Unravelling the Risk of Invasive Late-Onset Group B *Streptococcus* Disease and Defining a Threshold of Antibody-Mediated Immunity Resulting from Natural Exposure

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Submitted for the degree of Doctor of Philosophy

London, 2023

Declaration page

I, Konstantinos Karampatsas confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature:

Date: 1st June 2023

To Martoula,

Abstract

Introduction: Invasive Group B streptococcal disease (iGBS) remains among the most common causes of sepsis and meningitis among neonates and young infants. Risk factors of the late-onset form of iGBS disease (LOGBS) are not fully understood. Establishing a correlate of protection (CoP) through natural immunity studies will facilitate and accelerate vaccine licensure to prevent both early and late-onset iGBS disease.

Methods: Three studies were undertaken: 1) A systematic review of clinical risk factors associated with LOGBS. 2) An epidemiological study of cases with recurrence and in multiples from the United Kingdom (UK) and Republic of Ireland (national surveillance study 2014/15) and Germany and Switzerland (retrospective case collection). 3) A UK pilot cohort study (iGBS feasibility; 2018-2020) followed by a large-scale seroepidemiological study (iGBS3; still underway) to define protective levels of anti-GBS capsular polysaccharide (CPS) immunoglobulin G (IgG).

Results: 1) The odds ratios (OR) of LOGBS in infants born <37 weeks or to colonised women were 7.79 (95% CI, 6.56–9.24) and 2.18 (1.78–2.68), respectively. 2) The incidence in infants of multiple births with an affected sibling was 17%. Very low birth weight and a course of antibiotics <10 days were associated with recurrence (aOR 10.0 (2.7-37.4), P<0.001; and 4.5 (1.3-17.5), P=0.02, respectively). 3) The half-life of protective transplacental anti-GBS IgG was 27.4 (23.5-32.9) days. For serotype III, IgG geometric mean concentrations were higher in the cord sera of healthy infants compared to acute sera from cases (0.1 vs 0.01 µg/mL; P<0.001). The threshold associated with 90% disease risk reduction was 2.28 (1.16-7.03) µg/mL.

Conclusions: Prematurity, low birth weight and maternal colonisation are important risk factors for LOGBS. Persistent mucosal colonisation and acute horizontal transmission are the two most likely mechanisms underpinning recurrent iGBS disease and LOGBS in general. Relatively low levels of naturally acquired anti-GBS CPS IgG protect most infants against serotype III-associated iGBS disease.

Research Paper Declaration Form

referencing the doctoral candidate's own published work(s)

1. The material presented in Chapter 4 has been previously published in a peer-reviewed journal.

Karampatsas K, Davies H, Mynarek M, Andrews N, Heath PT, Le Doare K.

Clinical Risk Factors Associated With Late-Onset Invasive Group B Streptococcal Disease: Systematic Review and Meta-Analyses.

Clin Infect Dis. 2022 Sep 30;75(7):1255-1264.

doi: 10.1093/cid/ciac206. PMID: 35275986; PMCID: PMC9525091.

2. The material presented in Chapter 5 has been previously published in a peer-reviewed journal.

Freudenhammer M*, Karampatsas K*, Le Doare K, Lander F, Armann J, Acero Moreno D, Boyle M, Buxmann H, Campbell R, Chalker V, Cunney R, Doherty L, Davies E, Efstratiou A, Elling R, Endmann M, Essers J, Hentschel R, Jones CE, Kallsen S, Kapatai G, Krüger M, Ladhani S, Lamagni T, Lindsay D, Meehan M, O'Sullivan CP, Patel D, Reynolds AJ, Roll C, Schulzke S, Smith A, Stein A, von der Wense A, Voss E, Wieg C, Härtel C, Heath PT, Henneke P.

*Joint first authors

Invasive Group B Streptococcus Disease With Recurrence and in Multiples: Towards a Better Understanding of GBS Late-Onset Sepsis.

Front Immunol. 2021 Jun 2;12:617925.

doi: 10.3389/fimmu.2021.617925. PMID: 34149682; PMCID: PMC8208644.

3. Part of the material presented in Chapter 7 has been previously published as an abstract in a peer-reviewed journal.

Group B Strep in Pregnancy & Babies Conference, 12-13 July 2022: Abstracts.

Karampatsas K, Hall T, Ramkhelawon L, Andrews N, Heath PT, Le Doare K.

