	Diarrhoea		27	0.27 (0.18-0.36)	2	0.02 (0.002-0.07)		
12	96	Reduced feeding	All	50	0.52 (0.42-0.62)	1	0.01 (0.0003-0.06)		
	94	Reduced activity		50	0.53 (0.43-0.64)	5	0.05 (0.02-0.12)		
	96	Irritability		75	0.78 (0.69-0.86)	13	0.14 (0.07-0.22)		
	96	Crying		55	0.57 (0.47-0.67)	9	0.09 (0.04-0.17)		
	96	Vomiting		31	0.32 (0.23-0.43)	1	0.01 (0.0003-0.06)		
	97	Diarrhoea		29	0.30 (0.21-0.40)	2	0.02 (0.003-0.07)		

m= months

Chapter 7- Babies born Early Antibody Response to MenB vaccination: reactogenicity and safety

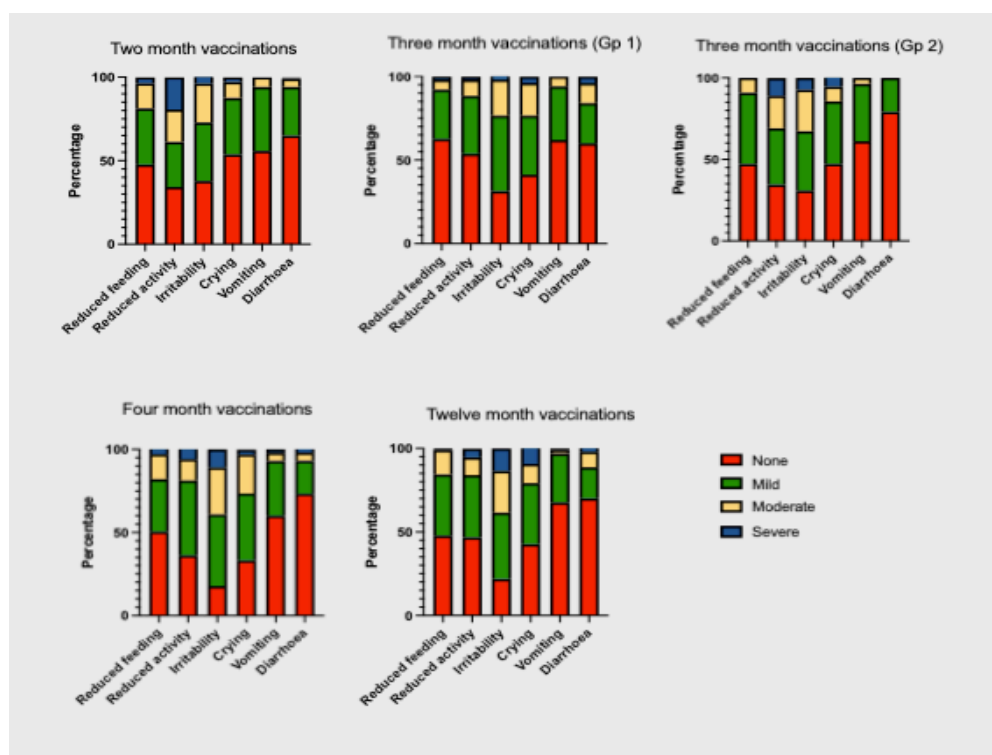


Figure 7.12. Classification of systemic reactions following vaccination.

Systemic reactions and gestational age

For several systemic reactions there was an increased odds ratio in those infants born at 30-34⁺⁶ WG compared to those born at less than 30 WG (table 7.14).

Table 7.14. Summary of the results for systemic reactions shown to have a significant relationship to gestational age (30-34⁺⁶ WG vs. <30+0 WG).

Age	Reaction	Odds Ratio (95% CI)	P value*
2 months	Reduced feeding	4.24 (1.87-9.58)	0.001
	Irritability	4.23 (1.8-9.9)	0.001
	Crying	3.59 (1.57-8.21)	0.002
3 months	Irritability	2.61 (1.12-6.05)	0.03
	Crying	2.82 (1.27-6.27)	0.01
4 months	Activity	2.57 (1.12-5.88)	0.03
	Reduced feeding	3.75 (1.62-8.69)	0.002
	Irritability	4.68 (1.52-14.4)	0.007
	Crying	3.38 (1.43-7.97)	0.005
	Diarrhoea	3.32 (1.20-9.15)	0.021
12 months	Reduced feeding	2.53 (1.10-5.80)	0.03
	Activity	2.55 (1.10-5.90)	0.03

*Logistic regression

7.2.1.3 Fever following vaccination

Within 7 days

There was an increase in frequency of fever reported with each subsequent vaccination (table 7.15, figure 7.13). At three months, when those in group two received an additional dose of 4CMenB, there were significantly more episodes of fever reported in group two than group one ($p=0.02$) although no difference in the number of infants experiencing a grade three fever of $\geq 39.0^{\circ}\text{C}$ ($p=1.0$). There was no difference seen between groups at the time of the four-month vaccines.

Table 7.15. Proportion of infants with fever up to seven days following vaccination.

Age (m)	Group	n	Within 7 days		Severe within 7 days (≥grade 3)		P value*	
			n	Proportion (95% CI)	n	Proportion (95% CI)	Any	Severe
2	All	122	7	0.06 (0.03-0.12)	1	0.008 (0.0002-0.04)		
3	Group 1	58	1	0.02(0.0004-0.09)	0	0 (0-0.06)	0.02	1.0
	Group 2	64	9	0.14 (0.07-0.25)	1	0.02 (-0.0004-0.08)		
4	Group 1	55	11	0.2 (0.10-0.33)	0		0.46	-
	Group 2	57	8	0.14 (0.06-0.26)	0			
12	All	102	27	0.26 (0.19-0.36)	7	0.07 (0.03-0.14)		

m= months. Greater than grade 3 fever defined as a temperature $\geq 39.0^{\circ}\text{C}$

*Fisher's exact test

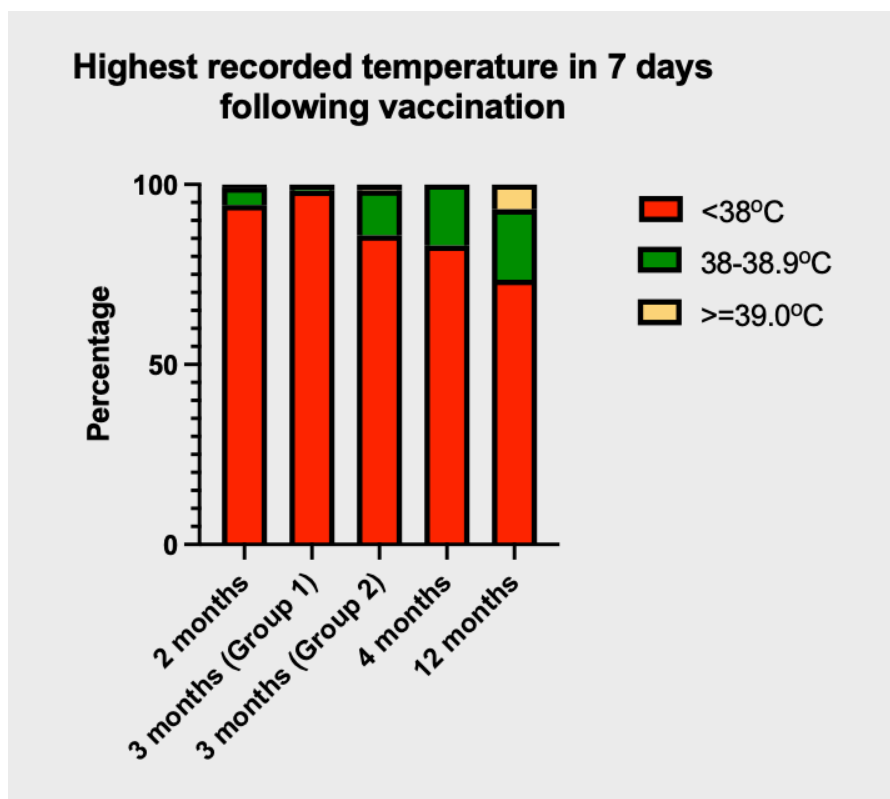


Figure 7.13. Highest recorded temperature in 7 days following vaccination.

7.2.1.4 Antipyretic use

The majority of infants received at least one dose of paracetamol on the day of vaccine administration if this included 4CMenB, but fewer infants were recorded as receiving three doses within 24 hours (table 7.16). The proportion of infants receiving paracetamol remained high following the booster vaccines at twelve months, and prophylaxis remained the predominant stated reason for paracetamol administration (prophylactic 76.7% (n=46), to treat fever 18.3% (n=11), both to prevent and treat 3.3% (n=2), to treat an unrelated symptom 1.7% (n=1)).

Only a small number of infants received any doses of ibuprofen: one infant in group one at three months, one at four months and seven at 12 months.

Table 7.16. Paracetamol received following vaccination.

		Received at least one dose in 24 hours			Received three doses within 24 hours		
Age (m)		n	Yes % (n)	No % (n)	n	Yes % (n)	No % (n)
2		114	91.2 (104)	8.8 (10)	97	58.8 (57)	41.2 (40)
3	Gp 1	54	64.8 (35)	35.2 (19)	45	31.1 (14)	68.9 (31)
3	Gp 2	59	94.9 (56)	5.1 (3)	50	66 (33)	34 (17)
4		99	96 (95)	4.0 (4)	85	71.8 (61)	28.2 (24)
12		91	94.5 (86)	5.5 (5)	75	54.7 (41)	45.3 (34)

At visit two infants in group two were significantly more likely to receive paracetamol on the day of vaccination ($p < 0.001$) and significantly more likely to receive three doses of paracetamol within 24 hours of vaccination ($p = 0.001$). There were no differences between groups at any of the other time points.

Logistic regression showed that neither gestational age category ($p = 0.33$), nor whether the vaccination visit took place in the neonatal unit ($p = 0.07$) were associated with having received three doses of paracetamol following primary vaccinations.

7.2.1.5 Investigation for sepsis

The number of infants being investigated for sepsis was highest following administration of the first vaccines, with six infants being investigated for sepsis within the first seven days following vaccination and a further six infants investigated in the following 21 days after vaccination. Following the second vaccines at three months, there was no difference in investigations for sepsis between those infants in group two who received 4CMenB and those in group one who did not (table 7.17).

Table 7.17. Percentage of participants investigated for sepsis within 7 and 28 days.

Age (m)	N	Investigated for sepsis within 7 days n (%)	P value*	Investigated for sepsis within 28 days n (%)	P value*
2	128	6 (4.7)	-	6 (4.7)	
3	Gp. 1	2 (3.2)	1.0	1 (1.6)	0.50
	Gp. 2	2 (3.1)		0 (0)	
4	128	0 (0)	-	1 (0.78)	
12	126	0 (0)	-	0 (0)	

m= months. *Fisher's exact test

7.2.2 Safety

7.2.2.1 Serious adverse events (SAEs)

There were three sudden unexpected serious adverse reactions (SUSARS) reported during the trial involving two participants, one from each study group. One SUSAR consisted of increasing episodes of desaturation, bradycardia and apnoea on the day following vaccination and the other two were episodes of apnoea after vaccination at two and four months. Although the summary of product characteristics (SmPC) includes apnoea as a possible adverse event following vaccination, this is only referred to in the context of infants born at less than 28 WG and it does not refer to an increase in desaturations and bradycardia. Both episodes of apnoea were in the same participant born at 32⁺⁵ WG and this was therefore considered to be unexpected and recorded as a SUSAR. The other participant experienced episodes of desaturation, bradycardia and apnoea soon after vaccination therefore despite being born at 26⁺¹ WG this was considered to be unexpected and recorded as a SUSAR.

Three babies died during the study (two from group one, one from group two), none of these deaths were considered to be related to vaccination. The first baby was born at 29⁺² WG and was admitted to hospital nine days after receiving his first vaccines with apnoeic episodes and was later diagnosed with RSV bronchiolitis; the second baby was

born at 24⁺⁵ WG and was admitted to hospital four months after completing her primary vaccinations with increased work of breathing and was later diagnosed with rhinovirus bronchiolitis; the third baby was born at 25⁺³ WG and experienced a sudden, unexpected collapse in hospital six weeks after receiving her first vaccines.

There were 100 SAEs reported as part of the study, one of these was reported in a baby who was withdrawn from the study prior to receiving their first vaccines and has therefore not been included. The large number of reported SAEs reflect the vulnerability of this preterm population, and, of note, the majority of the SAEs (n=72) occurred in those babies born at less than 30 WG. Six SAEs were determined to be definitely or probably related to vaccination (2 definitely: both elective admissions for monitoring following previous cardiorespiratory instability after vaccination; 4 probably: 3 apnoea, 1 fever). A summary of the recorded SAEs is shown in table 7.18.

Table 7.18. Recorded SAEs according to group.

Group 1	N= 48	Group 2	N= 51
Inguinal hernia	1	Infantile spasms	1
Coryzal, febrile and poor feeding	1	Inguinal hernia repair	3
Bronchiolitis	14	GORD	2
Respiratory distress	4	Apnoea	3
RTI	2	Respiratory distress	6
Cow's milk protein intolerance	1	Groin swelling	1
Anaemia	1	Cough	2
Vomiting and static growth	1	Gastroenteritis	2
UTI	2	Respiratory tract infection	1
Croup and bronchiolitis	1	PDA ligation	2
Refractory respiratory failure requiring ECMO	1	Bronchiolitis	5
Chronic lung disease and pulmonary hypertension	1	Tracheostomy formation	1
Increased work of breathing	2	Diarrhoea	1
Choking episode	1	Respiratory failure	1
Viral infection	1	Progression of ROP	1
Vomiting	1	Increased oxygen requirements	2
Sudden unexpected collapse	1	Constipation	1

Elective admission for vaccination following previous instability	1	Elective admission for vaccination following previous instability	1
Apnoea	5	Stridor	1
Increased work of breathing and groin swelling	1	Rash	1
Stridor	1	Increased work of breathing	2
Progression of ROP	1	Sleepy	1
Constipation	1	Right groin swelling and distress	1
Worsening post haemorrhagic hydrocephalus	1	Non-blanching rash	1
Vaccination reaction causing fever	1	Poor feeding	1
		Cardiac failure	2
		Hypertension	1
		Neuromuscular weakness	1
		Altered mental state secondary to hyponatraemia	1
		Intestinal prolapse through ileostomy	1
		Fever	1

GORD= gastro-oesophageal reflux disease; ROP= retinopathy of prematurity; ECMO= extracorporeal membrane oxygenation; RTI= respiratory tract infection; UTI= urinary tract infection; PDA= patent ductus arteriosus

7.3 Discussion

The population recruited for the BEAR Men B study included infants from a wide range of gestational ages with half born at less than 30 WG. The broad eligibility criteria mean that this is likely to be a representative sample of UK preterm infants.

Cardiorespiratory events

Cardiorespiratory events including apnoea, bradycardia and desaturation were common, particularly in the period following vaccination, although there was not a statistically significant difference between the periods before and after vaccination. As discussed in chapter four, there is significant variation in the reported rates of cardiorespiratory events in different studies due to differences in the included populations and the definitions used for the events. The percentage of infants experiencing apnoea, bradycardia and desaturation in our population was within the

large spectrum seen in other studies. To compare overall deterioration after vaccination, cardiorespiratory events and ventilatory requirements were combined to make a composite term for comparison. Using this, 41.8% and 45% of infants deteriorated after their first and second vaccines respectively, although this used a very conservative marker of deterioration.

Although 4CMenB is known to be a reactogenic vaccine, there was no statistically significant difference in cardiorespiratory events at three months between those who received an additional dose (group two) and those who did not (group one). The number of infants being monitored decreased at each vaccination time point, making it hard to draw firm conclusions about recurrence of cardiorespiratory events, but logistic regression did not identify deterioration at V1 or V2 to predict deterioration at V2 or V3 respectively. This is in contrast with the results reported by Flatz-Jequier who found that the only infants who experienced cardiorespiratory events following their second vaccines had experienced cardiorespiratory events following their first vaccines (187). This may be explained by the broader criteria used to define deterioration in the BEAR Men B trial. The number of infants who experienced deterioration following their second vaccines in this clinical trial were higher than that reported by some investigators (179, 188), but considerably lower than that reported by others (180). The current guidance suggests that monitoring should take place in hospital for the second vaccines if an infant experienced apnoea, bradycardia or desaturations following the first vaccines. Although our finding that deterioration following first vaccines did not predict deterioration after the second vaccines might challenge this recommendation, this may be because of the very broad criteria used to define

deterioration in BEAR Men B and a narrower definition for deterioration may allow this to be more predictive of future events.

Local reactions

Local reactions were frequently reported after all vaccine doses, with pain being the most common local reaction following each of the vaccines administered. This finding is in keeping with studies investigating term infants using both a 2+1 and 3+1 schedule (242, 249-252, 259). Pain was particularly common following the twelve-month booster dose, a finding which has also been described (250). 4CMenB is a reactogenic vaccine and some studies of term infants have reported higher rates of local reactions following 4CMenB compared with other vaccines (43, 203, 252). In the BEAR Men B cohort, local swelling was more common after the first 4CMenB vaccine compared with other vaccines given simultaneously, and following the booster there was more local erythema and swelling following 4CMenB than following MMR and Hib/MenC and more pain than was seen following Hib/MenC. When comparing the rates of local reactions with those seen in other published studies involving preterm infants there were lower rates of grade 3 local reactions for DTaP/IPV/Hib/HepB than had previously been reported (134), slightly lower rates of erythema, swelling and pain following PCV13 (152, 155) compared to some studies using a 7-valent and 13-valent pneumococcal vaccine, but slightly higher rates compared to those seen following a 10-valent pneumococcal vaccine (159).

Systemic reactions

Systemic reactions were common following vaccination. Irritability and reduced activity were the most commonly reported non-febrile systemic reactions reported following each vaccination. Irritability has been reported in some studies of term

infants as the most common systemic reaction (43, 249). Because these studies, along with this clinical trial, were not blinded, there is a considerable risk of observer bias, and it may be that the more subjective systemic reactions such as irritability and reduced activity are over-reported by caregivers who are aware that their infant has received vaccination.

Fever is well known as an adverse event following vaccination and this is particularly associated with 4CMenB administration. This can be of concern to parents, particularly in younger infants (254). The rate of fever within seven days following vaccination in this trial increased across subsequent vaccinations. 4CMenB is known as a particular cause of fever and it is therefore not surprising that at twelve weeks when the two study groups differed in whether they received 4CMenB or not, there was more fever reported in those who received the additional dose. There was not an increase in the rate of severe fever, nor was there any difference between groups in the incidence of fever at the subsequent vaccine dose at four months which is reassuring.

Since the introduction of 4CMenB to the routine schedule in the UK it has been recommended that three doses of paracetamol are given within the first 24 hours after vaccination for those doses given as part of the primary series (at two and four months). This is in contrast to the guidance prior to the introduction of 4CMenB to give paracetamol only for symptom relief, and there were concerns from some neonatologists that routine administration of paracetamol was unnecessary and potentially harmful, and some neonatal units initially decided not to give prophylactic paracetamol (202). In a UK survey of neonatal units which took place in 2015-2017, 81% of infants were found to have received at least one dose of prophylactic paracetamol with 68% having received all three doses (191). In this clinical trial where

paracetamol use was recorded, between 91.2 and 96% of infants were recorded as having received at least one dose of paracetamol on the day of 4CMenB vaccination given in the primary series and between 58.8 and 71.8% were reported to have received all three recommended doses. Interestingly the rates of prophylactic paracetamol administration remained high at 12 months when routine administration for prophylaxis is no longer recommended. A previous study showed that prophylactic paracetamol is associated with a significant reduction in primary antibody responses for pneumococcal vaccine serotypes, protein D, anti-polyribosylribitol phosphate, anti-diphtheria, anti-tetanus and anti-pertactin, with this also being seen after the booster dose for anti-tetanus, protein D and pneumococcal serotypes (260), however a follow up study suggested that use of prophylactic paracetamol did not affect immunological memory or nasopharyngeal carriage at four years (261). A subsequent study by the same group, which included 4CMenB, showed that whilst there was an impact of prophylactic paracetamol on anti-pneumococcal GMC responses, this was much less than seen in the previous trial and protective thresholds were reached for all pneumococcal serotypes irrespective of whether prophylactic paracetamol had been given (252). Overall, although prophylactic paracetamol is not recommended following the 12-month vaccines, its frequent use is unlikely to have any adverse impact.

Investigation for sepsis was most common following the first vaccines at two months, which may reflect both increased health care worker concern early in an infant's life and the increased caution recommended in guidelines for the management of fever in younger infants less than three months of age (262). Dubus reported a similar percentage of infants being investigated in the 48 hours after first or second vaccines

including 4CMenB vaccine when this was given with paracetamol prophylaxis (202).

Kent reported a slightly higher rate of 9% of infants having a blood culture collected in the 48 hours following vaccination including 4CMenB at eight weeks of age, although of note the percentage of infants who had received prophylactic paracetamol was slightly lower in this cohort (191). Mukherjee did not report on evaluations for sepsis but found that 33% of infants received antibiotics in the 48 hours following vaccine administration including 4CMenB (194), which suggests that the percentage of infants being investigated for sepsis would also be elevated, despite all the infants receiving prophylactic paracetamol. This study was small and reported on a period soon after the introduction of 4CMenB when there was less experience of the clinical effects of 4CMenB vaccination in preterm infants. At three months when only infants in group two received a dose of 4CMenB, with a statistically significant increase in fever seen in this group, there was no difference between groups in investigations for sepsis which again suggest a greater confidence from clinicians about the likely side effects of 4CMenB vaccination.

There were many SAEs reported in the BEAR Men B study, the majority of which were reported in infants born at less than 30 WG. Many of these were because of hospital readmission in the context of infection. Hospital readmission is common in preterm infants and this risk increases with decreasing gestation (263). The number of SAEs was higher than that reported in some older papers (91, 128), one of which included infants 24-36 WG and the other which included infants 27-36⁺⁶ WG, but similar to that seen in a more recent study (153). As survival of infants born at the earliest gestations increases, it may be that there is an increase in the number of clinically fragile infants

in the preterm population which may explain this increase in SAEs in the later study and in our population.

Limitations

There were several limitations in the investigation of the reactogenicity outcomes. The first of these is the subjective nature of the evaluations, both from the caregivers completing the diary cards and the nursing staff assessing the infants vaccinated as inpatients. A perception that vaccines are associated with adverse events may have led to over reporting of these events, particularly those with no objective component, for example local pain at vaccination site and systemic adverse events. Although a definition was provided for the cardiorespiratory events following vaccination, these were recorded by the research team based on the observations of the nursing staff according to whatever monitoring was used within that neonatal unit with no standardisation of this. There was also an unequal period of observation before and after vaccination which makes it hard to draw conclusions about any comparison of the periods before and after vaccination. Whilst an increase in events following vaccination may result from vaccination, in an unstable infant it may equally result from more events being detected as a product of a longer period of observation after vaccination (72 hours) compared with before (24 hours). Finally, no details were collected about how long any increase in cardiorespiratory events or ventilatory support lasted or what, if any, implications there were for the infant.

7.3 Conclusions

Some degree of cardiorespiratory deterioration after administration of the first and second vaccines was described in over 40% of infants vaccinated as inpatients, although this used very conservative criteria. Local and systemic reactions were

common following vaccination in this population, although rarely severe and in keeping with previous studies of term and preterm infants. Fever was less commonly reported than has previously been seen in preterm populations following 4CMenB vaccination and, importantly, although receiving an additional dose of 4CMenB at three months of age was associated with a higher rate of fever at this time point, it was not associated with any increase in investigations for sepsis. Overall, 4CMenB was well tolerated in this preterm population when given according to either a 2+1 or 3+1 schedule.

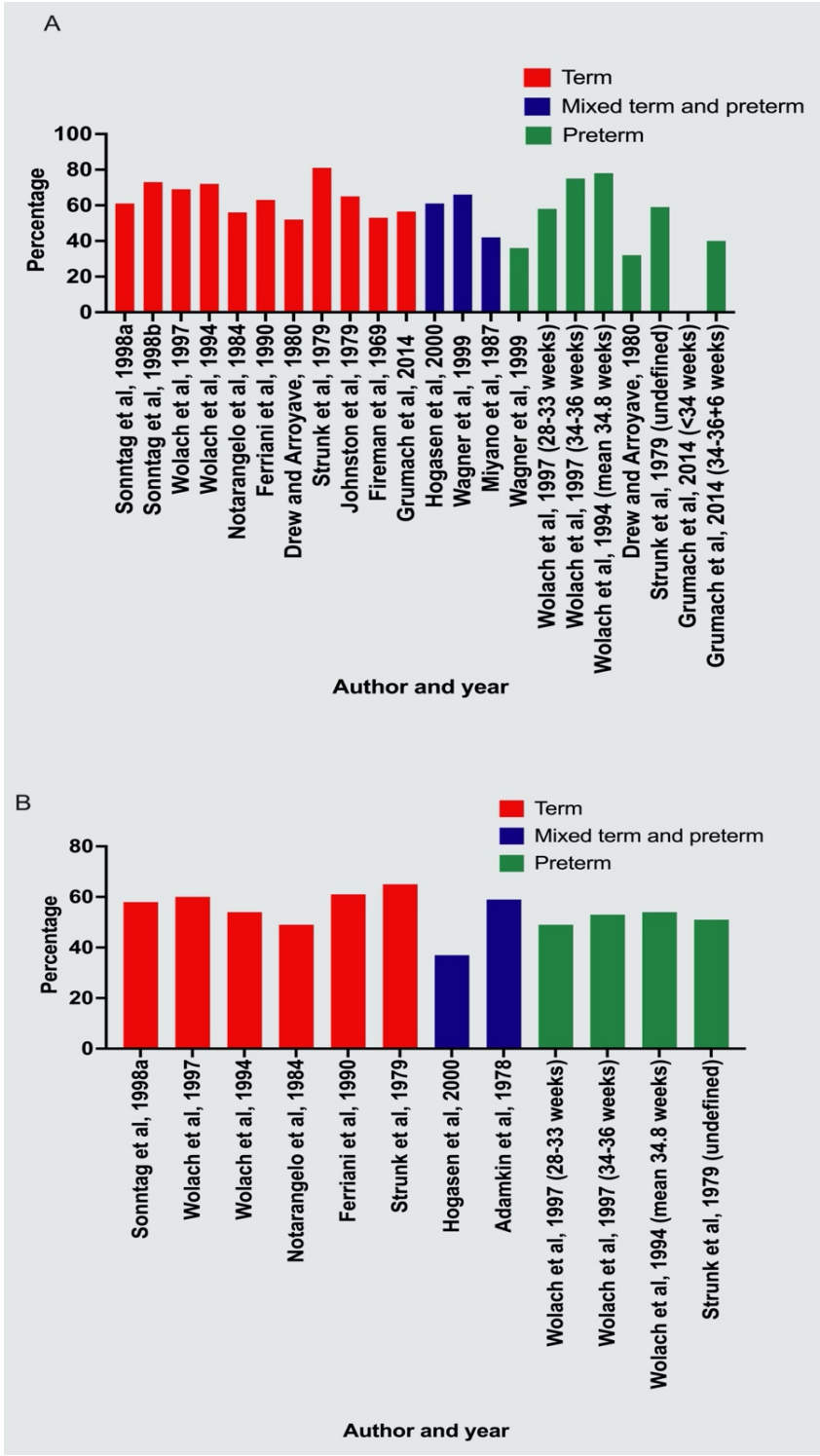
8 Sources of complement in serum bactericidal antibody assays

8.1 Background

Invasive Meningococcal Disease (IMD) is relatively rare, which makes efficacy and effectiveness studies of meningococcal vaccines challenging. Immunity to *N.*

meningitidis is primarily through the activity of complement and anti-meningococcal antibody, and serum bactericidal antibody (SBA) assays are the gold standard to assess meningococcal immunity. As discussed in chapter 1 ([section 1.4](#)), the source of complement used in the SBA assays is important. In assays assessing immunity against group B meningococcus for example, human complement must be used, as rabbit complement gives unacceptably high bactericidal titres (102, 103). This is primarily because, unlike human complement, rabbit complement does not bind to the factor H (fH) binding protein therefore downregulation of complement activation and the resulting reduction in bactericidal activity is not seen when rabbit complement is used (264). The human complement used in the SBA assays is from adults, with the donated serum or plasma either screened for low killing of the target strain or IgG and IgM-depleted to eliminate meningococcal-specific antibodies, whilst retaining complement activity (112).

There are marked differences in the complement profiles of adults and neonates. As discussed in [section 1.3.2](#), generally, both the total functional capacity of complement, measured by the CH50 assay, and the activity of the alternative pathway, measured by the AP50 assay is reduced in infants compared to adults and in most studies, this is further reduced in preterm infants (figure 8.1a and b).



SBA assays allow bactericidal killing mediated by a particular serum sample to be quantified. This functional test requires both heat-inactivated serum and a source of complement; the bactericidal antibody present in the serum and the complement work together to achieve bactericidal killing. When using adult complement to assess immunity in neonates it is possible that the estimation of bactericidal killing is overestimated.

The COMPAre (Sources of **C**omplement in **M**eningococcal and **P**ertussis serum bactericidal antibody **A**ssays) study was designed to collect cord blood samples from term and preterm neonates so that pooled complement sources could be produced, and a comparison could be made of SBA titres using adult, term neonatal and preterm neonatal complement sources. Neonatal complement is hard to obtain therefore using adult complement is preferable, providing that the assessment of immunity in assays using adult and neonatal complement are comparable.

8.1.1 Objectives

1. To investigate if the SBA assay results for group B meningococcus obtained from SBA assays using complement from a pooled adult complement source are comparable to those using a neonatal complement source.

8.1.2 Methods

A full description of the methods can be found in [section 2.4](#).

8.2 Results

8.2.1 Recruitment

We recruited 47 participants to the study. The recruitment was significantly disrupted by the Covid-19 pandemic. Although the planned number of participants were

recruited, the cumulative period for recruitment was reduced making it impossible to recruit the number of preterm deliveries which had been planned. Twelve babies were recruited who were born at less than 37 WG, of whom only one was born at less than 32 WG (figure 8.2).

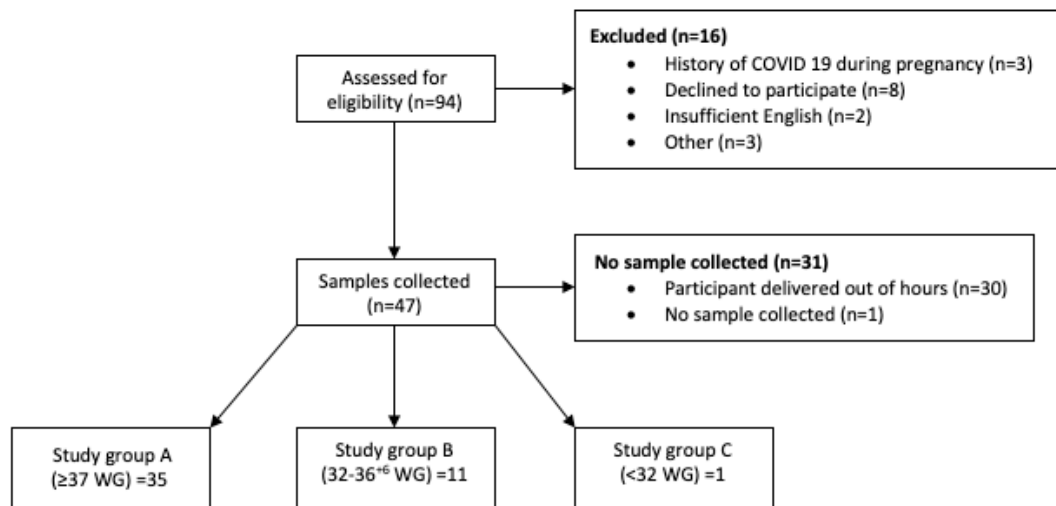


Figure 8.2. CONSORT flow diagram for the COMPARE study.

8.2.2 Demographics

Information about the participants is shown in table 8.1. The majority of infants were born by caesarean section (C/S).

Table 8.1. Participant demographics.

	Group A (≥37 ⁺⁰ WG) (n= 35)	Group B (32 ⁺⁰ -36 ⁺⁶ WG) (n= 11)	Group C (<32 ⁺⁰ WG) (n=1)
Gestational age median (range)	39 ⁺¹ (41 ⁺⁵ -38 ⁺⁰)	36 ⁺⁰ (33-36 ⁺⁶)	30 ⁺⁰
Caesarean Section % (n)	60 (21)	100 (11)	100 (1)
White ethnicity % (n)	57.1 (20)	72.7 (8)	100 (1)

WG= weeks of gestation

8.2.3 Antibiotic use

Overall, 70.2% of participants were recorded as having received at least one dose of antibiotics in the seven days prior to delivery which reflects the large number of

women recruited who delivered by C/S. All the infants born preterm were delivered by C/S which means that all of the preterm infants had been exposed to antibiotics around the time of delivery (table 8.2).

Table 8.2. Percentage of infants receiving antibiotics according to study group and mode of delivery.

Antibiotics prescribed	Group A ($\geq 37^{+0}$ WG) (n= 35)	Group B (32^{+0} - 36^{+6} WG) (n= 11)	Group C ($< 32^{+0}$ WG) (n=1)
Caesarean section % (n)	100 (21)	100 (11)	100 (1)
Vaginal delivery % (n)	0 (0)	-	-

WG= weeks of gestation

8.2.4 Laboratory analysis

8.2.4.1 Investigation of the complement components to be used in the assay

Radial immunodiffusion assays (RIDS) were performed on the individual samples prior to antibody depletion and pooling. Four infants had no CH50 activity detectable- three term and one preterm infant. Two of these had insufficient plasma remaining to be included in the pooled complement source and the other two were excluded. The absolute values and the values as a percentage of an adult reference value along with published term and preterm results presented as a percentage of adult reference values are shown in table 8.3.

Table 8.3. Results from RIDS showing absolute values and values as a percentage of the adult value, along with published term and preterm results presented as a percentage of adult values.

Test	Value		Normal adult value	% of normal value		Percentage of adult values in published literature Range (mean) (267)	
Overall	T	PT		T	PT	T	PT
CH50	370.2 U/mL	260.6 U/mL	728 U/mL ^a	50.9	35.8	52.0-81 (63.7)	0-78 (46.0)
AP50	76.3%	51.9%	97.5% ^d	78.3	53.2	49-65 (57.8)	49-54 (52)
Classical							
C3	836.4	604.4	1211.5 mg/L ^a	69.0	49.9	50-97 (72.9)	39-60 (49.5)

C1q	46.8	34.7	153 mg/L ^a	30.6	22.7	53-73 (64.8)	27-37 (33.5)
C2	15.8	15.4	20 mg/L ^d	79.0	77.0	62-79 (73.5)	69*
C4	157.3	133.0	274.5 mg/mL ^a	57.3	48.5	54-80 (66.3)	42-59 (50.5)
Alt							
C3	836.4	604.4	1211.5 mg/mL ^a	69.0	49.9	50-97 (72.9)	39-60 (49.5)
Factor B	98.4	66.0	298 ^a	33.0	22.1 4	36-73 (53.9)	36-53 (45.2)
Terminal							
C5	117.7	101.1	136.0 mg/mL ^a	86.5	74.3	67-79 (73.1)	60-71 (67)
C6	35.1	21.0	60 mg/L ^d	58.5	35.0	44-58 (51)	35-39 (36.7)
C7	78.1	65.9	65 mg/L ^d	120. 2	101. 3	67-120 (97)	73-75 (74.3)
C8	42.1	29.5	160 mg/L ^d	26.3	18.4	36-38 (37.3)	29 (29)
C9	49.1	33.9	150 mg/L ^d	32.7	22.6	14-55 (34.3)	47.3 (33-56)
Reg							
C4BP	49.3	16.0	365.5 µg/mL ^e	13.5	4.4	<15	None rep
C1ina	137.3	130.8	261 mg/mL ^a	52.6	50.1	None rep	None rep
Factor H	325.1	280.4	515.5 µg/mL ^e	63.1	54.4	56-68 (61.3)	None rep
Factor I	16.9	13.6	69.5 µg/mL ^e	24.3	19.6	39-58 (49.8)	None rep

Alt= alternative pathway; reg= regulators; T= term; PT= preterm; None rep= none reported; RIDS= radial immunodiffusion assay; ^aMedian value from 'The Binding Site'; ^b mean value from 'The Binding Site'; ^c mid range value from 'The Binding Site'; ^d mid range value from Protein Reference Unit; ^e Mid range values from (266)

*single value

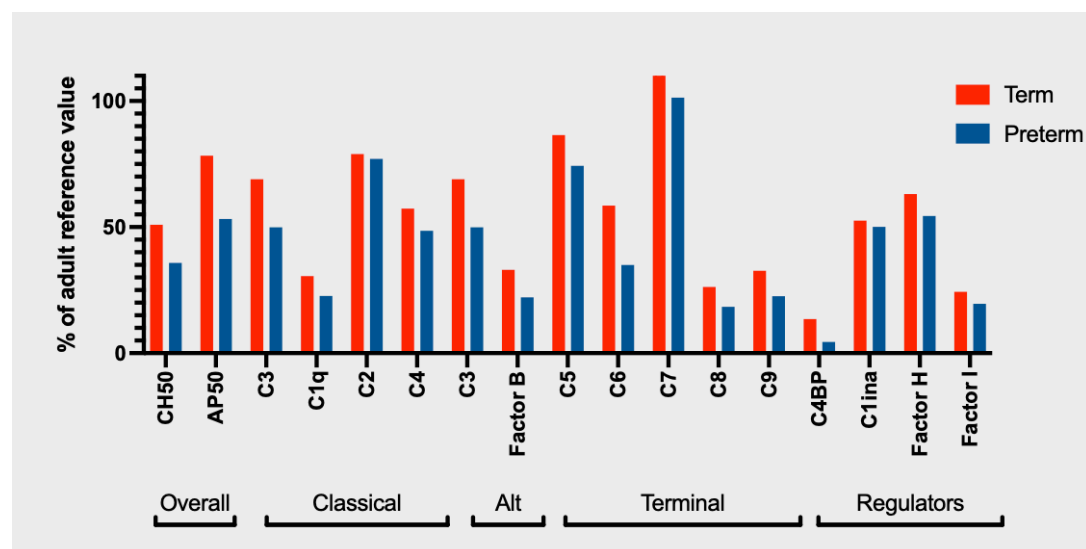


Figure 8.3. Complement components in term and preterm samples presented as a percentage of the adult reference value.

Alt= alternative pathway; CH50= Total haemolytic complement; AP50= alternative complement pathway

8.2.4.2 Investigation of the killing properties of the complement

SBA assays using serial complement dilutions

When SBA assays were performed using serial dilutions of complement, titres were unassignable using neonatal complement for the higher concentrations of 25% and 12.5% for *N. meningitidis* strain 5/99 (NadA), but for concentrations of 6.25% and 3.125% the titres assigned were 1:128 which is the same as that assigned using adult complement (figure 8.4).

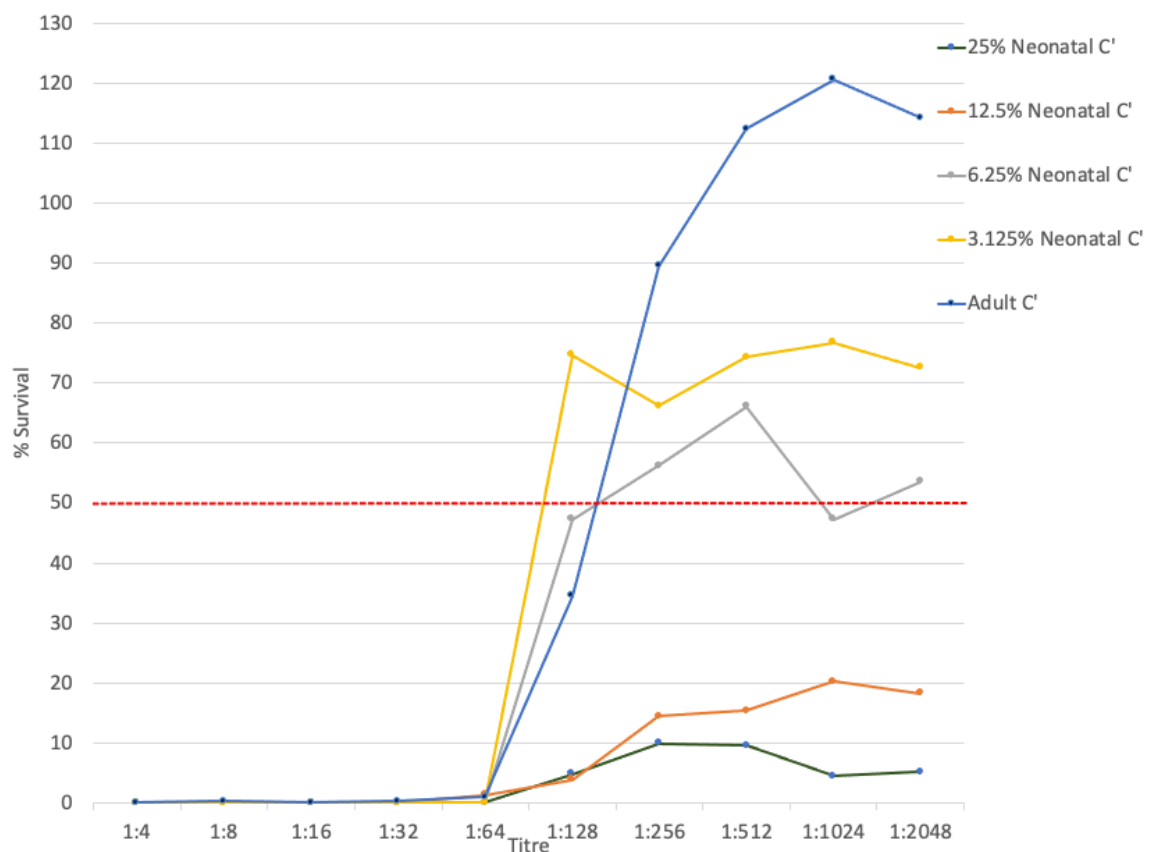


Figure 8.4. SBA curves for SBA assays performed using serial dilutions of term neonatal and adult complement and strain 5-99 (NadA).

When SBA assays were performed using neonatal complement and a dilution of 25% and 12.5%, the bacterial survival was too low to allow a titre to be assigned. The SBA titres using neonatal complement at 6.25% and 3.125% were comparable to those seen when using adult complement.

C'=complement; NadA= Neisserial adhesin A; SBA= serum bactericidal antibody.

Graph produced by Mrs Lauren Allen.

For *N. meningitidis* strain NZ98/254 (PorA) there was no assignable titre when 25% neonatal complement was used, titres were lower with subsequent dilutions, but were never as low as that seen for adult complement even at the lowest concentration of 3.25% (figure 8.5).

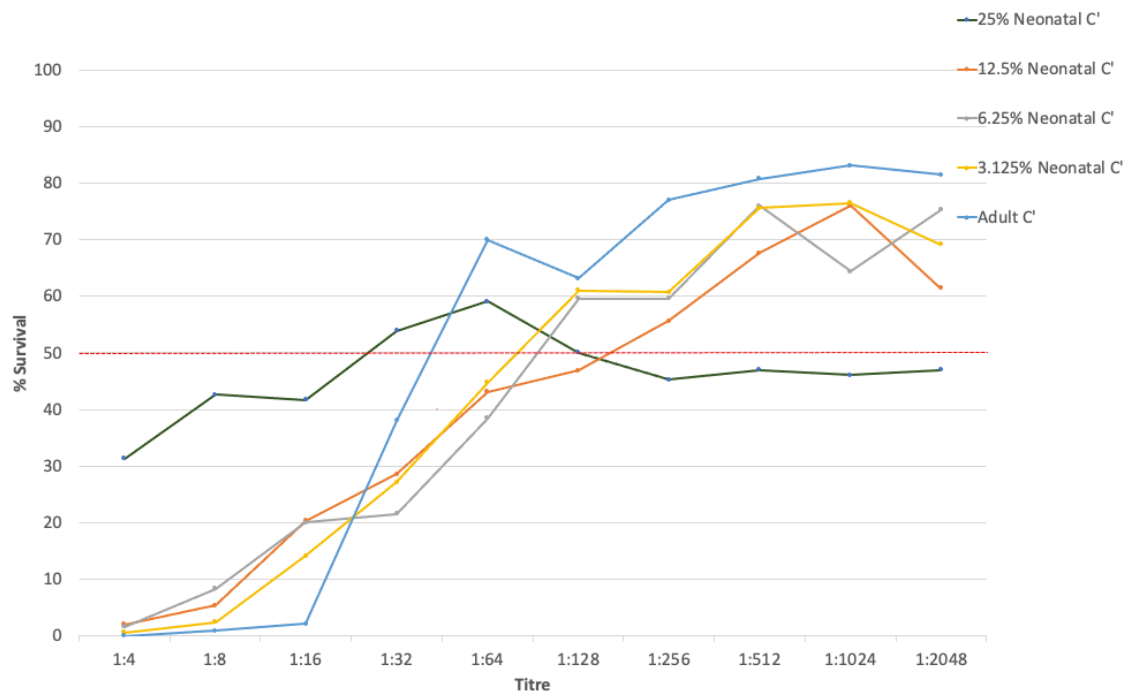


Figure 8.5. SBA results for SBA assays performed using serial dilutions of term neonatal complement and adult complement and strain NZ98/254 (PorA).

When SBA assays were performed using neonatal complement and a dilution of 25% there was no assignable titre. The titres were lower with subsequent dilutions, but never as low as seen with the adult complement. C'=complement; PorA= Porin A; SBA= serum bactericidal antibody.

Graph produced by Mrs Lauren Allen.

For *N. meningitidis* strain 44/76-SL (fHbp), there was no assignable titre with 25%

neonatal complement, but for all the other concentrations the titre assigned was the

same as that seen when using adult complement (figure 8.6).

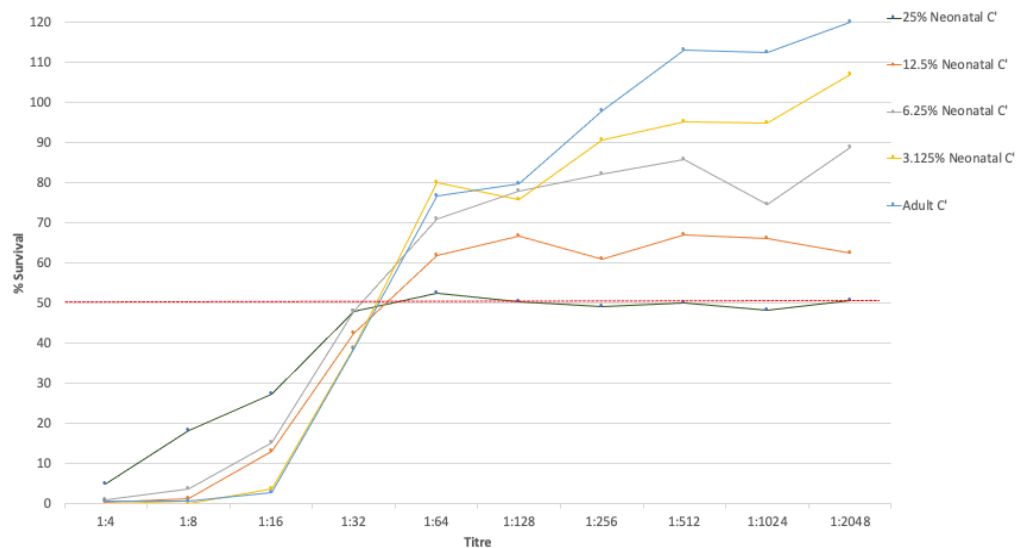


Figure 8.6. SBA results for SBA assays performed using serial dilutions of term neonatal complement and adult complement and *N. meningitidis* strain 44/76-SL (fHbp).

For *N. meningitidis* strain 44/76-SL (fHbp), there was no assignable titre for 25% neonatal complement, but for all the other concentrations of neonatal complement the titre assigned was the same as that seen when using adult complement. C'=complement; fHbp= factor H binding protein; SBA= serum bactericidal antibody. Graph produced by Mrs Lauren Allen.

Disc diffusion assays

Because greater killing was observed with neonatal complement, disc diffusion assays were employed to determine if complement alone would inhibit growth of *N. meningitidis*. The control was a disc infused with ampicillin which showed a clear ring of bacterial inhibition surrounding it (image 8.1).



Image 8.1. Ampicillin infused disc on meningococcal lawn.

Area of inhibition of bacterial growth on the meningococcal lawn surrounding the ampicillin infused disc

***N. meningitidis* 5/99 (NadA)**

Disc diffusion assays using strain 5/99 (NadA) showed an area of bacterial inhibition around the disc infused with neonatal complement from infants born by vaginal delivery. This was not seen surrounding the disc infused with complement from the same pooled complement which had been incubated with β -lactamase but was seen surrounding the disc infused with the same complement incubated with PBS (image 8.2).

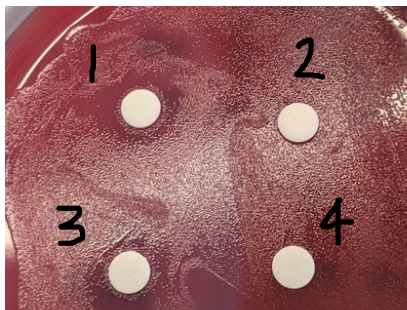
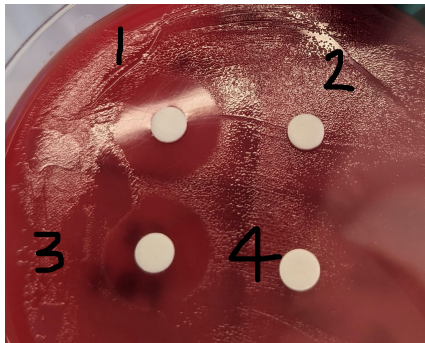


Image 8.2. Disc diffusion assay using *N. meningitidis* strain 5/99 (NadA) and neonatal complement from infants in the vaginal delivery group (no antibiotics reported).

1-neat neonatal complement, 2- neat adult complement, 3- neonatal complement pre-incubated with PBS, 4- neonatal complement pre-incubated with β -lactamase. There was an area of inhibition of bacterial growth around disc 1 (neonatal complement) which is not seen around disc 2 (adult complement) which suggests the presence of an inhibitory substance in the neonatal complement. This area of inhibition remained surrounding disc 3 (neonatal complement and PBS), but not surrounding disc 4 (neonatal complement and β -lactamase) which suggests that the effect of the inhibitory substance is reversed through the activity of β -lactamase.

There was a large area of bacterial inhibition surrounding the discs pre-incubated with complement derived from term and preterm infants born by caesarean section. This area of inhibition was not seen around the discs pre-incubated with these pooled complements and β -lactamase but was seen around the disc pre-incubated with these pooled complements and PBS (image 8.3).

A: Term



B: Preterm

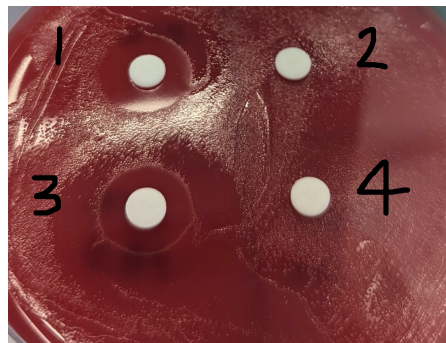


Image 8.3. a and b: Disc diffusion assay using *N. meningitidis* strain 5/99 (NadA) and neonatal complement from infants in term (A) and preterm (B) infants delivered by caesarean section (antibiotics reported).

1- neat neonatal complement, 2- neat adult complement, 3- neonatal complement pre-incubated with PBS, 4- neonatal complement pre-incubated with β -lactamase. There was a large area of inhibition of bacterial growth around disc 1 (neonatal complement) which is not seen around disc 2 (adult complement) which suggests the presence of an inhibitory substance in the neonatal complement. This was consistent with the known administration of antibiotics prior to delivery. This area of inhibition remained surrounding disc 3 (neonatal complement and PBS), but not surrounding disc 4 (neonatal complement and β -lactamase) which suggests that this effect is reversed through the action of β -lactamase.

***N. meningitidis* 44/76-SL (fHbp)**

There was no bacterial inhibition around the disc pre-incubated with complement derived from neonates born by vaginal delivery on a lawn of *N. meningitidis* strain 44/76-SL (fHbp) (image 8.4).

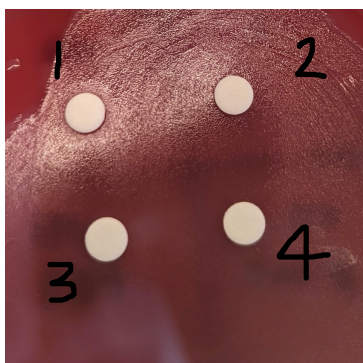


Image 8.4. Disc diffusion assay using *N. meningitidis* strain 44/76-SL (fHbp) and neonatal complement from infants delivered by vaginal delivery (no antibiotics reported).

1- neat neonatal complement, 2- neat adult complement, 3- neonatal complement pre-incubated with PBS, 4- neonatal complement pre-incubated with β -lactamase. There were no areas of inhibition around any of the discs in this assay which suggests the absence of any factors inhibiting bacterial growth.

***N. meningitidis* strain NZ98/254 (PorA)**

There was a ring of bacterial inhibition around the disc pre-incubated with neonatal complement derived from infants born by vaginal delivery and a smaller ring of inhibition around the disc pre-incubated with neonatal complement and PBS. This was not seen around the disc pre-incubated with adult complement or the disc pre-incubated with neonatal complement and β -lactamase (image 8.5).

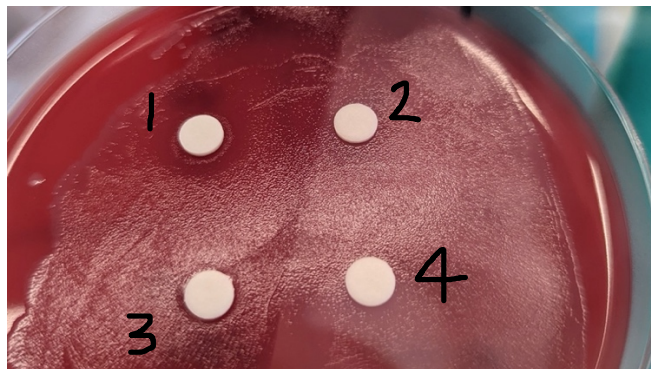


Image 8.5. Disc diffusion assay using *N. meningitidis* strain NZ98/254 (PorA) and neonatal complement from infants delivered by vaginal delivery (no antibiotics reported).

1- neat neonatal complement, 2- neat adult complement, 3- neonatal complement pre-incubated with PBS, 4- neonatal complement pre-incubated with β -lactamase. There is an area of inhibition of bacterial growth around disc 1 (neonatal complement) which is not seen around disc 2 (adult complement) which suggests the presence of an inhibitory substance in the neonatal complement. This area of inhibition remains, although much smaller, surrounding disc 3 (neonatal complement and PBS), but not surrounding disc 4 (neonatal complement and β -lactamase) which suggests that this effect is reversed through the action of β -lactamase.

SBA assay using *N. meningitidis* strain 5/99 (NadA) with β -lactamase.

When an SBA assay was performed using neat complement, complement with PBS and complement with β -lactamase, the survival curves were similar for adult complement (figure 8.7), but for neonatal complement, the survival was considerably higher for the complement which had been pre-incubated with β -lactamase (figure 8.8) and resulted in similar curves for the SBA assays using adult and neonatal complement.

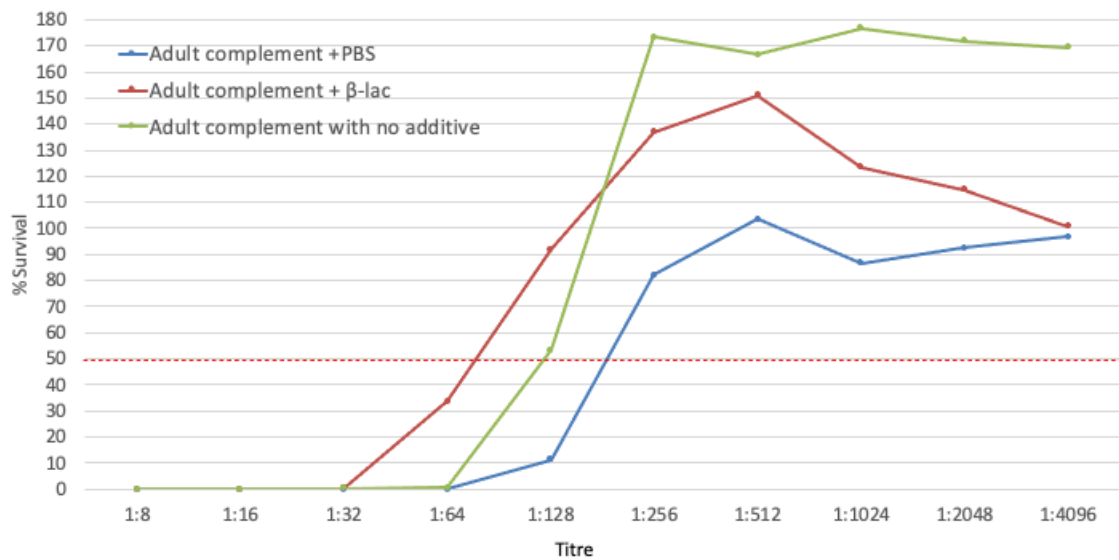


Figure 8.7. SBA assay results using adult complement neat, with added PBS or added β-lactamase and strain 5-99 (NadA).

The survival curves were similar for neat complement and complement with PBS and β-lactamase. PBS= phosphate buffered saline; β-lac= β-lactamase; NadA= neisserial adhesin A. Graph produced by Mrs Lauren Allen.



Figure 8.8. SBA assay results using neonatal complement neat, with added PBS or added β-lactamase and strain 5-99 (NadA).

Bacterial survival was markedly improved in the SBA assay using complement with added β-lactamase compared with that using complement alone or complement pre-incubated with PBS. PBS= phosphate buffered saline; β-lac= β-lactamase; NadA= neisserial adhesin A; SBA= serum bactericidal antibody. Graph produced by Mrs Lauren Allen.

8.2.4.3 Strain selection

In the SBA assays using neonatal complement and strain 5/99 (NadA) or NZ98/254 (PorA), the bacterial survival was markedly inhibited in comparison with that seen in the SBA assays using adult complement, suggesting that something other than complement-mediated bactericidal antibody activity was responsible (tables 8.4-8.7). This was much less marked when using 44/76-SL (table 8.8-8.9).

***N. meningitidis* strain 5/99 (NadA)**

Table 8.4. SBA assay results using strain 5/99 (NadA) and adult serum with adult and neonatal complement.

Serum dilution	Colony count					
	Adult serum					
	Adult complement			Neonatal complement		
1: 2	0	0	0	0	0	0
1: 4	0	0	0	0	0	0
1: 8	0	0	0	0	0	0
1: 16	0	0	0	0	0	0
1: 32	0	0	0	0	0	0
1: 64	0	1	0	0	0	0
1: 128	45	62	7	7	4	0
1: 256	162	178	155	17	8	19
1: 512	208	200	160	23	17	18
1: 1024	159	213	234	23	24	17
C' only	161	209	212	43	35	35
Bac+ HI C' only	169	130	148	47	48	50
T0	181	203	182	150	147	162
T0/2	90.5	101.5	91	75	73.5	81
Titre	1:128	1:128	1:128	>1:1024	>1:1024	>1:1024

Table 8.5. SBA assay results using strain 5/99 (NadA) and baby serum with adult and neonatal complement.

Serum dilution	Colony count					
	Baby serum					
	Adult complement			Neonatal complement		
1: 2	0	0	0	0	0	0
1: 4	0	0	0	0	2	0
1: 8	0	0	0	0	0	0
1: 16	0	0	0	0	0	2
1: 32	1	0	1	0	0	2
1: 64	0	0	0	0	2	0
1: 128	63	46	30	3	6	6
1: 256	231	149	152	13	16	12
1: 512	180	163	155	16	19	18
1: 1024	207	168	178	33	30	27
C' only	208	188	206	46	58	40
Bac+ HI C' only	196	165	164	44	59	27
T0	172	177	168	136	173	159
T0/2	86	88.5	84	68	86.5	79.5
Titre	1:128	1:128	1:128	>1:1024	>1:1024	>1:1024

N. meningitidis* strain NZ98/254 (PorA)*Table 8.6. SBA assay results using strain NZ98/254 (PorA) and adult serum with adult and neonatal complement.**

Serum dilution	Colony count					
	Adult serum					
	Adult complement			Neonatal complement		
1: 2	0	0	0	0	1	0
1: 4	0	0	0	0	0	0
1: 8	0	0	0	0	0	1
1: 16	0	0	0	9	5	5
1: 32	0	0	0	49	8	24
1: 64	42	45	14	75	18	44
1: 128	222	190	154	103	52	71
1: 256	288	192	209	91	90	88
1: 512	248	226	220	95	80	104
1: 1024	282	209	242	107	78	115
C' only	221	188	239	108	108	130
Bac+ HI C' only	228	200	232	113	127	19
T0	191	186	221	248	237	238
T0/2	95.5	93	110.5	124	118.5	119
Titre	1:64	1:64	1:64	>1:1024	>1:1024	>1:1024

Table 8.7. SBA assay results using strain NZ98/254 (PorA) and baby serum with adult and neonatal complement.

Serum dilution	Colony count					
	Baby serum					
	Adult complement			Neonatal complement		
1: 2	0	0	0	0	0	0
1: 4	0	0	0	0	0	0
1: 8	0	0	0	0	0	0
1: 16	0	0	0	1	0	0
1: 32	12	21	8	66	33	30
1: 64	139	146	84	126	102	97
1: 128		238	241	85	79	104
1: 256	255	233	255	102	91	101
1: 512	251	239	249	84	100	114
1: 1024	245	251	251	94	87	113
C' only	225	265	235	123	121	139
Bac+ HI C' only	192	231	219	134	110	158
T0	265	272	233	208	249	163
T0/2	132.5	136	116.5	104	124.5	81.5
Titre	1:32	1:32	1:64	1:32	NA	1:32

N. meningitidis* strain 44/76-SL (fHbp)*Table 8.8. SBA assay results using strain 44/76-SL and adult serum with adult and neonatal complement.**

Serum dilution	Colony count					
	Adult serum					
	Adult complement			Neonatal complement		
1: 2	0	0	2	0	1	0
1: 4	0	0	4	0	1	2
1: 8	5	0	2	3	4	5
1: 16	6	4	9	20	19	28
1: 32	29	60	87	71	56	95
1: 64	91	184	180	139	128	113
1: 128	216	284	306	152	153	145
1: 256	321	328	379	155	148	175
1: 512	337	365	407	151	151	166
1: 1024	341	337	402	167	231	197
C' only	377	341	343	280	258	222
Bac+ HI C' only	342	317	265	326	310	293
T0	368	306	285	317	301	335
T0/2	184	153	142.5	158.5	150.5	167.5
Titre	1:64	1:32	1:32	1:512	1:64	1:128

Table 8.9. SBA assay results using strain 44/76-SL and baby serum with adult and neonatal complement.

Serum dilution	Colony count					
	Baby serum					
	Adult complement			Neonatal complement		
1: 2	0	0	0	1	1	3
1: 4	2	1	0	2	3	6
1: 8	0	4	0	23	14	30
1: 16	7	0	7	76	63	78
1: 32	103	74	110	125	141	113
1: 64	200	264	194	174	139	182
1: 128	317	276	399	163	200	196
1: 256	378	406	441	184	231	179
1: 512	383	396	424	175	174	220
1: 1024	397	352	416	209	247	237
C' only	336	326	323	241	270	230
Bac+ HI C' only	314	304	325	247	281	266
T0	358	321	336	345	308	289
T0/2	179	160.5	168	172.5	154	144.5
Titre	1:32	1:32	1:32	1:32	1:64	1:32

8.2.4.4 SBA assays using *N. meningitidis* strain 44/76-SL

Because strain 44/76-SL (fHbp) appeared less affected by the neonatal complement, further SBA assays were performed with this strain only.

Of the 14 SBA assays which were performed, using 13 neonatal and one adult serum sample, 78.6% (11/14) had titres from the SBA assays performed with neonatal and adult complement within one dilution of each other and 92.9% (13/14) had titres within two dilutions of each other (table 8.10, figures for SBA assays for each of the tested sera in [appendix J](#)).

Table 8.10. Results from the SBA assays using adult and neonatal complement and a set of preterm infant sera and one adult serum samples.

	Adult complement	Neonatal complement	Within 1 dilution	Within 2 dilutions
Adult serum (PIP)	1: 32	1: 64	Yes	Yes
Neonatal serum 029B	<1:4	<1:4	Yes	Yes
Neonatal serum 049B	<1:8	<1:8	Yes	Yes
Neonatal serum 530B	<1:4	<1:4	Yes	Yes
Neonatal serum 541C	1:16	1:32	Yes	Yes
Neonatal serum 083A	<1:8	<1:8	Yes	Yes
Neonatal serum 066A	1:16	1:64	No	Yes
Neonatal serum 502A	1:16	<1:16	Yes	Yes
Neonatal serum 532A	1:8	<1:8	Yes	Yes
Neonatal serum 083C	1:64	1:512	No	No
Neonatal serum 527A	1:16	<1:8	Yes	Yes
Neonatal serum 530A	<1:16	<1:16	Yes	Yes
Neonatal serum 049C	1:16	1:16	Yes	Yes
Neonatal serum 599A	1:32	1:128	No	Yes

8.2.4.5 Antibody dependent complement deposition (ADCD) assays

***N. meningitidis* strain 5/99 (NadA)**

There were no significant differences seen in the C3b or C5b9 deposition between adult, term neonatal or preterm neonatal complement for strain 5/99 (figure 8.9).

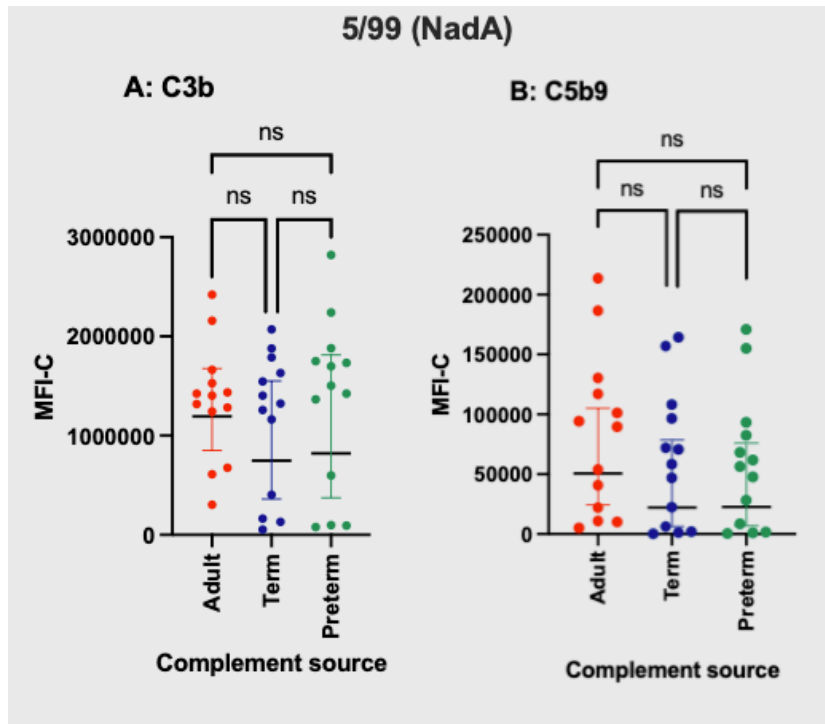


Figure 8.9. GMTs and 95% confidence intervals of MFI-C for C3b(A) and C5b9(B) comparing adult, term neonatal and preterm neonatal complement using strain 5/99.

There were no significant differences in the MFI-C between complement sources for either C3b(A) or C5b9(B) deposition. ns= not significant; MFI-C= MFI-C mean fluorescence index-control; NadA= neisserial adhesin A

There was a significant difference between the MFI-C values of the low and high SBA responders for C3b ($p \leq 0.01$) and between the low and high ($p \leq 0.0001$) and mid and high responders ($p \leq 0.01$) for C5b9 (figure 8.10).

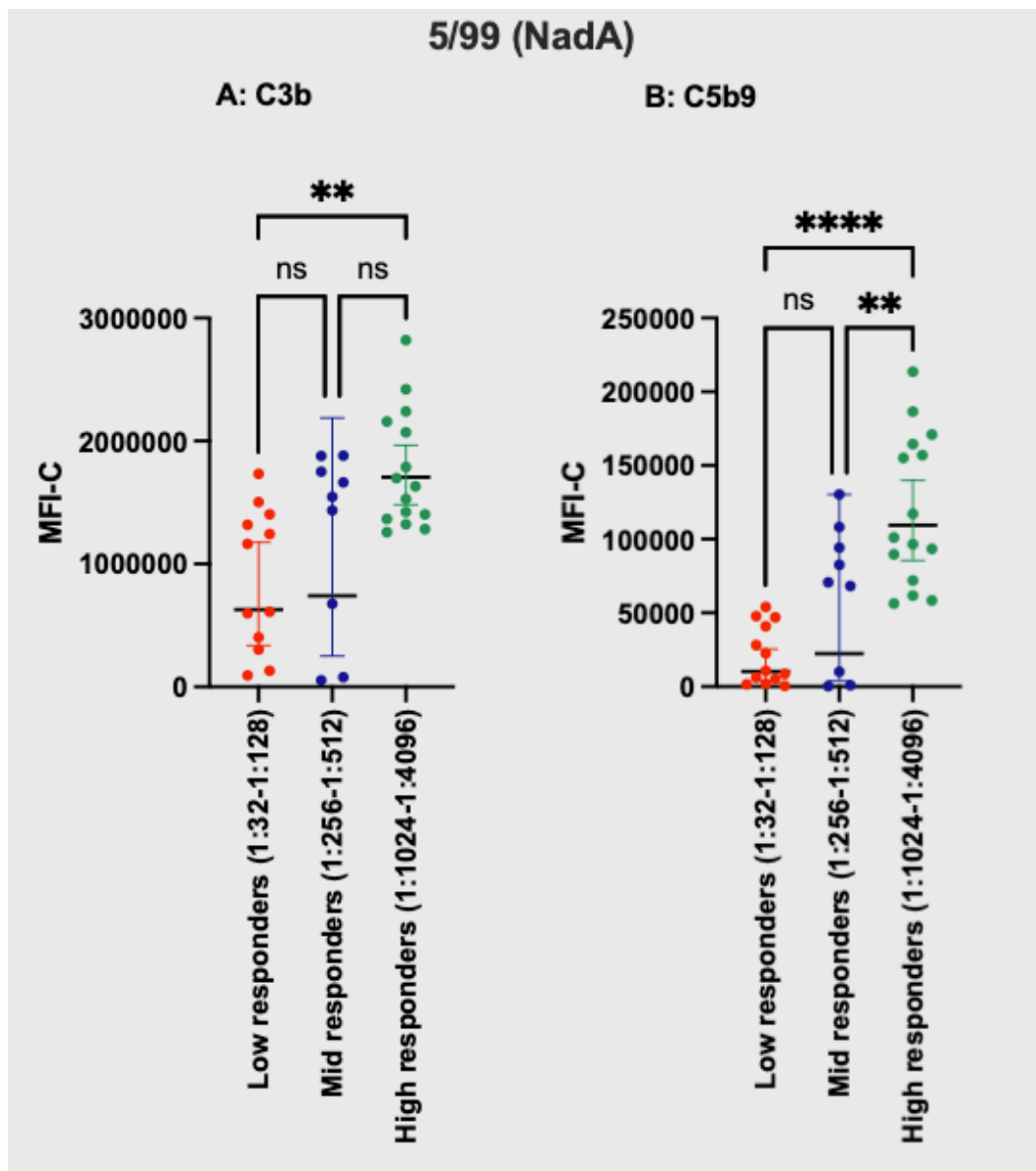


Figure 8.10. GMTs and 95% confidence intervals of MFI-C for C3b(A) and C5b9(B) comparing samples known to have low, mid and high SBA titres.

A: The MFI-C values were significantly higher for the high SBA responders compared with the low SBA responders ($p \leq 0.01$) for C3b. There were no significant differences between the low and mid or the mid and high responders. B: The MFI-C was significantly higher for the high SBA responders compared with the low SBA responders ($p \leq 0.0001$) for C5b9. The MFI-C was significantly higher for high compared with mid SBA responders ($p < 0.01$). ** ≤ 0.01 ; **** ≤ 0.0001 .

ns= not significant; MFI-C= MFI-C mean fluorescence index-control; NadA= neisserial adhesin A

***N. meningitidis* strain 44/76-SL (fHbp)**

There were no significant differences seen in the C3b or C5b9 deposition between adult, term neonatal and preterm neonatal complement (figure 8.11).

There were no significant differences in the MFI-C between complement sources for either C3b(A) or C5b9(B) deposition

There was a significant difference for the MFI-C values for C3b between the low and mid ($p \leq 0.0001$) and low and high SBA responders ($p \leq 0.0001$), but no significant difference between the mid and high SBA responders. For C5b9 there was a significant difference between the low and high ($p \leq 0.0001$), the mid and high ($p \leq 0.001$) and the low and mid ($p \leq 0.0001$) (figure 8.12).

A: The MFI-C values were significantly higher for the high SBA responders compared with the low SBA responders ($p \leq 0.0001$) for C3b. There was no significant difference between the mid and high SBA responders, but the mid SBA responders had significantly higher MFI-C values compared with low SBA responders ($p \leq 0.0001$). B: The MFI-C was significantly higher for the high SBA responders compared with the low SBA responders ($p \leq 0.0001$) and for the high compared to the mid ($p \leq 0.001$) and for the mid compared with the low ($p \leq 0.0001$). *** $P \leq 0.001$; **** ≤ 0.0001 ; ns= not significant
MFI-C= MFI-C mean fluorescence index-control; fHbp= factor H binding protein; GMT= geometric mean titre

***N. meningitidis* strain NZ98/264 (PorA)**

There were no significant differences seen in the C3b or C5b9 deposition between adult, term neonatal and preterm neonatal complement (figure 8.13 a and b).

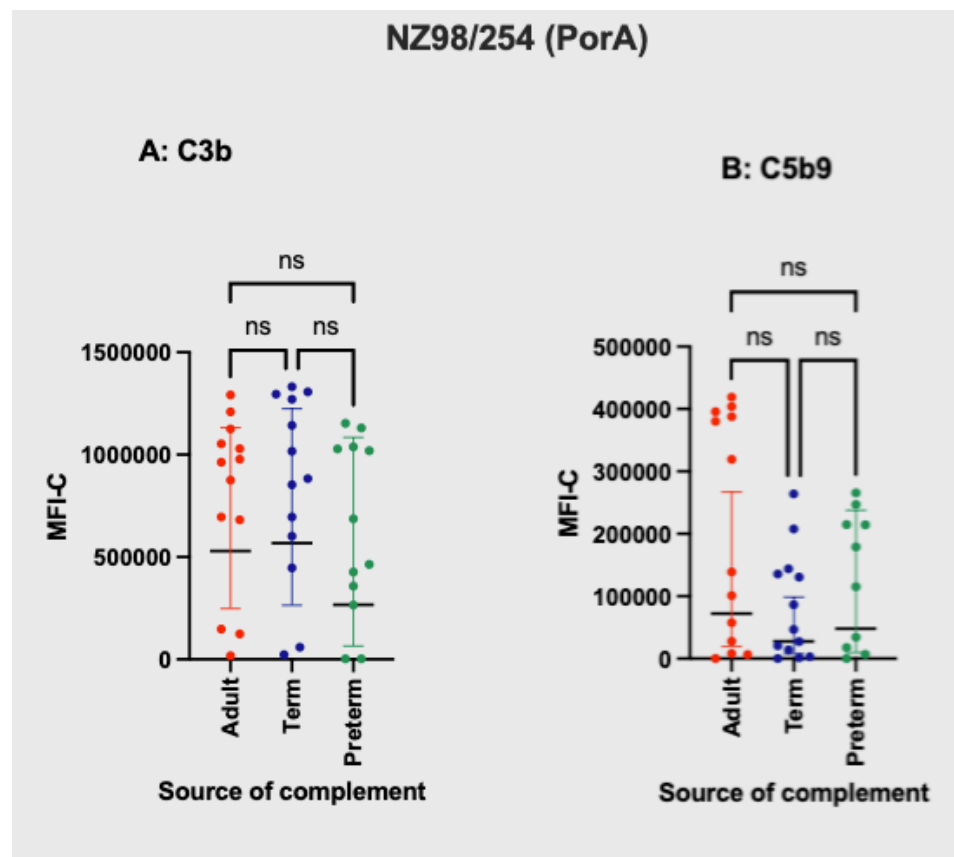


Figure 8.13. GMTs and 95% confidence intervals of MFI-C for C3b(A) and C5b9(B) comparing adult, term neonatal and preterm neonatal complement using strain NZ98/254.

There were no significant differences in the MFI-C between complement sources for either C3b(A) or C5b9(B) deposition.

ns= not significant; MFI-C= MFI-C mean fluorescence index-control; PorA= porin A; GMT= geometric mean titre

There was a significant difference for the MFI-C values for C3b between the low and mid ($p \leq 0.0001$) and low and high SBA responders ($p \leq 0.0001$), but no significant difference between the mid and high SBA responders. For C5b9 there was a significant difference between the low and high ($p \leq 0.0001$), the mid and low ($p \leq 0.001$), but not between the mid and high SBA responders (figure 8.14).

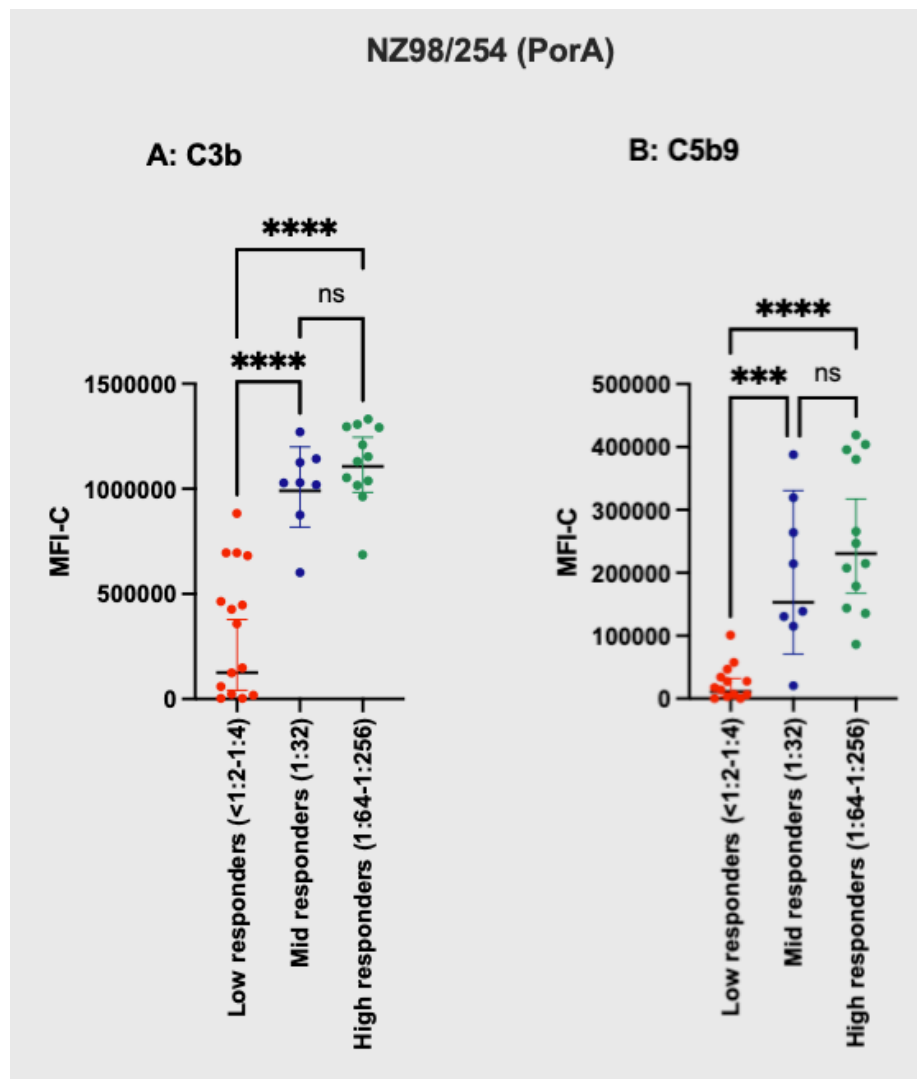


Figure 8.14. GMTs and 95% confidence intervals for MFI-C for strain NZ98/254 for C3b(A) and C5b9(B) comparing samples known to have low, mid and high SBA titres.

A: The MFI-C was significantly higher for the high SBA responders compared with the low SBA responders ($p \leq 0.0001$) for C3b. There was no significant difference between the mid and high SBA responders, but the mid SBA responders had significantly higher MFI-C values compared with low SBA responders ($p \leq 0.0001$); B: The MFI-C was significantly higher for the high SBA responders compared with the low SBA responders ($p \leq 0.0001$) and for the mid compared to the low responders ($p \leq 0.001$). MFI-C= MFI-C mean fluorescence index-control; PorA= porin A; GMT= geometric mean titre; SBA= serum bactericidal antibody). *** $P \leq 0.001$; **** ≤ 0.0001 ; ns= not significant; ns= not significant.

8.3 Discussion

The primary aim of this exploratory work was to compare the SBA titres obtained when using adult and neonatal complement. In the first part of this, it was necessary to produce a pooled complement source using cord blood samples. When investigating the complement activity in these samples, the findings were similar to those previously

published: overall CH50 and AP50 activity were reduced in term and preterm infants compared with adults, as were the quantities of complement components C3, C1q, C2, C4, factor B, C5, C6, C8 and C9 and the regulators C4BP, C1ina, factor H and factor I and the quantity of C7 was elevated in term and preterm infants compared to adults.

SBA assays are the established surrogate of protection for meningococcal vaccines (267), measuring functional bactericidal activity which is mediated by complement.

These assays quantify bactericidal activity in individual serum samples, and the results depend on the antibodies in the test serum and the activity of the complement used in the assay. Using complement from different sources can result in variability in titres achieved (247), and neonatal complement is functionally quite different from that of older children and adults.

In common with previous work, in this study the neonatal plasma contained less of several complement components, specifically C1q, C2, C3, C4, C5, C6, C8, C9 and factor B, which may render it functionally less active and may mean that SBA assays using adult complement overestimate bactericidal activity of the antibody in infant samples. Conversely, complement regulators were also found in lower quantities in infant samples compared with adult reference values, which may result in higher titres when using infant plasma as the complement source for SBA assays. When complement from baby rabbits is used in SBA assays for serogroup B meningococcus, the titres are unacceptably high because rabbit factor H (fH) is not bound by the fH binding protein on the meningococcus and down regulation therefore does not occur (97). It is possible that reduced levels of fH, as seen in neonates, may have a similar effect on the titres achieved.

The primary objective was to compare the SBA titres obtained using adult and neonatal complement. Originally, this was intended to include a third pooled complement source obtained from preterm infant samples, but this was not possible because all the preterm infants were delivered by caesarean section and had therefore received antibiotics.

The SBA results when using strain 5/99 (NadA) and, to a lesser extent NZ98/254 (PorA), showed significantly reduced bacterial survival in the presence of neonatal complement, such that titres for the SBAs using neonatal complement were extremely high or, in some cases unassignable. Two potential hypotheses explaining this were inhibition of bacterial growth because of the presence of a bactericidal factor, or the absence of a nutrient necessary for bacterial growth. To investigate this, SBA assays were performed using serial dilutions of neonatal complement. For both strains 5/99 (NadA) and NZ98/254 (PorA), serial dilutions reduced the inhibition of bacterial growth and allowed a titre to be assigned. This finding suggested that the issue was a bactericidal factor which was reduced by serial dilution, rather than a reduction in a nutrient which would not be expected to improve, or indeed would worsen, as the complement was diluted. From this hypothesis that the source of the bacterial inhibition was a bactericidal factor, and knowing the sensitivity of meningococci to antibiotics, the next step investigated if this inhibition of bacterial growth could have resulted from antibiotic contamination of the pooled neonatal complement from infants born by vaginal delivery. The disc diffusion assays supported this hypothesis: for the two strains most affected by the inhibition of bacterial growth, 5/99 (NadA) and NZ98/254 (PorA), the disc pre-incubated with neat neonatal complement was surrounded by a ring of bacterial inhibition. An area of bacterial inhibition was also

seen surrounding the disc which had been infused with neonatal complement and PBS but was not present around the disc infused with neonatal complement mixed with β -lactamase, an enzyme which breaks the β -lactamase ring and inactivates β -lactam antibiotics. For strain 44/76-SL (fHbp), which appeared unaffected by the inhibition of bacterial growth in SBA assays, there was no inhibition of bacterial growth around the disc infused with neat neonatal complement. It is surprising that strain 44/76-SL (fHbp) was not affected if the issue was antibiotic contamination, as meningococci are very sensitive to penicillin containing antibiotics, and the reasons for this difference are unclear. It may be that the minimum inhibitory concentration (MIC) is higher for strain 44/76-SL (fHbp) than for strains 5/99 (NadA) or NZ98/254 (PorA) meaning that the likely extremely low levels of antibiotic present in the pooled neonatal complement did not affect the assays using the strain 44/76-SL (fHbp). Because the disc diffusion assays were subjective, the final step to confirm this hypothesis of contamination was a quantitative SBA assay using strain 5/99 (NadA) and neat complement, complement mixed with PBS and complement mixed with β -lactamase. This showed that for neonatal complement, bacterial survival was much higher when using complement with β -lactamase compared with neat neonatal complement or neonatal complement with PBS.

This presumed contamination of the pooled neonatal complement from infants born vaginally, and the resulting impact on the SBA assays was unfortunate, as it limited the ability to investigate the main outcome of interest. However, the assays using strain 44/76-SL (fHbp) appeared to be unaffected and limited conclusions can therefore be drawn from the results using this strain only. The SBA titres achieved using neonatal

and adult complement were within one dilution of each other for 11/14 samples and within two dilutions of each other for 13/14 samples. This consistency in the results seen when adult and neonatal complement is used is reassuring as it suggests that using adult complement is not grossly over or underestimating the immunity of neonatal samples. However, as discussed previously there were significant limitations to this aspect of the work.

The finding that source of complement did not significantly affect the SBA titre achieved using an SBA assay is supported by the results from the antibody dependent complement deposition (ADCD) assays. These offer an alternative method to evaluate opsonophagocytic (C3) and bactericidal (C5b9) activity. Although SBA assays are the gold standard for meningococcal vaccine licensure, they require significant amounts of operator time and large volumes of sera. As well as having a much more rapid throughput and requiring considerably less sera, ADCD assays also offer the advantage of using killed bacteria. This is important in the context of this work as it allows the use of complement which has come from infants who have received antibiotics. This means that not only the presumed contaminated sera from term infants born by vaginal delivery can be used, but also the sera from term and preterm infants born by caesarean section. There were no significant differences between adult, term and preterm complement in the MFI-C obtained for any of the tested strains which supports the functional equivalence of these three complement sources.

For all the tested strains, the MFI-C values were significantly higher for C3b and C5b9 in the high SBA responders compared to the low SBA responders. This is expected for C5b9 because C5b9 is the membrane attack complex (MAC) and its presence can be

considered to be a proxy of bactericidal activity, but there are differences in the published literature about the association between deposition of C3b and bacterial lysis with some finding an association and others not.

Limitations

The major limitation was the finding that the neonatal complement from the cord blood of infants born by vaginal delivery appeared to have been contaminated with a bactericidal factor, most likely antibiotic(s), which made it impossible to compare the SBA results for two of the strains of interest. Furthermore, in the SBA assays using strain 44/76-SL and different test sera, the small volumes of sera necessitated a higher than usual starting dilution for some of the samples which resulted in less specific titres limiting the ability to compare titres between SBA assays using adult and neonatal complement.

8.4 Conclusions

Although there were limitations to the conclusions which could be drawn from the SBA assay results, for strain 44/76-SL for which a comparison could be made, the titres achieved using neonatal and adult complement were similar and this similarity is supported by the results of the ADCD assays in which there were no significant differences in the values for C3b or C5b9 deposition between the assays which used adult or neonatal complement. There is no evidence from this exploratory work that there is a significant difference in the results achieved when using adult or neonatal complement.

9 Conclusions and further work

9.1 Conclusions

Invasive meningococcal disease (IMD) is a devastating illness, with significant associated morbidity and mortality (269). The incidence of IMD has decreased in the UK over the past twenty years, however cases continue to occur, and infants aged less than one year remain at greatest risk. Recently there has been a significant reduction in cases of IMD following the social distancing measures implemented in response to the COVID-19 pandemic, however this has not been sustained and the number of cases began to increase again as these measures were withdrawn (25). In some countries there is concern about the potential for an increase in cases because of disruption to childhood vaccine programmes during the pandemic (270). Globally, the COVID-19 pandemic led to significant disruption of infant vaccine programmes (271), however although this was seen initially in the UK (272), rates improved through the pandemic and the most recent report for the epidemiological year 2021-22 showed that 91.5% of infants received two doses of 4CMenB, before 12 months of age (25).

It is recommended that preterm infants are vaccinated according to the same schedule as their term counterparts. Whilst the uptake of 4CMenB is very good in the UK, the information collected in the data capture system for vaccination (ImmForm) does not include details of gestational age which makes it impossible to know the details of the uptake in preterm infants. There is considerable evidence for other vaccines that preterm infants are often vaccinated later than term infants (78-85). This is often because of concerns that vaccines are not sufficiently immunogenic in preterm infants or result in a significant number of adverse events and clinical instability. In the

systematic review I found that, for the majority of vaccines, the proportion of infants who reached a protective antibody threshold was similar between term and preterm infants, but the absolute antibody concentrations were often lower in preterm infants. In some cases, this resulted in reduced persistence of antibody in preterm infants although there was no evidence of any reduction in efficacy or effectiveness of the vaccines in this population. In terms of reactogenicity, it is hard to draw definitive conclusions because of the lack of standardised case definitions used across the identified studies, the observational nature of the majority of the evidence and the significant potential for bias. There was no evidence however that vaccines should be delayed in preterm infants, and it is important that this message is clearly communicated to parents and health care professionals, whilst recognising the limitations of the evidence and seeking to fill this gap.

Preterm infants are at increased risk of several vaccine preventable infections and in this work, I have shown that preterm infants are also at increased risk of IMD, despite the introduction of 4CMenB into the routine programme. The clinical course and outcomes experienced by preterm infants were similar to term infants, apart from sequelae which were reported more frequently in preterm infants. In the light of the increased risk of IMD faced by preterm infants, it is vital to consider how they can better be protected, including through vaccination. In the clinical trial, for the first time in preterm infants a 2+1 schedule of 4CMenB was compared with a 3+1 schedule and, overall, immunogenicity was shown to be similar between the two schedules. A three-dose priming schedule was superior compared with a two-dose priming schedule for antibody against strain NZ98/254, but the results in preterm infants were similar to those seen in historical populations of term infants vaccinated with a two-dose priming

schedule. Whilst the results of the clinical trial did not suggest that a different schedule is required for preterm infants, particularly as following the booster dose there were no differences between schedules in GMT or proportions with titres $\geq 1:4$, the persistence of antibody after the booster dose was not studied. In term infants, the persistence of antibody is similar in those who received a 2+1 and a 3+1 schedule in infancy (273), and 76% of individuals who were vaccinated in infancy using a 2+1 or a 3+1 schedule maintained titres of $\geq 1:4$ to at least one of the vaccine antigens until 24-36 months after vaccination (274). This persistence needs to be investigated in preterm infants, particularly because in the clinical trial the GMTs one month following the booster were non-significantly lower for all three tested antigens in those infants receiving a 2+1 schedule, which may result in a more significant difference in immunity developing later in childhood.

In the clinical trial, we used SBA assays, the established gold standard, to investigate the immunogenicity of 4CMenB. In these assays, performed at the national meningococcal reference laboratory, exogenous human complement was used which had been derived from adult human volunteers before being depleted of antibody. The work investigating whether SBA assay results differed when using neonatal compared with adult complement was confounded by a marked reduction in the growth of meningococci for two of the tested strains (5/99 and, to a lesser extent, NZ98/254) which limited the ability to draw conclusions. One strain, 44/76-SL, was not affected by the reduction in meningococcal growth and for this strain, titres obtained when using neonatal or adult complement were similar, suggesting that the use of adult complement in SBA assays of infant serum provides a reliable assessment of neonatal immunity.

9.2 Further work

Although the work in this thesis has addressed the questions raised in the objectives, it has also revealed some areas for future work.

Firstly, there is work to be done to improve the overall knowledge base relating to vaccination in preterm infants. We do not have an accurate picture of the current vaccine uptake or timeliness of vaccination in UK preterm infants, which makes it hard to identify whether specific approaches need to be implemented for this group. Whilst some international studies investigating vaccine uptake and timeliness in preterm infants have found some factors likely to be associated with delayed vaccination, for example lack of information provided to parents, prolonged hospitalisation and larger number of children in the household (81, 82, 85) there has not been any detailed work with families to explore their decision-making framework for this important issue. A greater understanding of these issues may allow any weaknesses in vaccine coverage in preterm infants to be identified and an appropriate response made.

The perceived safety of vaccines is known to be an important element in parental decision making about vaccine uptake (275-277), and the provision of accurate information is a central part of fostering vaccine confidence. As discussed in chapter four, there are significant limitations in the quality of the data relating to adverse events following vaccination in preterm infants, particularly for cardiorespiratory stability in those infants vaccinated as inpatients. These limitations mean that most of the available evidence on which to make decisions and inform parents is based on results from observational studies with substantial potential for bias. Addressing this issue is a priority for future work. Cardiorespiratory stability could be investigated in a blinded study in which inpatient preterm infants are continuously monitored for

apnoea, bradycardia, desaturation and temperature, using a system in which pre-determined thresholds are set by the investigators and output recorded. This monitoring would take place for a period of two weeks around the time the vaccines are due to be given (i.e., from seven-nine weeks). During this period, at least three days after the monitoring had commenced, routine vaccines would be administered, and the details recorded. The monitoring output would be reviewed and summarised for each day of observation by two researchers independent of the clinical team with no knowledge of when the vaccines were administered. The period of 72 hours before and after vaccine administration could then be compared using this blinded monitoring data.

The surveillance programme in the UK is well established and, following the finding that the incidence of IMD is higher in preterm compared with term infants even after the introduction of 4CMenB, it is important that this data continues to be interrogated with respect to preterm infants, particularly for serogroups for which there is an available vaccine. Although the immunogenicity evidence does not currently support a change to the schedule for preterm infants, this would have to be reconsidered in a situation in which PorA strains became more common in preterm infants.

Using data from the enhanced surveillance programme in the UK allowed a comparison to be made of the incidence in term and preterm infants and a limited comparison of outcome and sequelae between these groups. Overall, at least one sequela was more common in preterm infants as were sequelae defined as 'other', although as I have discussed there are significant limitations to this finding. To investigate this more fully, it would be necessary to conduct a more detailed follow up study of affected infants. This could be done either retrospectively or prospectively. A

possible retrospective study design would involve all parents of surviving preterm infants who experienced IMD being contacted and invited to participate. If they consented, they would be asked to complete a detailed questionnaire about any sequelae which their child had experienced and to provide details of their development at the ages of two and five years. As this data collection would be extremely vulnerable to recollection bias, information about sequelae and development would, where possible, be cross-checked with information from outpatient follow up clinics, health visitor assessments and evaluations at school entry. For each recruited preterm infant, two controls would be recruited. The first of these would be a preterm control who had not experienced IMD, but who was matched for sex, gestation, and significant medical history; the second would be a term infant who had experienced IMD and was matched for sex, serogroup, significant medical history, presentation and age at onset of disease. The information that could be gathered in this design of study is likely to be incomplete and is open to significant recollection bias. An alternative design would be a prospective case control trial. According to this design, the family of every preterm infant with IMD would be contacted following completion of the enhanced surveillance questionnaire 3-6 months after disease and asked to participate. Recruited infants would have a detailed neurodevelopmental assessment performed at two and five years and the parents would be asked to complete a health assessment questionnaire at the same time. As previously described, two controls would also be recruited: one preterm infant not affected by IMD, and one term infant affected by IMD. This would allow a much more detailed assessment to be made of the sequelae experienced by preterm infants and allow an attempt to be made to separate those sequelae experienced as a consequence of

preterm birth and those as a consequence of IMD. Because IMD is relatively rare there would be only approximately ten cases per year whom it would be possible to recruit and therefore obtaining a meaningful sample is likely to take several years.

The persistence of the antibody response to 4CMenB in preterm infants is unknown. To investigate this persistence following 4CMenB vaccine given according to a 2+1 and a 3+1 schedule the participants of the clinical trial could be contacted to invite them to participate in a further study, which would involve collection of a single blood sample. Whilst this would provide interesting information, the participants of this study are now beyond the age of greatest risk so even if a difference were found, this finding would not inform decisions about the need for a further booster dose. An alternative approach would be to conduct a cohort study which would allow a longitudinal investigation of antibody response in children born preterm. In this study, recruitment would be in several UK neonatal units and blood sampling would be performed one month after the booster dose of 4CMenB, with further sampling time points at 2, 3 and 4 years.

The exploratory work on sources of complement in SBA assays has raised some interesting issues, as well as presenting some challenges resulting from the likely contamination of the complement derived from the plasma of infants born by vaginal delivery. Currently, only tentative conclusions have been possible, and these only for one strain. Further work is necessary to try to reverse the likely antibiotic contamination using β -lactamase, without enhancing the bacterial growth as a result of the sorbitol present in the β -lactamase. There may also be insights provided by conducting the same comparison of neonatal and adult complement in SBA assays of

pathogens less sensitive to the likely β -lactam antibiotics in the neonatal complement, for example *Bordetella pertussis*. This work is ongoing.

9.3 Final comments

IMD is serious and, although a relatively rare disease, has far reaching consequences for affected individuals, their families and society. Preterm infants have a higher incidence of IMD than their term counterparts and every effort should be made to optimise the protection of this group. Efforts need to be made to better understand the uptake of 4CMenB in preterm infants in the UK, timely vaccination with 4CMenB must be encouraged and gestational age should remain a factor of interest in surveillance so that any changes in the incidence in preterm infants can be identified and, if necessary, changes to the vaccination schedule for this group made.