Naturally acquired serotype-specific anti-GBS CPS IgG concentrations: A pilot UK prospective cohort study.

Therapeutic Advances in Infectious Disease. 2022;9.

doi:10.1177/20499361221112979

Acknowledgements

I would also like to thank all those who have made this research possible. This includes:

- My supervisors, Prof Kirsty Le Doare, Prof Paul Heath and Prof Nick Andrews, for their support, guidance and assistance throughout the project
- The lab members of Le Doare group at SGUL, Tom Hall, Olwenn Daniel, Dr Kay To, Robert Watts, Laxmee Ramkhelawon, Dr Suzy Lim, Simon Beach, Alexander Finnie and Dr Rossul Al-Bahadili for having me in the lab all this time, and their assistance with a large amount of the laboratory work
- The iGBS3 team at SGUL, Madeleine Cochet, Natalie Rouse, Rakan Musleh and Dr Mary Kyohere for coordinating all aspects of the study
- Prof Merryn Voysey from Oxford Vaccine Group, for her support with the statistical modelling
- Dr Mirjam Freudenhammer and Prof Philipp Henneke at the Institute for Immunodeficiency, University of Freiburg, for sharing precious clinical data and permitting me to utilise them
- Dr Hannah Davies at SGUL and Dr Maren Mynarek at the Center for Early Brain Development, Norwegian University of Science and Technology, Trondheim, for their help in conducting the systematic review
- Prof Andrew Gorringe, Stephanie Leung, Steve Thomas, Dr Stephen Taylor and the team at Pathogen Immunology Group, UK Health Security Agency, Porton Down for training and support with the opsonophagocytosis killing assay
- My partner Marta, our two children Ikaros and Enea, my mother Vaso, my brother Nikos and friends for their support and patience with me as I undertook this project

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Abbreviations

Abbreviation	Definition
4CMenB	Four-Component, protein-based Meningococcal serogroup B vaccine
aC	AlphaC
ADR	Absolute Disease Risk
AIC	Akaike's Information Criteria
Alp	Alpha-like protein
aOR	Adjusted Odds Ratio
BA	Columbia Agar with Horse Blood
BPAIIG	British Paediatric Allergy, Immunity and Infection Group
BPSU	British Paediatric Surveillance Unit
CI	Confidence Interval
CAG	Chromogenic agar
CO2	Carbon dioxide
CoP	Correlate of Protection/risk reduction
CovR/S	Control of virulence Regulator/Sensor
CPS	Capsular Polysaccharide
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
ECM	Enriched Culture Medium
ELBW	Extremely Low Birth Weight
EOGBS	Early-Onset Group B Streptococcal disease

FcRn	Neonatal Fc Receptor
GBS	Group B *Streptococcus*
GMC	Geometric Mean Concentration
GNN	German Neonatal Network
HIC	High-Income Countries
HIV	Human Immunodeficiency Virus
HvgA	Hypervirulent GBS Adhesin
HylB	Hyaluronidase
IAP	Intrapartum Antibiotic Prophylaxis
ICF	Informed Consent Form
IFN-γ	Interferon-gamma
iGBS	Invasive GBS
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ISF	Investigator Site File
LBW	Low Birth Weight
LIM Broth	Todd Hewitt Broth with nalidixic acid and colistin
LLOQ	Lower Limit of Quantitation
LMIC	Low- and Middle-Income Countries
LOGBS	Late-Onset Group B Streptococcal disease
MALDI-TOF MS	Matrix Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry
MFI	Median Fluorescent Intensities

MIA	Multiplex Immunoassay
MLST	Multilocus Sequence Typing
NDI	Neurodevelopment Impairment
neonIN	Neonatal Infection Surveillance Network
NHS	National Health Service
NICU	Neonatal Intensive Care Unit
NIHR	National Institute for Health Research
NOS	Newcastle-Ottawa Scale
OPkA	Opsonophagocytosis Killing Assay
OR	Odds Ratio
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
PHE	Public Health England
PIS	Patient Information Sheet
PROM	Prolonged Rupture of Membranes
RCD	Reverse Cumulative Disease
RCOG	Royal College of Obstetricians and Gynaecologists
RRR	Relative Risk Reduction
SA	South Africa
SGUL	St George's, University of London
Siglecs	Sialic acid-binding immunoglobulin-type lectins
Sip	Surface immunogenic protein