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Appendix A

Table 1: MEDLINE search teams used

1. exp Infant, premature 2. exp Premature birth 3. exp obstetric labour, premature 4. exp premature birth 5. (Preterm OR “pre term” OR “pre-term” OR prematur* OR “early delivery”).mp. 6. 1 or 2 or 3 or 4 or 5	Population
7. exp vaccination 8. exp immunization 9. (Vaccin* OR immunis* OR immuniz*).mp. 10. 7 or 8 or 9	Intervention
11. exp immunogenicity, vaccine 12. exp, safety 13. (“antibody response*” OR immunity OR immunogenicity OR “immune response*” OR “serologic response*” OR response OR “vaccine response*” OR “antibody titre” OR “vaccine effectiveness” OR effectiveness OR “vaccine efficacy” OR efficacy OR “adverse events” OR safety OR toxicity OR harm OR “side effects”).mp. 14. 11 OR 12 OR 13	Outcome
15. 6 AND 10 AND 14	Final search

Table 2: Embase search terms used

1. exp prematurity 2. exp premature labor 3. (Preterm OR “pre term” OR “pre-term” OR prematur* OR “early delivery”).mp. 4. 1 or 2 or 3	Population
5. exp vaccination 6. exp immunization 7. (Vaccin* OR immunis* OR immuniz*).mp. 8. 5 or 6 or 7	Intervention
9. exp vaccine immunogenicity 10. exp, safety 11. (“antibody response*” OR immunity OR immunogenicity OR “immune response*” OR “serologic response*” OR response OR “vaccine response*” OR “antibody titre” OR “vaccine effectiveness” OR effectiveness OR “vaccine efficacy” OR efficacy OR “adverse events” OR safety OR toxicity OR harm OR “side effects”).mp. 12. 9 OR 10 OR 11	Outcome
13. 4 AND 8 AND 12	Final search

Table 3: Web of science/Cochrane CENTRAL search terms

Preterm OR “pre term” OR “pre-term” OR prematur* OR “early delivery” (1)	Population
Vaccin* OR immunis* OR immuniz* (2)	Intervention
“Antibody response*” OR immunity OR immunogenicity OR “immune response*” OR “serologic response*” OR response OR “vaccine response*” OR “antibody titre” OR “vaccine effectiveness” OR effectiveness (3)	Outcome
1 AND 2 AND 3	Final search

Table 4: LILACS search terms

Preterm OR premature	Population
Vaccination OR immunization	Intervention

Appendix B

Document 1: MENSVO1



National epidemiological surveillance – confirmed invasive meningococcal disease*

Immunisation and Vaccine Preventable Diseases Division, 61 Colindale Avenue, London, NW9 5EQ.
Tel: 020 8327 7588 or 6421 Email: meningo@ukhsa.gov.uk

Form MENSVO1
Version: December 2022
Ref: [UKHSA National ref]

Please complete in block capital letters												IN CONFIDENCE		
Patient details														
Surname:					Forename:					Gender: ☐ Male ☐ Female				
Date of birth: DD/MM/YYYY				NHS number:				HPZone reference:				Onset date: DD/MM/YYYY		
Part A: Ethnicity – please tick below														
☐ White British ☐ White other ☐ Black-Caribbean ☐ Black African ☐ Indian ☐ Pakistani ☐ Bangladeshi ☐ Chinese ☐ Mixed* ☐ other*, *please specify:														
Part B: Vaccination history. This covers Men B, Men C and MenACWY vaccination.														
+ Please complete details for all vaccines below as fully as possible.														
Vaccine	Did this case receive any doses of each vaccine before disease onset?				1 st dose date	1 st dose batch number	1 st dose manufacturer/brand	2 nd dose date	2 nd dose batch number	2 nd dose manufacturer/brand	3 rd dose date	3 rd dose batch number	3 rd dose manufacturer/brand	
MenB vaccination ¹	Yes ☐	No ☐	NK ☐	Not eligible ☐	DD/MM/YYYY		Bexsero®	DD/MM/YY YY		Bexsero®	DD/MM/YY YY		Bexsero®	
MenC Vaccination ²	Yes ☐	No ☐	NK ☐	Not eligible ☐	DD/MM/YYYY			DD/MM/YY YY			DD/MM/YY YY			
MenC/Hib Vaccination ³	Yes ☐	No ☐	NK ☐	Not eligible ☐	DD/MM/YYYY		Menitorix®	All high-risk groups (complement deficiency or asplenia) should be offered MenB and MenACWY vaccination.						
MenACWY vaccination ⁴	Yes ☐	No ☐	NK ☐	Not eligible ☐	DD/MM/YYYY									
¹ MenB vaccine (Bexsero®) included in the routine infant programme since 01/09/2015 including any child born from 01/05/2015. Routinely offered at 8 weeks, 16 weeks and 1 year. ² MenC vaccine (Meningitec®, Menjugate® or Neissvac®) included in the routine infant programme from 01/11/1999-30/6/2016. Catch-up vaccination means all those born between 01/09/1981-31/03/2016 should have been offered at least one dose of MenC vaccine. MenC vaccine was offered to teenagers aged 13/14 years and Freshers June 2013-May 2015. ³ A single dose of Menitorix® vaccine (combined MenC-Haemophilus influenzae type B [Hib]) has been offered at 12-13 months of age from 01/09/2006 (DOB>01/08/2005). ⁴ MenACWY vaccine (Menveo®, Nimenrix®) replaced MenC vaccine for teenagers routinely at 13-15 years (all young people at least 13 years of age and born after 31/08/1996 would be eligible to 25th birthday). In addition, fresher doses for those eligible <25 years from 01/09/2015-31/08/2022 at university or another Higher Education Institution														

For meningococcal A, B, C, W or Y infection of the conjunctiva please complete this page for vaccine eligible individuals.

Appendix B

Part C: Clinical presentation 1) What was the clinical presentation? ☐ Meningitis ☐ Septicaemia ☐ Both meningitis and septicaemia ☐ Septic arthritis ☐ Epiglottitis ☐ Pneumonia ☐ Other ☐ Unknown Comments:	Part E: Co-morbidities and pregnancy 3) At the time of meningococcal disease, did the patient have any co-morbidities? ☐ Yes ☐ No ☐ Unknown 3.1) If yes, what were their co-morbidities? ☐ Chronic heart disease ☐ Congenital or chromosomal abnormality ☐ Chronic lung disease ☐ CNS disease (CSF leak, VP shunt etc) ☐ Chronic renal disease ☐ Chronic gastrointestinal disease ☐ Metabolic disease ☐ Other Comments:	Part G: Travel history 7) Was the patient born in the UK? ☐ Yes ☐ No ☐ Unknown 7.1) If no, when did they arrive in the UK DD/MM/YYYY 7.2) Country of birth: 8) Has the patient recently travelled abroad (returning in the last 28 days)? ☐ Yes ☐ No ☐ Unknown 8.1) If yes, where did they travel (town/ country)? 8.2) When did they return? DD/MM/YYYY						
Part D: Risk factors 2) At the time of onset did the patient have any known risk factors for meningococcal disease? ☐ Yes ☐ No ☐ Unknown 2.1) If yes, what were their risk factor/s? ☐ Asplenia/ splenic dysfunction ☐ Complement deficiency ☐ Malignancy/ immune deficiency ☐ Immunosuppressive drug (Including complement inhibitors, e.g. eculizumab) Comments:	4) Was the patient pregnant at the time? ☐ Not applicable ☐ Yes ☐ No ☐ Unknown Part F: Outcome 5) Was the patient admitted to ITU? ☐ Yes ☐ No ☐ Unknown 6) Is the patient currently alive? ☐ Yes ☐ No ☐ Unknown 6.1) If patient died, date of death DD/MM/YYYY	Part H: Is the case working at or attending any of these situations? Complete page 3 for university or other higher education settings ☐ child minder ☐ nursery ☐ school/college ☐ university ☐ barracks ☐ care/nursing home ☐ other _____ Part I: Please provide any further comment 						
<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 40%;">Completed by (full name):</td> <td style="width: 30%;">Contact Number:</td> <td style="width: 30%;">Date: DD/MM/YYYY</td> </tr> <tr> <td colspan="3">Surgery/hospital/HPT:</td> </tr> </table> Thank you for your time and assistance. Please return by post or secure email (both as detailed overleaf) or upload to HPZone.			Completed by (full name):	Contact Number:	Date: DD/MM/YYYY	Surgery/hospital/HPT:		
Completed by (full name):	Contact Number:	Date: DD/MM/YYYY						
Surgery/hospital/HPT:								

Document 2: MENSVO2

 Public Health England	Immunisation Department	T: 020 8327 7828 or 6688
	61 Colindale Avenue	F: 020 8327 7404
	London NW9 5EQ, UK	E: PHE.meningo@nhs.net
	www.gov.uk/phe	

Enhanced National Surveillance of Meningococcal Disease
Clinical Questionnaire Form **MENSVO2**, May 2019

Patient Details

Patient name: _____ Date of Birth:

NHS Number: _____

PHE reference: _____

Section A: Reporter Details

A1 Date of completion of questionnaire:

A2 Consultant responsible: _____

A3 Telephone number: _____ Email: _____

Section B: Demographics

☐ **B1 Ethnicity**
White / Black African / Black Caribbean / Indian / Pakistani / Bangladeshi / Chinese /
Mixed/Other (specify): _____

B2 If born prematurely, gestation at birth: _____ weeks

B3 Underlying Risk Factors

☐ None

☐ Asplenia / Splenic Dysfunction (including sickle cell disease)

☐ Known complement deficiency (including complement inhibitor therapy)

☐ Immunosuppression (including HIV)

Comments: _____

B4 Any other underlying medical condition: Yes ☐ No ☐

Comments: _____

B5 Travel abroad in the previous 28 days: Yes ☐ No ☐ NK ☐

If yes, where & date of return: _____

B6 Recently entered UK? Yes ☐ No ☐ NK ☐

If yes, country of origin & date of entry: _____

Section C: Presentation/Clinical featuresC1 Date of onset of illness: C2 Date of hospital admission: Time at Presentation: _____ : _____ am /pmC3 Date of hospital discharge:

C4 What was the clinical presentation?

- ☐ Meningitis ☐ Septicaemia ☐ Both meningitis & septicaemia ☐ Septic Arthritis ☐ Epiglottitis
☐ Pneumonia ☐ Other (please specify) _____

C5 Symptoms and signs at presentation: (please tick all that apply)

+

History *Tick all that apply*

☐ Fever ($\geq 38^{\circ}\text{C}$) ☐ Sore throat/ coryza ☐ Reduced feeding/appetite ☐ Thirst ☐ Nausea/vomiting ☐ Diarrhoea ☐ Abnormal skin colour ☐ Rash	☐ Lethargy ☐ Breathing difficulty ☐ Apnoea ☐ Floppy muscle tone ☐ Leg pain ☐ General aches ☐ Cold hands and feet ☐ Bone joint pain/swelling	☐ Irritability ☐ Bulging fontanelle ☐ Headache ☐ Neck Stiffness ☐ Photophobia ☐ Confusion/delirium ☐ Drowsy ☐ Seizures/Convulsions ☐ Unconscious
--	--	---

C6 Examination on admission *Tick all that apply*

- ☐ Fever: temp on admission $^{\circ}\text{C}$
☐ Rash: If yes, please tick all that apply:
 macular ☐ papular ☐ maculo-papular ☐ petechial ☐ purpuric ☐ fulminant ☐
☐ Reduced GCS (state score if reduced):
☐ Seizure: Total seizure duration _____ mins ☐ focal or ☐ generalised
 ☐ Required anticonvulsants in A&E
☐ Signs of shock*: ☐ Required fluid boluses: total volume: _____ ml/kg
 ☐ Required inotropes

*Signs of shock: Cap. Refill time > 2 secs, tachycardia > 160 beats/min, hypotension, pale/mottled skin, cold

Section D: Complete if admitted to PICU (attach discharge summary if available)

D1. Date of PICU admission: discharge:

D2. Reason for admission:

D3. Type of Support

	Yes	No	NK	
a) Ventilation	☐	☐	☐	If Yes No. days _____
b) Inotropes	☐	☐	☐	If Yes No of days _____
c) Haemofiltration	☐	☐	☐	If Yes No of days _____
d) Surgical procedures	☐	☐	☐	If Yes, explain: _____

Section E: Lumbar Puncture (cross out this section if not applicable)

E1. If LP done, date: Time taken: ____: ____am/pm

E2. LP performed before ☐ or AFTER ☐ antibiotics? *If after, how many hours after?* _____

E3. If No, state why: cardiovascular instability ☐ respiratory instability ☐ unable to ☐ other _____

E4. CSF WBC count _____ per mm³ Neutrophils ____% Lymphocytes ____%
CSF RBC count _____ per mm³ CSF protein _____mg/dl
CSF glucose _____mmol/l Plasma glucose _____mmol/l

Section F: Blood Investigations (on admission)

F1. Full Blood count: Hb: _____g/dL WBC count _____x10⁹/L Neutrophil count _____x10⁹/L
Platelets _____x10⁹/L **C-reactive protein:** _____mg/L Not done: ☐

F2. Liver Function Test: Bilirubin _____mg/dL Alanine Transaminase (ALT) _____IU/L Not done: ☐

F3. Urea and Electrolytes: Urea: _____mmol/L Creatinine: _____mg/dL Not done: ☐

F4. Clotting: APTT: _____seconds INR _____ Not done: ☐

Section G: Treatment

G1. Antibiotics on admission: _____

Time of FIRST antibiotic dose: _____: _____ am / pm

Total duration of antibiotics: IV _____ (days) then oral : _____ (days)

G2. Steroid given for meningitis diagnosis: Yes ☐ No ☐ NK ☐

If yes, how many hours after the first antibiotic dose? _____

Section H: Please complete if any imaging performed

Investigation	Performed			Scan Normal?			Date
	Yes	No	NK	Yes	No	NK	
H1 Cranial Ultrasound	☐	☐	☐	☐	☐	☐	
H2 CT Head	☐	☐	☐	☐	☐	☐	
H3 MRI Head	☐	☐	☐	☐	☐	☐	

H4 Major findings (you can please attach copy of report instead):

Section I: Outcomes

I1 Did the patient survive the infection? Yes ☐ No ☐

If died, date: Cause of death:

If survived:

I2 Date of last follow-up:

I3 At follow-up, did the patient have any of the following?:

	Yes	No	NK
a) Epilepsy	☐	☐	☐
b) Other Neurological complications	☐	☐	☐
c) Amputations	☐	☐	☐
d) Other	☐	☐	☐

If yes to (a)-(d) above, give details:

I4 Date of hearing test: Any Hearing loss? Yes ☐ No ☐ NK ☐

If yes: please indicate: RIGHT EAR: Mild ☐ Moderate ☐ Severe ☐
LEFT EAR: Mild ☐ Moderate ☐ Severe ☐

Section J: Additional Comments

Thank you for taking the time to complete the Questionnaire

Please return the completed form to: Immunisation Department, Public Health England, 61 Colindale Avenue, London NW9 5EQ, UK.

Any questions? Please call or email us using meningo@phe.gov.uk or the details provided on page 1.

Document 3: MENSVO3



UK Health
Security
Agency

Enhanced National Surveillance of Meningococcal Disease

Immunisation Division
61 Colindale Avenue
London NW9 5EQ, UK
www.gov.uk/ukhsa

T: 020 8327 7588 or 6058
F: 020 8327 7404
E: PHE.meningo@nhs.net

Consequences of disease questionnaire form, MENSVO3, January 2022, Ref: «Reflabno»

Patient name: _____

Date of Birth: «DOB» NHS Number: «NHSno»

Gestational age at delivery: _____ weeks

Section A: Reporter Details

A1 Completed by: _____ Date: dd / mm / yyyy

A3 Telephone number: _____ Email: _____

Section D: Outcomes

D1 Duration of hospital stay: Admission date: dd / mm / yyyy Discharge date: dd / mm / yyyy

D2 Was the patient admitted to PICU? Yes ☐ No ☐

Did the patient survive the infection? Yes ☐ No ☐

If died, date: dd / mm / yyyy Cause of death: _____

If survived:

I2 Date of last follow-up: dd / mm / yyyy

I3 At follow-up, did the patient have any of the following?

	Yes	No	NK
a) Epilepsy	☐	☐	☐
b) Other Neurological complications	☐	☐	☐
c) Amputations	☐	☐	☐
d) Other	☐	☐	☐

If yes to (a)-(d) above, give details:

I4 Date of hearing test: dd / mm / yyyy Any Hearing loss? Yes ☐ No ☐ NK ☐

If yes: please indicate: RIGHT EAR: Mild ☐ Moderate ☐ Severe ☐
LEFT EAR: Mild ☐ Moderate ☐ Severe ☐

Section E: Additional Comments

Please include the hospital discharge summary. Hospital discharge summary attached ☐

Thank you for taking the time to complete the questionnaire Please return the completed form to: Immunisation Division, UK Health Security Agency, 61 Colindale Avenue, London NW9 5EQ, UK. Any questions? Please call or email us using meningo@ukhsa.gov.uk.

Appendix C

Document 1: BEAR Men B study protocol



Babies born Early Antibody Response to Men B vaccination: BEAR Men B

Sponsor's Project Number: 16.0247

EudraCT number: 2017-001487-38

Publicly available Trial Registration Number ID: NCT03125616

IRAS ID: 223266

CHIEF INVESTIGATOR (CI):

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SPONSOR: St George's University of London

SPONSOR CONTACT:

Debbie Rolfe

St George's Joint Research and Enterprise Office (JREO)

Hunter Wing, Ground Floor

Cranmer Terrace

London SW17 0RE

Phone: 020 8725 5013

Email: [REDACTED]

Fax: [REDACTED]

Funding source: Meningitis Now and GSK

Information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the ethical/regulatory review of the study, without written authorisation from St George's Joint Research and Enterprise Office (JREO) or its affiliates.



Signature Page and Statement

The Chief Investigator (CI) and the Sponsor representative have discussed this protocol version. The investigators agree to perform the investigations and to abide by this protocol except in the case of medical emergency or where departures from the protocol are agreed in writing by the Sponsor.

The investigator agrees to conduct the trial in compliance with the approved protocol, EU GCP and UK Regulations for CTIMPs (SI 2004/1031; as amended), the UK Data Protection Act (1998), the St George's NHS Trust Information Governance Policy (or other local equivalent), UK Policy Framework for Health and Social Care Research v3.2 October 2017, the Sponsor's Standard Operating Procedures (SOPs), and other regulatory requirements as amended.

I also confirm that I will make the findings of the study publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

This protocol has been written in accordance to the Sponsor's procedure identified as: JREOSOP0039 'Protocol Design' and is intended for use at UK sites only.

Chief Investigator Professor Paul Heath Professor Paediatric Infectious Diseases St George's, University of London	Signature	Date
Sponsor Representative Subhir Bedi Head of Research Governance and Delivery St George's University of London		

Acknowledgements and Protocol contributories

This study was devised by Anna Calvert and Paul Heath and was developed by Anna Calvert, Paul Heath, Shamez Ladhani and Helen Findlow, and also incorporates comments and input from the prospective collaborating PIs. Paul Heath is the grant holder. Nick Andrews provided statistical expertise in clinical trial design and will be conducting primary statistical analysis. All authors contributed to refinement of the study protocol and approved the final version.

We are grateful for the funding from Meningitis Now and GlaxoSmithKline. These bodies have had no input into the design of this study and will have no role in data collection, management, analysis, or interpretation of the findings. The preparation of the report will be done by the study authors and will be entirely independent of the funding organisations. The funding organisations will have no influence over the content of the final report submitted for publication, or the decision to submit.



Contents

1. List of abbreviations	5
3. Study synopsis	9
4. Primary Objectives	11
5. Secondary Objectives	11
6. Background.....	11
Study disease.....	11
Non-Investigational Medicinal Products (NIMPs).....	12
Study Rationale and risk/benefit analysis	13
7. Trial design	14
7.1 Overall design.....	14
7.2 Schematic of trial design	16
8. IMP Dosage regimen and rationale	17
8.1 Source of IMP and NIMPs.....	17
8.2 Accountability procedures for the IMP	17
8.3 Post-trial IMP arrangements	17
8.4 Name and description of each non-IMP (NIMP)	17
8.5 Concomitant treatment	18
9 Participant Selection criteria	19
9.1 Inclusion criteria.....	19
9.2 Exclusion criteria	19
11 Study procedures	20
11.1 Informed consent.....	20
11.2 Randomisation procedure.....	21
11.5 Discontinuation/withdrawal of participants and stopping rules.....	22
11.6 Participant transfers	23
11.7 Lost to follow up.....	23
11.8 Definition of the End of Trial	23
12. Study Assessments	23
12.1 Screening assessments.....	23
12.2 Baseline assessments	24
12.3 Treatment procedure	24
12.4 Subsequent assessments.....	25
12.5 Summary flow chart of study assessments	26
Refer to Appendix 2	26
12.6 Methods	26
12.6.1 Samples.....	26
12.6.2 Obtaining, labelling, storing at local site	26
12.6.3 Laboratory procedures	27
13 Pharmacovigilance	27
13.1 Definitions	27
13.2 Investigator responsibilities relating to safety reporting	28



13.3	Notification of deaths.....	30
13.4	Annual Progress Reports (APRs).....	30
13.5	Reporting Urgent Safety Measures	30
13.6	Notification of Serious Breaches of GCP and/or the protocol.....	30
14	Data management and quality assurance	31
14.1	Confidentiality	31
14.2	Data collection tool	31
14.3	Data handling and analysis.....	31
15	Archiving arrangements	32
16	Statistical design.....	32
16.1	Statistical input in trial design	32
16.2	Endpoints	33
16.2.1	Primary endpoints.....	33
16.2.2	Secondary endpoints.....	33
16.3	Sample size and recruitment.....	33
16.3.1	Sample size calculation	33
16.3.2	Planned recruitment rate.....	34
16.4	Summary of baseline data and flow of patients.....	34
16.4.1	Primary endpoint analysis	34
16.4.2	Secondary endpoint analysis	35
16.4.3	Randomisation	35
16.5	Interim analysis	35
17	Committees involved in the trial	35
18	Direct access to source data	36
19	Site approval and ongoing Regulatory compliance	36
20	Monitoring plan for the trial.....	36
21	Finance	37
22	Insurance and indemnity.....	37
23	IP and development policy	38
24	Publication policy	39
24.1	Before the official completion of the Trial, ...	39
24.2	Up to 180 days after the official completion of the Trial	39
24.3	Beyond 180 days after the official completion of the Trial	40
25	Statement of compliance	40
26	List of Protocol appendices	41
27	References	41



1. List of abbreviations

AE	Adverse Event
AR	Adverse Reaction
CA	Competent Authority
CI	Chief Investigator
CRF	Case Report Form
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trial of Investigational Medicinal Product
DMC	Data Monitoring Committee
DSUR	Development Safety Update Report
EMA	European Medicines Agency
EU	European Union
EUCTD	European Clinical Trials Directive
EudraCT	European Clinical Trials Database
fHbp	factor H binding protein
GCP	Good Clinical Practice
GMT	Geometric Mean Titres
HRA	Health Research Authority
hSBA	human Serum Bactericidal Antibody
ICF	Informed Consent Form
IMP	Investigational Medicinal Product
ISF	Investigator Site File
ISRCTN	International Standard Randomised Controlled Trial Number
ITT	Intention to treat
JREO	Joint Research and Enterprise Office
MHRA	Medicines and Healthcare products Regulatory Agency
MoU	Memorandum of Understanding
NadA	Neisserial adhesinA
NIMP	Non- Investigational Medicinal Product
PHE	Public Health England
PI	Principal Investigator
PIS	Participant Information Sheet
PorA	Porin A
RCT	Randomised Control Trial
REC	Research Ethics Committee
RSI	Reference Safety Information
SAR	Serious Adverse Reaction
SAE	Serious Adverse Event
SDV	Source Document Verification
SOP	Standard Operating Procedure
SmPC	Summary of Product Characteristics
SSAR	Suspected Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction

Appendix C



TMF
TMG



Trial Master File|
Trial Management Group



2. Roles and Responsibilities:

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3. Study synopsis

Brief Title	BEAR MenB
Official title:	Babies born Early Antibody Response to Men B vaccination: BEAR MenB
Brief Summary	A phase IV multicentre study to evaluate the primary and booster immune responses in UK preterm infants receiving routine immunisations and incorporating a randomisation study of a three dose versus a two dose schedule of Bexsero® for primary immunisation.
Sponsor reference number:	16.0247
Public database Trial identifier number	ClinicalTrials. Gov NCT03125616
Study type & Phase	Phase IV randomisation study of two Men B vaccine schedules in preterm infants
Study Design	Preterm infants will be randomised to one of two 4CMenB schedules. Blood samples will be taken to assess vaccine responses at 5, 12 and 13 months of age and a diary card will be used to assess reactogenicity of the vaccines.
Chief Investigator:	Professor Paul Heath Professor Paediatric Infectious Diseases St George's, University of London
Study Population	Preterm infants born at <35 weeks gestation (i.e. up to 34 weeks and 6 days), 50% <30 weeks gestation (i.e. up to 29 weeks and 6 days)
Condition	Prematurity
Study Group/cohort (s)	Infants will be randomised to one of two 4CMenB schedules. One group will receive two 4CMenB vaccinations, i.e. at 8 and 16 weeks of age as per current UK schedule, while the other group will receive three 4CMenB vaccinations, i.e. at 8, 12 and 16 weeks of age. Both groups will receive a 4CMenB booster at 12 months of age.
Eligibility criteria:	Inclusion criteria: • Premature infant born at <35 weeks gestation • No contraindications to vaccination according to the 'Green Book' • Willing and able to comply with study procedures • Written informed consent
	Exclusion criteria: • Contraindication to vaccination according to the Green Book • Life-limiting congenital abnormality or condition • Prior diagnosis of an immunodeficiency syndrome • Considered unlikely to complete expected follow up until the end of the study

BEAR MenB. JREO Ref: 16.0247

9

Version 2.1 02.08.18

IRAS reference 223226

	• Child in care
Target number of participants:	132
Criteria for evaluation:	Primary outcome measure(s): (i) hSBA GMTs 1 month after completion of primary immunisations for relevant Bexsero® antigens: fHbp, NadA and PorA (ii) hSBA proportions $\geq 1:4$, at 1 month after completion of primary immunisations for relevant Bexsero® antigens: fHbp, NadA and PorA. Secondary outcome measure(s): (i) The percentage of infants presenting with fever, local reactions and non-febrile systemic reactions within the 7 days following each Bexsero® vaccine dose; (ii) The percentage of inpatients who have a change/deterioration in cardiorespiratory status within the 72 hours following each Bexsero® vaccine dose; (iii) The percentage of infants investigated for sepsis and commenced on antibiotics within 7 days of Bexsero® vaccination; (iv) hSBA GMTs at 12 months of chronological age (pre booster) for relevant Bexsero® antigens: fHbp, NadA and PorA; (v) hSBA proportions $\geq 1:4$, at 12 months of chronological age (pre booster) for relevant Bexsero® antigens: fHbp, NadA and PorA; (vi) hSBA GMTs at 13 months of chronological age (4-6 weeks post booster) for relevant Bexsero® antigens: fHbp, NadA and PorA; (vii) hSBA proportions $\geq 1:4$, at 13 months of age (post booster) for relevant Bexsero® antigens: fHbp, NadA and PorA.
Sources of funding:	Meningitis Now GSK
Anticipated start date:	June 2017
Anticipated primary completion date:	March 2020
Sponsor	St George's, University of London
Contact names	Sponsor Contact: Debbie Rolfe Tel: [REDACTED] Fax: [REDACTED] Email: [REDACTED] Chief Investigator: Professor Paul Heath Professor Paediatric Infectious Diseases St George's, University of London



4. Primary Objectives

To compare the immunological responses of infants born preterm to Bexsero® after two primary doses given at 2 and 4 months chronological age with three doses given at 2, 3 and 4 months chronological age.

5. Secondary Objectives

- To describe the safety of routine vaccines (including Bexsero®) in premature infants;
- To compare the persistence of immunological responses to Bexsero® at 12 months chronological age after two doses at 2 and 4 months of age with three doses at 2, 3 and 4 months of age;
- To compare the immunological responses following the 12-month Bexsero® booster in babies who received two doses at 2 and 4 months of age with those who received three doses at 2, 3 and 4 months of age.

6. Background

Study disease

Neisseria meningitidis (meningococcus) is a bacterium which causes meningitis and septicaemia. There are 13 serogroups of meningococcus, 6 (A, B, C, W, X and Y) cause most disease of which Men B is the most common cause of meningococcal disease in the UK. Babies are the most frequently affected group, with the main burden of disease falling in the second 6 months of life. In 2015 the UK became the first country in the world to introduce Men B vaccination into its routine schedule, with a two-dose priming regime with a booster at 12 months. A recent paper has shown this schedule to be highly effective in preventing Men B disease in infants (Parikh et al, 2016).

Premature delivery affects a significant minority of infants: around 7% in the UK and around 10% globally. These infants are more at risk from vaccine-preventable diseases and it is therefore very important that they are adequately vaccinated. Premature infants are vaccinated according to their chronological age (see table 1 for current vaccination schedule), but this may provide them with less protection than their term counterparts. There is existing evidence that preterm infants have a lower response to a range of routine vaccines including Hib (Heath et al, 2003), pneumococcal conjugates (Ruggeberg et al, 2005), Hepatitis B (Losonsky et al, 1999), and DTP (Robinson et al, 2004), and that the response to pneumococcal vaccination in premature infants is dependent on the primary schedule they receive (Kent et al, 2016). There are currently no data available on the response of preterm infants to the Bexsero® vaccine.

The vaccine schedule changed in August 2017 from a 5-in-1 to a 6-in-1 vaccine being given at each of the primary vaccinations. All babies born on or after the 1st August 2017 will receive the 6-in-1 vaccine.



Vaccine	Product names	Age (months)			
		2	3	4	12
DTaP/IPV/Hib/Hep B	Infanrix Hexa®	✓	✓	✓	
Rotavirus	Rotarix®	✓	✓		
MenB	Bexsero®	✓		✓	✓
PCV13	Prevenar13®	✓		✓	✓
MCC-TT/Hib-TT	Menitorix®				✓
MMR	Priorix®				✓

Table 1: UK infant vaccination schedule (from August 2017)

Investigational Medicinal Product (IMP)

- Bexsero® (Manufactured by Glaxo Smith Kline in Italy). (MenB): a four component vaccine that protects against *Neisseria meningitidis* serogroup B

Non-investigational Medicinal Products (NIMPs)

- Infanrix IPV/Hib® (DTaP/IPV/Hib): a 5-in-1 combination vaccine that protects against diphtheria, tetanus, pertussis, polio and *Haemophilus influenzae* serotype b (Hib)
- Pediacel®(DTaP/IPV/Hib): a 5-in-1 combination vaccine that protects against diphtheria, tetanus, pertussis, polio and *Haemophilus influenzae* serotype b (Hib)
- Infanrix hexa®(DTaP/IPV/Hib/Hep B): a 6-in-1 combination vaccine that protects against diphtheria, tetanus, pertussis, polio, *Haemophilus influenzae* serotype b (Hib) and hepatitis B.
- Prevenar 13® (PCV 13): a 13-valent pneumococcal conjugate vaccine that protects against 13 different pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F)
- Rotarix®: an oral vaccine containing live attenuated rotavirus that protects against rotavirus



- **Menitorix® (MCC-TT/Hib-TT):** a combined *Haemophilus influenzae* serotype b (Hib) and meningococcal serogroup C conjugate vaccine where the capsular polysaccharides of both organisms are covalently linked to tetanus toxoid carriers
- **Priorix® (MMR):** a live attenuated vaccine that protects against measles, mumps and rubella

All the above vaccines are licensed for routine childhood immunisation in the UK. The Summary of medical Product Characteristics (SmPC) for each of the vaccines will be included in the Investigator Site File (ISF) and the Trial Master File (TMF).

Study Rationale and risk/benefit analysis

This study is being undertaken to compare the immune responses of preterm infants who are vaccinated according to two different schedules. There is evidence that preterm infants respond less well to vaccinations than their term counterparts for some vaccines and this has not previously been studied for Bexsero®. The clinical trial will only use vaccines that have been licensed for routine childhood immunisation in the UK. One group will receive vaccinations according to the UK schedule and the other will receive vaccinations according to the original licence. Because the infants enrolled in the study would have received the same vaccines as part of their routine immunisation, this study is unlikely to be associated with any added risk of adverse events. Blood sampling will be performed by appropriately trained members of the research team and the volume of blood obtained at each visit will not exceed 1% of the infant's circulating blood volume, based on an estimated total circulating blood volume value for preterm infants of 90 mL/kg.

Assessment & management of potential risk

There is always a small risk of an adverse reaction following vaccination and this will be explained to parents, but the per dose risk will not be greater for infants in this study compared to those who receive routine care. The Bexsero® vaccine is particularly associated with fever following vaccination and parents will receive advice about this.

Vaccines will be given by experienced medical or nursing staff who are trained in how to manage an allergic reaction following vaccination and who will have with them emergency equipment according to their local SOPs.

The parents/legal guardians of the study infants will be asked to record in a diary card any symptoms or illness occurring up to 7 days after the Bexsero® vaccine is administered. This will include local reactions, general reactions and episodes of fever. Parents/legal guardians will also be provided with a 24-hour telephone number to contact in case of any queries or concerns and will be asked to contact the study team in the event of serious illness or illness requiring hospitalisation which will allow early identification of serious adverse events. In addition all illnesses will be enquired about at each study visit so that all adverse events, which are not



solicited in the diary card, can be recorded. Solicited adverse events recorded in the diary card (e.g. fever in the 7 days post vaccination, redness at the vaccine site etc.) do not need to be recorded in the adverse events log. Information about all SAEs occurring at any time within the study period (i.e. after consent has been taken) will be collected.

According to the MRC/DH/MHRA Joint Project on Risk-adapted Approaches to the Management of Clinical Trials of IMPs (October 2011), this trial is categorised as Type A (Risk no higher than that of standard medical care). Bexsero will be dispensed in accordance with a prescription given by an authorised healthcare professional and will be labelled in accordance with the requirements of Schedule 5 to the Medicines for Human Use (Marketing Authorisations Etc) Regulations 1994 (b) that apply in relation to dispensed relevant medicinal products. A Sponsor approved prescription template will be provided to participating sites to prompt appropriate documentation in the Pharmacy Site file.

7. Trial design

7.1 Overall design

This will be an open label, phase IV study. After appropriate consent, 132 premature infants born at <35 weeks gestation (i.e. up to 34 weeks and 6 days), 50% <30 weeks gestation (i.e. up to 29 weeks and 6 days) will be randomised to 1 of 2 4CMen B schedules (Table 1). Babies will remain in the study for around 12 months, from recruitment to 13 months of age. All visits can be performed at the participant's home or in clinic, depending on the preference of the parents and study team.

Blood samples will be obtained at 5 months of age (post primary sample), 12 months (persistence sample) and 13 months (post booster sample). Reactogenicity and safety will be assessed by caregiver completion of a 7-day diary after each vaccine dose. Inpatients will be monitored for cardiorespiratory events for 72 hours after vaccination by healthcare staff and this information will be collected on the CRF. This will include details of oxygen saturations, heart rate, respiratory rate and details of any episodes of desaturation, bradycardia or apnoea. If an infant is medically fit for discharge prior to the end of this 72 hours of observation the decision to discharge must be discussed with the PI, but will not be considered a protocol deviation. This decision must be documented on the appropriate CRF. Particular emphasis will be placed on rates, timing and intensity of fever and other adverse reactions in the first 24 hours after vaccination, because this remains a cause of great concern amongst neonatologists. Parents will be advised to give three doses of paracetamol after receiving Bexsero®, according to UK guidance unless the infant is an in-patient in which case local policy will be followed. If participants are outpatients at the time of Bexsero administration their parents will be provided with information about paracetamol administration following Men B vaccination and if the infant was born at less than 32 weeks an appropriate dose of paracetamol will be calculated by the study team and communicated to the parents. Administration of paracetamol will be systematically recorded on the diary card including number of doses given and whether given



prophylactically or in response to a fever.

Table 2. Timing of vaccinations and blood sampling

Visit Number	1	2	3	4	5	6
Age	2 months 49-84 days	3 months 28-42 days after V1	4 months 28-42 days after V2	5 months 28-42 days after V3	12 months 364- 393 days	13 months 28-42 days after V5
Group 1	Bexsero® RV	RV	Bexsero® RV	*	Bexsero® RV *	*
Group 2	Bexsero® RV	Bexsero® RV	Bexsero® RV	*	Bexsero® RV *	*

RV=Routine vaccines

*= Blood sampling

Ages of scheduled vaccination refer to chronological ages.



7.2 Schematic of trial design

Study procedures

Study procedures	Screening	2 months V1	3 months V2	4 months V3	5 months V4	12 months V5	13 months V6
Discuss study with parent/guardian	✓						
Check eligibility criteria	✓						
Arrange first appointment	✓						
Reconfirm eligibility		✓					
Informed Consent	✓ (Can be done at this visit)	✓					
Randomisation		✓					
Medical history		✓					
Record details of physical examination		✓					
Check for concomitant medications		✓	✓	✓	✓	✓	✓
Vaccine administration		✓	✓	✓		✓	
Post vaccination monitoring		✓	✓	✓		✓	
Ask about AEs/SAEs			✓	✓	✓	✓	✓
Blood sampling					✓	✓	✓
Distribution of diary card		✓	✓	✓		✓	
Checking diary card with parents			✓	✓	✓		✓



8. IMP Dosage regimen and rationale

Bexsero* (manufactured by Glaxo Smith Kline) is a four component vaccine that protects against *Neisseria meningitidis* serogroup B. It is indicated for use from 2 months of age and will be given according to the UK schedule at 2, 4 and 12 months or at 2, 3, 4 and 12 months for those babies randomised to receive a 3+1 regime.

8.1 Source of IMP and NIMPs

All vaccines will be obtained by local sites from national stock using the ImmForm system. Vaccines will be received by hospital clinical trials pharmacist or research teams as decided by individual sites and will be placed into quarantine until documentation of the cold chain had been reviewed to confirm that they may be released for use.

Vaccines used in this study will be labelled in accordance with the Medicines (Marketing Authorisations etc.) Regulations 1994 as amended and supplied by a pharmacy department without any need of special labelling.

8.2 Accountability procedures for the IMP and NIMPs

The research or hospital pharmacy department will be responsible for maintaining and updating the IMP/NIMP Accountability Log, filed in the pharmacy site file. IMP and NIMP(s) destruction will be conducted, once agreed by the Sponsor, in accordance to local pharmacy practice and will be documented on the Destruction Log filed in the pharmacy site file. All used/unused IMP/NIMP(s) must be returned to the trial pharmacist. Alternative arrangements relating to the accountability procedures for the IMP and NIMPs are to be agreed in writing with the Sponsor in advance of their implementation.

8.3 Post-trial IMP arrangements

Bexsero* is already part of the infant vaccine schedule in the UK and so no additional arrangements are required.

8.4 Name and description of each non-IMP (NIMP)

The following vaccines will be used as part of the study. All vaccines will be identified clearly with a participant number when they have been issued.

In babies born before 1st August 2017 **Infanrix IPV+Hib*** (manufactured by GSK) contains diphtheria toxoid, tetanus toxoid, three pertussis antigens (pertussis toxin, filamentous haemagglutinin and pertactin), three inactivated poliovirus strains and *Haemophilus influenzae* type b polysaccharide. This vaccine is indicated for use in infants from the age of 2 months and will be given according to the UK schedule of vaccination at 2, 3 and 4 months. **Infanrix IPV+Hib*** has been available to use in the UK since June 2014 at which time



it had been introduced alongside Pediacel® the vaccine which had originally been chosen to replace the whole cell pertussis vaccine. The routine vaccine programme changed from a 5-in-1 to a 6-in-1 vaccine on 1st August 2017 so the 5-in-1 vaccine will be given only to babies born before this date.

In babies born before 1st August 2017 Pediacel® (manufactured by Sanofi Pasteur) contains diphtheria toxoid, tetanus toxoid, five pertussis antigens (pertussis toxin, filamentous haemagglutinin, pertactin, fimbriae 2 and 3), three inactivated poliovirus strains, and *Haemophilus influenzae* type b polysaccharide. This vaccine is indicated from the age of 6 weeks and will be given according to the UK schedule of vaccination at 2, 3 and 4 months. Pediacel will be used interchangeably with Infanrix IPV/Hib according to availability in national stock. The routine vaccine programme changed from a 5-in-1 to a 6-in-1 vaccine on 1st August 2017 so the 5-in-1 vaccine will be given only to babies born before this date.

In babies born on or after 1st August 2017 Infanrix-hexa® (manufactured by GSK) contains diphtheria toxoid, tetanus toxoid, three pertussis antigens (pertussis toxin, filamentous haemagglutinin and pertactin), three inactivated poliovirus strains, *Haemophilus influenzae* type b polysaccharide and hepatitis B virus surface antigen recombinant. This vaccine is indicated for use in infants from the age of 2 months and will be given according to the UK schedule of vaccination at 2, 3 and 4 months to all babies born on or after 1st August 2017.

Prevenar 13® (manufactured by Pfizer) contains 13 pneumococcal serotypes and has been used in the UK infant schedule since 2010. The vaccine is indicated for use from 6 weeks of age and will be given according to the UK schedule at 2, 4 and 12 months.

Rotarix® (manufactured by GSK) is an oral vaccine which contains live, attenuated human rotavirus and has been used in the UK schedule since July 2013. It is indicated for use from 6 to 24 weeks of age and will be given according to the UK schedule at 2 and 3 months.

Menitorix® (manufactured by GSK) is a combined *Haemophilus influenzae* serotype b (Hib) and meningococcal serogroup C conjugate vaccine. It is indicated for use from 2 months to 2 years of age and will be given at 12 months according to the UK schedule.

Priorix® (manufactured by GSK) is a live attenuated vaccine that protects against measles, mumps and rubella. This is indicated for use from 9 months of age and will be given at 12 months according to the UK schedule.

8.5 Concomitant treatment

Receipt of immunoglobulin or corticosteroids during the neonatal course will not be an exclusion criterion. Medications of interest will be recorded at each visit on a concomitant medication form. These will include antibiotics, non-study vaccines, immunosuppressant medication and Palivizumab. Both medications currently being taken and a history of medication use since the previous visit will be recorded.



9 Participant Selection criteria

There will be no exceptions (waivers) to eligibility criteria prior to participant inclusion into the study. Any questions raised about eligibility should be addressed prior to inclusion.

The eligibility criteria have been carefully considered and are chosen to ensure both the safety of the participants and that the trial results can be appropriately used to make future decisions for babies born prematurely as a general group. It is therefore vital that exceptions are not made to the selection criteria. Deviations from the eligibility criteria are considered to be protocol violations and may be reported to the MHRA as a serious breach.

All participants that are screened for inclusion into the study must be entered onto the Sponsor screening log JREOLOG0001 and will be assigned a sequential number. Participants will be considered eligible for enrolment into this trial if they fulfil all of the inclusion criteria and none of the exclusion criteria as defined below.

Eligible participants will be entered onto the Sponsors Subject ID log JREOLOG0002 and assigned a Trial specific Identification number in a pre-agreed format in accordance with Site identifier and next sequential numerical value e.g. SG001

9.1 Inclusion criteria

- Premature infant born at <35 weeks gestation
- No contraindications to vaccination according to the 'Green Book'
- Willing and able to comply with study procedures
- Written informed consent

9.2 Exclusion criteria

- Contraindication to vaccination according to the Green Book
- Life-limiting congenital abnormality or condition
- Prior diagnosis of an immunodeficiency syndrome
- Considered unlikely to complete expected follow up until the end of the study
- Child in care

10 Subject/Patient Recruitment process

Patient recruitment at a site will only commence once evidence of the following are in place:

1. REC approval, MHRA Confirmation of Trial notification, Health Research Authority (HRA) approval
2. Signed Delegation of Duties and Sponsorship Agreement returned to the JREO
3. Final approval of the Sponsor (which may include evidence of local Pharmacy Green light) issued by Sponsor representative of the JREO
4. Confirmation of Capacity and Capability issued by the local R&D department,

5. The trial initiation procedure completed and the issue of the 'Open to recruitment' letter by the JREO

All sites participating in the trial will also be asked to provide a copy of the following:

1. Signed Clinical Trial Site Agreement (CTSA) or HRA Statement of Activities,
2. Confirmation of Capacity and Capability issued by the local R&D department

All potential participants whose parents wish them to enter the study will be fully screened and consented by the Principal Investigator, or one of the qualified clinicians involved in the study as Clinical Co-investigators. These may be medical or nursing staff.

132 participants will be recruited from suitable participating sites.

A variety of recruitment strategies will be used to recruit eligible participants. If babies are still on the neonatal unit the clinical team will identify potentially eligible babies and will check that families of these infants are happy to meet the research team to discuss the study. Parents will then be approached to discuss the study and be offered the chance of participation. There will be posters and leaflets about the study on the neonatal unit, in neonatal clinic waiting rooms and in the hospital more generally. Some units may choose to advertise the study on institutional websites or newsletters, or on posters in the community (e.g. in GP practices). Parents of recently discharged infants may be contacted by letter, email or phone informing them of the study. In this instance the information packs will be provided by the research team, but the patient details will be accessed by the direct care team and the packs despatched by them. We will have a twitter account to allow parents to follow the progress of the study, although this will not be used explicitly for recruitment.

11 Study procedures

11.1 Informed consent

Please note, it is essential that all trial personnel/staff undertaking the informed consent process have signed the Sponsor's Delegation of Responsibilities Log JREOLOG0004 to ensure that the person has been delegated the responsibility by the study CI/PI. All personnel taking informed consent must be GCP trained. Refer to Sponsor SOP JREOSOP0027

Informed consent from a parent or guardian must be obtained following explanation of the aims, methods, benefits and potential hazards of the trial and before any trial-specific procedures are performed. The only procedures that may be performed in advance of written informed consent being taken are those that would have been performed on all participants in the same situation as routine clinical practice. Consent will be re-sought for babies whose legal guardian has changed.

Consent can be taken by any member of the medical or nursing staff who is in possession of an up to date GCP qualification and who has been trained on the specifics of this study. Informed consent will be taken after the parents have had sufficient opportunity to read the information and ask questions about the study. There is no time limit on the period between receiving information and consenting to the study providing that the parents feel they have had all their questions answered. Screening for eligibility and informed consent can take place at the initial contact with parents if they feel they have had sufficient time to consider the study, or can take place at the first visit (V1).

The Investigator or designee will explain that the parents are under no obligation to have their infant enter the trial and that they can withdraw their consent at any time during the trial, without having to give a reason.

A copy of the signed Informed Consent Form (ICF) along with a copy of the most recent approved Parent Information Sheet (PIS) will be given to the parent/guardian of the study participant. The original signed and dated consent form will be retained in the site file, and a copy will be placed in the medical notes along with the corresponding version of the PIS.

If new safety information results in significant changes to the risk-benefit assessment, the consent form will be reviewed and updated if necessary. Parents/guardians of all participants, including of those already enrolled in the study, will be informed of the relevant new information, given a copy of the revised consent form, and asked to re-consent if they choose to continue in the study.

11.2 Randomisation procedure

Two computerised block randomisation lists (one for gestational age <30 weeks and one for 30-34+6 weeks) will be produced by the statistician at Public Health England for each site. These will be placed inside opaque envelopes bearing the corresponding participant number by a team of staff from SGUL who are not otherwise involved in the study. Each centre will be provided with the necessary envelopes. On recruitment to the study, each participant will be allocated, in order of inclusion, the next available participant number from the appropriate list depending on gestation. Following informed consent and prior to the first vaccinations the local study team will open the envelope and reveal the group number. This will define the group to which the baby is assigned and, therefore, the vaccination schedule the baby will receive. **Please note- randomisation must take place at the first visit even if consent has been obtained previously.**

Participants may be replaced if they are withdrawn from the study prior to receiving their first vaccinations. Participants will not be replaced if they are withdrawn from the study after they have received their first vaccinations.



11.3 Prescribing and Dispensing of IMP

A study specific prescription chart will be used for this study. This will be provided by the lead site, or individual sites may choose to use their own prescription charts and/or electronic prescribing system. For those participants who are inpatients, the vaccines will also be prescribed on their hospital prescription chart. The person prescribing the vaccines on the study specific prescription chart must have been signed off by the PI on the Staff delegation of duties log JREOLOG0004 for that task, however any member of the clinical team who normally prescribes vaccines may transcribe on the hospital prescription chart without being on the delegation log. A sample signature of all delegated prescribers must be provided for the Pharmacy Site File prior to receipt of the 1st trial prescription.

Vaccines will be obtained by individual sites from national stock using the ImmForm system or any other system used locally. Vaccines may be stored by sites in either a hospital or research pharmacy or in an appropriately monitored fridge in the clinical research site.

11.4 Overdose of Trial medication

In the event of an overdose of trial medication advice should be sought immediately from the PI in the institution or from the CI.

11.5 Discontinuation/withdrawal of participants and stopping rules

Because this study only involves licensed vaccines that would be routinely offered to premature infants as part of the national childhood immunisation programme, we do not foresee any reason for stopping the study prematurely. Parents/legal guardians may withdraw their infants from the study at any time without offering an explanation. Exceptionally, the local PI may also withdraw an individual participant if there are significant safety concerns regarding ongoing participation, though it is expected that as far possible there should have been prior discussion with the CI. This study is powered to allow for ~10% drop out of subjects over the course of the study and withdrawn subjects will not be replaced. If an infant is withdrawn from the study permission will be sought to contact the family by phone to ask about any adverse events 1 month after the final study visit in which IMP is administered, and the parent/guardian will also be asked if they will agree to contact the local research team should the infant have an illness requiring medical review.

At all stages it will be made clear to the parents that they remain free to withdraw their baby from the trial at any time without the need to provide any reason or explanation. Parents will be made aware that a decision to withdraw their baby will have no impact on any aspect of their baby's continuing care. If parents choose to withdraw their baby from trial participation, permission will be sought to complete data collection and use data up to the point of withdrawal from the trial.



11.6 Participant transfers

If a participant moves from the area every effort will be made to keep them within the study. If they move such a distance as to make continued follow up at their local trial site centre inappropriate, every effort should be made for them to be followed up at another Sponsor-approved trial centre. Written consent should be taken at the new centre and then a copy of the participant's CRF to date should be provided to the new centre. Original CRF paperwork will remain at the site responsible for completing that visit / data collection. Responsibility for the participant remains with the original consenting centre until the new consent process is complete at the new centre. Participants who are transferred between sites temporarily over study Visit window(s) will retain a copy of preceding CRF(s) together with original CRF(s) completed at the 'secondary' site. Should the participant be transferred back to the primary centre a copy of the CRFs completed at the new 'secondary' centre will be provided to complete the primary centre records.

11.7 Loss to follow up

Every effort will be made to maintain contact with participants. If unable to make contact after three consecutive phone calls or emails, the participant's GP will be contacted to check the contact details. Permission to allow this will be included within the consent form. If the details are incorrect then contact should be attempted on up to three further occasions using the correct details. If contact is not subsequently obtained despite three attempts a letter will be sent to the participant's home asking the parents to make contact with the study team. If there is no contact within 1 month of sending this letter the participant will be classified as lost to follow up.

11.8 Definition of the End of Trial

The end of trial for an individual infant is defined as being on completion of their final study visit (V6) schedule at age 13 months. The end of the trial overall will be defined as the time of final database lock following laboratory analysis of serum samples. The approving REC and the MHRA require notification of the end of trial within 90 days of its planned completion or within 15 days if the study is terminated early. The responsibility for submitting the Clinical Study Report will lie with the Chief Investigator and Sponsor. Refer to JREOSOP0015 and inform the JREO to facilitate assistance and compliance with requirements.

12. Study Assessments

12.1 Screening assessments

A standardised Case Report Form (CRF) will be completed after obtaining written consent which will check the infant's eligibility, medical history, concomitant medications and any contraindications to vaccination. A record of physical examination will be made. The intention of



this record of physical examination is to document that there are no clinical contraindications to vaccination and this may take a variety of forms: for participants who are still in-patients, recent documentation of physical examination and agreement from the clinical team that vaccination is appropriate is sufficient, for babies who were discharged at around 6-8 weeks, documentation of an examination at the time of discharge or evidence that the clinical team were happy for vaccination to take place (for example in discharge summary) is acceptable. For those discharged before 6 weeks of age in most cases the infant will have had the 6-8 week check performed by the GP and if this is the case a copy of this will suffice along with a check with the parents that they have no new concerns. If there is no evidence of a physical examination having taking place after the first few days of life an investigator trained to perform physical examinations on infants will perform a physical examination.

12.2 Baseline assessments

Baseline assessment will include collection of demographic information about the infant, contact details for the family, and for the GP. The temperature will be assessed using an axillary thermometer prior to administration of the vaccine. Vaccinations will not be given if the temperature is 38.0°C or above.

12.3 Treatment procedure

The study personnel will reconstitute and administer the vaccines according to the SmPC for each vaccine. The dose or route of administration will not be modified for any of the vaccines under any circumstances. Vaccines will be administered in hospital if the participant is still an in-patient. If vaccines are given in hospital these may be administered by a member of the clinical team who may not be on the delegation log but whom the PI is willing to take responsibility for. If this is the case the vaccines must be given according to the study specific instructions regarding anatomical locations for vaccination. A member of the research team should be present at the time of vaccination to assist in protocol compliance and to ensure that all other study tasks are performed. Following discharge, all vaccine doses administered to participants will be either in hospital or at home depending on family preference and local strategy. Before each dose is given, the infant's temperature will be checked. Vaccination will be deferred if the axillary temperature is $\geq 38.0^{\circ}\text{C}$, or if there is acute illness on the day of vaccination. Those infants who are still in hospital at the time of vaccination will have observations recorded in their CRF for a period of 24 hours prior to immunisation and for 72 hours after immunisation.

The vaccines will be administered by intramuscular injection into the antero-lateral thighs. These should be given according to the practice outline in The Green Book. If needles are provided with the vaccine these should be used, if needles are not provided, the person giving the vaccine should make a decision based on the size of the infant. It is recommended that a 25mm 23G (blue) or 25mm 25G (orange) needle should be used in most infants with the smaller 16mm 25G (orange) needle being more suitable for smaller preterm infants. For the 2, 3 and 4 month vaccinations, Bexsero® will be given in the left thigh, Infanrix IPV/Hib®/Pediace®/Infanrix hexa® will be given in the upper right thigh and Prevenar 13® will be given in the lower right thigh and Rotarix® will

BEAR MenB. JREO Ref: 16.0247

24

Version 2.1 02.08.18

IRAS reference 223226



be given orally. For the 12-month vaccination, Bexsero® will be given in the left thigh, Priorix® in the lower right thigh, Prevenar13® in the upper right thigh and Menitorix® in the right arm. The skin at the injection site should be stretched flat between the thumb and forefinger with the needle inserted at a 90° angle to the skin. Where more than one injection is to be given in the same limb, they will be administered at least 2.5 cm apart. Standard immunisation practices and appropriate precautions for any anaphylactic reaction will be followed. Subjects will be monitored in person for 20 minutes post-vaccination.

Immunisations given to infants participating in this study will still count for primary care target and remuneration purposes as though they had been given by the practice of the infant's General Practitioner (GP). GPs will be informed in writing of the participation of any of their participants in the study. A copy of the letter sent to the GP will be kept with the participant's identifiable study file. The research team will notify the GP of the vaccine details after every vaccination visit.

A diary card will be provided to parents at each vaccination visit to monitor for local and generalised reactions to Bexsero® and the other routine vaccinations. The information required will be clearly explained to the parents and the diary will be checked by research staff at the subsequent visits so that any necessary additional information can be sought.

Paracetamol administration following vaccination

Parents will be advised to give three doses of paracetamol following administration of Bexsero® at 4-6 hourly intervals according to UK guidance unless the infant is an in-patient in which case local policy will be followed. Parents may be provided with an information sheet about paracetamol following Bexsero®. If the infant was born at less than 32 weeks gestation an appropriate dose of paracetamol should be calculated and this should be clearly communicated to the parents along with a recommendation for 6-8 hourly administration. The paracetamol administration record in the pharmacy pack may be used for this purpose (Appendix 3). Administration of paracetamol will be systematically recorded on the diary card including number of doses given and whether given prophylactically or in response to a fever.

12.4 Subsequent assessments

Prior to administering subsequent doses of vaccines, the diary cards for each infant will be reviewed and the parents will be asked about any episodes of illness since the previous visit. Any adverse events will be recorded on the CRF and managed according to the protocol. Any concomitant medication of interest which is being taken or has been received since the last visit will also be recorded in the CRF. Peripheral temperature will then be measured and the vaccine will be administered after ensuring that the infant is fit for vaccination. Following administration of each set of vaccinations, infants will be observed in person for at least 20 minutes for any unexpected acute adverse events. Infants who are on a neonatal unit at the time of immunisation will have 24-hours pre-vaccination and 72-hours post-vaccination observations recorded in the CRF. AEs and SAEs will be solicited at each study visit and parents will be asked to contact the study team if the infant is seriously unwell or hospitalised between visits.



12.5 Summary flow chart of study assessments

Refer to Appendix 2

12.6 Methods

12.6.1 Samples

12.6.2 Obtaining, labelling, storing at local site

- a. Blood sampling will be postponed for at least 48 hours after the end of any course of antibiotics. If this is not possible, this will be recorded as a protocol deviation and a record should be taken of the antibiotic regime being received at the time of blood sampling.
- b. Blood sampling will be obtained by venepuncture where possible. Finger prick or heel prick may be attempted only as a last resort if venepuncture is not possible. Topical application of anaesthetic cream (EMLA or Ametop) and/or a milk feed or oral sucrose may be offered to all subjects aged 1 month corrected gestational age or above at the time of intended venepuncture site to minimise discomfort.
- c. Up to 3 mL of blood will be collected into a 5 mL serum separating tube.
- d. The research staff will clearly mark the tube with the participant's study number, visit number, and date of sampling. Dedicated study labelling stickers will facilitate this process

12.6.2.1 Transporting samples to SGUL

- a. Samples will be packaged in hard plastic containers within a cardboard box which will then be placed in Royal Mail packaging for guaranteed next day delivery. This will accord with regulations for posting of biological specimens through public post. The preference will be for samples to be sent on the same day they are obtained, however if this is not possible they should be stored in the fridge. Samples must be posted with consideration to ensure that they will arrive the next day on or between a Monday and a Friday (i.e. no samples to be sent on a Friday or Saturday).
- b. The samples will be transported at ambient temperature and will not need monitoring
- c. Before sending the sample local staff will email the coordinating team at SGUL to inform them that a sample is being despatched and the tracking number of the sample so that any missing samples can be traced.
- d. When samples arrive at SGUL, a standard email of receipt will be returned to the local site sending the sample

12.6.2.2 Processing samples at SGUL

- a. Samples will be centrifuged within 4 hours of being received by SGUL study staff and the serum will be pipetted into cryovials. The first 0.5 mL of serum will be placed in a cryovial for transfer to PHE laboratory, Manchester, and the remainder will be retained



at SGUL in case a sample is lost, or, subject to parental consent, to be used in future ethically-approved research. Parents will be asked specifically to consent for the retention of samples for such purposes. All cryovials will be immediately placed in the -80°C freezer.

- b. A log will be kept of samples being stored in this freezer

12.6.2.3 Transporting samples to PHE Manchester

- a. Samples will be sent in batches on dry ice by courier to the Public Health England laboratory in Manchester at various time points throughout the study.

12.6.3 Laboratory procedures

All laboratory testing for this study will be undertaken at the Public Health England laboratory in Manchester. Serum will be tested for functional antibody to the vaccine-containing antigens fHbp, NadA and PorA by Serum Bactericidal Assay (SBA) utilising human complement.

13 Pharmacovigilance

13.1 Definitions

Adverse Event (AE)—any untoward medical occurrence in a patient or clinical trial subject who is administered an IMP and which does not necessarily have a causal relationship with this treatment which may include an exacerbation of a pre-existing illness; increase in frequency or severity of pre-existing episodic condition; a condition (regardless of whether present prior to the start of the trial) that is detected after trial drug administration. (This does not include pre-existing conditions recorded as such at baseline).

Adverse Reaction (AR)—any untoward and unintended responses to an IMP related to any dose administered.

Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)—any Adverse Event or Reaction that at any dose:

- Results in death; or
- Is life-threatening (places the subject, in the view of the Investigator, at immediate risk of death)
- Requires hospitalisation or prolongation of existing hospitalisation (hospitalisation is defined as an inpatient admission, regardless of length of stay; even if it is a precautionary measure for observation; including hospitalisation for an elective procedure, for a pre-existing condition)
- Results in persistent or significant disability or incapacity (substantial disruption of one's ability to conduct normal life functions)



- Consists of a congenital anomaly or birth defect (in offspring of subjects or their parents taking the IMP regardless of time of diagnosis).
- Or is another important medical condition

Important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the outcomes listed in the definition of serious will also be considered serious.

Suspected Unexpected Serious Adverse Reaction (SUSAR)—an Adverse Reaction which is classed in nature as both serious and unexpected.

An 'Unexpected Adverse Reaction' is when both the nature and severity of the event is not consistent with the reference safety information available for the IMP in question.

13.2 Investigator responsibilities relating to safety reporting

All unsolicited adverse events which occur within 28 days of vaccination will be recorded on the CRF, as will all medically attended adverse events occurring at any time during the study i.e. until an individual infant has reached the end of the study. Unsolicited adverse events should also be recorded on the AE Log JREOLOG0007.

SAEs and SARs will be recorded throughout the study and must be notified to the Sponsor within 24 hours of the investigator becoming aware of the event. Refer to JREOSOP0006 and ensure the completed SAE report form JREODOC0012 is sent to the sponsor via fax on 020 8725 0794 or E-mailed to adverseevents@sgul.ac.uk.

In order to prevent a breach of patient confidentiality, SAE reports and any accompanying information should contain only anonymised or pseudo-anonymised patient data.

It is recognised that all information may not be available while the SAE report form is being completed, however the initial report must contain the following as a minimum:

- Identifiable event
- Identifiable patient
- Identifiable IMP (i.e. by providing batch number)
- Identifiable Reporter

If the information available is less than the specified minimum or if the SAE Form is not available for completion and reporting to meet the 24-hours deadline an initial report can be made verbally but must be followed within 48 hours by a detailed, written report.

All unforeseeable SAEs including SUSARs must be followed up by the PI/CI until satisfactory resolution and this should be recorded as a Follow Up report on the SAE form, and on the AE log. At each stage of follow up the PI/CI should sign and date the form.



The Sponsor will notify all SUSARs to the MHRA electronically and the REC utilising the eSUSAR system and within the required expedited reporting timescales:

- The Sponsor will inform the MHRA and the REC of fatal or life threatening SUSARs as soon as possible, but no later than 7 calendar days after the receipt of the SAE report form. Any additional information will be reported within 8 days of sending the initial report.
- The Sponsor must report all other SUSARs and safety issues to the MHRA and REC, as soon as possible but no later than 15 calendar days after the Sponsor has first knowledge of the minimum criteria for expedited reporting.

Causality Assessment—must be made by a medically qualified doctor as these decisions require medical and scientific judgment as well as knowledge of the participant concerned. The investigator must assess the causality of all SAEs or SARs in relation to the IMP using the following descriptions:

Definitely—there is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.

Probably—there is evidence to suggest a causal relationship, and the influence of other factors is unlikely.

Possibly—there is some evidence to suggest a causal relationship (e.g. the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (i.e. the patient's clinical condition, other concomitant events).

Unlikely—there is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, or other concomitant treatments).

Unrelated—there is no evidence of any causal relationship.

Not Assessable – note - if this description is used the sponsor will assume the event is related to the IMP until follow up information is received from the investigator to confirm a definitive causality assessment

Any SUSAR assessed as related to the IMP will need to be reported to the Sponsor irrespective of how long after IMP administration the reaction has occurred.

Expectedness should be based solely on the available RSI for the IMP and will be described using following categories:

Expected—an AE that is classed in nature as serious and which is consistent with the information about the IMP listed in the RSI or clearly defined in this protocol.

Unexpected—an AE that is classed in nature as serious and which is not consistent with the information about the IMP listed in the RSI



The completed AE Log JREOLOG0007 should be regularly reviewed and signed by the PI to detect any change in frequency or severity of reported events. The log will be sent to Sponsor upon request and/or every 2 months.

The Chief or Principal Investigator will respond to any SAE queries raised by the Sponsor as soon as possible. Follow up reports must continually be completed within acceptable time-frames and sent to the sponsor as detailed above until the reportable event is considered resolved.

All SAEs and SARs which occur at other sites will be notified to the Sponsor and to the CI within 24 hours. If there is safety information which needs to be disseminated relating to the study, the lead investigator at SGUL or a delegate will communicate with the PIs at the other sites. All SAEs will be communicated to GSK within 24 hours of Sponsor's first awareness of the event by the team at SGUL. Follow up information on safety reports will be provided within 24 hours of any request. Any emerging safety issues related to Bexsero and in connection with the study will be communicated to GSK within 48 hours of the Sponsor becoming aware of them.

13.3 Notification of deaths

All deaths will be reported to the Sponsor immediately upon first knowledge irrespective of whether the death is related to disease progression, the IMP, or an unrelated event.

13.4 Annual Progress Reports (APRs)

The Chief Investigator will prepare the APR in accordance with JREOSOP0043. Following review by the sponsor the report will be sent to the REC. The APR is due for submission annually within 30 days of the anniversary date on which the favourable opinion was given by the Ethics committee, until the trial is declared ended.

13.5 Reporting Urgent Safety Measures

The Sponsor and Investigator may take appropriate urgent safety measures in order to protect the participants against any immediate hazard to their health or safety. If such measures are taken the Sponsor shall immediately or no later than 3 days from the date the measures are taken give written notice to the MHRA and REC of the measures taken and the circumstances given rise to such measures. The CI must notify the Sponsor immediately to facilitate compliance with the regulations. The Sponsor will assist the CI in cascading relevant information to participating sites and relevant staff.

Refer to Sponsor SOP Management of Amendments JREOSOP0011 for guidance.

13.6 Notification of Serious Breaches of GCP and/or the protocol

Any Protocol Deviations, Violations will be documented using JREODOC0061, and entered onto the Sponsor's log JREOLOG0005. Potential Serious Breaches and Urgent Safety Measures will be



recorded both on the Sponsor's Log JREOLOG0005 and processed according to JREOSOP0012 and where necessary JREOSOP0032

A "serious breach" is a breach which is likely to effect to a significant degree:

- (a) The safety or physical or mental integrity of the participants of the trial; or
- (b) The scientific value of the trial.

The CI will notify the Sponsor immediately of any case where there exists a possible occurrence of a serious breach

14 Data management and quality assurance

14.1 Confidentiality

All data will be handled in accordance with the Data Protection Act 1998.

Contact details will be collected onto a data capture format the local site. This will contain patient identifiable information which will allow the local team to remain in touch with participants during the study. These will not be sent from other sites to SGUL. All CRFs sent to SGUL will be identified by the participant's trial identification number only.

14.2 Data collection tool

Case Report Forms will be designed by the CI and the final version will be approved by the Sponsor. All data will be entered legibly in black ink with a ball-point pen. If the Investigator makes an error, it will be crossed through with a single line in such a way to ensure that the original entry can still be read. The correct entry will then be clearly inserted. The amendment will be initialled and dated by the person making the correction immediately. Overwriting or use of correction fluid will not be permitted.

It is the Investigator's responsibility to ensure the accuracy of all data entered and recorded in the CRFs. The Staff Delegation of Responsibilities Log JREOLOG0004 will identify all trial personnel responsible for data collection, entry, handling and managing the database.

Data will be recorded directly onto specially designed CRFs for the study. CRFs will be photocopied by individual sites and the photocopies sent to SGUL for entering into the study database. Originals will be kept by the individual sites.

14.3 Data handling and analysis

A secure password-protected database will be designed and set up using REDCAP at SGUL. Copies of CRFs will be transferred from sites to SGUL by post or by email (BEARMenB@sgul.ac.uk) and identifiable patient information will not be transferred. Data from diary cards and CRFs will be

BEAR MenB, JREQ Ref: 16.0247

31

Version 2.1 02.08.18

IRAS reference 223226



entered into the database and checked by different members of the study team. Any queries will be resolved and corrected in the database by referring to the original documentation. Self-evident corrections will be amended by the team at SGUL without needing to contact the sites. If the original documentation is unclear clarification will be sought from sites. All paperwork relating to the study will be stored in secure locked cupboards/rooms in the sites participating in the study. At individual sites the CRFs will be contained within a plastic wallet in the patient files. No identifiable data will be transferred to SGUL. Access to the trials database will be restricted to the study team at SGUL and PIs from other sites if requested. Data analysis will be performed by the Clinical Trials Statistician at PHE Colindale. The Chief Investigator will have full access to the CRFs / diaries and trial database if required and PIs from other sites may access this information following a request to the CI. Any digital data transferred from the sites to SGUL will be documented at source and be fully anonymised and encrypted prior to transfer in strict accordance with the 1998 Data Protection Act as well as SGUL Information Governance Policies.

15 Archiving arrangements

The trial essential documents along with the trial database will be archived in accordance with the sponsor SOP JREOSOP0016. The agreed archiving period for this trial will be defined within the Delegation of Duties Sponsorship Agreement JREODOC0013

Each PI at any participating site will make arrangements for archiving of the trial essential documents generated at the site for the agreed archiving period in accordance with the signed Clinical Trial Site agreement

16 Statistical design

16.1 Statistical input in trial design

A formal statistical plan will be produced by the Trial Statistician prior to commencement of the study. Data will be analysed using a modified intention to treat analysis whereby all infants vaccinated and with at least one post vaccination blood sample taken will be included in the analysis. Per protocol analysis (whereby all vaccinated infants with at least one post vaccination blood sample taken and with no major protocol deviations) may also be performed if major protocol deviations are recorded. Infants with missing results (e.g. unable to obtain or insufficient blood sample) at the different time-points (e.g. post-primary immunisation, pre-booster and post-booster) will not be included in the analysis for that particular time-point). The primary objective of the study is to compare the hSBA GMT for Bexsero® antigens fHbp, NadA and PorA 1 month after completing the primary vaccinations according to two different schedules.



16.2 Endpoints

16.2.1 Primary endpoints

1. hSBA GMT one month after completing primary immunisations for relevant Bexsero® antigens: fHbp, NadA and PorA
2. hSBA proportions $\geq 1:4$, one month after completing primary immunisations for relevant Bexsero® antigens: fHbp, NadA and PorA.

16.2.2 Secondary endpoints

1. The percentage of infants experiencing fever, local reactions and non-febrile systemic reactions within the 7 days following each vaccine dose;
2. The percentage of inpatients experiencing change / deterioration in cardiorespiratory status within the 72 hours following each vaccine dose;
3. The percentage of infants investigated for sepsis and commenced on antibiotics within 7 days of vaccination;
4. The percentage of infants who experience fever and/or are investigated for sepsis and commenced on antibiotics within 28 days of vaccination;
5. The percentage of infants who experience a serious adverse event at any point within the study;
6. hSBA GMTs at 12 months of age (pre booster) for relevant Bexsero® antigens: fHbp, NadA and PorA;
7. hSBA proportions $\geq 1:4$, at 12 months of age (pre booster) for relevant Bexsero® antigens: fHbp, NadA and PorA;
8. hSBA GMTs at 13 months of age (post booster) for relevant Bexsero® antigens: fHbp, NadA and PorA;
9. hSBA proportions $\geq 1:4$, at 13 months of age (post booster) for relevant Bexsero® antigens: fHbp, NadA and PorA.

16.3 Sample size and recruitment

16.3.1 Sample size calculation

Sample size calculations are complicated because of the multiple components in Bexsero®. We will assess the immune responses to 3 antigens. We propose that a comparison of 2 schedules with respect to doses of Bexsero® will require 60 infants in each arm. Based on data from a



recent study (Gosger et al. 2012) the standard deviation of the GMT responses to the 3 antigens is expected to be around 1.0 log₁₀ units. Therefore, a sample size of 60 per group will allow a 1.7 fold difference between groups to be detectable at 80% power with 5% significance. Based on our experience from the PUNS (Premis Under a New Schedule) study, we expect a drop-out rate of around 10% so aim to recruit a total of 132 infants.

16.3.2 Planned recruitment rate

It is hoped recruitment to the study will be completed in a 6-12 month time frame. However, as with all paediatric studies, the recruitment rate depends on parental attitude and is largely unpredictable.

16.4 Summary of baseline data and flow of patients

At the end of the study, a flowchart will be used to summarise the number of infants approached, consented, recruited, assigned to the different study arms, receiving the intended vaccines, completing the study protocol and analysed for the primary outcome, as recommended by the CONSORT statement (<http://www.consort-statement.org/>). Baseline data comparing the two trial arms will be summarised in a table format and will compare median gestation age at birth, age at first vaccination, gender, recruiting study site, birth weight, underlying medical conditions, ventilation status, oxygen requirement, and receipt of immunoglobulins, blood transfusions, steroids, and other relevant concomitant medications or vaccines.

16.4.1 Primary endpoint analysis

For each schedule geometric mean titres (GMTs) of the hSBA titres to the three antigens (fHbp, NadA and PorA) will be calculated with 95% confidence intervals 1 month post primary vaccination. Schedules will be compared using unpaired t-tests on log-transformed titres or the Kruskal Wallis test if log-titres are not normally distributed. Proportions with titres $\geq 1:4$ will also be calculated with exact binomial 95% confidence intervals and compared between groups by Fisher's exact test. Significance will be at a 5% level with no adjustment for multiple comparisons since whilst there are three antigens there are only two groups being compared.

Analysis will be by modified intention to treat (mITT), meaning individuals are analysed according to the group they are randomised to, but only those with at least one blood taken and antibody result will be included, with missing data (e.g. from withdrawals, non-compliers) assumed missing at random. The mITT analysis will include blood samples taken outside the recommended timing. A per-protocol analysis will also be performed if there are major protocol deviations. Spurious data will be checked to source records and investigated, but included if no cause is identified.

The main subgroup analysis will be stratification by level of prematurity (<30weeks/ 30⁺⁰⁻³⁴+6 weeks). A multivariable normal errors regression on logged titres will be used to investigate the influence of prematurity, receipt of blood products or steroids, gestational age, gender, and birthweight.



16.4.2 Secondary endpoint analysis

Analysis of titres before and after the booster vaccination will be done in the same way as the analysis after primary vaccination. In addition, the geometric mean fold change between time points will be calculated with 95% confidence intervals.

For the safety end-points analysis will be As Treated (according to vaccine received) with proportions having each safety endpoint calculated with 95% exact confidence intervals. These proportions will be calculated combining across the two schedules except for when MenB is not given to group 1. Proportions will be according to the most severe level of the adverse event reached across the 72-hour, 7-day or 28 day follow up after each dose and will be by levels of any and severe as indicated on the diary card.

16.4.3 Randomisation

Two computerised block randomisation lists (one for gestational age <30 weeks and one for 30-34+6 weeks) will be produced by the statistician at Public Health England. The appropriate group number will be placed inside an opaque envelope bearing the corresponding participant number by a team of staff from SGUL who are not otherwise involved in the study. Each centre will be allocated blocks of sequential numbers in accordance with the block size used for randomisation and will be provided with the necessary envelopes. On recruitment to the study at Visit 1, each participant will be allocated, in order of inclusion, the next available participant number from the appropriate list depending on gestation. The local study team will then open the envelope and reveal the group number. This will define the group to which the baby is assigned and, therefore, the vaccination schedule the baby will receive. The randomisation procedure must take place at the first visit.

16.5 Interim analysis

As all vaccines used in this clinical trial are licensed for routine use in infants, interim analysis will not be performed. All data will be analysed at the overall end of the study.

17 Committees involved in the trial

A Trial Management Group (TMG) will be formed consisting of the CI, the study coordinator, the PIs and sub investigators at each site and the trial statistician. The TMG will supervise the trial on a day-to-day basis. The TMG meetings will be considered to be quorate if at least five members are able to attend. Information about the meetings will be circulated by the study coordinator or their delegate and the meetings will take place by teleconference at intervals of 3 months to discuss the progress of the study. The initial meeting will focus on the logistics of study set up and subsequent meetings will cover issues around recruitment, retention, protocol deviations and adverse events. Minutes will be taken by the study coordinator or their delegate and these will be distributed following the meeting. As all vaccines used within this study are part of routine care in the UK an Independent Data Monitoring Committee (IDMC) is not required.



18 Direct access to source data

The PI and their institutions will permit trial-related monitoring, audits, REC review, and regulatory inspection(s), providing direct access to source data/documents. Trial participants are informed of this during the informed consent discussion. Participants will consent to provide access to their medical notes.

19 Site approval and ongoing Regulatory compliance

Before any site can enrol patients into the trial, the Principal Investigator must ensure that written permission to proceed is in place and that confirmation of capacity and capability has been granted by that local Trust Research & Development (R&D) department. The site must conduct the trial in compliance with the protocol as agreed by the Sponsor and, by the regulatory authorities as appropriate, and which was given favourable opinion by the Research Ethics Committee (REC) and Health Research Authority (HRA)

The Chief Investigator will be provided (via the Sponsor) with file indexes i.e. JREODOC0003 TMF index and JREODOC0004 ISF index for use with SOP JREOSOP0019 'Preparation and Maintenance of the TMF' The CI will be responsible for the maintenance of the TMF and will delegate the responsibility of ISF file maintenance to the PI at each participating site.

It is the responsibility of the PI at each site to ensure that all subsequent REC-approved amendments gain the necessary local R&D approvals. Refer to JREOSOP0011 'Management of Amendments'. This does not affect the individual clinician's responsibility to take immediate action if thought necessary to protect the health and interest of individual patients.

Within 90 days after the overall end of the trial, the CI and Sponsor will ensure that the REC and the MHRA are notified that the trial has finished. If the trial is terminated prematurely, those reports will be made within 15 days after the end of the trial. Refer to JREOSOP0015 'End of study declaration'

The CI will supply an End of Study report of the clinical trial to the MHRA and REC within 1 year after the overall end of the trial. The Sponsor can provide JREODOC0059 End of study Report template. An End of Study report will be supplied to GSK and Meningitis Now within 60 days of study completion.

20 Monitoring plan for the trial

The CI will be requested to complete the JREODOC0032 Risk Assessment Questionnaire and forward to the Sponsor to facilitate appropriate costing and Sponsorship in Principle to be issued prior to REC application.



The trial will be monitored according to the risk-based monitoring plan JREODOC0030 agreed by the Sponsor. It is the responsibility of the CI to ensure that the Sponsor's self-monitoring template is completed and submitted as instructed (refer to the Study Monitoring Plan for detail). The JREO governance team will determine the initial project risk assessment and justify change as the study progresses.

The PI at each collaborating site in addition to permitting and facilitating site monitoring visits may also be required to complete self-monitoring form(s) and must return the form to the Sponsor for review and action. Failure for any PI to comply with reasonable requests on behalf of the Sponsor may be escalated in accordance with JREOSOP0031 Escalation Procedure; the site may also be selected for a GCP audit.

It is the Sponsor's responsibility to ensure that any findings identified in any monitoring report are actioned appropriately and in a timely manner and that any violations of GCP or the protocol are reported to the CI & Sponsor representative. Any serious breach will be handled according to JREOSOP0032 Serious Breach Reporting

Any urgent safety measures at either the CI or a PI site must be reported by that site Investigator within 3 days, as per UK Regulations.

The CI will be provided with a copy of the study monitoring plan during the Trial Initiation monitoring visit.

21 Finance

This study is funded by Meningitis Now and GSK.

22 Insurance and indemnity

St George's University of London holds insurance to cover participants for injury caused by their participation in the clinical trial. Participants may be able to claim compensation if they can prove that SGUL has been negligent. This includes negligence in the writing of the protocol, or selection of trial resources.

Where the Trial is conducted in a hospital, the hospital has a duty of care to participants. St George's University of London will not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. Hospitals selected to participate in this clinical trial shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to St George's University of London, upon request.



Participants may be able to claim compensation for injury caused by participation in this Trial without the need to prove negligence on the part of St George's University of London or another party.

If a participant indicates that they wish to make a claim for compensation, this needs to be brought to the attention of St George's University of London immediately.

Failure to alert St George's University of London without delay and to comply with requests for information by the Sponsor or any designated Agents may lead to a lack of insurance cover for the incident.

NHS bodies are liable for clinical negligence and other negligent harm to individuals covered by their duty of care. NHS Institutions employing researchers are liable for negligent harm caused by the design of studies they initiate.

23 IP and development policy

Unless otherwise specified in agreements, the following guidelines shall apply: All Intellectual Property Rights and Know How (IP) related to the Protocol and the Trial are and shall remain the property of the Sponsor excluding

- 1) pre-existing IP related to clinical procedures of any Hospital.
- 2) pre-existing IP related to analytical procedures of any external laboratory.

All contributors shall assign their rights in relation to all Intellectual Property Rights and in all Know How, not excluded above to the Sponsor and at the request and expense of the Sponsor, shall execute all such documents and do all such other acts as the Sponsor may reasonably require in order to vest fully and effectively all such Intellectual Property Rights and Know How in the Sponsor or its nominee.

All contributors shall promptly disclose to the Sponsor any Know How generated pursuant to this Protocol and not excluded above and undertake treat such Know How as confidential information jointly owned between it and the Sponsor

Nothing in this section shall be construed so as to prevent or hinder any medical professional from using Know How gained during the performance of the Trial in the furtherance of its normal business activities, to the extent such use does not result in the disclosure or misuse of Confidential Information or the infringement of any Intellectual Property Right of the Sponsor.



24 Publication policy

Publication: "Any activity that discloses, outside of the circle of trial investigators, any final or interim data or results of the Trial, or any details of the Trial methodology that have not been made public by the Sponsor including, for example, presentations at symposia, national or regional professional meetings, publications in journals, theses or dissertations."

All scientific contributors to the Trial have a responsibility to ensure that results of scientific interest arising from Trial are appropriately published and disseminated. The Sponsor has a firm commitment to publish the results of the Trial in a transparent and unbiased manner without consideration for commercial objectives.

To maximise the impact and scientific validity of the Trial, data shall be consolidated over the duration of the trial, reviewed internally among all investigators and not be submitted for publication prematurely. Lead in any publications arising from the Trial shall lie with the Sponsor in the first instance.

24.1 Before the official completion of the Trial,

All publications during this period are subject to permission by the Sponsor. If an investigator wishes to publish a sub-set of data without permission by the Sponsor during this period, the Trial Management Group/the Funders shall have the final say.

Exempt from this requirement are student theses that can be submitted for confidential evaluation but are subject to embargo for a period not shorter than the anticipated remaining duration of the trial.

24.2 Up to 180 days after the official completion of the Trial

During this period the Chief Investigator shall liaise with all investigators and strive to consolidate data and results and submit a manuscript for peer review with a view to publication in a reputable academic journal or similar outlet as the Main Publication.

The Authorship of the primary results manuscript ('Main Publication') arising will comprise all who have made a substantial intellectual contribution to the study (including the research question, design, analysis, interpretation), and so is expected to include all local PIs contributing to this study providing that they fulfil all four criteria of the ICMJE recommendations for authorship (www.icmje.org). The initial drafting of the paper will be the responsibility of the CI. All other contributors to the trial will be listed at the end of the manuscript, with their contribution identified and acknowledged.

- The Chief Investigator shall be senior and corresponding author of the Main Publication.
- Insofar as compatible with the policies of the publication outlet and good academic practice, the other Principal Investigators shall be listed in alphabetic order.

BEAR MenB, JREQ Ref: 16.0247

39

Version 2.1 02.08.18

IRAS reference 223226



- Providers of analytical or technical services shall be acknowledged, but will only be listed as co-authors if their services were provided in a non-routine manner as part of a scientific collaboration.
- Members of the Trial Management Group shall only be acknowledged as co-authors if they contributed in other capacities as well.
- If there are disagreements about the substance, content, style, conclusions, or author list of the Main Publication, the Chief Investigator shall ask the Trial Management Group to arbitrate.

24.3 Beyond 180 days after the official completion of the Trial

After the Main Publication or after 180 days from Trial end date any Investigator or group of investigators may prepare further publications. In order to ensure that the Sponsor will be able to make comments and suggestions where pertinent, material for public dissemination will be submitted to the Sponsor for review at least 60 days prior to submission for publication, public dissemination, or review by a publication committee. The Sponsor's reasonable comments shall be reflected. All publications related to the Trial shall credit the Chief and Co-Investigators as co-authors where this would be in accordance with normal academic practice and shall acknowledge the Sponsor and the Funders.

25 Statement of compliance

The trial will be conducted in compliance with the protocol, Sponsor's SOPs, GCP and the applicable regulatory requirements.

The study conduct shall comply with all relevant laws of the EU if directly applicable or of direct effect and all relevant laws and statutes of the UK country in which the study site is located including but not limited to, the Human Rights Act 1998, the Data Protection Act 1998, the Human Medicines Regulations 2012, the Medicines for Human Use (Clinical Trial) Regulations 2004, and with all relevant guidance relating to medicines and clinical studies from time to time in force including, but not limited to, the ICH GCP, the World Medical Association Declaration of Helsinki entitled 'Ethical Principles for Medical Research Involving Human Subjects' (2008 Version), the UK Policy Framework for Health and Social Care Research (Version 3.2, October 2017).

This study will be conducted in compliance with the protocol approved by the REC, HRA and according to GCP standards and UK Clinical Trials Regulation. No deviation from the protocol will be implemented without the prior review and approval of the Sponsor, HRA and REC except where it may be necessary to eliminate an immediate hazard to a research subject. In such case, the deviation will be reported to the Sponsor and REC as soon as possible.



26 List of Protocol appendices

Appendix 1 Protocol Amendment/Revision History (chronological order) or a statement "There are currently no amendments"

Appendix 2 Summary chart of study assessments

Appendix 3 Paracetamol administration record

27 References

Campbell H, Amirthalingam G, Andrews N et al. Accelerating control of pertussis in England and Wales. *Emerg Infect Dis* 2012; 18: 38-47.

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Losonsky GA, Wasserman SS, Stephens I, et al. Hepatitis B Vaccination of Premature Infants: A Reassessment of Current Recommendations for Delayed Immunization. *Pediatrics* 1999; 103:e14

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<http://webarchive.nationalarchives.gov.uk/20141205150130/http://www.mhra.gov.uk/home/groups/l-ctu/documents/websiteresources/con111784.pdf> (Accessed 12th February 2017)



Appendix 1

Protocol amendment /Revision History

Amendment ID/Protocol Version and Date	Justification for change/affected Protocol Sections
Amendment ID SAM01_AM04	Sponsor Representative changed
Amendment ID SAM02_AM05 Protocol Version 2.0, 21.03.18	Change of contact at central laboratory due to a change in staff. 2. Roles and responsibilities.
	Addition of 'child in care' to exclusion criteria at request from funder. 3. Study synopsis and 9.2 participant selection criteria.
	Clarification of new vaccine schedule because of a change in UK policy in August 2017. 6. Background and 8.4 Name and description of each non-IMP (NIMP).
	Change to adverse event recording so only those events which are not captured in the diary need to be recorded as adverse events and logged. This is to reduce the burden to sites of double reporting. 6. Background-assessment and management of potential risk. 13.2 Investigator responsibilities relating to safety reporting.
	Addition of wording clarifying the requirements for prescription of Bexsero. Clarifying existing practice. 6. Background-assessment and management of potential risk.
	Clarification of how alternative accountability arrangements are to be agreed with the Sponsor for clarity. 8.2 Accountability procedures for the IMPs and NIMPs.
	Addition of Palivizumab to concomitant medication of interest. 8.5 Concomitant medication.
	Addition of twitter account to allow parents to follow progress of study. 10. Subject/Patient Recruitment process.

BEAR MenB. JREO Ref: 16.0247

42

Version 2.1 02.08.18

IRAS reference 223226



	Clarification that randomisation must take place at first visit- no change to existing practice, just to reinforce. 11.2 Randomisation procedure and 16.4.3 Randomisation.
	Change to allow participants to be replaced if they are withdrawn from the study before receiving their first vaccination as no study procedure other than randomisation will have taken place. 11.2 Randomisation procedure.
	Change to allow hospital prescription of vaccines to be performed by a member of the clinical team not on the delegation log providing a member of the study team has prescribed the vaccine on the study prescription chart. This is to reduce obstacles in sites where the study team are not members of the inpatient clinical team and to ensure appropriate study and hospital records can be maintained. 11.3 Prescribing and Dispensing of IMP.
	Addition of information about the procedure which should be followed if a baby is temporarily transferred to another site (for example for surgical management). 11.6 Participant transfers.
	Clarification about the requirement to record physical examination in response to questions from sites. 12.1 Screening assessments.
	Change to allow inpatient administration of vaccine by a member of the clinical team who is not on the delegation log providing that a member of the study team is present and the PI is willing to take responsibility for the individual. This is to reduce obstacles in sites where the study team are not members of the inpatient clinical team. 12.3 Treatment procedure.
	Addition of more detail about administration of paracetamol following Bexsero to provide more information for sites. Inclusion of



	updated paracetamol administration record. 12.3 Treatment procedure and appendix 3.
	Change in amount of serum placed in cryovial to be sent to the central laboratory to 0.5mls in response to comments from the lab. 12.6.2.2 Processing samples at SGUL.
	Change to allow CRFs to be emailed as well as posted to facilitate the return of CRFs. 14.3 Data handling and analysis.
Amendment ID NSAM01_ Protocol Version 2.1, 02.08.18	Change of primary completion date to March 2020



Appendix 2. Summary chart of study assessments

Study Procedures	Screening	V1 2 months	V2 3 months	V3 4 months	V4 5 months	V5 12 months	V6 13 months
Informed consent	✓	✓					
Eligibility check	✓	✓					
Medical history		✓					
Concomitant medication		✓	✓	✓	✓	✓	✓
Record of physical examination		✓					
Randomisation		✓					
Dispensing of vaccines		✓	✓	✓		✓	
Administration of vaccinations		✓	✓	✓		✓	
Observation following vaccine administration		✓	✓	✓		✓	
Completion of red book and letter to GP		✓	✓	✓		✓	
Blood sampling					✓	✓	✓
Processing blood samples					✓	✓	✓
Distribution of diary card		✓	✓	✓		✓	
Checking diary card with parents			✓	✓	✓		✓
Checking for AEs/SAEs			✓	✓	✓	✓	✓



Appendix 3. Paracetamol administration record



BEAR MenB Trial

Paracetamol Administration Form (For babies born <32 weeks)

EudraCT Number: 2017-001487-38
Sponsor: St George's University of London
Investigator:

Patient Name: Subject Initials:
Hospital Number: Date of Birth:
Trial ID: Drug Allergies: Yes/No
(if yes, please specify:

	Visit 1	Visit 2	Visit 3
Most recent weight (kg)	kg	kg	kg
Dose = 15mg/kg every 8 hours (for 3 doses)			
Dose of paracetamol (mg)	mg	mg	mg
Amount of paracetamol (strength= 120mg/5ml) to be given (mls)	mls	mls	mls
Signature of study staff			
Name of study staff (print)			
Date of visit			
Signature that study staff have checked paracetamol to be used is in date, and strength 120mg/5ml			
Name of study staff (print)			

Document 2: BEAR Men B study parent information sheet



INSERT LOCAL HEADERS

Babies born Early Antibody Response to Men B Vaccination: BEAR Men B Parent Information Sheet

In 2015 the UK became the first country in the world to introduce the meningococcal group B (Men B) vaccine into its routine schedule for infants. This vaccine provides protection against meningitis (infection of the lining of the brain) and septicaemia (blood poisoning) caused by a subgroup (group B) of the meningococcus germ. In the UK babies are offered this vaccination at 2, 4 and 12 months.

We are working with Public Health England (PHE) to compare two schedules of the Men B vaccine in babies who were born prematurely. One group of babies will receive the Men B vaccine according to the current schedule and the other group will receive an additional dose at 3 months.

Why is this study being performed?

In the UK, babies receive their vaccinations according to a standard schedule, irrespective of their gestation at birth. This policy is designed so that all babies are protected as early as possible from vaccine preventable diseases such as polio, diphtheria, tetanus, rotavirus, pertussis (whooping cough), *Haemophilus influenzae* type B, pneumococcal disease and now meningococcal B disease. The Men B vaccination was added to the UK schedule in September 2015 and there has been no research looking at whether the vaccine gives the same protection to babies born early as it does to those born at term. We want to compare two different schedules of Men B vaccination and see if one gives better protection to babies born prematurely. It is possible that an extra Men B vaccine dose (i.e. three doses in early infancy instead of two) will offer better protection for premature babies. This is what we are trying to find out through this study.

Why has my baby been chosen and does my baby have to take part?

We are approaching you because your baby was born at less than 35 weeks of pregnancy and has not yet received their first vaccinations. If you decide to take part in the study you will be asked to sign a consent form, and we will give you a copy to keep. You would be able to withdraw your baby from the study at any time and without giving a reason. If you do not wish your child to take part in this study or withdraw your baby from the study before the end, their care will not be affected in any way and your baby would be offered vaccinations according to the routine UK schedule.

What happens if my baby takes part?

After you have signed the consent form your baby will be randomly (by chance) assigned to one of two groups: one group will receive all recommended vaccines according to the

standard UK schedule and the other will receive one additional dose of Men B vaccine at 3 months. There will be no other differences in the vaccines received. The diagram below shows the schedule of vaccinations and blood tests for this study:

Vaccine	Product names		Age (months)					
			2	3	4	5	12	13
DTaP/Hib/IP V/HepB	Infanrix hexa®		✓	✓	✓			
Rotavirus	Rotarix®		✓	✓				
PCV13	Prevenar13®		✓		✓		✓	
MCC/Hib	Menitorix®						✓	
MMR	Priorix®						✓	
4CMenB	Bexsero®	Group 1	✓		✓		✓	
		Group 2	✓	✓	✓		✓	
Blood tests						✓	✓	✓

In this study we want to compare the vaccine responses of premature babies who have received their vaccinations according to two different schedules and to do this we will take a blood sample from the babies at 5, 12 and 13 months. These time points have been chosen so that we can compare the response following the primary vaccinations and before and after the booster vaccination. A maximum of 3 mls will be taken on each occasion and a local anaesthetic cream and oral sucrose solution may be used to make the blood test as comfortable as possible.

What would I have to do?

If you agree for your baby to participate, you will be asked to sign a consent form. After every vaccination visit, you will be given a diary card to complete, which will be collected at the following visit. The diary card will be used to record daily temperatures and any redness or swelling that may occur around the injection site for up to 7 days after each vaccination. Your study doctor or nurse will explain how to fill in the diary. We will provide you with a telephone number to contact us at any time if you have any concerns about your baby during the study. We will also ask you to let us know by telephone if your baby has been unwell during the 7 days after immunisation, even if the illness seems unrelated to the vaccination. If you have to visit a doctor for the illness, we may contact them for further information. If your baby is still in hospital at the time of the vaccinations we will ask the nurses looking after them to provide us with some more detailed information for the first 48 hours after vaccination and for the remainder of the observation period the participant diary card will be used. We will request your permission for study staff to ask your GP for your contact details if we lose contact with you during the study.

What are the risks with these vaccines?

Because the vaccines used in this study are exactly the same ones being used for routine immunisation of other babies in the UK, we do not expect the risk to be any different than with the routinely given vaccines. As with all vaccinations, however, there may occasionally be some redness and/or swelling at the injection site and fever. Fever is a side effect particularly associated with the Men B vaccination and we will give you advice about steps you can take to try to prevent this. Some babies have been reported to feed less, be more irritable and cry more than usual after vaccinations. Severe allergic reactions, such as anaphylaxis, are extremely rare but can occur with any vaccine. Your baby would be observed for 20 minutes following each immunisation and the study staff are specifically trained and equipped to deal with this in the extremely unlikely event that it did occur.

What are the other possible disadvantages of taking part?

Blood tests may be uncomfortable for infants but we will try to minimise this by offering a local anaesthetic cream if appropriate to numb the skin before taking the blood sample.

What are the possible benefits of taking part?

We will check the antibody response your baby makes to the Men B vaccine. If we find that your baby has not made a sufficient response to the Men B vaccination they will be offered a booster. Unless your baby is an inpatient at the time of the vaccinations you will have the choice of coming to (insert local term here) or for us to come to your home to give the vaccinations and take the blood samples. If you attended at the local site, your travel expenses would be paid up to a cost of £10. You will have 24 hour telephone access to a member of the study team if you have any concerns about your baby's vaccinations.

What happens when the research stops or if new information becomes available?

Before the study ends, we will tell you your baby's blood test results and whether your baby has adequately responded to the vaccines. If new or relevant information becomes available during the course of the study, we will inform you as soon as possible.

What if there is a problem?

St George's, University of London has agreed that if you are harmed as a result of your participation in the study, you will be compensated, provided that, on the balance of probabilities, an injury was caused as a direct result of the intervention or procedures your baby received during the study. These special compensation arrangements apply where an injury is caused to your baby that would not have occurred if your baby was not in the trial. These arrangements do not affect your right to pursue a claim through legal action.

What if you wish to complain about the trial?

If you wish to complain, or have any concerns about the way you have been treated during this study, then you can talk to the BEAR Men B research team who will do their best to answer your questions or concerns (contact details at the end of Part 2). The National Health Service complaints mechanisms are also available to you.

Insert local information here

If you are still not satisfied with the response, you may contact:
The Sponsor Joint Research and Enterprise Office at St George's: 0208 725 4986.

Would my baby's participation in the study be kept confidential?

Personal data collected will include your baby's name, date of birth and address as well as your contact details and results of the blood tests and medical records. This information will be stored on secure password protected NHS or University computers. The only people with access to this information will be employees of the hospital where your baby was born, the study team, your GP, the Department of Health or regulatory authorities who may wish to check the study is being carried out within the appropriate guidelines. The data will only be used for the purposes of this study and any data released outside the above group will not contain any information that could be traced back to your baby. You have the right to obtain access to data relating to your child. If you wish to obtain any information, please contact us on the numbers listed below.

What would happen to any samples from my baby?

We will test the blood samples to check your baby's level of immunity against the Men B vaccine and we may also test antibody levels against other diseases. At the end of the study any remaining blood samples will be destroyed unless you provide your specific permission on the consent form to allow us to use any of the extra samples for future ethically-approved research studies looking at how vaccines work. All extra samples will be anonymised so that it will not be possible to link the results of any extra tests back to your baby. Please remember that your baby's participation in this study does not depend on whether you allow or don't allow us to use the remaining blood sample.

What would happen to the results of the research study?