Srr	Serine-rich repeat glycoprotein
ST	Serotype
STROBE	STrengthening the Reporting of OBservational studies in Epidemiology
TCS	Two-Component Systems
Th	T helper
UK	United Kingdom
UKHSA	UK Health Security Agency
UKPVG	UK Paediatric Vaccine Group
UKROI	United Kingdom and Republic of Ireland
UR	Uncertainty Range
USA	United States of America
VLBW	Very Low Birth Weight
WHO	World Health Organisation
β-H/C	β-Hemolysin/Cytolysin toxin

Chapter 1 – Background

1.1 Introduction

Streptococcus agalactiae, or Group B *Streptococcus* (GBS), is a Gram-positive diplococcus (1) frequently colonising human gastrointestinal and genitourinary tracts. (2) GBS was known to cause bovine mastitis, before it emerged as the leading cause of neonatal bacterial infections in America and Europe in the 1960s. (3,4) Although newborns and young infants under three months of age remain at the highest risk for invasive GBS (iGBS) disease (**Table 1**), (5) GBS can also cause urinary tract infections, chorioamnionitis and early postpartum infection in pregnant women. (6–9) GBS intrauterine infections are associated with a significant proportion of stillbirths and preterm births worldwide. (8,10,11) More recently, the incidence of GBS infection among non-pregnant adults has been better described, with adults older than 65 years and those with underlying conditions at higher risk of severe GBS infections. (7,12–15)

1.2 Current Epidemiology and Burden of Neonatal and Infant iGBS Disease

1.2.1 Global Estimates

Although child survival has substantially increased in the last 30 years, neonatal mortality (first 28 days of life) remains high, accounting for 47% of all children deaths under five years, up 7% since 1990.(16) Infections cause most deaths within the first 28 days of birth, (16) and GBS is the most common cause of sepsis and meningitis in neonates and young infants in most countries. (8,17) In 2020, it was estimated that 231,800 (95% uncertainty range (UR) 114,100–455,000) infants had early-onset Group B streptococcal disease (EOGBS, occurring in infants aged <7 days) and 162,200 (95% UR 70,200–394,400) infants had late-onset Group B streptococcal disease (LOGBS, occurring in infants aged 7-89 days) worldwide. (8) Moreover, it was estimated that 91,900 (95% UR 44,800–187,800) deaths occurred globally in children with iGBS disease, with a disproportionate burden in sub-Saharan Africa and Asia. (8)

The disease burden is even higher when an estimated number of 46,200 (95% UR 20,300–111,300) stillbirths associated with GBS infection are included. (8)

1.2.2 Geographical Variation

In high-income contexts, where there is good capture of cases and routine laboratory surveillance, GBS has been well-recognised as one of the leading causes of early and late-onset disease in neonates and young infants since the 1990s. (5) However, there remains uncertainty regarding the incidence of iGBS in low- and middle-income countries (LMIC). In the late 1990s, a large prospective aetiology study of serious infections in infants younger than 90 days old, the World Health Organisation (WHO) Young Infants study, conducted across Ethiopia, the Gambia, Papua New Guinea and the Philippines, has not identified GBS among the common neonatal pathogens. (18) In contrast, more recent facility studies in South Africa (SA), Kenya, and The Gambia have shown that GBS is an important neonatal pathogen in those settings. (19–21) A systematic review and meta-analysis of the burden of iGBS disease in sub-Saharan Africa that included nine studies reported an incidence of EOGBS as 1.3 per 1000 births (95% confidence interval (CI) 0.81-1.9) and that of LOGBS 0.73 per 1000 births (95% CI 0.48-1.0). (22) Similarly, a 2017 global systematic review and meta-analysis estimated the incidence of iGBS disease in Africa at 1.12 per 1000 live births, two times higher than in developed countries at 0.46 per 1000 live births. (23) In the same review, the incidence of iGBS disease was strikingly low in Asia at 0.31 per 1000 live births. (23) A recent population-based pregnancy surveillance at five sites in Bangladesh, India, and Pakistan also showed a four-fold lower proportion of early onset sepsis cases attributed to GBS in South Asia than that reported in a SA study that used the same inclusion criteria and identical molecular methodology for pathogen identification. (24,25)