We will write to you at the end of the study to inform you of our findings. This will be around 12 months after the end of the study. This may be some time after your child's participation in the study has ended. We will retain your contact details in a secure database at the local site to enable us to contact you. . We plan to publish the results in a medical journal so that other doctors can learn about the findings of the study. If you wish, you will also be sent a copy of the published research.

Who is organising and funding the research?

The study is funded by Meningitis Now and GlaxoSmithKline (GSK) and is sponsored by St. George's, University of London. This study has been reviewed and approved by Yorkshire & The Humber- Sheffield Research Ethics Committee and by the NHS Health Research Authority.

Further information

If you have any questions, or would like further information please contact:

(INSERT LOCAL INFORMATION).

We do hope that you and your family will take part in this study. Your contribution would be an important step towards the continual improvement of vaccine policy in the UK and would provide important information that should help us know what the best vaccination schedule should be for premature babies born in the future.

Thank you for reading this leaflet and thinking about taking part in the study.

Document 3: BEAR Men B study informed consent form



INSERT LOCAL HEADERS

Babies born Early Antibody Response to Men B

Vaccination: BEAR Men B

INFORMED CONSENT FORM

Baby's participant number:

Baby's name:

Baby's date of birth:

	Please initial
I have read and considered the Parent Information Sheet (version X, XX.XX.XX) about the study on infant vaccines. The information in the leaflet provides enough information so that I understand what is involved and any questions I had about the study have been answered to my satisfaction.	
I consent to my baby's vaccinations being given by the study nurse or doctor and to the blood samples being taken from my baby.	
I consent to my GP being informed about my baby's participation in this study and of the vaccinations being given to my baby. I agree that if the study team is unable to contact me during this study they may ask my GP for my contact details.	
I understand that relevant sections of medical notes and data collected during the study relating to my baby may be looked at by the researchers and by the Sponsor, Funders, regulatory authorities or by my NHS Trust. I give permission for these individuals to have access to these notes where it is relevant to taking part in this research.	
I understand that if the blood tests looking at antibody levels against Meningococcal group B show that my baby does not have adequate protection, I will be offered an opportunity for my baby to have further vaccinations as appropriate.	
I understand that participation is voluntary and that I may withdraw my baby from the study at any time without having to give a reason and that this will not affect my baby's routine care.	
I agree to the processing of my baby's personal data as explained in the section on "Would my baby's participation in the study be kept confidential?" of the Parent Information Sheet (version X, XX.XX.XX) which I have read and understood.	
I agree that my baby may take part in this study	

Appendix C

<i>OPTIONAL: I agree that any remaining blood may be used for further ethically approved research to help improve the understanding of vaccines and how they work. I understand that the sample will be used anonymously and it will not be possible to link the results of any tests back to my baby.</i>		
Signed _____ Date: _____ Parent / Guardian (*Please delete as appropriate) Name (block capitals)		
I have explained and discussed the study with the above parent/guardian. I have answered all their questions regarding the study and I am satisfied that the above signature denotes their informed consent for the above subject to take part in the trial. Signature of Study Doctor/Nurse: _____ Date: _____ Name (block capitals)		

1 copy to Parent, 1 copy in Medical notes and 1 original in ISF

Appendix C

Document 4: BEAR Men B study participant diary

BEAR Men B

Study number _____

Babies born Early Antibody Response to Men B vaccination:

BEAR Men B

Version 2.0, 04/12/17, IRAS reference 223266, REC reference 17/YH/0150

Participant Initials: _____

Study group (circle):

Group 1 (2 +1 schedule)

Group 2 (3+1 schedule)

Diary (please circle) 1 2 3 4

DATE OF VACCINATION (DAY 0): ____/____/____

If you have any questions about the study or how to complete this diary please contact a member of the study team.

(Insert local contact details)

IMPORTANT

PLEASE HAVE THIS DIARY WITH YOU AT YOUR NEXT VISIT

Next visit date: ____/____/____ Time _____

1

BEAR Men B

Study number _____

Episodes of illness between now and the next visit

If your child becomes unwell, if he/she is seen by a doctor or a nurse or if he/she receives any new medication between visits please record the details below. If you have any questions please contact a member of the study team (details on the front page).

Date	Problem	Action taken	Were any new medicines started?	Details of medication
Start date: ____/____/____ End date: ____/____/____		Phoned for advice (GP/practice nurse/NHS 111)? Y/N Seen by a GP? Y/N Seen in A+E? Y/N Admitted to hospital? Y/N	Y/N	Name: Dose: Route: Start date: End date:
Start date: ____/____/____ End date: ____/____/____		Phoned for advice (GP/practice nurse/NHS 111)? Y/N Seen by a GP? Y/N Seen in A+E? Y/N Admitted to hospital? Y/N	Y/N	Name: Dose: Route: Start date: End date:

Comments Please use this space to record any other information which you feel might be helpful for the study

2

Appendix C

BEAR Men B

Study number _____

Temperature

Please measure your child's temperature on the day of vaccination and for 7 days afterwards. For the first 3 days please measure the temperature twice daily and once daily thereafter.

Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Before vaccination ____: ____ °C	AM ____: ____ °C	AM ____: ____ °C	____: ____ °C	____: ____ °C	____: ____ °C	____: ____ °C	____: ____ °C
4-6 hrs after vaccination ____: ____ °C	PM ____: ____ °C	PM ____: ____ °C					
Did your child receive: Paracetamol Y/N No of doses: ____ Ibuprofen Y/N No of doses: ____ Was this: To prevent fever/in response to fever (delete as applicable)	Did your child receive: Paracetamol Y/N No of doses: ____ Ibuprofen Y/N No of doses: ____ Was this: To prevent fever/in response to fever (delete as applicable)	Did your child receive: Paracetamol Y/N No of doses: ____ Ibuprofen Y/N No of doses: ____ Was this: To prevent fever/in response to fever (delete as applicable)	Did your child receive: Paracetamol Y/N No of doses: ____ Ibuprofen Y/N No of doses: ____ Was this: To prevent fever/in response to fever (delete as applicable)	Did your child receive: Paracetamol Y/N No of doses: ____ Ibuprofen Y/N No of doses: ____ Was this: To prevent fever/in response to fever (delete as applicable)	Did your child receive: Paracetamol Y/N No of doses: ____ Ibuprofen Y/N No of doses: ____ Was this: To prevent fever/in response to fever (delete as applicable)	Did your child receive: Paracetamol Y/N No of doses: ____ Ibuprofen Y/N No of doses: ____ Was this: To prevent fever/in response to fever (delete as applicable)	Did your child receive: Paracetamol Y/N No of doses: ____ Ibuprofen Y/N No of doses: ____ Was this: To prevent fever/in response to fever (delete as applicable)

If after the first 7 days your child feels hot, please measure their temperature and record it below if it is elevated (>37.5 °C):

Date ____/____/____	Time: _____	Temperature: ____: ____ °C
Date ____/____/____	Time: _____	Temperature: ____: ____ °C
Date ____/____/____	Time: _____	Temperature: ____: ____ °C
Date ____/____/____	Time: _____	Temperature: ____: ____ °C

3

BEAR Men B

Study number _____

Reactions at the site of vaccination Please record any local reactions your child had at the site of vaccination

NB: To grade tenderness please use this scale
 0 None
 1 Mild child whimpers when the site is touched
 2 Moderate child cries when the site is touched
 3 Severe child cries when the limb is moved

Left thigh	Day 0 (4-6 hours after vaccination)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Date reaction ended (if continuing beyond day 7)
Redness Use ruler to measure redness and record the widest part (in millimetres). If none present write 0.									
Swelling Use ruler to measure swelling and record the widest part (in millimetres). If none present write 0.									
Tenderness 0 None 1 Mild 2 Moderate 3 Severe									
Lower right thigh	Day 0 (4-6 hours after vaccination)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Date reaction ended (if continuing beyond day 7)
Redness Use ruler to measure redness and record the widest part (in millimetres). If none present write 0.									
Swelling Use ruler to measure swelling and record the widest part (in millimetres). If none present write 0.									
Tenderness 0 None 1 Mild 2 Moderate 3 Severe									

4

Appendix C

BEAR Men B

Study number _____

Upper right thigh	Day 0 (4-6 hours after vaccination)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Date reaction ended (if continuing beyond day 7)
Redness Use ruler to measure redness and record the widest part (in millimetres). If none present write 0.									
Swelling Use ruler to measure swelling and record the widest part (in millimetres). If none present write 0.									
Tenderness 0 None 1 Mild 2 Moderate 3 Severe									
Right arm	Day 0 (4-6 hours after vaccination)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Date reaction ended (if continuing beyond day 7)
Redness Use ruler to measure redness and record the widest part (in millimetres). If none present write 0.									
Swelling Use ruler to measure swelling and record the widest part (in millimetres). If none present write 0.									
Tenderness 0 None 1 Mild 2 Moderate 3 Severe									

Other Local Reactions (If your child has experienced any other local reactions, please describe and give dates):

5

BEAR Men B

Study number _____

General symptoms Please circle the appropriate number/s. If your child has symptoms, please indicate severity (mild or moderate or severe) of the symptom(s).

	Day 0 (vaccination)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Date reaction ended i.e. if reaction continues beyond day 7 please fill in date reaction ended
Has your child been feeding less than usual? 0 None 1 Mild Feeding less than normal for 1-2 feeds 2 Moderate Missed 1-2 feeds completely 3 Severe Refused most or all feeds	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	____/____/____
Has your child been less active than usual? 0 None 1 Mild Less interested in surroundings, toys etc. 2 Moderate No interest in above and sleeping through feeds ... 3 Severe Sleeping most of the time	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	____/____/____
Has your child been more irritable than usual? 0 None 1 Mild Continuously irritable for less than 1 hour 2 Moderate Continuously irritable for 1 to less than 3 hours 3 Severe Continuously irritable for 3 or more hours	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	____/____/____
Has your child cried persistently ? 0 None 1 Mild Cried continuously for less than 1 hour 2 Moderate Cried continuously for 1 to less than 3 hours 3 Severe Cried continuously for 3 or more hours	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	____/____/____
Has your child been sick ? 0 None 1 Mild 1-2 episodes without interfering with routine 2 Moderate Several episodes & cannot keep any food down ... 3 Severe Frequent episodes & taking nothing by mouth	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	____/____/____
Has your child had diarrhoea ? 0 None 1 Mild More loose stools than usual 2 Moderate Frequent runny stools without much solid material... 3 Severe Multiple liquid stools without much solid material....	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	____/____/____

6

**BEAR MenB CASE REPORT
FORM**



Participant number

Case Report Form (CRF)

BEAR Men B

Version 1, 15.07.17

A phase IV multicentre study to evaluate the primary and booster immune responses in UK preterm infants receiving routine immunisations and incorporating a randomisation study of a 3 dose versus a 2 dose schedule of 4CMenB for primary immunisation.

Short Title: BEAR Men B

Sponsor: St. George's University of London

Chief Investigator: Professor Paul Heath

Sponsor's R&D Number: 16.0247

Eudract Number: 2017-001487-38



Study Sponsor: St George's, University of London

I am confident that the information supplied in this case report form is complete and accurate. I confirm that the study was conducted in accordance with the protocol and any protocol amendments. I confirm that written informed consent was obtained prior to any study related procedures taking place.

Principal Investigator Signature:

Date of Signature: ____/____/____

BEAR Men B
Case Report Form version 1.0
15.07.17

BEAR Men B CASE REPORT FORM		Study No		Group	
INCLUSION / EXCLUSION CRITERIA					
Male ☐ Female ☐					
Hospital Name					
Birth Gestation +					
Degree of prematurity (tick as applicable): Up to 29+6 ☐ 30+0 to 34+6 ☐					
Birth Weight (g)		Most recent weight (g)		Date of Weight	
ETHNIC ORIGIN					
☐ White	☐ Chinese	☐ Pakistani			
☐ Black African	☐ Black Caribbean	☐ Black-Other:			
☐ Indian	☐ Bangladeshi	☐ Asian-Other:			
☐ Mixed ethnic group:					
INCLUSION CRITERIA					
<35 week gestation at birth:			Yes ☐	No ☐	
No contraindication to vaccination <i>as per Green Book</i> :			Yes ☐	No ☐	
Willing and able to comply with study procedures:			Yes ☐	No ☐	
Written informed consent obtained:			Yes ☐	No ☐	
Date of consent:					
EXCLUSION CRITERIA					
Life-limiting congenital abnormality or condition:			Yes ☐	No ☐	
Prior diagnosis of an immunodeficiency syndrome:			Yes ☐	No ☐	
Considered unlikely to complete expected follow up until the end of the study:			Yes ☐	No ☐	

Appendix C

BEAR Men B CASE REPORT FORM	<table border="1" style="width: 100%; border-collapse: collapse;"><tr><td style="width: 50%; text-align: center; padding: 2px;">Study No</td><td style="width: 50%; text-align: center; padding: 2px;">Group</td></tr></table>	Study No	Group
Study No	Group		

RANDOMISATION

Study No. Group

Letter sent to GP informing them of participant entry to study? Yes ☐ No ☐

Consent obtained for storage of residual sample: Yes ☐ No ☐

Completed by: (Signature) _____ Name: _____

Completion date of inclusion/exclusion criteria: ____/____/____

(NB: Study staff must have signed delegation log prior to completing any study activities)

**BEAR Men B CASE REPORT
FORM**
Study
No

Group

PARTICIPANT MEDICAL HISTORY

 Does the participant have any significant medical history? Yes ☐ No ☐ *If Yes, please enter below*

Diagnosis	Onset Date	End Date	Ongoing?

VENTILATORY SUPPORT HISTORY

 Ventilatory support required at any time? Yes ☐ No ☐

Type of ventilatory support: High Frequency Oscillation: Yes ☐ No ☐ No. of days:

Conventional: Yes ☐ No ☐ No. of days:

CPAP: Yes ☐ No ☐ No. of days:

High flow: Yes ☐ No ☐ No. of days:

Low flow oxygen: Yes ☐ No ☐ No. of days:

Other: No. of days:

Appendix C

BEAR Men B CASE REPORT FORM		Study No	Group																
VACCINATION IN PREGNANCY Did mother have pertussis vaccination in pregnancy? Yes ☐ No ☐ If yes, what was the date of pertussis vaccination:																			
INFANT NON-STUDY VACCINES BCG vaccine given? Yes ☐ No ☐ Date Hep B vaccine given? Yes ☐ No ☐ Dates																			
INTRAVENOUS IMMUNOGLOBULIN Immunoglobulins given? Yes ☐ No ☐ <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Product:</td> <td style="width: 33%;">Date:</td> <td style="width: 33%;">Volume: ml/kg</td> </tr> <tr> <td>Product:</td> <td>Date:</td> <td>Volume: ml/kg</td> </tr> </table>				Product:	Date:	Volume: ml/kg	Product:	Date:	Volume: ml/kg										
Product:	Date:	Volume: ml/kg																	
Product:	Date:	Volume: ml/kg																	
BLOOD PRODUCTS <table style="width: 100%; border: none;"> <tr> <td style="width: 35%;">Red blood cell transfusions given?</td> <td style="width: 10%;">Yes ☐</td> <td style="width: 10%;">No ☐</td> <td style="width: 45%;">No. of transfusions: </td> </tr> <tr> <td>Exchange transfusions given?</td> <td>Yes ☐</td> <td>No ☐</td> <td>No. of transfusions: </td> </tr> <tr> <td>Platelet transfusions given?</td> <td>Yes ☐</td> <td>No ☐</td> <td>No. of transfusions: </td> </tr> <tr> <td>Fresh frozen plasma (FFP) given?</td> <td>Yes ☐</td> <td>No ☐</td> <td>No. of transfusions: </td> </tr> </table>				Red blood cell transfusions given?	Yes ☐	No ☐	No. of transfusions:	Exchange transfusions given?	Yes ☐	No ☐	No. of transfusions:	Platelet transfusions given?	Yes ☐	No ☐	No. of transfusions:	Fresh frozen plasma (FFP) given?	Yes ☐	No ☐	No. of transfusions:
Red blood cell transfusions given?	Yes ☐	No ☐	No. of transfusions:																
Exchange transfusions given?	Yes ☐	No ☐	No. of transfusions:																
Platelet transfusions given?	Yes ☐	No ☐	No. of transfusions:																
Fresh frozen plasma (FFP) given?	Yes ☐	No ☐	No. of transfusions:																

**BEAR Men B CASE REPORT
FORM**

 Study
No

Group

STEROID HISTORY
ANTENATAL STEROIDS

 Antenatal steroids given? Yes ☐ No ☐

 Product: Date: Gestation: Dose:

 Product: Date: Gestation: Dose:
POSTNATAL STEROIDS

Course 1:

 Product: Date started: Date finished:

 Cumulative dose (mg): Weight during course: (g)

Course 2:

 Product: Date started: Date finished:

 Cumulative dose (mg): Weight during course (g): (g)

CONCOMITANT MEDICATION

 Is the participant taking regular medication? Yes ☐ No ☐
If Yes, please complete Concomitant Medication CRF

Completed by: (Signature) _____ Name: _____

Completion date of participant medical history: ____/____/____

(NB: Study staff must have signed delegation log prior to completing any study activities)

 BEAR CRF
Version 1.0
15.07.17

Page 6 of 22

**BEAR Men B CASE REPORT
FORM**

 Study
No

Group

VISIT ONE (2 months): VACCINATION (49-84 days old)

 Is participant in NNU at time of vaccination? Yes ☐ No ☐
If Yes, please complete pre-vaccination observations
PRE-VACCINATION OBSERVATIONS: ALL INPATIENTS

 Was the participant observed for 24 hours pre-vaccination? Yes ☐ No ☐

 If no, reason:

 Ventilatory Support: Yes ☐ No ☐ *If Yes, please specify type below*

 HFO ☐ Conventional ☐ CPAP ☐ High flow ☐ Low flow O₂ ☐ Max. O₂ Req

Other (please specify):

 Apnoea (cessation of respiration ≥ 20 s) Yes ☐ No ☐ Number of episodes:

 Bradycardia (HR ≤ 100 for ≥ 20 s) Yes ☐ No ☐ Number of episodes:

 Desaturation (O₂ sats $< 88\%$) Yes ☐ No ☐ Number of episodes:
VACCINATION: ALL INFANTS RECEIVE INFANRIX IPV/HIB/(HEP B), PREVENAR 13, BEXSERO & ROTARIX

 Has the participant started any new medication since screening? Yes ☐ No ☐
If yes please record on concomitant medication form

 Is the baby breastfeeding? Totally ☐ Partially ☐ Not at all ☐

 Axillary temp prior to vaccination (°C): Temp vaccine stored at prior to vaccination (°C):

 Infanrix IPV/Hib/(Hep B) (UPPER RIGHT THIGH) YES/NO (circle) Batch No: Expires:

 Prevenar13 (LOWER RIGHT THIGH) YES/NO (circle) Batch No: Expires:

 Bexsero (LEFT THIGH) YES/NO (circle) Batch No: Expires:

 Rotarix (ORAL) YES/NO (circle) Batch No: Expires:

Comments:

 Date of vaccination: Time of vaccination:

 Vaccine given by: Red book completed: YES/NO

**BEAR Men B CASE REPORT
FORM**

Study No	Group
-------------	-------

VISIT ONE OBSERVATIONS
Please complete Part A if participant is an inpatient at time of vaccination
PART A: POST-VACCINATION OBSERVATIONS

 Was the participant observed for 72 hours post-vaccination: Yes ☐ No ☐
If no please complete a protocol deviation form.

 Ventilatory Support: Yes ☐ No ☐ *If Yes, please specify type below*

 HFO ☐ Conventional ☐ CPAP ☐ High flow ☐ Low flow O₂ ☐ Max. O₂ Req (%)

Other (please specify):

 Apnoea (cessation of respiration ≥ 20 s) Yes ☐ No ☐ Number of episodes:

 Bradycardia (HR ≤ 100 for ≥ 20 s) Yes ☐ No ☐ Number of episodes:

 Desaturation (O₂ sats $< 88\%$) Yes ☐ No ☐ Number of episodes:
Please complete Part B if participant is an outpatient at time of vaccination
PART B: POST-VACCINATION OBSERVATIONS

 Was the participant observed for 20 minutes post-vaccination: Yes ☐ No ☐

If no, reason:

Time observation completed:

Completed by: (Signature) _____ Name: _____

Completion date of visit 1 CRF: ____/____/____

(NB: Study staff must have signed delegation log prior to completing any study activities)

**BEAR Men B CASE REPORT
FORM**
Study
No

Group

VISIT TWO (3 months): VACCINATION (28-42 days after V1)
DIARY
Diary collected? Yes ☐ No ☐Diary completed? Yes ☐ No ☐

If no, reason: _____

ADVERSE EVENTS
Has the participant experienced any adverse events since the previous visit? Yes ☐ No ☐

If Yes, please complete AE section below.

Symptoms	Severity	Start date	Stop date / ongoing	Medical review	New medication	Hospitalised
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No

Severity: 1=Mild 2=Moderate 3=Severe

Please ensure new medication documented on Concomitant Medication CRF

Please ensure SAE notification procedures followed if hospitalised or prolongation of existing hospital stay

Is participant in NNU at time of vaccination? Yes ☐ No ☐

If Yes please complete pre-vaccination observations

PRE-VACCINATION OBSERVATIONS: ALL INPATIENTS
Was the participant observed for 24 hours pre-vaccination? Yes ☐ No ☐

If no, reason:

Ventilatory Support: Yes ☐ No ☐ If Yes, please specify type belowHFO ☐ Conventional ☐ CPAP ☐ High flow ☐ Low flow O₂ ☐ Max. O₂ Req (%)

Other (please specify):

Apnoea (cessation of respiration ≥ 20 s) Yes ☐ No ☐ Number of episodes: Bradycardia (HR ≤ 100 for ≥ 20 s) Yes ☐ No ☐ Number of episodes: Desaturation (O₂ sats $< 88\%$) Yes ☐ No ☐ Number of episodes: Version 1.0
15.07.17

**BEAR Men B CASE REPORT
FORM**

Study No.	Group
--------------	-------

VISIT TWO VACCINATION

VACCINATION: GROUP 1 INFANRIX IPV/HIB(HEP B) & ROTARIX
GROUP 2 BEXSERO, INFANRIX IPV/HIB/(HEP B) & ROTARIX

Has the participant started any new medication since screening? Yes ☐ No ☐

If yes please record on concomitant medication form

Is the baby breastfeeding? Totally ☐ Partially ☐ Not at all ☐

Axillary temperature prior to vaccination (°C):

Temp vaccine stored at prior to vaccination (°C):

****ALL INFANTS****

Infanrix IPV/Hib/(Hep B) (RIGHT THIGH) YES/NO (circle) Batch No: _____ Expires: _____

Rotarix (ORAL) YES/NO (circle) Batch No: _____ Expires: _____

****GROUP 2 ONLY****

Bexsero (LEFT THIGH) YES/NO (circle) Batch No: _____ Expires: _____

Comments:

Date of vaccination:

Time of vaccination:

Vaccine given by: _____

**BEAR Men B CASE REPORT
FORM**
Study
No

Group

VISIT TWO OBSERVATIONS

Please complete Part A if participant is an inpatient at time of vaccination

PART A: POST-VACCINATION OBSERVATIONS

Was the participant observed for 72 hours post-vaccination: Yes ☐ No ☐

If no please complete a protocol deviation form.

Ventilatory Support: Yes ☐ No ☐ *(If Yes, please specify type below)*

HFO ☐ Conventional ☐ CPAP ☐ High flow ☐ Low flow O₂ ☐ Max. O₂ Req (%)

Other (please specify):

Apnoea (cessation of respiration ≥ 20 s) Yes ☐ No ☐ Number of episodes:

Bradycardia (HR ≤ 100 for ≥ 20 s) Yes ☐ No ☐ Number of episodes:

Desaturation (O₂ sats $< 88\%$) Yes ☐ No ☐ Number of episodes:

Please complete Part B if participant is an outpatient at time of vaccination

PART B: POST-VACCINATION OBSERVATIONS

Was the participant observed for 20 minutes post-vaccination: Yes ☐ No ☐

If no, reason:

Time observation completed:

Completed by: (Signature) _____ Name: _____

Completion date of visit 2 CRF: ____/____/____

(NB: Study staff must have signed delegation log prior to completing any study activities)

BEAR Men B CASE REPORT FORMStudy
No

Group

VISIT THREE (4 months): VACCINATION (28-42 days after V2)**DIARY**Diary collected? Yes ☐ No ☐Diary completed? Yes ☐ No ☐

If no, reason: _____

ADVERSE EVENTSHas the participant experienced any adverse events since the previous visit? Yes ☐ No ☐

If Yes, please complete AE section below.

Symptoms	Severity	Start date	Stop date / ongoing	Medical review	New medication	Hospitalised
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No

Severity: 1=Mild 2=Moderate 3=Severe

Please ensure new medication documented on Concomitant Medication CRF

Please ensure SAE notification procedures followed if hospitalised or prolongation of existing hospital stay

Is participant in NNU at time of vaccination? Yes ☐ No ☐ If Yes, please complete pre-vaccination observations**PRE-VACCINATION OBSERVATIONS: ALL INPATIENTS**Was the participant observed for 24 hours pre-vaccination? Yes ☐ No ☐

If no, reason: _____

Ventilatory Support: Yes ☐ No ☐ If Yes, please specify type belowHFO ☐ Conventional ☐ CPAP ☐ High flow ☐ Low flow O₂ ☐ Max. O₂ Req (%)

Other (please specify): _____

Apnoea (cessation of respiration ≥20s) Yes ☐ No ☐ Number of episodes: Bradycardia (HR≤100 for ≥20s) Yes ☐ No ☐ Number of episodes: Desaturation (O₂ sats<88%) Yes ☐ No ☐ Number of episodes:

15.07.17

**BEAR Men B CASE REPORT
FORM**

 Study
No

Group

VISIT THREE VACCINATION
VACCINATION: ALL GROUPS INFANRIX IPV/HIB(HEP B), PREVENAR 13 & BEXSERO

 Has the participant started any new medication since previous visit? Yes ☐ No ☐
If yes please record on concomitant medication form

 Is the baby breastfeeding? Totally ☐ Partially ☐ Not at all ☐

 Axillary temperature prior to vaccination (°C):

 Temp vaccine stored at prior to vaccination (°C):

Infanrix IPV/Hib/(Hep B) (UPPER RIGHT THIGH) YES/NO (circle) Batch No: _____ Expires: _____

PREVENAR 13 (LOWER RIGHT THIGH) YES/NO (circle) Batch No: _____ Expires: _____

BEXSERO (LEFT THIGH) YES/NO (circle) Batch No: _____ Expires: _____

Comments:

 Date of vaccination:

 Time of vaccination:

Vaccine given by: _____

**BEAR Men B CASE REPORT
FORM**

Study
No

Group

VISIT THREE OBSERVATIONS

Please complete Part A if participant is an inpatient at time of vaccination

PART A: POST-VACCINATION OBSERVATIONS

Was the participant observed for 72 hours post-vaccination: Yes ☐ No ☐

If no please complete a protocol deviation form.

Ventilatory Support: Yes ☐ No ☐ *If Yes, please specify type below*

HFO ☐ Conventional ☐ CPAP ☐ High flow ☐ Low flow O₂ ☐ Max. O₂ Req (%)

Other (please specify):

Apnoea (cessation of respiration ≥ 20 s) Yes ☐ No ☐ Number of episodes:

Bradycardia (HR ≤ 100 for ≥ 20 s) Yes ☐ No ☐ Number of episodes:

Desaturation (O₂ sats $\leq 88\%$) Yes ☐ No ☐ Number of episodes:

Please complete Part B if participant is an outpatient at time of vaccination

PART B: POST-VACCINATION OBSERVATIONS

Was the participant observed for 20 minutes post-vaccination: Yes ☐ No ☐

If no, reason:

Time observation completed:

Completed by: (Signature) _____ Name: _____

Completion date of visit 3 CRF: ____/____/____

(NB: Study staff must have signed delegation log prior to completing any study activities)

**BEAR Men B CASE REPORT
FORM**

Study
No

Group

VISIT FOUR (5 months): BLOOD SAMPLING (28-42 days after V3)

If the participant has received any antibiotics in the past 7 days, please delay this visit by at least 48 hours after the last dose of antibiotics. If the appointment cannot be rescheduled please complete a protocol deviation

DIARY

Diary collected? Yes ☐ No ☐

Diary completed? Yes ☐ No ☐

If no, reason: _____

ADVERSE EVENTS

Has the participant experienced any adverse events since the previous visit? Yes ☐ No ☐

If Yes, please complete AE section below.

Symptoms	Severity	Start date	Stop date / ongoing	Medical review	New medication	Hospitalised
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No

Severity: 1=Mild 2=Moderate 3=Severe

Please ensure new medication documented on Concomitant Medication CRF

Please ensure SAE notification procedures followed if hospitalised or prolongation of existing hospital stay

CONCOMITANT MEDICATION

Has the participant started any new medication since previous visit? Yes ☐ No ☐

If yes please record on concomitant medication form

BEAR CRF
Version 1.0
15.07.17

**BEAR Men B CASE REPORT
FORM**

Study No	Group
-------------	-------

BLOOD SAMPLE A

Blood sample taken? Yes ☐ No ☐ Method: Venous ☐ Capillary ☐ Volume (ml)

Name of person taking sample: _____

Date of sampling: Date sample sent to SGUL:

If no or late sample, reason:

Completed by: (Signature) _____ Name: _____

Completion date of visit 4 CRF: ____/____/____

(NB: Study staff must have signed delegation log prior to completing any study activities)

**BEAR Men B CASE REPORT
FORM**

Study
No

Group

VISIT FIVE (12 months): BLOOD SAMPLING AND VACCINATION (364-393 days old)

If the participant has received any antibiotics in the past 7 days, please delay this visit by at least 48 hours after the last dose of antibiotics. If the appointment cannot be rescheduled please complete a protocol deviation

ADVERSE EVENTS

Has the participant experienced any adverse events since the previous visit? Yes ☐ No ☐

If Yes, please complete AE section below.

Symptoms	Severity	Start date	Stop date / ongoing	Medical review	New medication	Hospitalised
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No

Severity: 1=Mild 2=Moderate 3=Severe

Please ensure new medication documented on Concomitant Medication CRF

Please ensure SAE notification procedures followed if hospitalised or prolongation of existing hospital stay

BLOOD SAMPLE B

Blood sample taken? Yes ☐ No ☐ Method: Venous ☐ Capillary ☐ Volume (ml)

Name of person taking sample:

Date of sampling: Date sample sent to SGUL:

If no or late sample, reason:

**BEAR Men B CASE REPORT
FORM**

Study
No

Group

VISIT FIVE VACCINATION

VACCINATION: ALL GROUPS BEXSERO, PRIORIX, MENITORIX & PREVENAR13

Has the participant started any new medication since previous visit? Yes ☐ No ☐

If yes please record on concomitant medication form

Is the baby breastfeeding? Totally ☐ Partially ☐ Not at all ☐

Axillary temperature prior to vaccination (°C):

Temperature vaccine stored at prior to vaccination (°C):

PRIORIX (LOWER RIGHT THIGH) YES/NO (circle) Batch No: _____ Expires: _____

PREVENAR 13 (UPPER RIGHT THIGH) YES/NO (circle) Batch No: _____ Expires: _____

BEXSERO (LEFT THIGH) YES/NO (circle) Batch No: _____ Expires: _____

MENITORIX (RIGHT ARM) YES/NO (circle) Batch No: _____ Expires: _____

Comments:

Date of vaccination:

Time of vaccination:

Vaccine given by: _____

VISIT FIVE OBSERVATIONS

Was the participant observed for 20 minutes post-vaccination: Yes ☐ No ☐

If no, reason:

Time observation completed:

Completed by: (Signature) _____ Name: _____

Completion date of visit 5 CRF: ____/____/____

(NB: Study staff must have signed delegation log prior to completing any study activities)

BEAR CRF
Version 1.0
15.07.17

Page 18 of 22

**BEAR Men B CASE REPORT
FORM**

Study
No

Group

VISIT SIX (13 months): BLOOD SAMPLING (28-42 days after V5)

If the participant has received any antibiotics in the past 7 days, please delay this visit by at least 48 hours after the last dose of antibiotics. If the appointment cannot be rescheduled please complete a protocol deviation.

DIARY

Diary collected? Yes ☐ No ☐

Diary completed? Yes ☐ No ☐

If no, reason: _____

ADVERSE EVENTS

Has the participant experienced any adverse events since the previous visit? Yes ☐ No ☐

If Yes, please complete AE section below.

Symptoms	Severity	Start date	Stop date / ongoing	Medical review	New medication	Hospitalised
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No
	1 2 3			Yes / No	Yes / No	Yes / No

Severity: 1=Mild 2=Moderate 3=Severe

Please ensure new medication documented on Concomitant Medication CRF

Please ensure SAE notification procedures followed if hospitalised or prolongation of existing hospital stay

CONCOMITANT MEDICATION

Has the participant started any new medication since previous visit? Yes ☐ No ☐

If yes please record on concomitant medication form

**BEAR Men B CASE REPORT
FORM**

Study
No

Group

BLOOD SAMPLE C

Blood sample taken? Yes ☐ No ☐ Method: Venous ☐ Capillary ☐ Volume (ml)

Name of person taking sample: _____

Date of sampling: Date sample sent to SGUL:

If no or late sample, reason:

Completed by: (Signature) _____ Name: _____

Completion date of visit 6 CRF: ____/____/____

(NB: Study staff must have signed delegation log prior to completing any study activities)

BEAR CRF
Version 1.0
15.07.17

Page 20 of 22

**BEAR Men B CASE REPORT
FORM**

Study
No

Group

CHANGE OF STUDY STATUS

Please complete this form if a patient is withdrawn from the study

Change in participant status

Withdrawn from study.

☐

If yes, circle as appropriate:

Agrees to contact for SAE enquiry / Does NOT agree to contact for SAE enquiry

Agrees to use of previous data and sample / does NOT agree to use of previous data and samples

Lost to follow up

☐

Died

☐

Other

☐

Please provide details:

Reason for change in participant status

PI decision

☐

Parental decision

☐

Withdrawn because of SAE (Please refer to section 13.1 of protocol)

☐

If an SAE has occurred please ensure that an SAE form has been completed and the sponsor has been informed

Died

☐

If died date of death _____

Completed by: (Signature) _____ Name: _____

Completion date of change of study status: ____/____/____

(NB: Study staff must have signed delegation log prior to completing any study activities)

BEAR CRF
Version 1.0
15.07.17

Page 21 of 22

**BEAR Men B CASE REPORT
FORM**

Study No. _____	Group _____
--------------------	----------------

CONCOMITANT MEDICATION

*Please check concomitant medication at each visit and record any antibiotics,
immunosuppressants or non-study vaccines received since last visit*

	Medication	Indication	Dose and frequency	Route of administration	Start date	Stop date/ongoing	Ongoing at enrolment
1							
2							
3							
4							
5							
6							
7							

Completed by: (Signature) _____ Name: _____

Completion date of concomitant medication: ____/____/____

(NB: Study staff must have signed delegation log prior to completing any study activities)

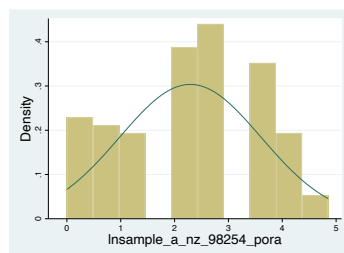
Appendix D

Figures showing histograms of distributions of the GMT results for each tested strain at each time point along with results of the Shapiro Wilks test

All participants at five months

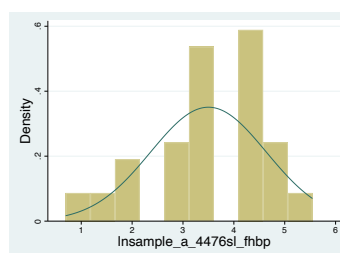
NZ98/254

Normally distributed
distributed
Shapiro Wilks = 0.055



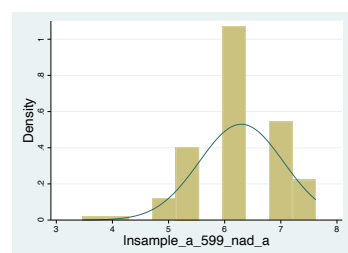
44/76-SL

Not normally distributed
Shapiro Wilks = 0.02



5/99

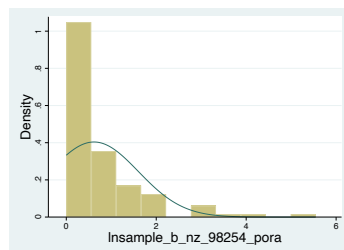
Not normally
Shapiro Wilks = 0.002



All participants at 12 months

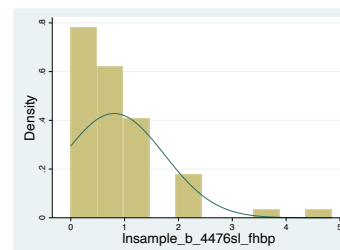
NZ98/254

Not normally distributed
Shapiro Wilks = 0.00



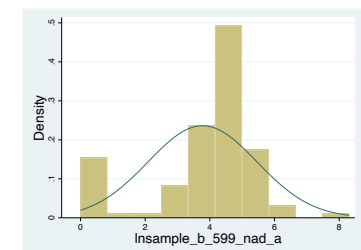
44/76-SL

Not normally distributed
Shapiro Wilks = 0.00



5/99

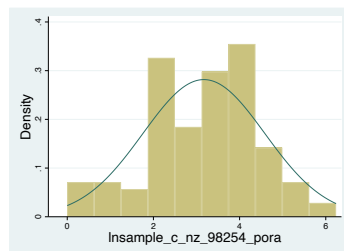
Not normally distributed
Shapiro Wilks = 0.00



All participants at 13 months

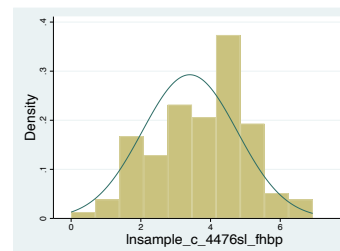
NZ98/254

Normally distributed
Shapiro Wilks = 0.50



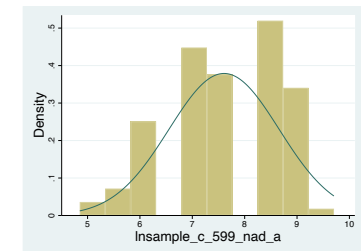
44/76-SL

Normally distributed
Shapiro Wilks = 0.50



5/99

Not normally distributed
Shapiro Wilks = 0.02



Appendix E

Document 1: COMPAre study protocol

1



FULL/LONG TITLE OF THE STUDY	Sources of ~~Complement~~ in Meningococcal and Pertussis serum bactericidal antibody Assays
SHORT STUDY TITLE / ACRONYM	~~COMPARE~~ study
PROTOCOL VERSION NUMBER AND DATE	Version 5.0, 14.06.2021
IRAS Number:	266052
JRES Reference Number	2019.0116
This protocol has regard for the HRA guidance and order of content	

~~COMPARE~~ protocol
Version 5.0, 14.06.2021
IRAS 266052

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature:

Date:

...../...../.....

.....
Name (please print):

.....
Position:

.....
Chief Investigator:

Signature:

Date:

.....
Name: (please print):

KEY STUDY CONTACTS	
Chief Investigator	Dr Kirsty Le Doare Paediatric Infectious Diseases Research Group Institute of Infection and Immunity St George's, University of London Cranmer Terrace London SW170RE [REDACTED] [REDACTED]
Principal Investigator	Dr Anna Calvert Paediatric Infectious Diseases Research Group Institute of Infection and Immunity St George's, University of London Cranmer Terrace London SW170RE [REDACTED] [REDACTED]
Sponsor	St George's, University of London Subhir Bedi Head of Research Governance and Delivery St Georges Joint Research & Enterprise Service (JRES) Cranmer Terrace SW17 ORE researchgovernance@sgul.ac.uk
Funder(s)	This study is supported by a European Society for Paediatric Infectious Diseases (ESPID) Research Training Fellowship

COMPAre protocol

Version 5.0, 14.06.2021

IRAS 266052

Key Protocol Contributors	Dr Anna Calvert Dr Kirsty Le Doare Professor Paul Heath Dr Stephen Taylor Professor Asma Khalil
Committees	Trial Management Group: Dr Kirsty Le Doare Dr Anna Calvert Professor Paul Heath Dr Stephen Taylor Professor Asma Khalil

STUDY SUMMARY	
Study Title	Sources of CO mplement in Me ningococcal and Pe rtussis serum bactericidal antibody Ass ays
Internal ref. no. (or short title)	COMPA re
Study Design	This study is designed to allow cord blood sample collection from the cords of babies born in three gestational age windows: ≥ 37 gestational weeks, 32-36+6 gestational weeks and less than 32 gestational weeks.
Study Participants	Pregnant women
Planned Size of Sample (if applicable)	Approximately 45 cord blood samples (15 samples in three gestational age groups)
Follow up duration (if applicable)	No follow up
Planned Study Period	October 2020 – February 2022
Research Question/Aim(s)	Are the assay results for meningococcal B, pertussis, GBS and covid obtained from assays using complement from a pooled adult complement source comparable to a those using a gestation matched complement source?

FUNDING AND SUPPORT	
FUNDER(S)	FINANCIAL AND <u>NON FINANCIAL</u> SUPPORT GIVEN
N/A	This study is supported by a European Society for Paediatric Infectious Diseases (ESPID) Research Training Fellowship

ROLES AND RESPONSIBILITIES OF STUDY MANAGEMENT COMMITTEES/GROUPS & INDIVIDUALS

Trial Management Group

We will have a Trial Management Group to monitor the progress of the study. Meetings will take place in person or by teleconference at intervals throughout the study.

Trial Steering Group	
Chair	Dr Kirsty Le Doare
Member	Dr Anna Calvert
Member	Professor Paul Heath
Member	Dr Stephen Taylor
Member	Professor Asma Khalil
Member	Uzma Khan

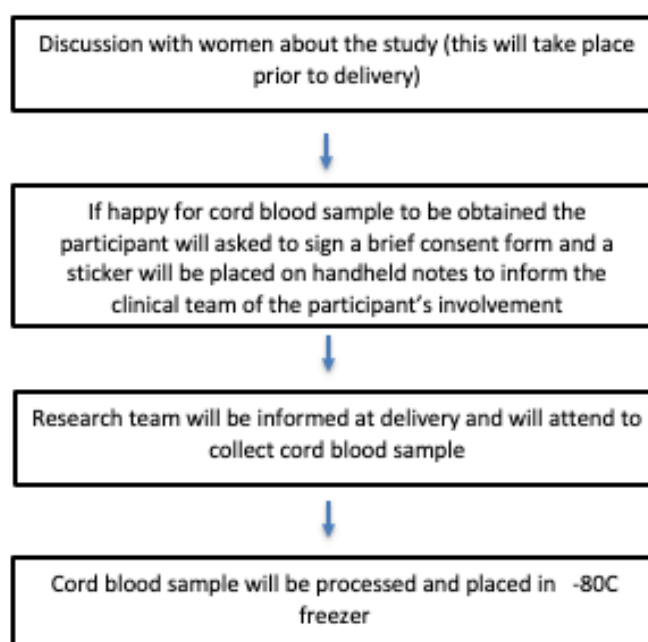
Patient & Public Involvement Group

This study has been discussed with the maternal and neonatal infections PPI group run by the Vaccine Institute at St. George's, University of London.

PROTOCOL CONTRIBUTORS

The protocol was written by Dr Anna Calvert under guidance from Dr Kirsty Le Doare, Professor Paul Heath, Dr Stephen Taylor and Professor Asma Khalil.

STUDY Schematic



STUDY PROTOCOL

Sources of Complement in Meningococcal and Pertussis serum bactericidal antibody Assays
(COMPAre)

1 BACKGROUND

Newborn infants, particularly those who are born preterm, are vulnerable to infection because of their immature immune systems. Invasive meningococcal disease (IMD) and pertussis both represent significant risks to the newborn infant.

Invasive meningococcal disease (IMD), caused by *Neisseria meningitidis*, is a devastating illness with a case fatality rate of 10-20% even with intensive treatment. Even infants who survive IMD experience significant disability in 10-20% of cases. Serogroups A, B, C, Y, W and X cause almost all cases of IMD, and in Europe, North America and parts of Latin America serogroup B is responsible for the majority of cases. In the UK there were 747 cases of IMD in 2016/17 and Men B was responsible for 396 (53%) of these. The major burden of disease is in infants under the age of 1 year. Pertussis is a highly infectious respiratory illness caused by *Bordetella pertussis* which can cause significant morbidity and mortality in young infants. There has been an increase in the incidence of pertussis in the UK, along with other high income countries in recent years which has disproportionately affected young infants.

Infants in the UK are vaccinated against meningococcal group B disease at 2, 4 and 12 months with Bexsero® and against pertussis at 2,3 and 4 months as part of the 6-in-1 vaccine Infanrix hexa®. Additionally, since 2012 pregnant women in the UK have been routinely offered pertussis vaccination during pregnancy to protect the infant in the first few months of life prior to them receiving their own vaccinations.

Serum bactericidal antibody (SBA) assays are important in the assessment of immunity following vaccination and are used in the production, release and licensure of some vaccines and the evaluation of the function of others. SBA assays for pertussis and meningococcal B typically use adult complement from a healthy adult donor pool. There is some concern that using adult complement may not allow an adequate assessment of neonatal immunity. The concentration of most complement components in the neonate is around 10-80% of that in the adult and circulating regulator levels are also reduced. Differences in complement function are more pronounced in preterm infants and the differences in the quality and activation of complement in neonates raises questions about whether an SBA assay using adult complement sources allows an accurate assessment of neonatal immunity.

In this study we will create a pooled complement source for three different gestational ages, ≥37 gestational weeks, 32-36+6 gestational weeks and less than 32 gestational weeks, which will allow us to compare the results of the pertussis and meningococcal SBA assays when using both standard adult complement and a gestational age appropriate complement source. To do this we will collect cord blood samples from deliveries within the three gestational age groups.

COMPAre protocol

Version 5.0, 14.06.2021

IRAS 266052

2 RATIONALE

Vaccination plays a vital role in protecting infants against pertussis and meningococcal disease. SBA assays are extremely useful in assessing post vaccination immunity and it is very important that these are optimised to allow the most accurate assessment for the population under consideration. We wish to investigate whether there is a difference in the assessment of immunity in infants when assays are performed using human adult complement compared to a gestational age matched source of complement.

3 RESEARCH QUESTION/AIM(S)

Are the assay results for meningococcal B, pertussis, GBS and covid obtained from assays using complement from a pooled adult complement source comparable to those using a gestation matched complement source?

3.1 Objectives

To investigate if the assay results for meningococcal B, pertussis, GBS and covid obtained from assays using complement from a pooled adult complement source are comparable to those using a gestation matched complement source.

3.2 Outcome

Assay results using adult human complement and a gestation matched complement source.

4 STUDY DESIGN and METHODS of DATA COLLECTION AND DATA ANALYSIS

We will recruit approximately 45 women from our institution into one of three groups: delivery at term (>37 gestational weeks), at 32-36+6 gestational weeks and at <32 gestational weeks. We will collect cord blood following delivery which will allow us to create a pooled complement source for these three gestational ages which can then be used in the SBA assays for pertussis and meningococcal B.

All participants will be given a participant identification number at the time of sample collection. This will be a chronological number followed by a letter: A- >37 gestational weeks, B- 32-36+6 gestational weeks, C- <32 gestational weeks. Participant identification numbers will be entered into an excel database along with details of gestational age at delivery, ethnicity and gender of the infant.

COMPAre protocol

Version 5.0, 14.06.2021

IRAS 266052

5 STUDY SETTING

This study will be performed at St George's University Hospitals NHS Foundation Trust. We will recruit participants prior to delivery. This may involve a woman being approached by a member of the research team or a woman seeing information about the study and approaching the research team.

6 SAMPLE AND RECRUITMENT

6.1 Eligibility Criteria

6.1.1 Inclusion criteria

- Pregnant and planning to deliver at St George's University Hospitals NHS Foundation Trust

6.1.2 Exclusion criteria

- Aged less than 16 years
- Known complement deficiency

6.2 Sampling

Cord blood samples will be obtained following delivery after the cord has been clamped and cut. As much blood as possible will be collected from the cord after samples have been obtained for any clinical use.

6.2.1 Size of sample

We estimate that 15 cord samples in each group will allow us to obtain a large enough volume of serum to create a sufficient pooled complement source. The amount of cord blood which will be obtained from each cord is uncertain and it is possible, particularly for the very preterm infants, that we may not obtain sufficient blood from 15 cords. We will continue to recruit until we have obtained sufficient blood to allow us to have sufficient pooled complement.

6.3 Recruitment

Participants will be recruited according to gestational age into three groups term (>37 gestational weeks), 32-36+6 gestational weeks and <32 gestational weeks.

6.3.1 Participant identification

Women will be identified by members of the research team or by clinical members of staff when they attend the hospital prior to delivery. No one will be approached about the study without discussing this with a member of the clinical team to ensure that the approach is appropriate. Some women will self-

COMPAre protocol

Version 5.0, 14.06.2021

IRAS 266052

identify having seen information about the study in the clinical environment. They will contact a member of the research team to discuss the study.

6.3.2 Consent

We will ask participants to sign a brief consent form after the study has been explained to them. Consent can be taken by any appropriately trained members of the research team and there is no minimum period between receiving information about the study and being able to provide consent.

If a woman is willing to have a cord blood sample taken a sticker will be placed on the front of her notes to notify the clinical team of her participation so that the research team can be contacted at the time of delivery.

6.3.3 Data collection tool

Case Report Forms will be designed by the CI.

- On paper CRFs all data should be entered legibly in black ink. If the Investigator makes an error, it will be crossed through with a single line in such a way to ensure that the original entry can still be read. The correct entry will then be clearly inserted. The amendment will be initialled and dated by the person making the correction immediately. Overwriting or use of correction fluid will not be permitted.

It is the Investigator's responsibility to ensure the accuracy of all data entered and recorded in the CRFs. The Staff Delegation of Responsibilities Log should identify all trial personnel responsible for data collection, entry, handling and managing the database.

The only information being collected on the CRF will be the gestational age at delivery, sex and ethnicity of the infant, mode of delivery and information about any antibiotics received by the mother in the week prior to delivery, the time of sample collection and the time of sample processing.

6.3.4 Biological Sample Handling

A cord blood sample consisting of as large a volume as possible will be obtained from each participant. The sample will be collected by a member of the research team using a plastic syringe and the blood will be transferred immediately into a hirudin containing tube. The sample will be centrifuged and the plasma aliquoted into cryovials labelled with the participant number, gestational age and date of sampling. These samples will then be frozen at -80C and stored in a freezer in the Vaccine Institute at St George's, University of London accessible only to members of the University.

At the end of sample collection all samples will be couriered to the laboratory at PHE Porton Down where three functional pools of complement will be generated and the analysis will take place. Prior to generating the functional pools, the individual complement components will be analysed to ensure suitability to be included in the functional pool and specific antibody levels in each of the samples will

COMPAre protocol

Version 5.0, 14.06.2021

IRAS 266052

be measured. Assays, including serum bactericidal antibody assays and complement deposition assays will be performed using the pooled complement sources and sera from vaccinated adults. Residual sera from the BEAR Men B study (REC reference 17/YH/0150; IRAS 223266) will be used as an alternative sera source. These samples will come from those participants whose parents had given permission for their samples to be used in future ethically approved research to help improve the understanding of vaccines and how they work.

7 ETHICAL AND REGULATORY CONSIDERATIONS

We will be approaching women prior to delivery, some of whom may be close to the time of delivery. This will require us to be sensitive in our approach. We will not discuss the study with anyone without a discussion with the clinical team looking after the woman to ensure that our approach is appropriate.

7.2 Research Ethics Committee (REC) and other Regulatory review & reports

Before the start of the study, a favourable opinion will be sought from an appropriate REC for the study protocol and other relevant documents e.g. advertisements.

For HRA- NHS REC reviewed research

- Substantial amendments that require review by NHS REC will not be implemented until that review is in place and other mechanisms are in place to implement at site.
- It is the Chief Investigator's responsibility to produce the annual reports and submit the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the study is declared ended.
- The Chief Investigator will notify the REC of the end of the study within one year after the end of the study.
- If the study is ended prematurely, the Chief Investigator will notify the REC, including the reasons for the premature termination.

Regulatory Review & Compliance

Before any site can enrol patients into the study, the Chief Investigator/Principal Investigator or designee will ensure that appropriate approvals from participating organisations are in place. Specific arrangements on how to gain approval from participating organisations are in place and comply with the relevant guidance.

Amendments

For any amendment to the study, the Chief Investigator or designee, in agreement with the sponsor will submit information to the appropriate body in order for them to issue approval for the amendment. The Chief Investigator or designee will work with sites (R&D departments at NHS sites as

COMPAre protocol

Version 5.0, 14.06.2021

IRAS 266052

well as the study delivery team) so they can put the necessary arrangements in place to implement the amendment to confirm their support for the study as amended.

7.3 Peer review

This study has been discussed within the Paediatric Infectious Diseases Research Group including researchers who are very experienced in maternal and paediatric studies.

7.4 Patient & Public Involvement

This study has been discussed with the maternal and neonatal infections PPI group run by the Vaccine Institute at St. George's, University of London. We will continue to discuss the study throughout the period of recruitment.

7.5 Protocol compliance

Protocol deviations, non-compliances, or breaches are departures from the approved protocol.

All protocol deviations must be adequately documented on the relevant forms and reported to the Chief Investigator and Sponsor immediately.

Deviations from the protocol which are found to frequently recur are not acceptable, will require immediate action and could potentially be classified as a serious breach.

7.6 Data protection and patient confidentiality

All data should be handled in accordance with the Data Protection Act 2018 (UK implementation of the EU General Data Protection Regulation (GDPR)).

The only information we will collect as part of this study is the sex and ethnicity of infants along with their gestational age at delivery, the mode of delivery and information about antibiotics received by the mother in the week prior to delivery. No identifiable information will be collected on the CRF. Participants' names will be collected on the consent forms and these will be retained for a period of five years.

7.7 Indemnity

St George's University of London sponsored research:

St George's University of London holds insurance to cover participants for injury caused by their participation in the clinical trial. Participants may be able to claim compensation if they can prove that St George's has been negligent. This includes negligence in the writing of the protocol, or selection of trial resources.

COMPAre protocol

Version 5.0, 14.06.2021

IRAS 266052

Where the Trial is conducted in a hospital, the hospital has a duty of care to participants. St George's University of London will not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees.

If a participant indicates that they wish to make a claim for compensation, this needs to be brought to the attention of St George's University of London immediately.

Failure to alert St George's University of London without delay and to comply with requests for information by the sponsor or any designated Agents may lead to a lack of insurance cover for the incident.

7.8 Access to the final study dataset

- The Trial Management Group will have access to the final study dataset.

8 DISSEMINATION POLICY

8.1 Dissemination policy

Publication: "Any activity that discloses, outside of the circle of trial investigators, any final or interim data or results of the Trial, or any details of the Trial methodology that have not been made public by the Sponsor including, for example, presentations at symposia, national or regional professional meetings, publications in journals, theses or dissertations."

All scientific contributors to the Trial have a responsibility to ensure that results of scientific interest arising from Trial are appropriately published and disseminated. The Sponsor has a firm commitment to publish the results of the Trial in a transparent and unbiased manner without consideration for commercial objectives.

To maximise the impact and scientific validity of the Trial, data shall be consolidated over the duration of the trial, reviewed internally among all investigators and not be submitted for publication prematurely. Lead in any publications arising from the Trial shall lie with the Sponsor in the first instance.

Before the official completion of the Trial,

All publications during this period are subject to permission by the Sponsor. If an investigator wishes to publish a sub-set of data without permission by the Sponsor during this period, the Trial Management Group shall have the final say.

Exempt from this requirement are student theses that can be submitted for confidential evaluation but are subject to embargo for a period not shorter than the anticipated remaining duration of the trial.

Up to 180 days after the official completion of the Trial

During this period the Chief Investigator shall liaise with all investigators and strive to consolidate data and results and submit a manuscript for peer-review with a view to publication in a reputable academic journal or similar outlet as the Main Publication.

- The Chief Investigator shall be senior and corresponding author of the Main Publication.

COMPAre protocol
Version 5.0, 14.06.2021
IRAS 266052

- Insofar as compatible with the policies of the publication outlet and good academic practice, the other Investigators shall be listed in alphabetical order.
- Providers of analytical or technical services shall be acknowledged, but will only be listed as co-authors if their services were provided in a non-routine manner as part of a scientific collaboration.
- Members of the Trial Management Group shall only be acknowledged as co-authors if they contributed in other capacities as well.
- If there are disagreements about the substance, content, style, conclusions, or author list of the Main Publication, the Chief Investigator shall ask the Trial Management Group to arbitrate.

Beyond 180 days after the official completion of the Trial

After the Main Publication or after 180 days from Trial end date any Investigator or group of investigators may prepare further publications. In order to ensure that the Sponsor will be able to make comments and suggestions where pertinent, material for public dissemination will be submitted to the Sponsor for review at least sixty (60) days prior to submission for publication, public dissemination, or review by a publication committee. Sponsor's reasonable comments shall be reflected. All publications related to the Trial shall credit the Chief and Co-Investigators as co-authors where this would be in accordance with normal academic practice and shall acknowledge the Sponsor and the Funders.

8.2 Archiving Arrangements

Each site will be responsible for their onsite level study archiving. The trial essential TMF along with any central trial database will be archived according to SGUL policy and Standard operating procedure JREOSOP0016 in a purpose built off-site archiving facility (currently Iron Mountain).

9. APPENDICES

9.1 Appendix 1

Procedures	Visit 1	Visit 2
Discussion of study and consent	x	
Collection of details of gestational age, ethnicity and gender of infant		x
Collection of sample		x

9.2 Appendix 2

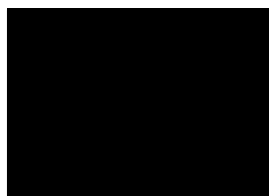
COMPAre protocol
Version 5.0, 14.06.2021
IRAS 266052

Appendix E

There are currently no amendments

Amendment Log				
Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
NSA_01	3.0	30/11/19	A.Calvert	Change to allow collection of information about mode of delivery and antibiotics received.
NSA_02	4.0	13/10/20	A.Calvert	Change to study end date to October 2021 due to delayed start of study Addition of funding information
NSA_03	5.0	14/06/21	A. Calvert	Change to extend the range of assays to include group B streptococcus (GBS) and covid. Clarification about the individual assessment of complement components which will be undertaken before the individual samples are added to the pooled complement source. Addition to allow use of retained sera from another study for which we have permission to use retained samples, as an alternative source of sera for the assays. Extension to study dates included.

Document 2: Participant information sheet and informed consent form COMPAre trial



Would you like to help researchers understand more about immunity in infants?

Researchers at St George's University Hospitals NHS Foundation Trust would like to invite you to take part in the COMPAre study, a study investigating the way that infant immunity to infection is measured.

What does the study involve?

We are investigating some tests which are currently used to assess infant immunity to infection. For this we need samples of cord blood.

In this study researchers will take a sample of blood from the umbilical cord attached to the placenta after your baby and the placenta have been delivered. We do not need any blood samples from your baby or from you.

What information would be collected about myself or my baby?

For this study we do not need to collect any identifiable information about you or your baby. The only information we will collect is the gestation your baby was born at, their gender and ethnicity, the type of delivery you have, and information about any antibiotics which you have recently received. We will retain a copy of your consent form which will include your name for a period of five years.

Who can take part in the study?

If you are pregnant and plan to deliver at St George's University Hospitals NHS Foundation Trust you are eligible to take part in the study.

If you might be interested in taking part please contact the research team:

vaccinesforchildren@sgul.ac.uk

Telephone: [REDACTED]

Consent

	Please initial
I _____ have read and considered the information above and I am happy to have a cord blood sample obtained by a member of the research team	
I understand that a copy of this consent form will be retained by the study team for a period of five years	

Participant signature _____ Date _____

Researcher signature _____ Date _____

Researcher name _____

COMPAre
Information leaflet and ICF
V3.0, 30.11.19
IRAS: 266052

Document 3: Case report form COMPAre trial

COMPAre CASE REPORT
FORM

Participant number

Case Report Form (CRF)

COMPAre: Sources of Complement in Meningococcal and Pertussis serum bactericidal antibody Assays

IRAS: 266052

JRES reference number: 2019.0116

VISIT ONE

Date of visit one

/ /

Location of visit one

Labour ward

☐

Antenatal ward

☐

Antenatal clinic

☐Fetal medicine unit☐

Other (please specify)

☐**INCLUSION / EXCLUSION CRITERIA****INCLUSION CRITERIA**

Pregnant and planning to deliver at St George's hospital

Yes ☐ No ☐**EXCLUSION CRITERIA**Aged less than 16 yearsYes ☐ No ☐

Known complement deficiency

Yes ☐ No ☐

Appendix E

Date of consent

Completed by: (Signature) _____ Name: _____

Completion date: ____/____/____

VISIT TWO

Date of delivery

Time of delivery

Gestational age at delivery

 weeks days

Mode of delivery

Vaginal delivery ☐ C/section ☐

Study group

>37 GWs ☐ 32-36+6 GWs ☐ <32 GWs ☐

Sex of infant

Male ☐ Female ☐

Ethnicity of infant

Have any antibiotics been received in the past 7 days? Yes ☐ No ☐

If yes, please provide details

(Please include name of antibiotic, dose,
number of doses received and time of
last dose administration)

Time of sample collection

Participant identification number (number and letter)

Completed by: (Signature) _____ Name: _____

Completion date: ____/____/____

Appendix F

Table 1: Summary of full texts reviewed in systematic review with decision, and reason for rejection if relevant

Author, year	Title	Journal	Decision	Comments
Adam, 2008	Safety and effectiveness against respiratory tract infections for pneumococcal conjugate vaccine co-administered with routine vaccine combinations	Vaccine	Exclude	Unable to separate term and preterm results
Anderson, 2013	Apnoea after the 2-month immunisation in extremely preterm infants: What happens with the 4-month immunisation?	Journal of Paediatrics and Child Health	Include	
Anonymous, 2011	Preterm infants may need a boost to protect against invasive pneumococcal disease	International Journal of Virology	Exclude	Commentary
Asturias, 2013	Post-authorization safety surveillance of a liquid pentavalent vaccine in Guatemalan children	Vaccine	Exclude	Unable to separate term and preterm results
Baxter, 2010	Vaccinating premature infants in a Special Care Baby Unit in the UK: Results of a prospective, non-inferiority based, pragmatic case series study	Human Vaccines	Include	
Bednarek, 2018	Persistence of vaccine-induced immunity in preschool children: Effect of gestational age	Medical Science Monitor	Include	
Ben Jmaa, 2017	Cardio-respiratory Events and Inflammatory Response after Primary Immunization in Preterm Infants < 32 Weeks Gestational Age: A Randomized Controlled Study	Pediatric Infectious Disease Journal	Include	
Berrington, 2006	Haemophilus influenzae type b immunization in infants in the United Kingdom: Effects of diphtheria/tetanus/acellular pertussis/Hib combination vaccine, significant prematurity, and a fourth dose	Pediatrics	Include	
Berrington, 2007	Avidity of Haemophilus influenzae type b antibody in UK infants	Archives of Disease in Childhood	Include	
Berrington, 2007	Reduced anti-PRP antibody response to Hib immunisation in preterm (< 32 weeks) UK infants who received inactivated polio (eIPV)	Vaccine	Include	
Black, 2002	Safety and efficacy of the seven-valent pneumococcal conjugate vaccine: Evidence from Northern California	European Journal of Pediatrics, Supplement	Exclude	Review
Bohnhorst, 2021	Cardiorespiratory Events Following the Second Routine Immunization in Preterm Infants: Risk Assessment and Monitoring Recommendations	Vaccines	Include	

Appendix F

Briggs-Steinberg, 2021	Clinical Tolerance of In-Neonatal Intensive Care Unit Administration of Rotavirus Vaccine	Am J Perinatol	Include	
Carbone, 2008	Absence of an Increase in Cardiorespiratory Events after Diphtheria-Tetanus-Acellular Pertussis Immunization in Preterm Infants: A Randomized Multicenter Study	Pediatrics	Include	
Chaplin, 2014	Rotarix: Rotavirus vaccine for infants of two months	Prescriber	Exclude	Guidelines
Chlebna-Sokol, 2015	Evaluation of the immune response to vaccination against pneumococcus in children born prematurely including the influence of perinatal factors	Hong Kong Journal of Paediatrics	Exclude	Entirely produced in another paper
Clarke, 2003	Effect of a fourth Haemophilus influenzae type b immunisation in preterm infants who received dexamethasone for chronic lung disease	Archives of Disease in Childhood: Fetal and Neonatal Edition	Include	
Clarke, 2006	Response of steroid-treated former preterm infants to a single dose of meningococcal C conjugate vaccine	Vaccine	Include	
Clarke, 2004	DTP immunisation of steroid treated preterm infants	Archives of Disease in Childhood-Fetal and Neonatal Edition	Include	
Clifford, 2011	Recurrent apnoea post immunisation: Informing re-immunisation policy	Vaccine	Include	
Collins, 2005	Immunogenicity and immunologic memory of meningococcal C conjugate vaccine in premature infants	Pediatric Infectious Disease Journal	Include	
D'Angio, 2007	Measles-mumps-rubella and varicella vaccine responses in extremely preterm infants	Pediatrics	Include	
D'Angio, 2010	Heptavalent pneumococcal conjugate vaccine immunogenicity in very-low-birth-weight, premature infants	Pediatric Infectious Disease Journal	Include	
D'Angio, 2013	Immunogenicity of Haemophilus influenzae Type b Protein Conjugate Vaccines in Very Low Birth Weight Infants	Pediatric Infectious Disease Journal	Include	
Demeo, 2015	Adverse Events After Routine Immunization of Extremely Low-Birth-Weight Infants	Jama Pediatrics	Include	
Dubus, 2020	Prophylactic Paracetamol After Meningococcal B Vaccination Reduces Postvaccination Fever and Septic Screens in Hospitalized Preterm Infants	Pediatric Infectious Disease Journal	Include	
Ellison, 2005	Adverse reactions to immunization with newer vaccines in the very preterm infant	Journal of Paediatrics and Child Health	Include	

Appendix F

Esposito, 2007	Immunogenicity, safety and tolerability of meningococcal C CRM197 conjugate vaccine administered 3, 5 and 11 months post-natally to pre- and full-term infants	Vaccine	Include	
Esposito, 2002	Long-term pertussis-specific immune responses to a combined diphtheria, tetanus, tricomponent acellular pertussis and hepatitis B vaccine in pre-term infants	Vaccine	Include	
Esposito, 2005	Immunogenicity, safety and tolerability of heptavalent pneumococcal conjugate vaccine administered at 3, 5 and 11 months post-natally to pre- and full-term infants	Vaccine	Include	
Faldella, 2007	Safety of DTaP-IPV-Hib-HBV hexavalent vaccine in very premature infants	Vaccine	Include	
Ferreira, 2018	Humoral immune response to measles and varicella vaccination in former very low birth weight preterm infants	Brazilian Journal of Infectious Diseases	Include	
Flatz-Jequier, 2008	Recurrence of cardiorespiratory events following repeat DTaP-based combined immunization in very low birth weight premature infants	Journal of Pediatrics	Include	
Fortunato, 2022	Safety evaluation of the DTaP5-IPV-Hib-HepB vaccine: a review	Expert Opin Drug Saf	Exclude	Review
Fortmann, 2022	Immunization of preterm infants: current evidence and future strategies to individualized approaches	Semin Immunopathol	Exclude	Review
Furck, 2010	Very low birth weight infants have only few adverse events after timely immunization	Journal of Perinatology	Include	
Goveia, 2007	Safety and efficacy of the pentavalent human-bovine (WC3) reassortant rotavirus vaccine in healthy premature infants	Pediatric Infectious Disease Journal	Include	
Hacking, 2010	Frequency of respiratory deterioration after immunisation in preterm infants	Journal of Paediatrics and Child Health	Include	
Heath, 2003	Hib vaccination in infants born prematurely	Archives of Disease in Childhood	Include	
Hviid, 2009	Effectiveness of two pertussis vaccines in preterm Danish children	Vaccine	Include	
Ichikawa, 2013	Effect of early measles vaccination (AIK-C strain) for preterm infants	Pediatrics International	Include	
Kabir, 2022	Effectiveness of 7-Valent Pneumococcal Conjugate Vaccine Against Invasive Pneumococcal Disease in Medically At-Risk Children in Australia: A Record Linkage Study	J Pediatric Infect Dis Soc	Exclude	PT infants can not be separated
Kent, 2019	Safety of meningococcal group B vaccination in hospitalised premature infants	Archives of Disease in Childhood-Fetal and Neonatal Edition	Include	
Kent, 2016	Schedules for pneumococcal vaccination of preterm infants: An RCT	Pediatrics	Include	

Appendix F

Kirmani, 2002	Seven-year follow-up of vaccine response in extremely premature infants	Pediatrics	Include	
Klein, 2010	Preterm Infants' T cell Responses to Inactivated Poliovirus Vaccine	J Infect Dis	Include	
Klein, 2008	Risk factors for developing apnea after immunization in the neonatal intensive care unit	Pediatrics	Exclude	Unable to separate term and preterm data
Kulkarni-Munje, 2021	Immune Response of Indian Preterm Infants to Pentavalent Vaccine Varies With Component Antigens and Gestational Age	Frontiers in Immunology	Include	
Ladhani, 2014	Timely immunisation of premature infants against rotavirus in the neonatal intensive care unit	Archives of Disease in Childhood-Fetal and Neonatal Edition	Exclude	Commentary
Lee, 2006	Frequency of apnea, bradycardia, and desaturations following first diphtheria-tetanus-pertussis-inactivated polio-Haemophilus influenzae type B immunization in hospitalized preterm infants	BMC Pediatrics	Include	
Martinon-Torres, 2015	13-Valent pneumococcal conjugate vaccine (PCV13) in preterm versus term infants	Pediatrics	Include	
Martinon-Torres, 2017	Circulating antibody 1 and 2 years after vaccination with the 13-valent pneumococcal conjugate vaccine in preterm compared with term infants	Pediatric Infectious Disease Journal	Include	
McArdle, 2016	Vaccinating preterm infants in resource-poor settings: what is the incidence of apnoea, bradycardia and need for respiratory support?	BMJ	Exclude	Commentary
Meinus, 2012	Adverse cardiorespiratory events following primary vaccination of very low birth weight infants	Jornal De Pediatria	Exclude	Full text not available in English
Mialet-Marty, 2013	Possible Predictors of Cardiorespiratory Events after Immunization in Preterm Neonates	Neonatology	Include	
Miralha, 2013	Response to the complete hepatitis B vaccine regimen in infants under 12 months of age: A case series	Brazilian Journal of Infectious Diseases	Exclude	Not pertinent
Mollah, 2012	Antibody titre against 3 doses of hepatitis B vaccine among preterm and term babies	Mymensingh Med J	Exclude	Unable to obtain
Monk, 2014	Safety of rotavirus vaccine in the NICU	Pediatrics	Exclude	Unable to separate safety outcomes for the seven days following vaccination
Moss, 2010	Responses to a conjugate pneumococcal vaccine in preterm infants immunized at 2, 3, and 4 months of age	Clinical & Vaccine Immunology: CVI	Include	
Mukherjee, 2018	Men B (bexsero) immunisation side effects in extremely premature infants (<28 weeks)	Arch Dis Child Fetal Neonatal Ed	Include	

Appendix F

Nieminen, 2019	Effectiveness of the 10-valent pneumococcal conjugate vaccine among girls, boys, preterm and low-birth-weight infants - Results from a randomized, double-blind vaccine trial	Vaccine	Include	
Omenaca, 2011a	Combined Haemophilus Influenzae Type B-Neisseria Meningitidis Serogroup C Vaccine Is Immunogenic and Well Tolerated in Preterm Infants When Co-administered With Other Routinely Recommended Vaccines	Pediatric Infectious Disease Journal	Include	
Omenaca, 2010	Hepatitis B response of premature infants after primary and booster immunisation with a diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated poliovirus/ haemophilus influenzae type B vaccine	Infectious Diseases in Obstetrics and Gynecology	Exclude	Entirely replicated in another paper
Omenaca, 2005	Response of preterm newborns to immunization with a hexavalent diphtheria-tetanus-acellular pertussis-hepatitis B virus-inactivated polio and Haemophilus influenzae type b vaccine: first experiences and solutions to a serious and sensitive issue	Pediatrics	Include	
Omenaca, 2007a	Antipolyribosyl ribitol phosphate response of premature infants to primary and booster vaccination with a combined diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio virus/Haemophilus influenzae type b vaccine	Pediatrics	Include	
Omenaca, 2007b	Antibody persistence and booster vaccination during the second and fifth years of life in a cohort of children who were born prematurely	Pediatric Infectious Disease Journal	Include	
Omenaca, 2011b	Immunization of preterm infants with 10-valent pneumococcal conjugate vaccine	Pediatrics	Include	
Omenaca, 2012	Safety, reactogenicity and immunogenicity of the human rotavirus vaccine in preterm European Infants: a randomized phase IIIb study	Pediatric Infectious Disease Journal	Include	
Perin, 2012	Immune response to tetanus booster in infants aged 15 months born prematurely with very low birth weight	Vaccine	Include	
Pfister, 2004	Safety of DTaP-based combined immunization in very-low-birth-weight premature infants: Frequent but mostly benign cardiorespiratory events	Journal of Pediatrics	Include	
Pourcyrous, 2007	Primary immunization of premature infants with gestational age < 35 weeks: Cardiorespiratory complications and C-reactive protein responses associated with administration of single and multiple separate vaccines simultaneously	Journal of Pediatrics	Include	
Riise, 2017	Risk of Pertussis in Relation to Degree of Prematurity in Children Less Than 2 Years of Age	Pediatric Infectious Disease Journal	Include	
Riise, 2018	Preterm infants have higher risk than full-term children of invasive pneumococcal disease during the first 2 years of life	Pediatric Infectious Disease Journal	Include	

Appendix F

Robinson, 2004	Antibody Response to Diphtheria-Tetanus-Pertussis Immunization in Preterm Infants Who Receive Dexamethasone for Chronic Lung Disease	Pediatrics	Include	
Roué, 2014	Impact of rotavirus vaccine on premature infants	Clinical & Vaccine Immunology	Include	
Rouers, 2020	Association of Routine Infant Vaccinations with Antibody Levels among Preterm Infants	JAMA Journal of the American Medical Association	Include	
Ruckinger, 2010	Effect of heptavalent pneumococcal conjugate vaccination on invasive pneumococcal disease in preterm born infants	BMC Infectious Diseases	Include	
Ruggeberg, 2007	Immunogenicity and induction of immunological memory of the heptavalent pneumococcal conjugate vaccine in preterm UK infants	Vaccine	Include	
Sadarangani, 2017	Four Component Meningococcal Capsular Group B Vaccine in Preterm Infants	Journal of the Pediatric Infectious Diseases Society	Include	
Schulzke, 2005	Apnoea and bradycardia in preterm infants following immunisation with pentavalent or hexavalent vaccines	European Journal of Pediatrics	Include	
Sen, 2001	Adverse events following vaccination in premature infants	Acta Paediatrica, International Journal of Paediatrics	Include	
Sheridan, 2022	Pertussis burden and acellular pertussis vaccine effectiveness in high-risk children	Vaccine	Include	
Shinefield, 2002	Efficacy, immunogenicity and safety of heptavalent pneumococcal conjugate vaccine in low birth weight and preterm infants	Pediatric Infectious Disease Journal	Exclude	Different definition of preterm
Sikora, 2006	Haemophilus influenzae type b and pertussis vaccinations in preterm infants	Archivum Immunologiae et Therapiae Experimentalis	Exclude	Some infants commenced their primary vaccines at greater than 18 months
Sikora, 2005	Evaluation of immunogenicity of parenteral inactivated poliomyelitis vaccine in preterm infants	International Review of Allergology and Clinical Immunology	Include	
Sikora, 2004	Assessment of the efficacy of vaccination against pertussis in prematurely born infants after the full series of basal immunization	International Review of Allergology and Clinical Immunology	Include	
Slack, 2003	Acellular pertussis and meningococcal C vaccines: cardio-respiratory events in preterm infants	Eur J Pediatr	Include	
Slack, 2005	DT5aP-Hib-IPV and MCC vaccines: Preterm infants' response to accelerated immunisation	Archives of Disease in Childhood	Include	

Appendix F

Slack, 2001	Immune response of premature infants to meningococcal serogroup C and combined diphtheria-tetanus toxoids-acellular pertussis-Haemophilus influenzae type b conjugate vaccines	Journal of Infectious Diseases	Include	
Slack, 2004	Acellular pertussis vaccine given by accelerated schedule: Response of preterm infants	Archives of Disease in Childhood: Fetal and Neonatal Edition	Include	
Slack, 2004	Responses to a fourth dose of Haemophilus influenzae type B conjugate vaccine in early life	Archives of Disease in Childhood: Fetal and Neonatal Edition	Exclude	Not pertinent
Soans, 2021	Vaccination in preterm and low birth weight infants in India	Human Vaccines & Immunotherapeutics	Exclude	Review
Szynczewska, 2014	Immunogenicity of heptavalent conjugate vaccine against streptococcus pneumoniae in premature babies with low birth weight	Pediatrics and Neonatology	Include	
Szynczewska, 2011	Immunogenicity and safety of heptavalent conjugate vaccine against Streptococcus pneumoniae in pre-term Polish infants	Vaccine	Exclude	Entirely replicated in another paper
Tsuda, 2012	Immune response to Haemophilus influenzae type b conjugate vaccine in preterm infants	Pediatrics International	Include	
Van den berg, 2013	Neutral and acidic oligosaccharides supplementation does not increase the vaccine antibody response in preterm infants in a randomized clinical trial	PLoS One	Exclude	Entirely replicated in another paper
Van den berg, 2015	Response on pneumococcal vaccine in preterm infants after neutral and acidic oligosaccharides supplementation	Pediatric Infectious Disease Journal	Include	
Van Der Maas, 2019	Pertussis hospitalizations among term and preterm infants: Clinical course and vaccine effectiveness	BMC Infectious Diseases	Include	
Vazquez, 2008	Immunogenicity and reactogenicity of DTPa-HBV-IPV/Hib vaccine as primary and booster vaccination in low-birth-weight premature infants	Acta Paediatrica	Include	
Vermeulen, 2013	Persistence at one year of age of antigen-induced cellular immune responses in preterm infants vaccinated against whooping cough: comparison of three different vaccines and an effect of a booster dose	Vaccine	Include	
Vermeulen, 2010	Cellular immune responses of preterm infants after vaccination with whole-cell or acellular pertussis vaccines	Clinical and Vaccine Immunology	Include	
Wilck, 2021	Safety and immunogenicity of a fully-liquid DTaP-IPV-Hib-HepB vaccine (Vaxelis (TM)) in premature infants	Human Vaccines & Immunotherapeutics	Exclude	Review
Wilinska, 2016	Risk factors for adverse events after vaccinations performed during the initial hospitalization of infants born prematurely	Developmental period medicine	Include	

Appendix F

Wilson, 2012	Incidence of adverse events in premature children following 2-month vaccination	Human vaccines & Immunotherapeutics	Include	
Zahedpash, 2012	Immune response in preterm infants to hepatitis B vaccine	Journal of Babol University of Medical Sciences	Exclude	Full text not in English

Appendix G

Table 1: Summary of all included reports in the systematic review

Reports relating to the same trial are shaded in blue in the first column

Author, year	Location	Study design	Focus of paper	Objectives	Outcomes	Details of outcome assessment	Population	Vaccine(s) used	Results
Anderson, 2013	Australia (single centre)	Retrospective review	R	Describe outcome after 4-month vaccinations in extremely PT infants who had clinically significant apnoea after 2-month vaccinations	-CREs: apnoea	48 hours following vaccine administration	203 inpatient PT infants ≤ 28 WG receiving first vaccines; nine receiving second vaccines	DTaP-HBV, Hib, PCV, DTaP-IPV/Hib-HBV, rotavirus	Following first vaccines 8.4% (n=17) of infants had a likely or possible apnoeic episode and 5.9% (n=12) required an increase in respiratory support. Babies with apnoea were of lower gestation and birthweight, but no difference in postnatal age. 9/17 infants were monitored for second vaccines. No infants experienced an episode of apnoea following the second vaccine dose.
Baxter, 2010	UK (single centre)	Case series	I	Evaluate antibody response in PT infants to diphtheria, tetanus, Hib, MenC and pneumococcal conjugate vaccines. Determine the effect of GA on antibody	-IgG for diphtheria, tetanus, Hib and pneumococcus and SBA for MenC -Prop of infants reaching protective thresholds	26-45 days after third vaccination	131 PT infants ≤ 32 WG	DTwP-Hib, DTaP-Hib/IPV and MCC, PCV	Diphtheria: 98.3% of infants achieved a titre ≥ 0.01 IU/ml and 88% a titre ≥ 0.1 IU/ml. Tetanus: 100% of infants developed a minimum protective response ≥ 0.01 IU/ml and 98.3% an optimum response ≥ 0.1 IU/ml. Hib: 67.8% of infants produced an antibody titre $\geq 0.15\mu\text{g/ml}$; 32.2% had an undetectable antibody response. MenC: 86.6% developed an SBA titre ≥ 8 . Pneumococcus: Response to vaccination depended on serotype. Highest number

Appendix G

				response to these vaccines					achieving a protective level seen with 19F (92.5%) and the lowest number achieving a protective level seen with 6B (67.5%). General: There was a significant inverse association between age and antibody response for diphtheria; no significant association for any of the other antigens.
Bednarek, 2018	Poland (single centre)	Cross sectional study	I	To verify at 2.5-3 years after the last primary vaccine dose if there is a difference between PT and FT infants in their post vaccination antibody titres	-IgG against diphtheria, tetanus, pertussis, Hib, polio, measles, mumps and rubella	Single measurement on second day after admission	352 children aged 4.5-5.9 admitted to hospital with a non-infectious condition: 46 born at 32-36 WG and 306 FT	HBV, poliomyelitis, Hib, DTaP, HBV-DTaP-IPV-Hib, MMR	All children, FT and PT, maintained seroprotective antibody levels against polioviruses type one, two and three and against measles antigens. No statistically significant differences were found in the proportions of pre-schoolers born PT and at FT who were seroprotected against other vaccine antigens.
Ben Jmaa, 2017	Canada (single centre)	RCT	R	To examine the occurrence of CRE following the first dose of pentavalent vaccines in infants <32 WG and the impact of inflammatory response inhibition	-CREs: apnoea, bradycardia, desaturation	24 hours before and 48 hours after	56 inpatient PT infants <32WG	DTaP-IPV-Hib, PCV	There were no significant differences in CRE before and after vaccination in either the placebo (CRE/patient/24 hours= 8.6+/- 11.1 before vaccination vs 14.0 +/-12.8 after vaccination; p=0.08)) or ibuprofen (CRE/patient/24 hours= 6.7 +/- 7.7 before vaccination and 6.8 +/-9.7 after vaccination; p=0.9)) group. When comparing the proportion of infants with an increase in CREs following vaccination there were significantly more in the placebo group. There was a significant increase in CRP level in both ibuprofen and placebo groups.

Appendix G

Berrington, 2006	UK (four centres)	Prospective cohort study	I	To study the effect of significant PT delivery and DTaP-Hib use on Hib antibody responses; To measure the response to a booster dose of PRP-T in those who had an anti PRP antibody level <1.0µg/mL	-Anti-PRP and anti- TT antibody	Before and 6-8 weeks after completion of the primary series and 6-8 weeks following a booster dose if given	166 PT infants <32WG, 45 FT infants. 97 infants (15 FT and 82 PT) had a suboptimal PRP response and were offered a booster	DTP-Hib, DTaP, DTwP, Hib, MenC, eIPV and OPV	51% of PT and 67% of FT infants had an anti-PRP response ≥1.0µg/ml. PT infants were less likely to have achieved titres of ≥0.15µg/ml or ≥1.0µg/ml compared with FT infants. Having received one or more acellular vaccine doses was associated with lower anti-PRP antibody titres ≥1.0µg/ml. Of those infants who received a booster vaccination, 93% achieved a titre of ≥1.0µg/ml after booster vaccination. All infants in both groups achieved protective tetanus titres.
Berrington, 2007	UK (four centres)	Prospective cohort study	I	To measure the geometric mean avidity index (GMAI) in patients where the antibody level was >0.7µg/mL	-Geometric mean avidity index (GMAI)	6-8 weeks after completion of the primary series and 6-8 weeks following a booster dose if given	166 PT infants <32WG, 45 FT infants. 97 infants (15 FT and 82 PT) had a suboptimal PRP response and were offered a booster	DTP-Hib, DTaP, DTwP, Hib, MCC, eIPV and OPV	If an anti-PRP level of >0.7µg/ml is reached in a PT infant following a three-dose priming schedule, the avidity of the antibody is not likely to be significantly reduced compared with a FT infant. If an anti-PRP level of <1.0µg/ml is reached after primary vaccination, a fourth vaccine dose results in high antibody levels with similar avidity indices in FT and PT infants.
Berrington, 2007	UK (four centres)	Re-analysis of results from prospective cohort study	I	To evaluate the effect of eIPV on anti-PRP response	-Anti-PRP and anti-tetanus antibody assessed according to receipt of eIPV	N/A	64 PT infants (<32 WG) in the original study received eIPV	DTP-Hib, DTaP, DTwP, Hib, MCC, eIPV and OPV	There was a significant reduction in anti-PRP antibody in those infants who received eIPV, but no decrease in anti-tetanus antibody.
Bohnhorst, 2021	Germany (two centres)	Case series	R	To prospectively investigate the frequency and	-CREs: apnoea, bradycardia,	Six hours before and 48 hours	71 PT infants (<31 WG)	DTaP-HBV-IPV/Hib, PCV13	Overall, there was an increase in episodes of bradycardia, desaturation, apnoea and CREs requiring tactile

Appendix G

				timing of CREs in response to the second routine immunisations in PT infants who had experienced CREs following the first routine immunisation	desaturation , increased ventilatory support	following vaccine administration			stimulation in the first 24 hours following second vaccination, this significantly fell in the second 24 hours of monitoring. Only 9.9% (n=7) did not have any increase in CREs following second vaccination. 4.2% of infants required respiratory support (supplementary oxygen only) following second vaccination. All infants who presented with CREs requiring treatment following second vaccinations had required respiratory support or analeptic treatment at first vaccination. GA and weight at birth and at second vaccinations were significantly associated with the occurrence of CREs requiring treatment. There was no association in CREs requiring treatment with comorbidities.
Briggs-Steinberg, 2021	US (single centre)	Case control study	R	Determine the tolerance of NICU-based administration of rotavirus vaccine compared with the same infant after receipt of 2-month vaccine series and to historical, unimmunised controls	-CREs: apnoea, bradycardia, increased ventilatory support -Systemic adverse events: diarrhoea, vomiting -Sepsis evaluation	- All outcomes: Observation for 7 days before and after vaccination	201 PT infants <36WG, 189 PT control infants <36WG	Pentavalent rotavirus vaccine	Comparing the period before and after vaccination: no difference in the proportion of infants with an increase in apnoea and bradycardia or in episodes of loose stool, but an increase in total number of stools and non-bilious vomiting. Compared to two-month series: In comparison with routine vaccinations, fewer infants who received rotavirus vaccine alone experienced fever, an increase in number of apnoeic episodes, apnoea requiring stimulation or bradycardia requiring stimulation. Compared to historic controls: More infants with an increase in episodes of apnoea, apnoea with stimulation and bradycardia with stimulation following

Appendix G

									rotavirus vaccination. No increase in number of infants who were evaluated for sepsis, had increase in ventilatory requirements or presence of feed intolerance. No increase in sepsis evaluations in rotavirus vaccinated infants compared with the period pre-vaccination, with those who had received two-month vaccinations or with historic controls.
Carbone, 2008	US (10 centres)	RCT	R	Examine the relationship between DTaP and CRE in PT infants	-CREs: apnoea, bradycardia	48 hours after vaccine administration	191 inpatient PT infants <37WG	DTaP	16.1% of infants in the vaccinated group had at least one episode of apnoea and 58.1% at least one episode of bradycardia. No difference between the vaccinated and control groups.
Clarke, 2003	UK (Three centres)	Case series	I	To assess whether a fourth PRP-T vaccine would improve antibody response in PT infants previously treated with dexamethasone	-Anti PRP IgG -GMAI	Before and eight weeks following fourth dose of PRP-T	12 infants <30 WG	PRP-T	No significant increase in anti-PRP GMT following the fourth dose of PRP-T. Despite poor antibody rise, there was a significant increase in avidity following a fourth dose.
Clarke, 2004	UK (Three centres)	Case series	I	To investigate whether four, rather than three DTP doses may be preferable for primary immunisation of	-IgG against diphtheria, tetanus, FIM, PT and FHA	Before and eight weeks following a fourth dose of DTP vaccine	12 PT infants (<30 WG) who had received dexamethasone	DTP-Hib	Excellent response to three doses of DTP vaccine in steroid treated PT infants, a fourth dose did not further improve the antibody response.

Appendix G

				steroid treated infants					
Clarke, 2006	UK (Four centres)	Historical cohort study	I	To study the immune response of former PT infants to a single MenC dose given after infancy, including those who received corticosteroids as part of their early neonatal treatment	-SBA and IgG against MenC	One month following vaccination.	49 infants born at <33 WG. Historical cohort of 70 FT toddlers.	DTwP, Hib, oral polio, MCC.	Following a single MenC dose, the SBA GMT for former PT infants was 249 (111-558) which was not significantly different from that seen in the historic term cohort 141 (89-224). A significantly lower proportion of former PT infant achieved a protective SBA of ≥ 8 compared with FT infants (77% vs 91%; $p=0.03$). Previous steroid treatment resulted in significantly higher SBA GMTs than non-steroid treated infants, but there were no differences in proportions achieving protective titres.
Clifford, 2011	Australia (regional surveillance)	Retrospective review	R	Determine recurrence rates for apnoea temporally associated with vaccination in PT and FT infants and to explore risk factors for recurrence	-CREs: apnoea	48 hours before and after vaccine administration	38 PT infants <37 WG, 8 FT infants	DTaP-Hib-IPV-HBV, PCV, rotavirus; DTap-IPV, Hib-HBV	18% (n=7) of PT infants had a recurrent apnoea following four-month vaccinations. Lower birthweight ($p=0.04$) and ongoing hospitalisation for complications ($p=0.01$) increased the odds of a recurrent apnoea. None of the seven infants who experienced apnoea following vaccination at four-months had recurrence of apnoea following six-month vaccinations.
Collins, 2005	UK (Two centres)	Prospective cohort study	I	Compare the antibody response to three doses of MenC vaccine administered as a primary course to PT infants with	-SBA and IgG against MenC	2, 5 and 12 months of age and 2-4 weeks after the booster vaccination	62 PT (<37 WG) and 60 FT controls	MCC	No significant difference between FT and PT infants who achieved a protective SBA of ≥ 8 or in SBA GMTs achieved. Persistence at 12 months was similar in both groups. Post-booster GMTs were significantly lower in the PT group, but the proportion achieving GMTs ≥ 8 was not significantly different between groups. No correlation

Appendix G

				that of FT infant controls					between antibody responses and GA or BW.
D'Angio, 2007	USA (single centre)	Prospective cohort study	I	To measure the immunogenicity of measles, mumps and rubella and varicella vaccines in PT and FT children	-Neutralising antibody for measles and IgG against mumps and rubella	Before vaccination and 3-6 weeks after vaccination	16 PT infants <29 WG; 16 FT infants	MMR	PT and FT infants had similar titres after vaccination for mumps, rubella, measles and varicella and no difference in proportions achieving protective titres.
D'Angio, 2010	USA (9 centres)	Case series	I, S, R	To assess PCV-7 immunogenicity in VLBW, PT infants	-IgG against PCV7 serotypes - Opsonisation titres against serotypes 4, 6B, 14 and 23F - Rehospitalisation - CREs- apnoea	Immunogenicity: 4-6 weeks following the third vaccine dose Reactogenicity: Diary card for 7 days after vaccine administration Safety: Questionnaire completed at 18-22 months	369 infants <32WG and ≤1500g enrolled, 244 infants completed primary vaccinations and had serum obtained	PCV7	Immunogenicity: Infants with a lower BW (401-1000g) were less likely than those with a higher BW (1001-1500) to achieve a titre ≥0.15 microg/ml against serotypes 6B and 23F. Postnatal steroid administration was associated with reduced antibody concentrations for serotypes 6B and 23F. Safety: High rate of adverse events and rehospitalisation reported, but only three rehospitalisations took place within seven days of vaccination. Reactogenicity: 4% of hospitalised infants experienced apnoea.
D'Angio, 2013	USA (9 centres)	Case series	I	To assess the anti-PRP titres among PT infants	Anti PRP IgG	4-6 weeks following the third vaccine dose	161 infants <32WG and ≤1500g	Hib	79% of infants had post vaccination titres ≥1.0µg/ml; 96% had titres ≥0.15 µg/ml. No statistically significant difference between those with a birthweight of < and >1000g in antibody titres.
DeMeo, 2015	US (348 centres)	Retrospective review	R, S	Compare the incidence of	- Sepsis evaluation	3 days	13,926 PT infants ≤28	DTaP, IPV, HBV, Hib,	Increase in rate of sepsis evaluation ARR 3.7 (3.2-4.4), need for increased

Appendix G

				sepsis evaluations, need for increased respiratory support, intubation, seizures and death among immunised ELBW infants in the 3 days before and after vaccination	-CREs: Increased respiratory support, intubation		WG and BW <1000g	PCV, DTaP-IPV-HBV, DTaP-IPV-Hib	respiratory support ARR 2.1 (1.9-2.5), need for intubation ARR 1.7 (1.3-2.2) and seizure ARR 0.3 (0.1-1.1). Infants at 23-24WG had an increased risk of sepsis evaluation and intubation compared with infants with a greater GA. Postnatal age, small for gestational age and postnatal weight at vaccination were not associated with any of the outcomes. No difference in rates of events in those who received single or multiple vaccinations.
Dubus, 2020	UK (single centre)	Retrospective review	R	Evaluate whether 4CMenB is associated with an increase in AE in the 48 hours after vaccination in PT infants and the impact of prophylactic paracetamol on AEs	- Fever - Sepsis evaluation - Receipt of antibiotics	48 hours after vaccine administration	95 vaccination events involving 83 inpatient PT infants <37WG	4CMenB alongside routine vaccinations	When 4CMenB was given without prophylactic paracetamol there was an increased chance of infants developing fever (RR 4.04; 1.23-13.35), being evaluated for sepsis (RR 21.94; 1.32-364.10) and receiving IV antibiotics (RR 18.56; 1.10-314.02), which reduced significantly when paracetamol given at the same time.
Ellison, 2005	Australia (single centre)	Retrospective review	R	Study the frequency and types of adverse reactions to currently available vaccines in very PT infants	- CREs: apnoea, respiratory support - Fever - Sepsis evaluation	48 hours before and 48 hours after vaccination	48 inpatient PT infants ≤30w	Hib-HBV, DTaP-IPV	Fever occurred in 0% prior to vaccination and in 33% following vaccination, there was little change in the proportion of infants who required supplemental oxygen, but there was a small statistically significant increase in mean oxygen concentration required post immunisation. There was no statistically significant difference in

Appendix G

									apnoea before and after vaccination. Evaluation for sepsis in 8%.
Esposito, 2007	Italy (single centre)	Prospective cohort study	I, R, S	To evaluate the immunogenicity , safety and reactogenicity of MenC-CRM ₁₉₇ conjugate vaccine administered at 3, 5 and 11 months to PT and term infants	-SBA and IgG against MenC -Local and systemic reactions -Unsolicited AEs/SAEs	Immunogenicity sampling: before first vaccine dose, one month after second and before and after the third (booster) dose. Reactogenicity and safety: 7-day diary card following vaccine administration	79 PT infants 32-36 GW and 74 FT infants	MCC	Immunogenicity: No statistically significant difference in GMC of antibody against MenC. Very high proportions of infants in both groups achieved protective titres after primary and booster doses. Reactogenicity: Some varied differences between PT and FT infants in local reactions reported; PT infants experienced more systemic side effects, but these were not of clinical significance. Safety: No vaccine related SAEs reported in either group.
Esposito, 2002	Italy (single centre)	Cross sectional study	I	To compare pertussis specific humoral responses and cell mediated immunity five years after primary vaccination with a combined DTaP-HBV	-IgG against PT, FHA and PRN and CMI	5-6 years following vaccination	38 children aged 5-6: 13 PT born at 27-31 WG, 10 PT born at 32-37 WG and 15 born at FT.	DTaP-HBV	A positive response to PT, FHA and PRN was less common in PT than FT infants, with the lowest percentages being obtained in participants born at ≤ 31 weeks. Low IgG and T cell responses in all children, both FT and PT.

Appendix G

				vaccine in children with different gestational ages					
Esposito, 2005	Italy (single centre)	Prospective cohort study	I, R	-To assess the immunogenicity, safety and tolerability of PCV7 administered 3, 5 and 11 months postnatally to PT and FT infants	-IgG against PCV7 serotypes -Local and systemic reactions -Fever	Immunogenicity: Baseline, one month after second dose, pre- and one month after dose 3 of PCV7 Reactogenicity: Details of any local or systemic reactions was actively collected for the three days following vaccination and passively for 30 days	46 PT (32-36 WG) and 46 FT infants	PCV7	Immunogenicity: After two doses of PCV there was a significant increase in antibody concentrations against all the serotypes. The magnitude of the response varied from serotype to serotype, but there was no significant difference between PT and FT infants. No difference between groups prior to or following the booster. Proportions achieving protective titres also showed no differences between groups. Reactogenicity: No statistically significant difference between PT and FT infants in local or systemic side effects.
Faldella, 2007	Italy (single centre)	Case series	R	Assess the clinical safety of DTaP-IPV-Hib-HBV hexavalent vaccination in very PT infants and to verify if the	- CREs: apnoea, desaturation, bradycardia	3 days before and 3 days after	45 PT infants <31WG: 23 with chronic disease and 22 in good health	DTaP-IPV-Hib-HBV	11.1% (n=5) of all infants, all with BPD and/or GORD, had CREs. This represents 21.7% of the chronic disease group. 2.2% of infants experienced apnoea, 4.4% desaturation and 4.4% bradycardia and desaturation.