These differences could reflect an actual regional difference but may also be due to incomplete case ascertainment. Low case ascertainment in LMIC can result from cases being “missed” through lack of access to healthcare, inadequate clinical assessment and suspicion of infection, lack of diagnostic testing, and lack of appropriate laboratory detection methods such as high-quality blood cultures. (24,26) In settings where most births are at home, most EOGBS cases with onset on

day zero may be missed since home deliveries have the lowest likelihood of receiving medical attention and the highest likelihood of unreported deaths. Even where treatment is delivered, only a tiny proportion of hospital admissions may have a microbiological specimen taken since, in many low-resource settings, laboratories have restricted operating hours and limited availability of qualified personnel and microbiological culture facilities, particularly for blood and Cerebrospinal Fluid (CSF) culture. Previous antibiotic therapy also influences culture detection, especially in areas where "over-the-counter" antibiotics are widely used. However, case ascertainment biases due to different medical practices might not fully account for the observed geographical variation in the iGBS disease burden. These differences may also be related to strain differences (low prevalence of serotype III and clonal complex 17 among colonising and invasive strains in Asian countries), level of natural acquired protective maternal antibody, or other host, environmental, or behavioural factors. (23,27)

1.2.3 Neurodevelopment Impairment

Recently published cohort follow-up studies beyond early childhood from LMIC and high-income countries (HIC) suggest a high risk of neurodevelopment impairment (NDI) among children with iGBS sepsis or meningitis. (28–30) It was estimated that 37,100 children (95% UR 14,600–96,200) who recovered from iGBS disease developed moderate or severe NDI, a considerably higher number than previously recognised. (8,31)

Table 1. Definitions

Newborn and young infant invasive GBS (iGBS) disease	Laboratory isolation of GBS from a normally sterile site (e.g. blood, CSF, joint fluid, peritoneal fluid) in an infant aged 0–89 days with signs of clinical disease
Early-onset Group B streptococcal disease (EOGBS)	iGBS disease occurring in infants aged <7 days age
Late-onset Group B streptococcal disease (LOGBS)	iGBS disease occurring in infants aged 7-89 days age
Correlate of protection/risk reduction (CoP) against iGBS disease	The amount of placentally transferred maternal GBS antibody (whether from natural exposure to GBS (colonisation or infection) or from maternal GBS vaccine) that is related to protection against newborn and young infant iGBS disease
GBS: Group B *Streptococcus*	

1.3 Epidemiology and Burden of Neonatal and Infant iGBS Disease in the United Kingdom (UK)

In 2014–2015, an enhanced surveillance study of laboratory-confirmed cases identified through a capture-recapture methodology (primary care notifications enhanced by microbiology reference laboratories notifications, existing microbiology laboratory surveillance, and public health agencies) conducted in the UK and the Republic of Ireland (UKROI) showed that the incidence of iGBS disease was 0.94 per 1000 live births (95% confidence interval (CI) 0.88–1.00). (32) This indicated an increase in the incidence of both EOGBS and LOGBS compared to a 2000–2001 surveillance study which used the same methodology (0.57 per 1000 live births in

2014–15 vs 0.48 per 1000 live births in 2000–01; 0.37 per 1000 live births vs 0.24 per 1000 live births; respectively). (33) These findings were also confirmed by studies that used a different methodology. A laboratory-only surveillance study in England and Wales reported a rise in EOGBS (0.41 per 1000 live births in 2010 vs 0.28 per 1000 live births in 2000) and LOGBS (0.29 per 1000 live births in 2010 vs 0.11 per 1000 live births in 1991). (34) A study in England that analysed hospitalisation records between 1998 and 2017 showed a relatively stable incidence of 1.28 per 1000 live births (95% CI 1.26–1.30) for the study period. (35)

Notably, this study relied on clinical information-derived disease coding; it may have included probable cases that did not have positive blood or CSF cultures, so the overall incidence was higher. On the other hand, the fact that all data were collected longitudinally over a continuous period removes the possibility of a bias associated with changes in clinical practices after the implementation of risk-based intrapartum antibiotic prophylaxis recommendations and, more recently, improved laboratory surveillance that could have partially accounted for the differences seen between 2000-2001 and 2014-2015 studies. A genomic data analysis for this period has not been performed; therefore, it is not known whether the invasiveness of the circulating GBS strains changed, as was noted in the Netherlands with the emergence of two clonal complex 17 sub-lineages. (36) Overall, besides their methodological differences, the UK studies suggest that the existing preventive strategies have not led to a decrease in the incidence of iGBS disease in the UK.