Appendix G

				administration of first vaccines is a reason for close monitoring hospitalised infants <31 WG.					
Ferreira, 2018	Brazil (single centre)	Prospective cohort study	I	-To compare the immune response to measles and varicella vaccination in PT with FT infants and to analyse factors associated with measles and varicella antibody levels	-Measles antibody titres	Before MMR vaccination and at 15 months	65 PT (<37 GW) and 56 FT infants	MMR	Similar immunogenicity in FT and PT infants- 100% of infants in both groups achieved protective titres. Measles antibody levels were negatively associated with antenatal corticosteroid use.
Flatz-Jequier, 2008	Switzerland (single centre)	Retrospective review	R	Evaluate the tolerance to vaccination of PT infants	- CREs: apnoea, bradycardia, desaturation	24 hours before and 48 hours after vaccine administration	64 inpatient PT infants <32 WG who were observed at the time of second vaccines	DTaP-IPV/Hib, DTaP-IPV-HepB/Hib	34 infants had a significant CRE after first vaccines (25.2%). Six monitored infants had a significant CRE after the second vaccinations (9.4%) which represents 18.2% of those who had had a significant CRE after first vaccines. The only risk factor for significant CRE after second vaccines was CRE following first vaccinations.
Furck, 2010	Germany (single centre)	Case series	R	Analyse AEs associated with immunisation of LBW infants	-CREs: apnoea, bradycardia, desaturation -Fever	Up to 48 hours after vaccine administration	473 inpatient PT (<35WG) infants with a birthweight <1500g	DTaP-IPV-Hib, HBV, DTaP-IPV-Hib-HBV, PCV	Local reactions occurred in 2.8% (n=13) of infants, fever in 2.8% (n=13), apnoea in 10.8% (n=51) and bradycardia in 10.8% (n=51). The incidence of apnoea was higher in the group which received more antigens, lower in those with increasing GA and those of greater BW.

Appendix G

									In those infants who received their second vaccines in hospital 4.3% (n=2) had apnoea and bradycardia, neither of whom had had AEs following the first vaccines.
Goveia, 2007	Global (11 countries ; >500 centres)	RCT	S, E, R	Assess safety and efficacy of pentavalent human-bovine (WC3) reassortment rotavirus vaccine in PT infants	Safety: - Intussusception - SAEs - Fever - Vomiting - Diarrhoea - Behavioural change Efficacy: - Hospitalisation - ED visits	Safety: Infants were followed up for relevant events for 42 days Reactogenicity: Diary card for seven days Efficacy: Hospitalisation and ED visits assessed up to two years after vaccine administration	2,070 PT infants $\leq$ 36WG in the reactogenicity and safety analysis; 153 in the efficacy analysis	Rotavirus	Safety: No case of confirmed intussusception in any PT infants. No difference in rates of SAEs between infants who received vaccine or placebo. Reactogenicity: No difference between vaccine and placebo groups for fever, vomiting, diarrhoea or irritability Efficacy: Vaccination prevented 73% (-2.2-95.2%) of rotavirus gastroenteritis of any severity. 100% rate reduction in ED and hospital visits in those infants who received the vaccine.
Hacking, 2010	Australia (single centre)	Retrospective review	R	Determine the relationship between the initiation of respiratory support and first routine vaccines at two months	- CREs: increased ventilatory support	Seven days following vaccination	409 PT infants (mean GA 27WG) with birthweight <1000g	DTap, HBV, Hib, OPV/IPV, PCV, rotavirus	5.4% (n=22) infants experienced apnoea following vaccination which required respiratory support. Risk factors for requiring respiratory support following vaccination were previous episodes of sepsis and longer period of CPAP prior to vaccination. No association seen with GA or BW.
Heath, 2003	UK (single centre)	Historical cohort study	I, E	To document the immunogenicity	-Anti PRP antibodies	Immunogenicity: 2, 5 and 12 months	Immunogenicity:	Hib	Immunogenicity: Compared with FT infants, the PT infants had lower anti PRP GMC and/or lower

Appendix G

				and persistence of antibody to PRP in PT infants vaccinated according to the routine schedule. To determine if PT infants were more likely than FT infants to experience vaccine failure	-Cases of invasive Hib after Hib vaccination	and a follow up sample taken at five years Efficacy: Details of Hib vaccine failures collected for eight-year period	41 PT infants (≤ 32 WG). 107 healthy FT historic control infants Efficacy: 147 term, 18 PT		proportion reaching protective titres at all ages. Lower GA was associated with lower antibody concentrations. Efficacy: No statistically significant increase in vaccine failures in PT infants.
Hviid, 2009	Denmark (national surveillance)	Retrospective cohort study	E	To evaluate the effectiveness of pertussis vaccination in PT and FT Danish children	-Pertussis hospitalisation	Until two years	820,113 term infants and 59,311 PT infants	DTwP/DTaP	After completion of the primary series there was no significant difference between PT and FT infants in vaccine effectiveness, whether the vaccine received was acellular or whole cell. The first dose of whole cell pertussis vaccination provided no protection for PT infants.
Ichikawa, 2013	Japan (single centre)	Case series	I	To investigate early measles vaccination in PT infants	- Neutralisation assays	1, 3, 6 and 12 months following vaccination.	17 PT infants (< 34 WG)	Measles	Positive titres were detected in all infants one month following vaccination and remained positive for the 12 month follow up period.
Kent, 2019	UK (19 centres)	Historical cohort study	R	To assess the risk of significant adverse events in PT infants receiving 4CMenB	- Fever - CRE - Sepsis evaluation - Receipt of antibiotics	48 hours before and after vaccine administration	133 PT infants <35 WG	4CMenB, DTaP-IPV-Hib PCV, rotavirus	Fever reported in 7% (n=8) of those infants who received paracetamol and 20% (n=5) of those who did not. 5% received antibiotics for suspected sepsis. Infants born at <28 WG were more likely to have an increase in desaturations and bradycardias than those >28 WG. No significant differences between infants vaccinated prior to addition of 4CMenB,

Appendix G

									or infants given 4CMenB with or without paracetamol in proportion of infants with apnoea, bradycardia, desaturation or requiring respiratory support after vaccination.
Kent, 2016	UK (12 centres)	RCT	I, S, R	Assess the immunogenicity of three commonly used PCV13 priming schedules in PT infants and their responses to a 12-month booster dose	-IgG for PCV serotypes -SAEs -Local and systemic AEs	Immunogenicity: prior to first vaccines, one month after primary and before and one month after booster vaccines Safety: Follow up of infants until the end of the study at 13 months	210 PT infants (<35 WG) recruited, 199 participants completed the primary phase and 194 completed the study	PCV 13	Immunogenicity: After the primary vaccination, 75%, 88% and 97% of participants had protective antibodies for at least one half of the PCV13 serotypes for the reduced, accelerated and extended schedules respectively. After the booster vaccination, participants receiving the extended schedule had significantly lower GMCs compared with reduced and accelerated schedules, but nearly all participants had seroprotective IgG concentrations. Reactogenicity: No significant differences in frequency or severity of local or systemic AEs between vaccine schedules at any timepoint. Safety: One SUSAR reported from each group- all infants made a full recovery.
Kirmani, 2002	USA (single centre)	Prospective cohort study	I	To assess the immune response of seven year old former extremely PT infants to routine childhood immunisations	-Anti PRP, anti-diphtheria, anti-tetanus, pertussis toxoid, filamentous haemagglutinin, pertactin IgG, HBsAb	Single blood sampling at age of six years	16 PT infants (<29 WG) and 16 FT controls	DTwP and Hib vaccines	<u>Diphtheria:</u> GMTs lower in PT than FT infants, all FT children and 13/16 PT had maintained protective titres. Avidity similar between FT and PT infants. <u>Tetanus:</u> GMTs lower in PT than FT infants, all children in both groups maintained protective titres <u>Pertussis:</u> No difference between GMTs in the PT and FT groups <u>Hib:</u> Anti-Hib antibodies were lower in PT than FT infants; all of the children in both groups maintained antibody levels

Appendix G

					and neutralising antibody to polio				≥0.15µg/ml, 10/16 PT and 12/16 FT infants maintained antibody level ≥1.0µg/ml. Avidity similar between FT and PT infants. <u>Polio</u>: GMTs for polio serotype 1, 2 and 3 showed no statistical difference between groups; all children maintained protective titres for ST1, ST2 and ST2 <u>Hep B</u>: No difference in HBsAg between PT and FT groups; 11/16 FT and 12/14 maintained protective levels
Klein, 2010	US (single centre)	Prospective cohort study	I, S	-To compare T cell responses of PT infants after IPV with those of FT infants	-Cytokine flow cytometry assay, flow cytometry staining and analysis, stimulation of PBMCs and measurement of neutralising antibody titres. -ED presentation/hospitalisation	Immunogenicity : One month following third dose Safety : 30-day post-vaccination period	33 PT infants (≤33 WG), 50 FT infants	DTaP-IPV-HBV, PCV, Hib	Immunogenicity: After immunisation, PT and FT infants had comparable frequencies of poliovirus specific CD4+CD45RO+CD69+IFN gamma memory T cells. PBMCs from PT infants had diminished poliovirus specific lymphoproliferation. Although all infants developed seroprotective poliovirus antibody titres, serotype 1 titres were lower among PT infants. Reactogenicity: No increase in fever, rash or irritability in FT or PT infants. Safety: No difference in ED attendance between term and PT infants. No hospital admission in either group.
Kulkarni-Munje, 2021	India (single centre)	Prospective cohort study	I	To examine antibody and cellular responses of PT infants to the pentavalent	- IgG against diphtheria, tetanus, Hib, pertussis and hepatitis B	Before vaccination and one month after the third dose	113 PT (<36 WG) and 80 FT infants	DTwP-HBV-Hib	Tetanus: All infants, both FT and PT achieved titres ≥0.1 IU/ml. There was a significant association between GA and antibody titre. Diphtheria: All infants, both FT and PT achieved titres ≥0.01 IU/ml, but lower

Appendix G

				vaccine in comparison with their FT counterparts	-Stimulated PBMC recall responses				rates of infants 28-32WG achieving protective titres of 0.1 IU/ml. Hib: High proportions of infants had protective titres ≥ 0.15, but almost half of infants 28-32WG did not achieve protective titres $\geq 1\mu\text{g/ml}$ compared with 80% in PT infants 32-36WG and term infants. Cell mediated recall responses were much lower in PT than FT infants. Hep B: FT infants developed higher antibody titres than PT infants, but most infants in both groups were protected. Cellular response was dependent on gestational age with those PT infants 28-32 WG unable to make a T cell response. Pertussis: Low rates of putative protection in both PT groups and FT infants. Cell mediated recall responses were much lower in PT than FT infants. Reduced memory responses in PT infants whether or not they had antibody responses in comparison to FT infants.
Lee, 2006	Canada (two centres)	Case control study	R	-To determine the frequency of CREs among PT infants following their first immunisation with DTP-IPV-Hib vaccine and to compare the frequency of events following whole cell	-CREs: apnoea, bradycardia, desaturation	72 hours before and 72 hours after first vaccine administration	124 PT infants ≤ 32 WG and 124 term infants	DTwP-IPV-Hib, DTaP-IPV-Hib	There was a significant difference in CRE between vaccinated cases and unvaccinated controls: 45.1% of cases and 29% of controls had an increase in CREs following vaccination. No difference between infants receiving wP and aP vaccines. Lower weight at time of vaccination was a risk factor for increased CREs.

Appendix G

				versus acellular pertussis vaccines					
Martinon-Torres, 2015	Poland and Spain (11 sites)	Prospective cohort study	I, R, S	To evaluate the immune response and safety profile of PCV13 in PT compared with FT infants	-IgG against PCV13 serotypes -Local and systemic reactions -SAEs	Immunogenicity: one month after the primary series and one month after the booster dose Reactogenicity: A diary card was completed for a week following vaccine administration. Safety: Infants were followed up until one month after the booster dose.	99 PT infants (<37 WG) and 98 FT infants	PCV13	Immunogenicity: One month after completion of the primary series >85% of infants achieved an IgG ≥ 0.35, but the proportion of responders was significantly lower in PT infants for serotypes 5, 6A and 6B. One month after the booster dose, the proportion of participants in both the FT and PT groups achieving the 0.35μg/ml threshold was >97% for all STs except ST3 with no statistically significant between group differences. Reactogenicity: Proportion of infants with local reactions within seven days following vaccination were comparable between FT and PT infants. Systemic reactions occurred at a comparable rate between term and PT infants apart from decreased appetite, irritability or decreased sleep, all of which were more frequent in the PT group. Safety: SAEs occurred more commonly in PT than FT infants. Infections were the most commonly reported SAEs.
Martinon-Torres, 2017	Poland and Spain (11 centres)	Prospective cohort study	I	To assess the persistence of antibody response to PCV-13 in formerly PT vs FT infants	-IgG against PCV13 serotypes	Measured one and two years after final dose of PCV13	88 PT (<37 WG) and 88 FT participants completed the one year follow up; 81 PT and 80 FT	PCV13	The proportion of participants with pneumococcal IgG concentration $\geq 0.35\mu$ g/ml was statistically significantly lower at one year in formerly PT infants for serotypes 3, 4, 9V, 18C, 19A and 23F and at the two-year follow up for serotypes 18C, 19A and 23F.

Appendix G

							infants completed the two-year follow up		
Mialet-Martin, 2013	France (single centre)	Case series	R	To describe the changes induced by immunisation on heart and respiratory rate variability and to test a potential association between preimmunisation profiles and postimmunisation CREs	-CREs: bradycardia and desaturations	24 hours before and 48 hours after vaccine administration	31 inpatient PT infants <32 WG	DTap-Hib-HepB, PCV13	Significant increase in all three assessed outcomes in period following vaccination compared with the period before vaccination.
Moss, 2010	UK (single site)	Prospective cohort study	I	To investigate the immunogenicity of PCV7 when administered to PT infants and to compare this to the immunogenicity seen in a FT cohort	IgG against PCV7 serotypes and two control serotypes (1 and 5)	Prior to first vaccine, four weeks following the third vaccine and either at 12 months of age or four weeks after a booster dose of PCV7	165 PT infants (<36 WG) and 55 FT infants recruited	PCV7	For all infants, GMCs increased from pre- to postimmunisation for all seven vaccine serotypes, but not for the two control serotypes. Only 36% FT infants and 34% PT infants achieved protective levels against all seven vaccine serotypes. There were no significant differences in the odds of being protected against all seven vaccine serotypes between FT and PT infants or with increasing GA. Post booster there was a significant increase in GMCs for all infants for all serotypes including the control serotypes. 28/29 FT and 22/32 PT infants who received a booster dose had protective titres against all seven vaccine serotypes.

Appendix G

Mukherjee, 2018	UK (single centre)	Retrospective review	R	To compare tolerability of vaccination in extremely PT infants before and following introduction of 4CMenB	-CREs: apnoea, desaturations, bradycardia, increase in ventilatory support -Fever -Treatment with antibiotics	48 hours before and after vaccine administration	Inpatient PT infants <28 WG, <1200g. 17 pre-vaccination, 18 post-vaccination	Routine vaccinations before and after the addition of 4CMenB	After introduction of 4CMenB 22% experienced apnoea, 72% desaturations, 61% bradycardia, 28% fever, 33% received antibiotics and 28% required an increase in respiratory support. There was an increase in apnoea, desaturations, fever and bradycardia in the period following vaccine administration compared with the period before.
Nieminen, 2019	Finland (139 centres)	RCT	E	To evaluate the vaccine effectiveness by sex, GA and BW	-Laboratory confirmed IPD	2-3 years	30, 527 infants (1,519 PT)	PHiD	Point estimates suggest protection in PT and low BW infants, but insufficient power for statistically significant results.
Omenaca, 2005	Spain (single centre)	Prospective cohort study	I, R, S	To evaluate the immunogenicity, reactogenicity and safety of a hexavalent DTaP-HBV-IPV/Hib vaccine in PT infants	-IgG against diphtheria, tetanus, PT, FHA, PRN, PRP and HBV -Local adverse events -Systemic adverse events -CREs -SAEs	Immunogenicity: before the first dose and 30-35 days after the third dose Reactogenicity: Diary cards for four days after vaccine administration Safety: Infants were followed up through the entire study period	93 PT infants (24-36WG) and 92 FT infants	DTaP-HBV-IPV/Hib	Immunogenicity: <u>Hib:</u> Following primary vaccination 92.5% of PT and 97.8% of FT infants achieved a titre of $\geq 0.15\mu\text{g/ml}$ and 76.3% of PT and 86.5% of FT infants achieved a titre $\geq 1.0\mu\text{g/ml}$. <u>Diphtheria:</u> Following primary vaccination all infants had seroprotective titres. <u>Tetanus:</u> Following primary vaccination all infants had seroprotective titres. <u>Polio:</u> Following primary vaccination all infants had seroprotective titres for all three serotypes. ST3 GMTs were higher in FT infants. <u>HBV:</u> 93.4% of PT infants and 95.2% of FT infants had seroprotective titres for hep B. <u>Pertussis:</u> GMTs were similar in FT and PT infants

Appendix G

									Reactogenicity: No differences in reactogenicity between FT and PT infants. Some infants experienced transient CREs within 72 hours of the first vaccination with no clinical repercussions. Safety: 17 SAEs reported (16 in PT infants and 1 in FT infants; none related to vaccination).
Omenaca, 2007a	Spain (single centre)	Prospective cohort study	I, R, S	To investigate the immunogenicity of a fourth dose of DTaP-HBV-IPV/Hib administered during the second year of life, and a fifth dose of DTaP vaccine given at 4 years of age to children who had been born prematurely and their FT counterparts	-IgG against diphtheria, tetanus, PT, FHA, PRN and HBV -Local and systemic reactions -SAEs	Immunogenicity: Before and one month after the booster dose in the second year of life and one month after the booster dose in the fifth year of life. Reactogenicity: Diary card for four days after vaccination. Safety: SAEs collected at any point in the study	85 PT and 70 FT infants participated in the booster study in the second year of life; 74 PT and 46 FT infants participated in the booster study at four years of age	DTaP-HBV-IPV/Hib, DTaP	Immunogenicity: No significant difference seen in antibody titre prior to the booster vaccination for tetanus, diphtheria, polio ST1 and 2 and pertussis, but significantly higher in FT infants for Hib, hep B and polio ST3. Following the second-year booster only hep B showed a significant difference in those protected, being lower in PT infants. There was no difference in the response to the booster at four years of age. Reactogenicity: Booster doses well tolerated with minimal differences between groups. Safety: No SAEs were reported which were associated with vaccine administration.

Appendix G

Omenaca, 2007b	Spain (single centre)	Prospective cohort study	I	To perform a detailed analysis of the immune response of a PT cohort to the Hib component of a hexavalent vaccine	-IgG against PRP	Measured before and one month after the primary vaccination and before and one month after the booster dose	93 PT and 89 FT infants were eligible for inclusion in this analysis of the primary schedule and 84 PT and 68 FT infants eligible for inclusion in this analysis following the booster dose	DTaP-HBV-IPV/Hib	Post-primary sero-protection rates $\geq 0.15\mu\text{g/ml}$ were lower in PT than term infants (92.5% vs 97.8%) with anti PRP GMC of 2.241 vs 4.247. No differences in response following booster vaccination.
Omenaca, 2011a	Spain (seven centres)	Prospective cohort study	I, R, S	To evaluate the immunogenicity and safety of Hib-MenC-TT in formerly PT infants, when co-administered with other routinely recommended paediatric vaccines	-SBA against MenC, IgG against Hib and hep B -Local and systemic reactions	Immunogenicity: prior to the administration of the first vaccines, one month following dose three and before and one month after the booster dose Reactogenicity: Diary card for seven days after vaccine administration	163 PT infants <37 WG, 150 FT infants	Hib-MenC-TT	Immunogenicity: One month after completion of the three dose primary series the percentage of infants with seroprotective anti-PRP antibody was $\geq 99\%$. Booster vaccination induced marked increases in anti-PRP antibody GMCs. At least 99% of infants in each age group achieved post-primary vaccination titres $>1:8$. At least 97.5% of infants in each age group had anti-HBs concentrations $\geq 10\text{mIU/ml}$ one month following dose 3. Hib-MenC-TT was well tolerated in all age groups. Reactogenicity: There were no differences between FT and PT infants for systemic symptoms; redness and swelling were more frequently seen in FT infants than PT infants after all vaccine doses Safety:

Appendix G

						Safety: SAEs throughout the study period			No SAEs were found to be related to vaccination
Omenaca 2011b	Spain and Greece (four centres)	Prospective cohort study	I, R	To assess the safety and immunogenicity of PHiD-CV primary and booster vaccination in PT infants >27WG	-IgG against pneumococcal vaccine serotypes -Local and systemic reactions -Fever -AEs	Immunogenicity: Measured before and one month after primary vaccination and before and one month after booster vaccination Reactogenicity: Diary cards were completed for four days after vaccine administration	137 PT infants <37 WG, 149 term infants	PHiD-CV	Immunogenicity: PHiD was immunogenic for all pneumococcal serotypes with a trend for lower postprimary GMCs and OPA titres in PT infants for some pneumococcal serotypes. Reactogenicity: The incidence of solicited general symptoms was similar across groups. Reported redness and swelling was lower in PT infants.
Omenaca, 2012	France, Portugal, Poland and Spain	RCT	I, R, S	To assess the safety, reactogenicity and immunogenicity of two oral doses of rotavirus vaccine as	-Anti-rotavirus IgA -Fever, irritability, diarrhoea, vomiting, loss of appetite -SAEs	Immunogenicity: sampling prior to vaccination in 300 infants, and	1,009 PT infants (20% 27-30WG; 80% 31-36WG)	Rotavirus	Immunogenicity: Seroconversion rates were 85.7% in the vaccine group and 16.0% in the placebo group. Babies in the group born at earlier gestations had a seroconversion rate of 75.9% compared with 88.1% in those born at later PT gestations. Similarly, the GMCs of vaccinated babies born at an earlier gestation were 236.5

Appendix G

				compared with placebo in PT infants		30-83 days after second vaccine dose Reactogenicity: Infants followed for 15 days following vaccine administration Safety: Infants followed up for duration of the study			U/ml compared with 359.1 U/ml in those born at later gestations. Reactogenicity: No difference in systemic AEs between infants in the placebo and vaccine groups. Safety: No difference in rates of SAEs or solicited adverse events between vaccine and placebo recipients.
Perin, 2012	Brazil (single centre)	Prospective cohort study	I	To compare humoral and cellular immune responses to tetanus booster vaccination in PT and FT infants and identify factors associated with the humoral response	-Anti-tetanus IgG and cellular immunity	Measured prior to booster vaccination at 15 months and post booster at 18 months	64 PT (<35 WG) and 54 FT infants were enrolled	DTP	Anti-tetanus antibody concentration was lower in PT infants prior to booster vaccination, but similar following booster vaccination. Proportions with protective titres were comparable both before and after vaccination. The cellular immunity was similar both before and after the booster vaccination.
Pfister, 2004	Switzerland (single centre)	Case series	R	Assess the clinical safety of DTap-IPV-Hib vaccination in the most PT and LBW infants	-CRE: apnoea, bradycardia, desaturation, increased ventilatory requirement -Fever	CRE and fever: 24 hours before and 48 hours after	78 inpatient PT infants <35 WG or ≤1500g	DTaP-IPV-Hib	Vaccination resulted in resurgence or increase in CRE in 47% of infants. Most CRE resolved spontaneously or after minimal stimulation. RR was 5 to 8-fold higher in infants with a severe clinical course or persistence of cardiorespiratory symptoms at the time of vaccination. Fever occurred in 3.8%.

Appendix G

Pourcyrous, 2007	US (single centre)	Case series	R	Determine the incidence of CREs associated with administration of single or multiple vaccinations	-CRE: apnoea, bradycardia, desaturation -Evaluation for sepsis	Three days following vaccination	239 inpatient PT infants <35 WG	DTaP, Hib, IPV, hep B, PCV	Overall, 16% of infants had CREs associated with vaccination. 5% of infants were investigated for sepsis.
Riise, 2017	Norway (national surveillance)	Prospective cohort study	E	To determine whether there is a higher risk of reported pertussis and pertussis hospitalisations among PT than FT infants during the first 2 years of life	-Cases of pertussis -Hospital admission with pertussis	2 years	713,166 children (36,913 PT)	DTaP	VE similar in term and PT infants: FT 88.8% (95% CI: 1.47-2.71); PT 93.0% (95% CI 85.8-96.5).
Riise, 2018	Norway (national surveillance)	Prospective cohort study	E	To compare the rate of IPD in FT and PT infants in the period before and after the addition of PCV7 to the routine vaccination schedule	-Cases of IPD	10 years	644,595 children (41,931 PT)	PCV7	The introduction of PCV7 reduced the rate of IPD in children from six months of age to a similar extent in FT and PT infants.
Robinson, 2004	UK (three centres)	Prospective cohort study	I	To study the effect of dexamethasone in PT infants with CLD on antibody response to	IgG against diphtheria toxin, tetanus toxin, pertussis toxin,	Before first vaccination and 6-8 weeks after the third vaccination	93 PT infants <33 WG, 67 of these infants were treated with dexamethasone	DTP	GMT following vaccination was significantly lower for tetanus, diphtheria, PT, FIM 2&3, but not FHA or PRN, in the dexamethasone treated group. No difference in percentage protected when the lower limit of protection used for diphtheria and

Appendix G

				routine immunisation against diphtheria, tetanus and pertussis.	filamentous haemagglutinin, fimbrial agglutinogens 2+3, and pertactin				tetanus, but reduced protection in dexamethasone treated infants when the higher limit of protection is used.
Roué, 2014	France (regional surveillance)	Case series	E, S	To investigate the impact of the pentavalent rotavirus virus vaccine on the number of hospitalisations for rotavirus diarrhoea in PT infants	- Hospitalisation with rotavirus diarrhoea -SAEs	Effectiveness: Hospitalisation in the period 2009-2010 with rotavirus diarrhoea in infants born at <37 WG, aged less than three years and living within the catchment area Safety: All vaccinated infants followed until 42 days after the last dose	201 PT infants <37 WG	Rotavirus	Significant reduction in hospitalisations in PT infants (by a factor of 2.6 in the first and second seasons and by a factor of 11 in the third season). Safety: No significant difference in rates of SAEs between PT and FT infants.
Rouers, 2020	Netherlands (8 centres)	Historical cohort study	I	To determine the immunogenicity of the Dutch routine national immunisation	-IgG against pertussis toxin, filamentous haemagglutinin,	Before vaccination and one month following the final	296 PT infants (<37 WG), 66 FT infants	DTaP-IPV-Hib-HepB; PCV	After the primary series, the proportion of PT infants across all gestational age groups who achieved protective IgG antibody levels against PT, diphtheria, tetanus and 6/10 pneumococcal serotypes varied between 83-100%, Hib

Appendix G

				programme in the first year of life in PT infants	pertactin, diphtheria toxoid, tetanus toxin, PRP and pneumococcal serotypes	dose of the primary series			40.6%, and pneumococcal serotypes 4, 6B, 18C and 23F between 45.8% and 75.1%. After the booster dose, protective antibody levels were achieved in more than 95% of all PT groups, except for Hib (88.1%). In general, GMCs in PT infants were significantly lower than in FT infants except for pertussis toxin and pneumococcal serotypes 4 and 19F after the primary series and booster vaccination.
Ruckinger, 2010	Germany (national surveillance)	Surveillance	E	To evaluate the impact of PCV7 on IPD in PT born infants in Germany comparing those born in 2000 with those born in 2007	- Hospitalisation with IPD	1-2 years	58 cases of IPD in 2007 birth cohort, 115 cases in 2000 birth cohort	PCV	Similar reduction in incidence of IPD in FT and PT infants between 2000 and 2007.
Ruggeberg, 2007	UK (Four centres)	Prospective cohort study	I	To assess the immunogenicity of PCV7 in PT infants who were vaccinated at two, three and four months of age	IgG against the seven PCV7 pneumococcal vaccine serotypes and 2 PPV23 serotypes not included in PCV7	Before the first vaccine, 4 weeks after the third vaccine dose, before and 3 weeks after PPV23 vaccination	69 FT and 68 PT infants (<37 WG)	PCV7	IgG GMC was significantly lower in PT infants to six vaccine serotypes following primary vaccination, to five vaccine serotypes at 12 months of age and to three serotypes at 13 months of age. A significantly lower proportion of PT infants achieved IgG levels $\geq 0.35\mu\text{g/ml}$ to ST4, 6B, 9V following primary vaccinations and to ST4, 6B, 18C, 19F and 23F at 12 months of age.
Sadarangani, 2017	UK (single centre)	Prospective cohort study	R	Compare reactions in PT infants who did and did not	-Fever -Systemic reactions	Seven days	17 inpatient PT infants	4CMenB	Participants who received 4CMenB were significantly more likely to have temperature instability.

Appendix G

				receive 4CMen B					
Schulzke, 2005	Switzerland (single centre)	Retrospective review	R	Report on the incidence and clinical significance of post immunisation apnoea or bradycardia in former PT infants not requiring respiratory support or supplemental oxygen	-CREs: apnoea, bradycardia -Fever	CRE and fever: 72 hours before and after vaccine administration	53 inpatient PT infants ≤ 30 WG	DTaP-Hib-IPV, DTaP-IPV+Hib, DTaP-Hib-IPV+HBV	13% (n=7) of infants had recurrent or increased apnoea or bradycardia episodes following vaccination; four of these infants required new respiratory support. 9.4% of infants experienced fever.
Sen, 2001	UK (single centre)	Retrospective review	R	To study the frequency, severity and types of adverse reactions following DTP/Hib vaccination in very PT infants	-CREs: apnoea, bradycardias, desaturations, increased ventilatory support -Fever -Systemic reactions: poor handling, feed intolerance	Three days before and seven days after	45 inpatient PT infants <32WG.	DTP, Hib	Overall, 37.8% had some form of AE. 20% experienced major problems including four (8.9%) with apnoea, bradycardias and desaturations, and five (11.1%) with bradycardias and desaturations alone. 17.8% experienced minor problems including seven (15.6%) with temperature instability, six (13.3%) with poor handling, four (8.9%) with increased oxygen requirement and 5 (11.1%) with feed intolerance. Babies with major side effects were significantly younger than babies with minor or no symptoms and all babies who developed major symptoms were 70 days or less at the time of vaccination. No difference between those babies who had major problems and those who had minor or no problems with regard to their mean

Appendix G

									BW, GA, duration of ventilation, duration of oxygen dependency or frequency of Hib administration.
Sheridan, 2021	Australia (regional surveillance)	Retrospective cohort study	E	To compare notification rates of pertussis and estimate VE by comorbidity status including PT status	-Confirmed pertussis cases	Period monitored 1 st July 2001 and 31 st December 2012 in children up to 18 months	>1,300,000 children of whom 8396 were <32 WG	DTaP	Children born at <28 WG had a markedly increased risk of pertussis notification following dose two (RR 10.85; 95% CI 3.95-23.91) and dose three (RR 6.78; 95% CI 3.70-11.42) compared to children born at term.
Sikora, 2005	Poland (single centre)	Case series	I	To evaluate serologic efficiency of primary immunisation of PT infants with IPV	-Anti-polio serotype 1 antibody titre measured using neutralisation assay	Measured before vaccination and four weeks after the third and fourth doses of IPV	44 PT infants (24-36 WG); 17 received a booster dose	IPV	There was a statistically significant increase in antibody titres after vaccination, with 95.5% of PT infants achieving protective antibody titres. There were no significant correlations between BW or GA. There was a significant increase in antibody concentration following receipt of a fourth dose in the 17 infants who received this.
Sikora, 2004	Poland (single centre)	Case series	I	To determine anti-pertussis antibody titres after primary vaccination against diphtheria, tetanus and pertussis (three doses) and booster dose (the fourth dose) in PT infants.	IgG against PT and FHA	Measured before vaccination and four weeks after the third and fourth consecutive doses.	19 PT infants (24-35 WG)	DTaP	'Seroconversion' (defined as antibody concentrations in virotech units >11.0) was seen in 94.7% of PT infants.

Appendix G

Slack, 2005	UK (three centres)	Prospective cohort study	I	To investigate the response of PT infants to a combined DTaP-Hib-IPV vaccine given at the same time as a MenC vaccine	-IgG against PRP, diphtheria, tetanus FHA, FIM, PRN and PT, and SBA for MenC	Prior to and 4-6 weeks after the third vaccine	60 term infants and 50 PT infants <32 WG	DTaP-Hib-IPV, MCC	PT infants vaccinated with DTaP-Hib-IPV achieved PRP ≥ 0.15 in 80% of cases and ≥ 1.0 in 60% of cases, 100% of infants achieved SBA ≥ 8 for MenC and protective titres to diphtheria, tetanus, polio, and $\geq 80\%$ achieving protective titres against the five pertussis antigens
Slack, 2001	UK (five centres)	Prospective cohort study	I	To determine the immune response of premature infants to MenC and DTaP-Hib conjugate vaccines	IgG against Hib and SBA for MenC	Measured one month after third vaccination	105 PT infants (<32 WG)	DTaP-Hib; MCC	Significant difference in Hib GMCs- $0.27\mu\text{g/ml}$ in PT infants compared with $0.81\mu\text{g/ml}$ in term infants. GMCs of ≥ 1.0 were significantly higher in term compared to PT infants (21% of PT infants vs 46% of term infants). A protective SBA of ≥ 8 was achieved in 99% of PT and 98% of term infants.
Slack, 2004	UK (five centres)	Prospective cohort study	I	Describe the immune response of PT infants to a DTaP vaccine under an accelerated schedule, and the effects of steroids on these responses	-IgG against diphtheria, tetanus, FHA, PRN, PT was measured prior to first vaccination and 4-6 weeks following third vaccination	4-6 weeks following third vaccination	128 PT (<32WG) and 54 term infants	DTaP-Hib; MCC	After the third immunisation, PT infants had similar GMCs to control to diphtheria, tetanus, filamentous haemagglutinin and pertactin, but a significantly lower GMC to pertussis toxin. No association between responses and postnatal steroids.
Slack, 2003	UK (single centre)	Case series	R	To investigate the incidence of CREs following immunisation with DTaP-Hib vaccine given concurrently	-CREs: apnoea, bradycardia and desaturation	24 hours before and after vaccine administration	76 inpatient PT infants <32WG	DTaP-Hib, MCC	There was no statistically significant increase in the number of CREs in the period post-vaccination compared with the period pre-vaccination

Appendix G

				with MenC vaccine					
Szynczewska, 2014	Poland (single centre)	Case series	I	To assess the immune response of PT infants to PCV7	IgG against vaccine specific pneumococcal serotypes	Measured prior to the first vaccination, four weeks after completion of the primary series, prior to the booster dose and four weeks following booster dose administration	60 PT infants (24-34 WG)	PCV7, DTP-IPB-Hib, hep B	Serotypes 6B and 23F were the least immunogenic. The antibody titres following booster vaccination were significantly higher than those seen after completion of the primary series.
Tsuda, 2012	Japan (single centre)	Case series	I	To evaluate the immunogenicity of Hib vaccine in Japanese PT infants	-Anti PRP IgG	Before first vaccination and one month after the third vaccination	54 PT (<37WG) infants	PRP-T	85.2% of infants achieved a protective titre of $\geq 1.0\mu\text{g/ml}$.
van den Berg, 2015	Netherlands (single centre)	RCT	I	Investigate the effect of neutral and acidic oligosaccharides on antibody concentrations after PCV in very PT infants	-IgG against vaccine specific pneumococcal serotypes	Measured 4-6 weeks after the third pneumococcal vaccination and 4-8 weeks after	113 PT infants (<32WG)	PCV7	-Following the primary vaccinations, antibody concentrations and proportions protected were lower in the oligosaccharide group compared to placebo. No difference following booster vaccination. -Antibody concentrations were lower in term than the placebo group, but

Appendix G

						the booster vaccination			comparable with the oligosaccharide treated group
van der Maas, 2019	Netherlands (national surveillance)	Surveillance study	E	Compare the VE against hospitalisation for pertussis between FT and PT infants	-Pertussis hospitalisation	Two years	1,187 cases were identified, for which 676 had data available	DTP-IPV-Hib/ DTP-IPV-Hib-HepB	12% of hospitalised cases for pertussis were in PT infants, in contrast to the national rate of prematurity in the Netherlands of 8%. PT infants were older at the time of admission, with lower VE for their first vaccination and a higher need for intensive treatment.
Vazquez, 2008	Countries unknown (eight centres)	Case series	I, R, S	To assess suitability of a combined DTaP-HBV-IPV/Hib vaccine for immunisation of LBW PT infants, with particular focus on the hep B response	IgG against HBs, PRP, diphtheria, tetanus and polio -Local reactions -Systemic reactions -Adverse events	Immunogenicity: Before the first dose, one month after the third dose, before and one month after the booster dose. Reactogenicity: completion of a diary card for three days following vaccine administration	169 PT infants (24-36WG) enrolled; 161 and 119 included in the primary and booster vaccination immunogenicity cohorts respectively	DTaP-HBV-IPV/Hib	Immunogenicity: One month after the three-dose primary vaccination GMCs for anti HBs were similar in LBW and VLBW infants, and 93.7% and 94.9% of VLBW and LBW infants respectively had anti HBs antibody concentrations of >10miU/ml. At least 98% of infants reached a protective threshold for diphtheria, tetanus, Hib and poliovirus types one, two and three although the higher protective threshold for Hib was reached in significantly more LBW compared with VLBW infants and the anti-PT GMC and anti-polio type 3 GMT were significantly higher in the LBW group compared with the VLBW group and there was a trend towards a lower response to pertussis toxin in the VLBW group compared with the LBW group. After the booster dose no significant difference in reaching protective thresholds between groups for hep B, Hib, diphtheria and tetanus although there was a trend for lower antibody GMC/Ts in the VLBW group for all antigens apart from Hib.

Appendix G

									Reactogenicity: Grade three local reactions were reported in 10.7% of infants in the LBW group and 11.8% in the VLBW group. Safety: 34 SAEs reported in the primary phase, one of which could have been related to vaccination. Five SAEs reported in the booster phase, none of which were related to vaccination.
Vermeulen, 2010	Belgium (two centres)	Case series	I	To assess the specific cellular immune responses, together with the humoral responses in PT infants with a very low GA	-IFN gamma responses -IgG against FHA and PT	One month after the first vaccine and two months after the third vaccine	48 PT infants (<31WG)	Acellular or whole cell pertussis	Most PT infants are able to mount a specific cellular immune response to the administration of the first doses of an acellular or whole cell pertussis vaccine
Vermeulen, 2013	Belgium (two centres)	Case series	I	To assess at one year of age the specific cellular immune responses in the PT infants investigated in a previous study (Vermeulen, 2010)	IgG against FHA and PT stimulation of PBMCs	Before and 1-2 months after the booster vaccination	68 PT infants (<31 WG)	DTaP-IPV or DTwP-IPV and Hib vaccine	Antigen specific cellular immune responses persisted in one year old children born prematurely and vaccinated during infancy at their chronological age and a booster dose did not significantly boost the cellular immune responses.
Wilinksa, 2016	Poland (single centre)	Case series	R	Describe the frequency and kinds of AEs following immunisation in PT infants	-Local and systemic reactions -Fever -CREs: apnoea, desaturation	72 hours following vaccination	138 inpatient PT infants <36WG (73 ≤28WG; 65 >28WG)	Diphtheria, tetanus, pertussis, Hib, polio, pneumococcus	No local reactions reported. Fever in 0.7%, activity dysfunction in 9.4%, apnoea in 4.3%, respiratory support 2.2%, desaturations in 2.2%, new caffeine requirement 0.7%.

Appendix G

					s, increase in ventilatory support				
Wilson, 2012	Canada (regional)	Case control study	S	Examine rate of ED visits and hospital admissions following two month vaccinations	-ED visits -Hospital admissions	Within three days of vaccine administration	119,899 PT infants, 714,841 FT infants	DTaP, Hib, IPV, PCV	Relative incidence of events, defined as ED visits or hospitalisation, was less than one in all groups. There was a trend towards a lower incidence of events as gestational age decreased, a trend which was strengthened when weight was adjusted for.

AEs= adverse events; ARR= absolute risk reduction; BPD= bronchopulmonary dysplasia; BW= birthweight; CMI= cell mediated immunity; CPAP= continuous positive airway pressure; CREs= cardiorespiratory events; CRP= C reactive protein; DTaP= diphtheria, tetanus, acellular pertussis; DTwP= diphtheria, tetanus, whole cell pertussis; ED= emergency department; FHA= filamentous haemagglutinin; FT= full term; GA= gestational age; GMT= geometric mean titre; GORD= gastro-oesophageal reflux disease; HBsAG= Hepatitis B surface antigen; HBV= hepatitis B vaccine; Hib= haemophilus influenzae type B; i= immunogenicity; IFN= interferon; IgG= immunoglobulin; IPV= inactivated polio vaccine; IU= international unit; IV= intravenous; LBW= low birthweight; MCC= meningococcal c conjugate vaccine; MMR= measles, mumps and rubella; NICU= neonatal intensive care unit; PBMC= peripheral blood mononuclear cells; PCV= pneumococcal conjugate vaccine; PHiD-CV= pneumococcal polysaccharide protein D-conjugate vaccine; PPV= pneumococcal polysaccharide vaccine; PRN= pertactin; PRP= polyribosylribitol phosphate; PT= preterm; r= reactogenicity; RR= risk reduction; s= safety; SAEs= serious adverse events; SBA= serum bactericidal antibody; ST= serotype; SUSAR= suspected unexpected serious adverse reaction; TT= tetanus toxoid; VE= vaccine effectiveness; VLBW= very low birthweight; WG= weeks of gestation

Appendix H

Table 1: Summary of risk of bias assessments for each included study

Author, year	Assessment tool	Assessment domain	Outcome	
			Outcome: Reactogenicity	
Anderson, 2013	NIH quality assessment tool for before-after (pre-post) studies with no control group	Was the study question or objective clearly stated?	Yes	
		Were eligibility/selection criteria for the study population prespecified and clearly described?	Yes	
		Were the participants in the study representative of those who would be eligible for the test/service/intervention in the general or clinical population of interest?	Yes	
		Were all eligible participants that met the prespecified entry criteria enrolled?	Yes	
		Was the sample size sufficiently large to provide confidence in the findings?	Yes	
		Was the test/service/intervention clearly described and delivered consistently across the study population?	No	
		Were the outcome measures prespecified, clearly defined, valid, reliable, and assessed consistently across all study participants?	No	
		Were the people assessing the outcomes blinded to the participants' exposures/interventions?	No	
		Was the loss to follow-up after baseline 20% or less? Were those lost to follow-up accounted for in the analysis?	No	
		Did the statistical methods examine changes in outcome measures from before to after the intervention? Were statistical tests done that provided p values for the pre-to-post changes?	N/A	
		Were outcome measures of interest taken multiple times before the intervention and multiple times after the intervention (i.e., did they use an interrupted time-series design)?	Yes	
		If the intervention was conducted at a group level (e.g., a whole hospital, a community, etc.) did the statistical analysis take into account the use of individual-level data to determine effects at the group level?	N/A	
		Overall	Poor	

Appendix H

			Outcome: Immunogenicity	
Baxter, 2010	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	
		Was the study population clearly and fully described, including a case definition?	No	
		Were the cases consecutive?	Yes	
		Were the subjects comparable?	Yes	
		Was the intervention clearly described?	Yes	
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	
		Was the length of follow-up adequate?	Yes	
		Were the statistical methods well-described?	Yes	
		Were the results well-described?	No	
		Overall	Fair	
			Outcome: Immunogenicity	
Bednarek, 2018	Robins-I	Bias due to confounding	Critical risk of bias	
		Bias in selection of participants into the study	Serious risk of bias	
		Bias in classification of interventions	Moderate risk of bias	
		Bias due to deviations from intended interventions	Moderate risk of bias	
		Bias due to missing data	Low risk of bias	
		Bias in measurement of outcomes	Low risk of bias	
		Bias in selection of the reported results	Low risk of bias	
		Overall	Critical risk of bias	
			Outcome: Reactogenicity	
Ben Jmaa, 2017	ROB	Bias arising from the randomisation process	Low risk of bias	
		Bias due to deviations from intended interventions	Low risk of bias	
		Bias due to missing outcome data	Low risk of bias	
		Bias in measurement of the outcome	Low risk of bias	
		Bias in selection of the reported result	Low risk of bias	
		Overall	Low risk of bias	
			Outcome: Immunogenicity	
Berrington, 2006; Berrington,	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Moderate risk of bias	

Appendix H

2007; Berrington, 2007		Bias due to deviations from intended interventions	Low risk of bias	
		Bias due to missing data	No information	
		Bias in measurement of outcomes	Low risk of bias	
		Bias in selection of the reported results	Moderate risk of bias	
		Overall	Serious risk of bias	
			Outcome: Reactogenicity	
Bohnhorst, 2021	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	
		Was the study population clearly and fully described, including a case definition?	No	
		Were the cases consecutive?	NR	
		Were the subjects comparable?	Yes	
		Was the intervention clearly described?	Yes	
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	
		Was the length of follow-up adequate?	Yes	
		Were the statistical methods well-described?	Yes	
		Were the results well-described?	Yes	
		Overall	Fair	
			Outcome: Reactogenicity	
Briggs- Steinberg, 2021	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Moderate risk of bias	
		Bias due to deviations from intended interventions	Low risk of bias	
		Bias due to missing data	Low risk of bias	
		Bias in measurement of outcomes	Serious risk of bias	
		Bias in selection of the reported results	Low risk of bias	
		Overall	Serious risk of bias	
			Outcome: Reactogenicity	
Carbone, 2008	ROB	Bias arising from the randomisation process	Low risk of bias	
		Bias due to deviations from intended interventions	Low risk of bias	
		Bias due to missing outcome data	Moderate risk of bias	
		Bias in measurement of the outcome	Low risk of bias	
		Bias in selection of the reported result	Low risk of bias	

Appendix H

		Overall	Moderate risk of bias	
			Outcome: Immunogenicity	
Clarke 2003; 2004	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	
		Was the study population clearly and fully described, including a case definition?	Yes	
		Were the cases consecutive?	No	
		Were the subjects comparable?	Yes	
		Was the intervention clearly described?	Yes	
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	
		Was the length of follow-up adequate?	Yes	
		Were the statistical methods well-described?	Yes	
		Were the results well-described?	Yes	
		Overall	Fair	
			Outcome: Immunogenicity	
Clarke, 2006	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Serious risk of bias	
		Bias due to deviations from intended interventions	Moderate risk of bias	
		Bias due to missing data	Low risk of bias	
		Bias in measurement of outcomes	Low risk of bias	
		Bias in selection of the reported results	Low risk of bias	
		Overall	Serious risk of bias	
Clifford, 2011	Robins-I*		Outcome: Reactogenicity	
		Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Critical risk of bias	
		Bias in classification of interventions	Moderate risk of bias	
		Bias due to deviations from intended interventions	Moderate risk of bias	
		Bias due to missing data	No information	
		Bias in measurement of outcomes	Serious risk of bias	
		Bias in selection of the reported results	Moderate risk of bias	
		Overall	Critical risk of bias	
			Outcome: Immunogenicity	

Appendix H

Collins, 2005	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Low risk of bias	
		Bias due to deviations from intended interventions	Low risk of bias	
		Bias due to missing data	Low risk of bias	
		Bias in measurement of outcomes	Low risk of bias	
		Bias in selection of the reported results	Low risk of bias	
		Overall	Serious risk of bias	
			Outcome: Immunogenicity	
D'Angio, 2007	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Low risk of bias	
		Bias due to deviations from intended interventions	Moderate risk of bias	
		Bias due to missing data	Low risk of bias	
		Bias in measurement of outcomes	Low risk of bias	
		Bias in selection of the reported results	Moderate risk of bias	
		Overall	Serious risk of bias	
			Outcome: Immunogenicity	Outcome: Safety/reactogenicity
D'Angio, 2010; D'Angio, 2013	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	No
		Was the study population clearly and fully described, including a case definition?	Yes	Yes
		Were the cases consecutive?	NR	NR
		Were the subjects comparable?	Yes	Yes
		Was the intervention clearly described?	Yes	Yes
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	No
		Was the length of follow-up adequate?	Yes	Yes
		Were the statistical methods well-described?	Yes	Yes
		Were the results well-described?	Yes	No
		Overall	Good	Fair
De Meo, 2015	NIH quality assessment tool for before-after (pre-	Was the study question or objective clearly stated?	Yes	
		Were eligibility/selection criteria for the study population prespecified and clearly described?	Yes	

Appendix H

	post) studies with no control group	Were the participants in the study representative of those who would be eligible for the test/service/intervention in the general or clinical population of interest?	Yes	
		Were all eligible participants that met the prespecified entry criteria enrolled?	Yes	
		Was the sample size sufficiently large to provide confidence in the findings?	Yes	
		Was the test/service/intervention clearly described and delivered consistently across the study population?	Yes	
		Were the outcome measures prespecified, clearly defined, valid, reliable, and assessed consistently across all study participants?	Yes	
		Were the people assessing the outcomes blinded to the participants' exposures/interventions?	No	
		Was the loss to follow-up after baseline 20% or less? Were those lost to follow-up accounted for in the analysis?	Yes	
		Did the statistical methods examine changes in outcome measures from before to after the intervention? Were statistical tests done that provided p values for the pre-to-post changes?	Yes	
		Were outcome measures of interest taken multiple times before the intervention and multiple times after the intervention (i.e., did they use an interrupted time-series design)?	No	
		If the intervention was conducted at a group level (e.g., a whole hospital, a community, etc.) did the statistical analysis take into account the use of individual-level data to determine effects at the group level?	NA	
		Overall	Fair	
			Outcome: Reactogenicity	
Dubus, 2020	NIH quality assessment tool for before-after (pre-post) studies with no control group	Was the study question or objective clearly stated?	Yes	
		Were eligibility/selection criteria for the study population prespecified and clearly described?	Yes	
		Were the participants in the study representative of those who would be eligible for the test/service/intervention in the general or clinical population of interest?	Yes	
		Were all eligible participants that met the prespecified entry criteria enrolled?	Unsure	
		Was the sample size sufficiently large to provide confidence in the findings?	Yes	
		Was the test/service/intervention clearly described and delivered consistently across the study population?	Yes	

Appendix H

		Were the outcome measures prespecified, clearly defined, valid, reliable, and assessed consistently across all study participants?	No	
		Were the people assessing the outcomes blinded to the participants' exposures/interventions?	No	
		Was the loss to follow-up after baseline 20% or less? Were those lost to follow-up accounted for in the analysis?	Yes	
		Did the statistical methods examine changes in outcome measures from before to after the intervention? Were statistical tests done that provided p values for the pre-to-post changes?	Yes	
		Were outcome measures of interest taken multiple times before the intervention and multiple times after the intervention (i.e., did they use an interrupted time-series design)?	No	
		If the intervention was conducted at a group level (e.g., a whole hospital, a community, etc.) did the statistical analysis take into account the use of individual-level data to determine effects at the group level?	N/A	
		Overall	Fair	
			Outcome: Reactogenicity	
Ellison, 2005	NIH quality assessment tool for before-after (pre-post) studies with no control group	Was the study question or objective clearly stated?	Yes	
		Were eligibility/selection criteria for the study population prespecified and clearly described?	Yes	
		Were the participants in the study representative of those who would be eligible for the test/service/intervention in the general or clinical population of interest?	Yes	
		Were all eligible participants that met the prespecified entry criteria enrolled?	NK	
		Was the sample size sufficiently large to provide confidence in the findings?	No	
		Was the test/service/intervention clearly described and delivered consistently across the study population?	Yes	
		Were the outcome measures prespecified, clearly defined, valid, reliable, and assessed consistently across all study participants?	No	
		Were the people assessing the outcomes blinded to the participants' exposures/interventions?	Yes	
		Was the loss to follow-up after baseline 20% or less? Were those lost to follow-up accounted for in the analysis?	Yes	
		Did the statistical methods examine changes in outcome measures from before to after the intervention? Were statistical tests done that provided p values for the pre-to-post changes?	Yes	

Appendix H

		Were outcome measures of interest taken multiple times before the intervention and multiple times after the intervention (i.e., did they use an interrupted time-series design)?	No	
		If the intervention was conducted at a group level (e.g., a whole hospital, a community, etc.) did the statistical analysis take into account the use of individual-level data to determine effects at the group level?	NA	
		Overall	Fair	
			Outcome: Immunogenicity	Outcome: Reactogenicity/safety
Esposito, 2007	Robins-I	Bias due to confounding	Serious risk of bias	Serious risk of bias
		Bias in selection of participants into the study	Moderate risk of bias	Moderate risk of bias
		Bias in classification of interventions	Moderate risk of bias	Moderate risk of bias
		Bias due to deviations from intended interventions	Moderate risk of bias	Moderate risk of bias
		Bias due to missing data	Moderate risk of bias	Moderate risk of bias
		Bias in measurement of outcomes	Low risk of bias	Serious risk of bias
		Bias in selection of the reported results	Low risk of bias	Low risk of bias
		Overall	Serious risk of bias	Serious risk of bias
Esposito, 2002	Robins-I		Outcome: Immunogenicity	
		Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Serious risk of bias	
		Bias in classification of interventions	Serious risk of bias	
		Bias due to deviations from intended interventions	Moderate risk of bias	
		Bias due to missing data	Moderate risk of bias	
		Bias in measurement of outcomes	Low risk of bias	
		Bias in selection of the reported results	Moderate risk of bias	
Esposito, 2005	Robins-I	Overall	Critical risk of bias	
			Outcome: Immunogenicity	Outcome: Reactogenicity/safety
		Bias due to confounding	Serious risk of bias	Serious risk of bias
		Bias in selection of participants into the study	No information	No information
		Bias in classification of interventions	Low risk of bias	Low risk of bias
		Bias due to deviations from intended interventions	Low risk of bias	Low risk of bias
		Bias due to missing data	Moderate risk of bias	Moderate risk of bias
		Bias in measurement of outcomes	Low risk of bias	Serious risk of bias
		Bias in selection of the reported results	Moderate risk of bias	Moderate risk of bias
		Overall	Serious risk of bias	Serious risk of bias

Appendix H

			Outcome: Reactogenicity	
Faldella, 2007	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	
		Was the study population clearly and fully described, including a case definition?	Yes	
		Were the cases consecutive?	Yes	
		Were the subjects comparable?	Yes	
		Was the intervention clearly described?	Yes	
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	
		Was the length of follow-up adequate?	Yes	
		Were the statistical methods well-described?	Yes	
		Were the results well-described?	Yes	
		Overall	Good	
			Outcome: Immunogenicity	
Ferreira, 2018	Robins-I	Bias due to confounding	Moderate risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Low risk of bias	
		Bias due to deviations from intended interventions	Low risk of bias	
		Bias due to missing data	Moderate risk of bias	
		Bias in measurement of outcomes	Low risk of bias	
		Bias in selection of the reported results	Moderate risk of bias	
		Overall	Moderate risk of bias	
			Outcome: Reactogenicity	
Faltz-Jequier, 2008	NIH quality assessment tool for before-after (pre-post) studies with no control group	Was the study question or objective clearly stated?	Yes	
		Were eligibility/selection criteria for the study population prespecified and clearly described?	Yes	
		Were the participants in the study representative of those who would be eligible for the test/service/intervention in the general or clinical population of interest?	Yes	
		Were all eligible participants that met the prespecified entry criteria enrolled?	Yes	
		Was the sample size sufficiently large to provide confidence in the findings?	Yes	
		Was the test/service/intervention clearly described and delivered consistently across the study population?	Yes	

Appendix H

		Were the outcome measures prespecified, clearly defined, valid, reliable, and assessed consistently across all study participants?	Yes	
		Were the people assessing the outcomes blinded to the participants' exposures/interventions?	No	
		Was the loss to follow-up after baseline 20% or less? Were those lost to follow-up accounted for in the analysis?	Yes	
		Did the statistical methods examine changes in outcome measures from before to after the intervention? Were statistical tests done that provided p values for the pre-to-post changes?	Yes	
		Were outcome measures of interest taken multiple times before the intervention and multiple times after the intervention (i.e., did they use an interrupted time-series design)?	No	
		If the intervention was conducted at a group level (e.g., a whole hospital, a community, etc.) did the statistical analysis take into account the use of individual-level data to determine effects at the group level?	NA	
		Overall	Fair	
			Outcome: Reactogenicity	
Furck, 2010	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	
		Was the study population clearly and fully described, including a case definition?	Yes	
		Were the cases consecutive?	Yes	
		Were the subjects comparable?	Yes	
		Was the intervention clearly described?	Yes	
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	
		Was the length of follow-up adequate?	Yes	
		Were the statistical methods well-described?	Yes	
		Were the results well-described?	Yes	
		Overall	Good	
			Outcome: Safety	Outcome: Efficacy
Goveia, 2008	ROB	Bias arising from the randomisation process	Low risk of bias	Low risk of bias
		Bias due to deviations from intended interventions	Low risk of bias	Low risk of bias
		Bias due to missing outcome data	Low risk of bias	Low risk of bias
		Bias in measurement of the outcome	Moderate risk of bias	Low risk of bias
		Bias in selection of the reported result	Low risk of bias	Low risk of bias
		Overall	Moderate risk of bias	Low risk of bias

Appendix H

			Outcome: Reactogenicity	
Hacking, 2010	NIH quality assessment tool for before-after (pre-post) studies with no control group	Was the study question or objective clearly stated?	Yes	
		Were eligibility/selection criteria for the study population prespecified and clearly described?	Yes	
		Were the participants in the study representative of those who would be eligible for the test/service/intervention in the general or clinical population of interest?	Yes	
		Were all eligible participants that met the prespecified entry criteria enrolled?	Yes	
		Was the sample size sufficiently large to provide confidence in the findings?	Yes	
		Was the test/service/intervention clearly described and delivered consistently across the study population?	Yes	
		Were the outcome measures prespecified, clearly defined, valid, reliable, and assessed consistently across all study participants?	Yes	
		Were the people assessing the outcomes blinded to the participants' exposures/interventions?	No	
		Was the loss to follow-up after baseline 20% or less? Were those lost to follow-up accounted for in the analysis?	Yes	
		Did the statistical methods examine changes in outcome measures from before to after the intervention? Were statistical tests done that provided p values for the pre-to-post changes?	Yes	
		Were outcome measures of interest taken multiple times before the intervention and multiple times after the intervention (i.e., did they use an interrupted time-series design)?	No	
		If the intervention was conducted at a group level (e.g., a whole hospital, a community, etc.) did the statistical analysis take into account the use of individual-level data to determine effects at the group level?	NA	
		Overall	Fair	
			Outcome: Immunogenicity	Outcome: Effectiveness
Heath, 2003	Robins-I	Bias due to confounding	Serious risk of bias	Moderate risk of bias
		Bias in selection of participants into the study	Moderate risk of bias	Serious risk of bias
		Bias in classification of interventions	Low risk of bias	Moderate risk of bias
		Bias due to deviations from intended interventions	Low risk of bias	Moderate risk of bias
		Bias due to missing data	Serious risk of bias	Moderate risk of bias
		Bias in measurement of outcomes	Moderate risk of bias	Moderate risk of bias
		Bias in selection of the reported results	Low risk of bias	Moderate risk of bias
		Overall	Serious risk of bias	Serious risk of bias

Appendix H

			Outcome: Effectiveness	
Hviid, 2009	Robins-I	Bias due to confounding	Moderate risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Moderate risk of bias	
		Bias due to deviations from intended interventions	Moderate risk of bias	
		Bias due to missing data	Moderate risk of bias	
		Bias in measurement of outcomes	Moderate risk of bias	
		Bias in selection of the reported results	Moderate risk of bias	
		Overall	Moderate risk of bias	
			Outcome: Immunogenicity	
Ichikawa, 2013	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	
		Was the study population clearly and fully described, including a case definition?	Yes	
		Were the cases consecutive?	No	
		Were the subjects comparable?	Yes	
		Was the intervention clearly described?	Yes	
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	
		Was the length of follow-up adequate?	Yes	
		Were the statistical methods well-described?	Yes	
		Were the results well-described?	Yes	
		Overall	Fair	
			Outcome: Reactogenicity	
Kent, 2019	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Serious risk of bias	
		Bias in classification of interventions	Moderate risk of bias	
		Bias due to deviations from intended interventions	Moderate risk of bias	
		Bias due to missing data	Moderate risk of bias	
		Bias in measurement of outcomes	Serious risk of bias	
		Bias in selection of the reported results	Moderate risk of bias	
		Overall	Serious risk of bias	
			Outcome: Immunogenicity	Outcome: Reactogenicity/safety

Appendix H

Kent, 2016	ROB	Bias arising from the randomisation process	Low risk of bias	Low risk of bias
		Bias due to deviations from intended interventions	Low risk of bias	Low risk of bias
		Bias due to missing outcome data	Low risk of bias	Low risk of bias
		Bias in measurement of the outcome	Low risk of bias	Low risk of bias
		Bias in selection of the reported result	Low risk of bias	Moderate risk of bias
		Overall	Low risk of bias	Moderate risk of bias
			Outcome: Immunogenicity	
Kirmani, 2002	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Low risk of bias	
		Bias due to deviations from intended interventions	Low risk of bias	
		Bias due to missing data	Low risk of bias	
		Bias in measurement of outcomes	Low risk of bias	
		Bias in selection of the reported results	Low risk of bias	
		Overall	Serious risk of bias	
Klein, 2010	Robins-I		Outcome: Immunogenicity	Outcome: Reactogenicity/safety
		Bias due to confounding	Serious risk of bias	Serious risk of bias
		Bias in selection of participants into the study	Moderate risk of bias	Moderate risk of bias
		Bias in classification of interventions	Low risk of bias	Low risk of bias
		Bias due to deviations from intended interventions	Low risk of bias	Low risk of bias
		Bias due to missing data	Moderate risk of bias	Moderate risk of bias
		Bias in measurement of outcomes	Low risk of bias	Serious risk of bias
		Bias in selection of the reported results	Moderate risk of bias	Moderate risk of bias
Kulkarni-Munje, 2021	Robins-I	Overall	Serious risk of bias	Serious risk of bias
			Outcome: Immunogenicity	
		Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Low risk of bias	
		Bias due to deviations from intended interventions	Low risk of bias	
		Bias due to missing data	Serious risk of bias	
		Bias in measurement of outcomes	Low risk of bias	
		Bias in selection of the reported results	Low risk of bias	
		Overall	Serious risk of bias	
			Outcome: Reactogenicity	

Appendix H

Lee, 2006	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Serious risk of bias	
		Bias in classification of interventions	Low risk of bias	
		Bias due to deviations from intended interventions	Low risk of bias	
		Bias due to missing data	No information	
		Bias in measurement of outcomes	Serious risk of bias	
		Bias in selection of the reported results	Moderate risk of bias	
		Overall	Critical risk of bias	
			Outcome: Immunogenicity	Outcome: Reactogenicity/safety
Martinon-Torres, 2015	Robins-I	Bias due to confounding	Serious risk of bias	Serious risk of bias
		Bias in selection of participants into the study	Moderate risk of bias	Moderate risk of bias
		Bias in classification of interventions	Low risk of bias	Low risk of bias
		Bias due to deviations from intended interventions	Low risk of bias	Low risk of bias
		Bias due to missing data	Low risk of bias	Low risk of bias
		Bias in measurement of outcomes	Low risk of bias	Serious risk of bias
		Bias in selection of the reported results	Low risk of bias	Low risk of bias
		Overall	Serious risk of bias	Serious risk of bias
			Outcome: Immunogenicity	
Martinon-Torres, 2017	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Low risk of bias	
		Bias due to deviations from intended interventions	Low risk of bias	
		Bias due to missing data	Moderate risk of bias	
		Bias in measurement of outcomes	Low risk of bias	
		Bias in selection of the reported results	Low risk of bias	
		Overall	Serious risk of bias	
			Outcome: Reactogenicity	
Mialet-Marty, 2013	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	
		Was the study population clearly and fully described, including a case definition?	Yes	
		Were the cases consecutive?	NR	
		Were the subjects comparable?	Yes	
		Was the intervention clearly described?	Yes	

Appendix H

		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	
		Was the length of follow-up adequate?	Yes	
		Were the statistical methods well-described?	Yes	
		Were the results well-described?	Yes	
		Overall	Good	
			Outcome: Immunogenicity	
Moss, 2010	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Critical risk of bias	
		Bias due to deviations from intended interventions	Moderate risk of bias	
		Bias due to missing data	Low risk of bias	
		Bias in measurement of outcomes	Low risk of bias	
		Bias in selection of the reported results	Low risk of bias	
		Overall	Critical risk of bias	
Mukherjee, 2018	NIH quality assessment tool for before-after (pre-post) studies with no control group		Outcome: Reactogenicity	
		Was the study question or objective clearly stated?	Yes	
		Were eligibility/selection criteria for the study population prespecified and clearly described?	No	
		Were the participants in the study representative of those who would be eligible for the test/service/intervention in the general or clinical population of interest?	Yes	
		Were all eligible participants that met the prespecified entry criteria enrolled?	NR	
		Was the sample size sufficiently large to provide confidence in the findings?	No	
		Was the test/service/intervention clearly described and delivered consistently across the study population?	Yes	
		Were the outcome measures prespecified, clearly defined, valid, reliable, and assessed consistently across all study participants?	NR	
		Were the people assessing the outcomes blinded to the participants' exposures/interventions?	No	
		Was the loss to follow-up after baseline 20% or less? Were those lost to follow-up accounted for in the analysis?	Yes	

Appendix H

		Did the statistical methods examine changes in outcome measures from before to after the intervention? Were statistical tests done that provided p values for the pre-to-post changes?	No	
		Were outcome measures of interest taken multiple times before the intervention and multiple times after the intervention (i.e., did they use an interrupted time-series design)?	No	
		If the intervention was conducted at a group level (e.g., a whole hospital, a community, etc.) did the statistical analysis take into account the use of individual-level data to determine effects at the group level?	NA	
		Overall	Poor	
			Outcome: Effectiveness	
Nieminen, 2019	ROB	Bias arising from the randomisation process	Low risk of bias	
		Bias due to deviations from intended interventions	Low risk of bias	
		Bias due to missing outcome data	Low risk of bias	
		Bias in measurement of the outcome	Low risk of bias	
		Bias in selection of the reported result	Low risk of bias	
		Overall	Low risk of bias	
			Outcome: Immunogenicity	
Omenaca, 2007a	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Moderate risk of bias	
		Bias due to deviations from intended interventions	Moderate risk of bias	
		Bias due to missing data	Moderate risk of bias	
		Bias in measurement of outcomes	Moderate risk of bias	
		Bias in selection of the reported results	Moderate risk of bias	
		Overall	Serious risk of bias	
			Outcome: Immunogenicity	Outcome: Reactogenicity/safety
Omenaca, 2005	Robins-I	Bias due to confounding	Serious risk of bias	Serious risk of bias
		Bias in selection of participants into the study	Moderate risk of bias	Moderate risk of bias
		Bias in classification of interventions	Low risk of bias	Low risk of bias
		Bias due to deviations from intended interventions	Low risk of bias	Low risk of bias
		Bias due to missing data	Low risk of bias	Low risk of bias
		Bias in measurement of outcomes	Low risk of bias	Serious risk of bias
		Bias in selection of the reported results	Low risk of bias	Low risk of bias

Appendix H

		Overall	Serious risk of bias	Serious risk of bias
			Outcome: Safety	Outcome: Immunogenicity
Omenaca, 2011b	Robins-I	Bias due to confounding	Serious risk of bias	Serious risk of bias
		Bias in selection of participants into the study	Moderate risk of bias	Moderate risk of bias
		Bias in classification of interventions	Low risk of bias	Low risk of bias
		Bias due to deviations from intended interventions	Low risk of bias	Low risk of bias
		Bias due to missing data	Moderate risk of bias	Moderate risk of bias
		Bias in measurement of outcomes	Serious risk of bias	Low risk of bias
		Bias in selection of the reported results	Moderate risk of bias	Moderate risk of bias
		Overall	Serious risk of bias	Serious risk of bias
			Outcome: Safety/reactogenicity	Outcome: Immunogenicity
Omenaca, 2011(a)	Robins-I	Bias due to confounding	Serious risk of bias	Serious risk of bias
		Bias in selection of participants into the study	Moderate risk of bias	Moderate risk of bias
		Bias in classification of interventions	Low risk of bias	Low risk of bias
		Bias due to deviations from intended interventions	Low risk of bias	Low risk of bias
		Bias due to missing data	Low risk of bias	Low risk of bias
		Bias in measurement of outcomes	Serious risk of bias	Low risk of bias
		Bias in selection of the reported results	Moderate risk of bias	Low risk of bias
		Overall	Serious risk of bias	Serious risk of bias
			Outcome: Reactogenicity	Outcome: Immunogenicity
Omenaca, 2007b	Robins-I	Bias due to confounding	Serious risk of bias	Serious risk of bias
		Bias in selection of participants into the study	Moderate risk of bias	Moderate risk of bias
		Bias in classification of interventions	Low risk of bias	Low risk of bias
		Bias due to deviations from intended interventions	Low risk of bias	Low risk of bias
		Bias due to missing data	Low risk of bias	Low risk of bias
		Bias in measurement of outcomes	Serious risk of bias	Low risk of bias
		Bias in selection of the reported results	Low risk of bias	Low risk of bias
		Overall	Serious risk of bias	Serious risk of bias
			Outcome: Immunogenicity	Outcome: Reactogenicity/safety
Omenaca, 2012	ROB	Bias arising from the randomisation process	Low risk of bias	Low risk of bias
		Bias due to deviations from intended interventions	Low risk of bias	Low risk of bias
		Bias due to missing outcome data	Low risk of bias	Low risk of bias
		Bias in measurement of the outcome	Low risk of bias	Low risk of bias
		Bias in selection of the reported result	Low risk of bias	Low risk of bias
		Overall	Low risk of bias	Low risk of bias
			Outcome: Immunogenicity	
Perin 2012	Robins-I	Bias due to confounding	Moderate risk of bias	

Appendix H

		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Moderate risk of bias	
		Bias due to deviations from intended interventions	Moderate risk of bias	
		Bias due to missing data	Moderate risk of bias	
		Bias in measurement of outcomes	Moderate risk of bias	
		Bias in selection of the reported results	Moderate risk of bias	
		Overall	Moderate risk of bias	
			Outcome: Reactogenicity	
Pfister, 2004	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	
		Was the study population clearly and fully described, including a case definition?	Yes	
		Were the cases consecutive?	NR	
		Were the subjects comparable?	Yes	
		Was the intervention clearly described?	Yes	
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	No	
		Was the length of follow-up adequate?	Yes	
		Were the statistical methods well-described?	Yes	
		Were the results well-described?	Yes	
		Overall	Good	
			Outcome: Reactogenicity	
Pourcyrus, 2007	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	
		Was the study population clearly and fully described, including a case definition?	Yes	
		Were the cases consecutive?	Yes	
		Were the subjects comparable?	Yes	
		Was the intervention clearly described?	Yes	
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	
		Was the length of follow-up adequate?	Yes	
		Were the statistical methods well-described?	Yes	
		Were the results well-described?	Yes	
		Overall	Good	
			Outcome: Effectiveness	

Appendix H

Riise, 2017	Robins-I	Bias due to confounding	Moderate risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Moderate risk of bias	
		Bias due to deviations from intended interventions	Moderate risk of bias	
		Bias due to missing data	Moderate risk of bias	
		Bias in measurement of outcomes	Moderate risk of bias	
		Bias in selection of the reported results	Moderate risk of bias	
		Overall	Moderate risk of bias	
			Outcome: Effectiveness	
Riise, 2018	Robins-I	Bias due to confounding	Moderate risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Moderate risk of bias	
		Bias due to deviations from intended interventions	Moderate risk of bias	
		Bias due to missing data	Moderate risk of bias	
		Bias in measurement of outcomes	Moderate risk of bias	
		Bias in selection of the reported results	Moderate risk of bias	
		Overall	Moderate risk of bias	
			Outcome: Immunogenicity	
Robinson, 2004	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Low risk of bias	
		Bias due to deviations from intended interventions	Low risk of bias	
		Bias due to missing data	Low risk of bias	
		Bias in measurement of outcomes	Low risk of bias	
		Bias in selection of the reported results	Low risk of bias	
		Overall	Serious risk of bias	
			Outcome: Effectiveness	Outcome: Safety
Roué, 2014	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	Yes
		Was the study population clearly and fully described, including a case definition?	Yes	Yes
		Were the cases consecutive?	Yes	Yes
		Were the subjects comparable?	Yes	Yes
		Was the intervention clearly described?	Yes	Yes

Appendix H

		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes
		Was the length of follow-up adequate?	Yes	Yes
		Were the statistical methods well-described?	Yes	Yes
		Were the results well-described?	Yes	Yes
		Overall	Good	Good
			Outcome: Immunogenicity	
Rouers 2020	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Serious risk of bias	
		Bias in classification of interventions	Low risk of bias	
		Bias due to deviations from intended interventions	Low risk of bias	
		Bias due to missing data	Low risk of bias	
		Bias in measurement of outcomes	Low risk of bias	
		Bias in selection of the reported results	Moderate risk of bias	
		Overall	Serious risk of bias	
			Outcome: Effectiveness	
Ruckinger, 2010	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Serious risk of bias	
		Bias due to deviations from intended interventions	Moderate risk of bias	
		Bias due to missing data	Moderate risk of bias	
		Bias in measurement of outcomes	Moderate risk of bias	
		Bias in selection of the reported results	Moderate risk of bias	
		Overall	Serious risk of bias	
			Outcome: Immunogenicity	
Ruggeberg 2007	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Low risk of bias	
		Bias in classification of interventions	Low risk of bias	
		Bias due to deviations from intended interventions	Low risk of bias	
		Bias due to missing data	Moderate risk of bias	
		Bias in measurement of outcomes	Low risk of bias	
		Bias in selection of the reported results	Low risk of bias	
		Overall	Serious risk of bias	

Appendix H

			Outcome: Reactogenicity	
Sadarangani 2017	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Serious risk of bias	
		Bias in classification of interventions	Moderate risk of bias	
		Bias due to deviations from intended interventions	Moderate risk of bias	
		Bias due to missing data	Moderate risk of bias	
		Bias in measurement of outcomes	Serious risk of bias	
		Bias in selection of the reported results	Moderate risk of bias	
		Overall	Critical risk of bias	
			Outcome: Reactogenicity	
Schulzke, 2005	NIH quality assessment tool for before-after (pre- post) studies with no control group	Was the study question or objective clearly stated?	Yes	
		Were eligibility/selection criteria for the study population prespecified and clearly described?	Yes	
		Were the participants in the study representative of those who would be eligible for the test/service/intervention in the general or clinical population of interest?	Yes	
		Were all eligible participants that met the prespecified entry criteria enrolled?	Yes	
		Was the sample size sufficiently large to provide confidence in the findings?	Yes	
		Was the test/service/intervention clearly described and delivered consistently across the study population?	Yes	
		Were the outcome measures prespecified, clearly defined, valid, reliable, and assessed consistently across all study participants?	Yes	
		Were the people assessing the outcomes blinded to the participants' exposures/interventions?	No	
		Was the loss to follow-up after baseline 20% or less? Were those lost to follow-up accounted for in the analysis?	Yes	
		Did the statistical methods examine changes in outcome measures from before to after the intervention? Were statistical tests done that provided p values for the pre-to-post changes?	Yes	
		Were outcome measures of interest taken multiple times before the intervention and multiple times after the intervention (i.e., did they use an interrupted time-series design)?	No	
		If the intervention was conducted at a group level (e.g., a whole hospital, a community, etc.) did the statistical analysis take into	N/A	

Appendix H

		account the use of individual-level data to determine effects at the group level?		
		Overall	Fair	
Sen, 2001	NIH quality assessment tool for before-after (pre-post) studies with no control group		Outcome: Reactogenicity	
		Was the study question or objective clearly stated?	Yes	
		Were eligibility/selection criteria for the study population prespecified and clearly described?	Yes	
		Were the participants in the study representative of those who would be eligible for the test/service/intervention in the general or clinical population of interest?	Yes	
		Were all eligible participants that met the prespecified entry criteria enrolled?	Yes	
		Was the sample size sufficiently large to provide confidence in the findings?	Yes	
		Was the test/service/intervention clearly described and delivered consistently across the study population?	Yes	
		Were the outcome measures prespecified, clearly defined, valid, reliable, and assessed consistently across all study participants?	Yes	
		Were the people assessing the outcomes blinded to the participants' exposures/interventions?	No	
		Was the loss to follow-up after baseline 20% or less? Were those lost to follow-up accounted for in the analysis?	Yes	
		Did the statistical methods examine changes in outcome measures from before to after the intervention? Were statistical tests done that provided p values for the pre-to-post changes?	No	
		Were outcome measures of interest taken multiple times before the intervention and multiple times after the intervention (i.e., did they use an interrupted time-series design)?	No	
		If the intervention was conducted at a group level (e.g., a whole hospital, a community, etc.) did the statistical analysis take into account the use of individual-level data to determine effects at the group level?	N/A	
		Overall	Fair	
			Outcome: Effectiveness	
Sheridan, 2022	Robins-I	Bias due to confounding	Low risk	
		Bias in selection of participants into the study	Low risk	
		Bias in classification of interventions	Low risk	
		Bias due to deviations from intended interventions	Low risk	

Appendix H

		Bias due to missing data	Low risk	
		Bias in measurement of outcomes	Low risk	
		Bias in selection of the reported results	Low risk	
		Overall	Low risk	
			Outcome: Immunogenicity	
Sikora, 2005	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	
		Was the study population clearly and fully described, including a case definition?	No	
		Were the cases consecutive?	NR	
		Were the subjects comparable?	Yes	
		Was the intervention clearly described?	Yes	
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	
		Was the length of follow-up adequate?	Yes	
		Were the statistical methods well-described?	Yes	
		Were the results well-described?	Yes	
		Overall	Fair	
			Outcome: Immunogenicity	
Sikora, 2004	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	
		Was the study population clearly and fully described, including a case definition?	Yes	
		Were the cases consecutive?	NR	
		Were the subjects comparable?	Yes	
		Was the intervention clearly described?	Yes	
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	
		Was the length of follow-up adequate?	Yes	
		Were the statistical methods well-described?	Yes	
		Were the results well-described?	Yes	
		Overall	Fair	
			Outcome: Immunogenicity	
Slack 2001	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Low risk of bias	
		Bias in classification of interventions	Low risk of bias	

Appendix H

		Bias due to deviations from intended interventions	Low risk of bias	
		Bias due to missing data	Low risk of bias	
		Bias in measurement of outcomes	Low risk of bias	
		Bias in selection of the reported results	Low risk of bias	
		Overall	Serious risk of bias	
			Outcome: Immunogenicity	
Slack 2005	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Low risk	
		Bias in classification of interventions	Low risk	
		Bias due to deviations from intended interventions	Low risk	
		Bias due to missing data	Low risk	
		Bias in measurement of outcomes	Low risk	
		Bias in selection of the reported results	Low risk	
		Overall	Serious risk of bias	
			Outcome: Immunogenicity	
Slack 2004	Robins-I	Bias due to confounding	Moderate risk of bias	
		Bias in selection of participants into the study	Low risk of bias	
		Bias in classification of interventions	Low risk of bias	
		Bias due to deviations from intended interventions	Low risk of bias	
		Bias due to missing data	Low risk of bias	
		Bias in measurement of outcomes	Low risk of bias	
		Bias in selection of the reported results	Low risk of bias	
		Overall	Moderate risk of bias	
			Outcome: Reactogenicity	
Slack 2003	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	No	
		Was the study population clearly and fully described, including a case definition?	Yes	
		Were the cases consecutive?	NR	
		Were the subjects comparable?	Yes	
		Was the intervention clearly described?	Yes	
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	
		Was the length of follow-up adequate?	Yes	

Appendix H

		Were the statistical methods well-described?	No	
		Were the results well-described?	Yes	
		Overall	Fair	
			Outcome: Immunogenicity	
Szynczewska 2013	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	
		Was the study population clearly and fully described, including a case definition?	Yes	
		Were the cases consecutive?	NR	
		Were the subjects comparable?	Yes	
		Was the intervention clearly described?	Yes	
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	
		Was the length of follow-up adequate?	Yes	
		Were the statistical methods well-described?	Yes	
		Were the results well-described?	Yes	
		Overall	Good	
			Outcome: Immunogenicity	
Tsuda, 2012	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	
		Was the study population clearly and fully described, including a case definition?	Yes	
		Were the cases consecutive?	NR	
		Were the subjects comparable?	Yes	
		Was the intervention clearly described?	Yes	
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	
		Was the length of follow-up adequate?	Yes	
		Were the statistical methods well-described?	Yes	
		Were the results well-described?	Yes	
		Overall	Good	
			Outcome: Effectiveness	
Van den berg, 2015	ROB	Bias arising from the randomisation process	Low risk of bias	
		Bias due to deviations from intended interventions	Low risk of bias	
		Bias due to missing outcome data	Low risk of bias	
		Bias in measurement of the outcome	Low risk of bias	

Appendix H

		Bias in selection of the reported result	Low risk of bias	
		Overall	Low risk of bias	
			Outcome: Effectiveness	
van der Maas, 2019	Robins-I	Bias due to confounding	Serious risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Moderate risk of bias	
		Bias due to deviations from intended interventions	Moderate risk of bias	
		Bias due to missing data	Serious risk of bias	
		Bias in measurement of outcomes	Serious risk of bias	
		Bias in selection of the reported results	Moderate risk of bias	
		Overall	Critical risk of bias	
			Outcome: Immunogenicity	Outcome: Reactogenicity and safety
Vazquez 2008	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	Yes
		Was the study population clearly and fully described, including a case definition?	Yes	Yes
		Were the cases consecutive?	NR	NR
		Were the subjects comparable?	Yes	Yes
		Was the intervention clearly described?	Yes	Yes
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	No
		Was the length of follow-up adequate?	Yes	Yes
		Were the statistical methods well-described?	Yes	No
		Were the results well-described?	Yes	Yes
		Overall	Good	Fair
Vermeulen, 2010	NIH quality assessment tool for case series studies		Outcome: Immunogenicity	
		Was the study question or objective clearly stated?	Yes	
		Was the study population clearly and fully described, including a case definition?	Yes	
		Were the cases consecutive?	NR	
		Were the subjects comparable?	Yes	
		Was the intervention clearly described?	Yes	
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	
		Was the length of follow-up adequate?	Yes	

Appendix H

		Were the statistical methods well-described?	Yes	
		Were the results well-described?	Yes	
		Overall	Good	
			Outcome: Immunogenicity	
Vermeulen, 2013	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	
		Was the study population clearly and fully described, including a case definition?	Yes	
		Were the cases consecutive?	Yes	
		Were the subjects comparable?	Yes	
		Was the intervention clearly described?	Yes	
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	
		Was the length of follow-up adequate?	Yes	
		Were the statistical methods well-described?	Yes	
		Were the results well-described?	Yes	
		Overall	Good	
			Outcome: Reactogenicity	
Wilinska 2016	NIH quality assessment tool for case series studies	Was the study question or objective clearly stated?	Yes	
		Was the study population clearly and fully described, including a case definition?	Yes	
		Were the cases consecutive?	NR	
		Were the subjects comparable?	Yes	
		Was the intervention clearly described?	Yes	
		Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?	No	
		Was the length of follow-up adequate?	Yes	
		Were the statistical methods well-described?	Yes	
		Were the results well-described?	Yes	
		Overall	Fair	
			Outcome: Safety	
Wilson, 2012	Robins-I	Bias due to confounding	Moderate risk of bias	
		Bias in selection of participants into the study	Moderate risk of bias	
		Bias in classification of interventions	Moderate risk of bias	
		Bias due to deviations from intended interventions	Moderate risk of bias	

Appendix H

		Bias due to missing data	Low risk of bias	
		Bias in measurement of outcomes	Serious risk of bias	
		Bias in selection of the reported results	Moderate risk of bias	
		Overall	Serious risk of bias	

Appendix I

Table 1: Summary of number of participants in included studies for each outcome of interest along with criteria for GRADE assessment

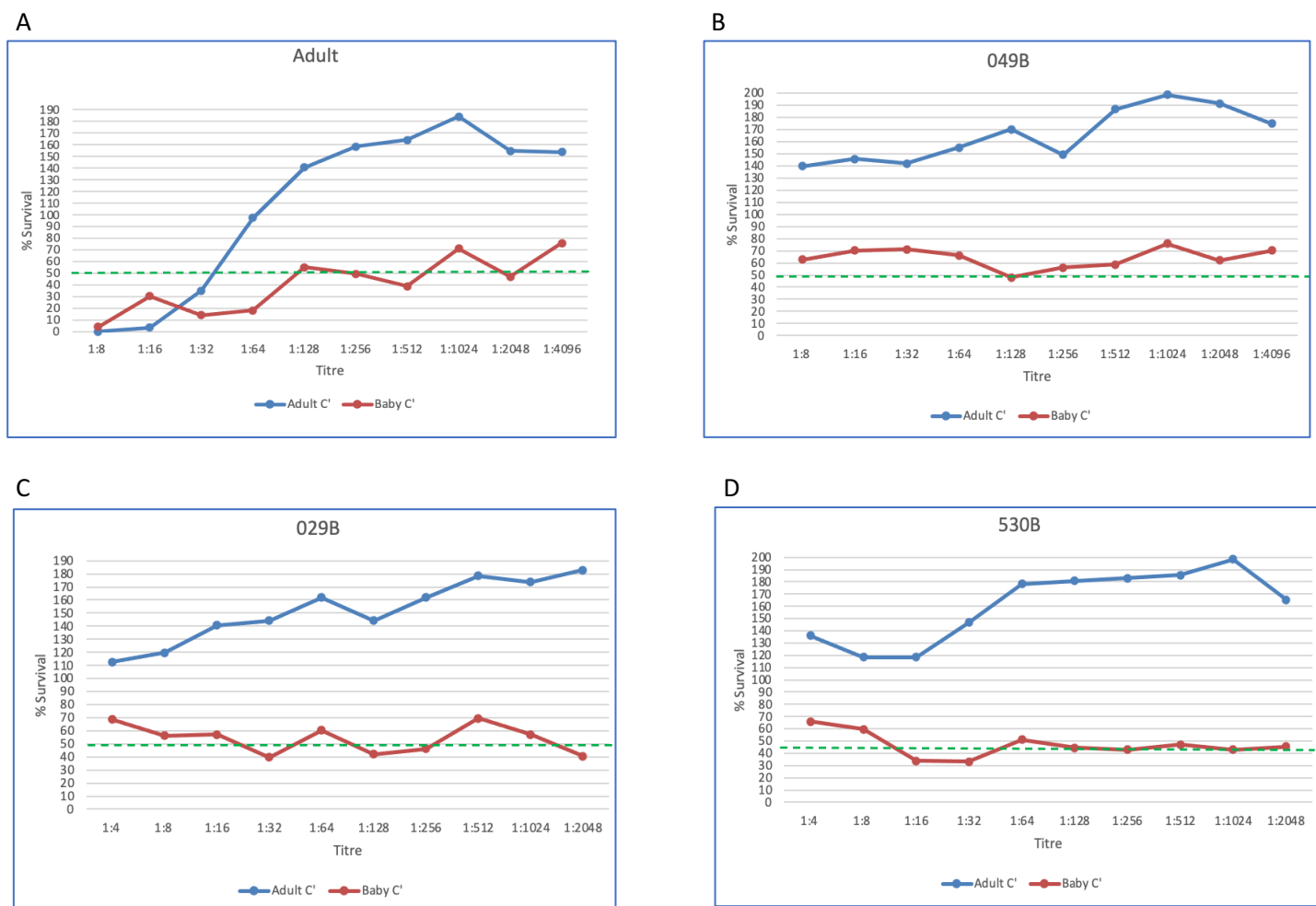
Outcomes		Number of participants (studies)	Risk of bias	Inconsistency of results	Indirectness of evidence	Publication bias	Quality of the evidence
Immunogenicity	Hib	2,861: PT 1,780; FT 1,081 (18)	High	High	Moderate	Low	⊕⊕○○
	Diphtheria	1,868: PT 1,184; FT 684 (11)	High	Moderate	Moderate	Low	⊕⊕○○
	Tetanus	2,197: PT 1,414; FT 783 (13)	High	Low	Moderate	Low	⊕⊕○○
	Hep B	854: PT 526; FT 328 (5)	High	Low	Moderate	Low	⊕⊕○○
	MenC	1,107: PT 639; FT 468 (7)	High	Low	Moderate	Low	⊕⊕○○
	Polio	1,130: PT 536; FT 594 (8)	High	Low	Moderate	Low	⊕⊕○○
	Pertussis	2,020: PT 1,261; FT 759 (15)	High	High	Moderate	Low	⊕⊕○○
	MMR	522: PT 144; FT 378 (4)	High	Low	Moderate	Low	⊕⊕○○
	Rotavirus	300: PT 300 (1)	Low	-	Low	Moderate	⊕⊕⊕○
	Pneumococcal	2,217: PT 1,646; FT 571 (12)	High	High	Moderate	Low	⊕⊕○○
Reactogenicity	Local and systemic reactions	5,525: PT 4,747; FT 779 (15)	High	High	Moderate	Low	⊕⊕○○
	Cardiorespiratory events	17,214: PT 17,206; FT 8 (24)	High	High	Moderate	Low	⊕⊕○○
	Sepsis evaluation and IV abx	14,899: PT 14,899 (8)	High	Moderate	Moderate	Low	⊕⊕○○
	Fever	4,966: PT 4,580; FT 386 (16)	High	High	Moderate	Low	⊕⊕○○
Safety	Hospital admission	83,5017: PT 120,176; FT 714,841 (3)	High	Low	Moderate	Low	⊕⊕○○
	Adverse events	5,126: PT 4,517; 609 (12)	High	Low	Moderate	Low	⊕⊕○○

Appendix I

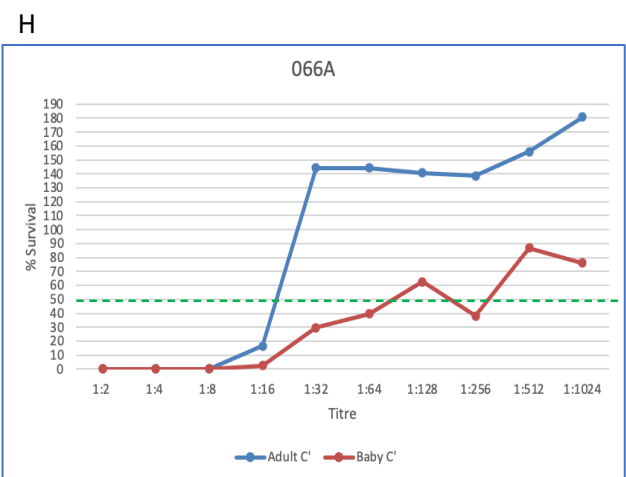
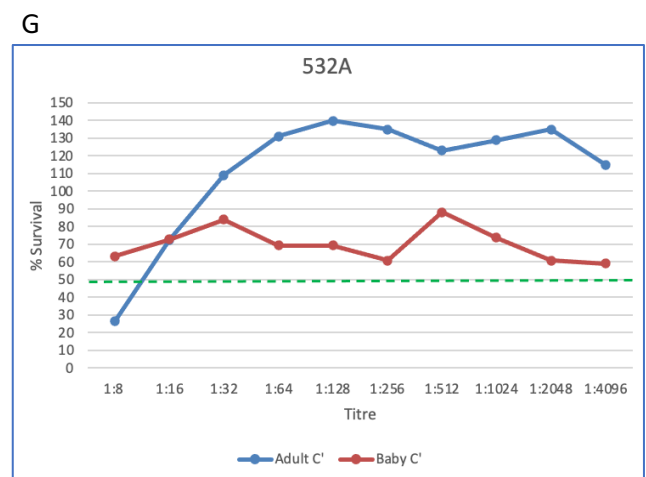
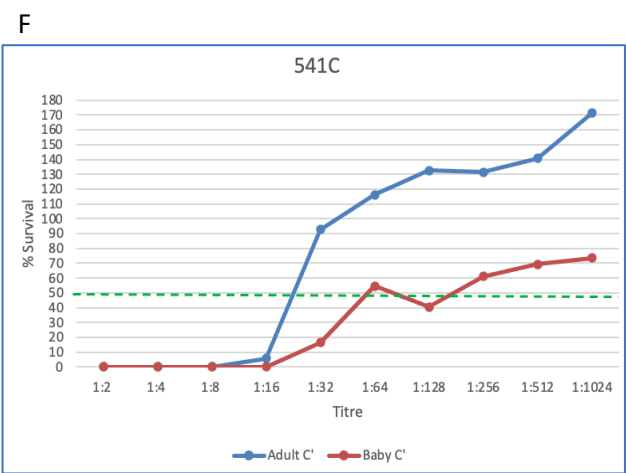
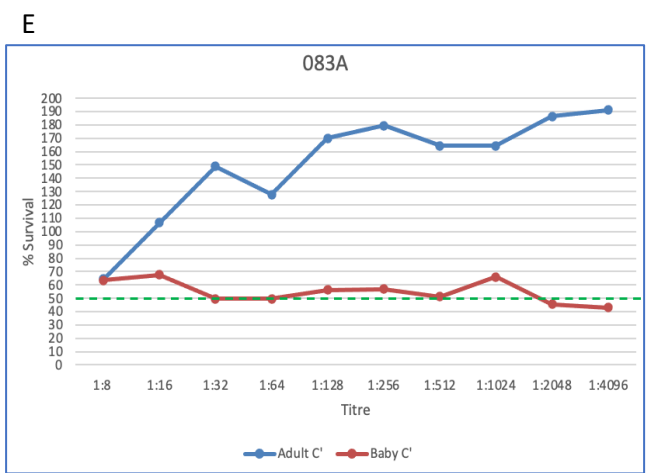
Efficacy/effectiveness	Rotavirus	354: PT 354 (2)	Low	Low	Moderate	Low	⊕⊕⊕○
	Hib	165: PT 18; FT 147 (1)	High	-	Moderate	Moderate	⊕⊕○○
	Pertussis	2,905,365: PT 96,304; 2,809,061 (4)	High	Low	Moderate	Low	⊕⊕○○
	Pneumococcal	31,111: PT 1591; FT 29520 (3)	High	Low	Moderate	Low	⊕⊕○○

Appendix J

Figures A-N: SBA curves for SBA assays using neonatal and adult complement and *N. meningitidis* strain 44/76-SL

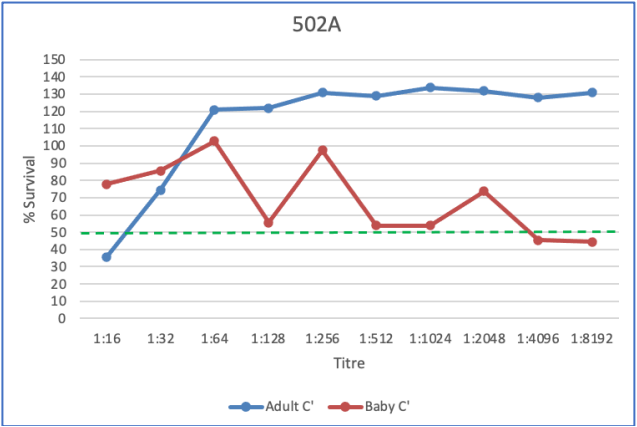


Appendix J

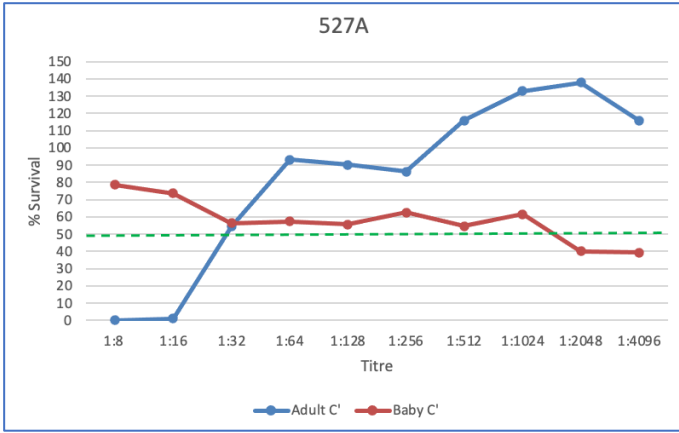


Appendix J

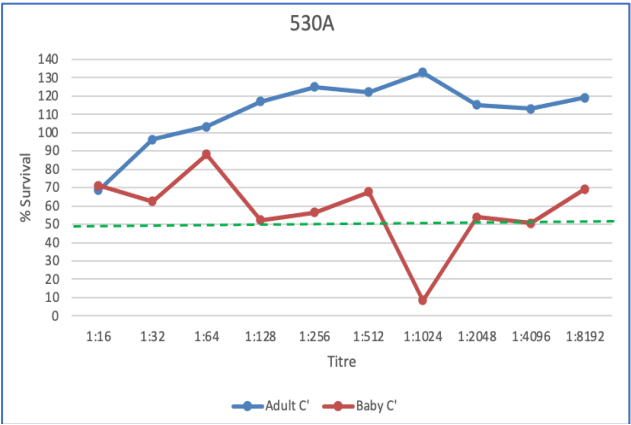
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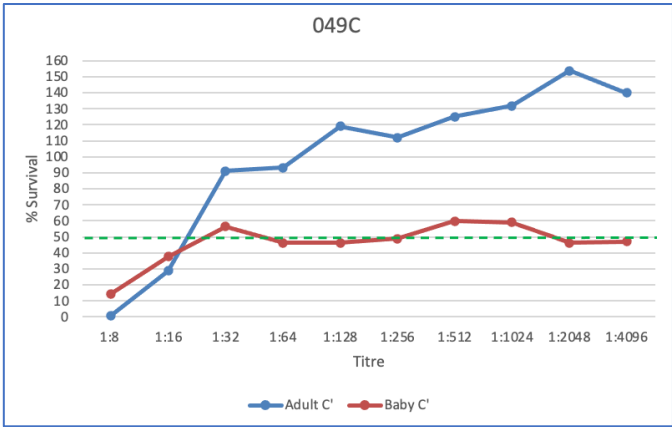
J



K

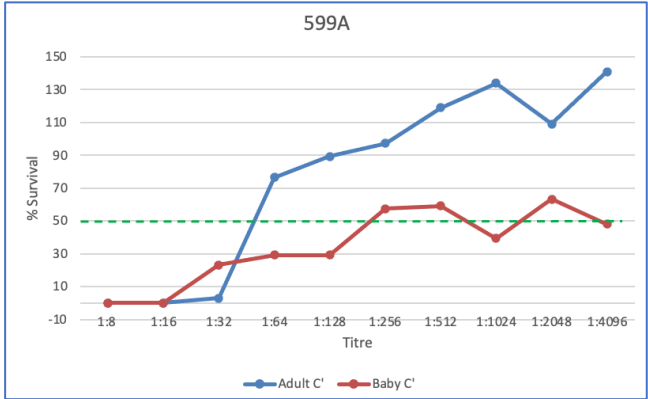


L



Appendix J

M



N