Moreover, GBS remains the leading pathogen causing neonatal deaths in the UK (7% of all infection-related deaths in 2013-2015), (37) although the case fatality rate was reduced by 70% between 2001 and 2017 (0.044 (95% CI 0.029–0.065) per 1000 live births vs 0.014 (95% CI 0.010–0.026)). (35) Furthermore, in the UK, half of all bacterial meningitis cases in infants less than 90 days old were caused by GBS, (38) and almost a third (31%) of infants diagnosed with iGBS sepsis had NDI. (39)

1.4 Pathogenesis and risk factors for Neonatal and Infant iGBS Disease

1.4.1 Comparison of EOGBS and LOGBS

EOGBS and LOGBS differ in their clinical presentation, morbidity, and serotype distribution (**Table 2**). EOGBS manifests as sepsis without a focus of infection in 80-85% of cases. (40) In contrast, LOGBS can present with a broader clinical spectrum, including focal infections such as pneumonia, osteoarticular infections, urinary tract infection, cellulitis, and submandibular or parotid adenitis. (41) A higher proportion of LOGBS cases are diagnosed with meningitis compared to EOGBS (31% vs 10% in the United States of America (USA); 41% vs 13% globally). (8,40) Infants with GBS meningitis are at the greatest risk for NDI (20.7% (95% UR 16.1–25.6)) compared to infants with GBS sepsis in HIC (3.3% (95% UR 1.0–7.6)) and infants with GBS sepsis in LMIC (9.2% (95% UR 2.4–22.8)). The differences in NDI after iGBS sepsis between HIC and LMIC could reflect real differences in co-morbidities or healthcare and education but could also be due to misclassification of meningitis as sepsis that is more common in resource-limited settings and for very sick neonates where lumbar puncture may not be done, or done after antibiotic administration. (29) The studies on NDI outcomes after GBS meningitis and sepsis in LM were small and done retrospectively, highlighting the need for better data. (8,42–44)

GBS is usually subdivided based on its capsular polysaccharide (CPS) into ten serotypes (Ia, Ib, II, III, IV, V, VI, VII, VIII, and IX). A recent systematic review and meta-analysis found that six serotypes (Ia, Ib, II, III, IV and V) account for 99% of infant iGBS disease. (27) Serotype III strains account for 52% of EOGBS and 77% of LOGBS cases, although it is only found in 28% of colonised women. (27) The predominance of serotype III is associated with the emergence and expansion of the highly virulent sequence type 17 strains (most are CPS serotype III). (36)

Table 2. Comparison of EOGBS and LOGBS

	EOGBS	LOGBS
Onset (Age)	<7 days	7-89 days
Median Age (interquartile range)	0 days (0–1 days) (40)	34 days (20–49 days) (40)
Transmission	Vertical	Vertical, Horizontal, Hospital
Risk factors	Well described	Not well defined
Clinical Syndromes	Sepsis (80-85%), pneumonia (10%), or meningitis (5-10%) (40)	Sepsis (65%), meningitis (25-30%), focal infection (pneumonia, septic arthritis, osteomyelitis, urinary tract infection, cellulitis-adenitis) (40)
Serotype Distribution	52% STIII (27)	77% STIII (27)
Mortality	7% (40)	5% (40)
NDI	Lower risk	Higher risk
Impact of IAP	Incidence reduced	No impact
EOGBS: Early-onset Group B streptococcal disease; LOGBS: Late-onset Group B streptococcal disease; ST: Serotype; NDI: Neurodevelopment impairment; IAP: Intrapartum antibiotic prophylaxis		

1.4.2 Pathogenesis and Risk Factors for EOGBS

EOGBS is caused by vertical transmission of GBS to the newborn from the maternal gastrointestinal and genitourinary flora. As shown in studies from the 1970s, heavy colonisation increases the risk of vertical transmission. (45,46) The most common pathway is ascending colonisation of the amniotic cavity that leads to skin or mucous colonisation of the foetus or aspiration of infected amniotic fluid. (47) For term infants, transmission primarily occurs during labour, after the rupture of membranes, and during passage through the birth canal. (48) Transmission might occur antenatally in preterm infants for whom intraamniotic infection may cause premature rupture of membranes and preterm labour. (49) GBS invades the respiratory epithelium and the lung parenchyma of the neonate and gains entry to

the bloodstream, causing bacteraemia and a cytokine storm characteristic of sepsis. (50) (**Figure 1**).

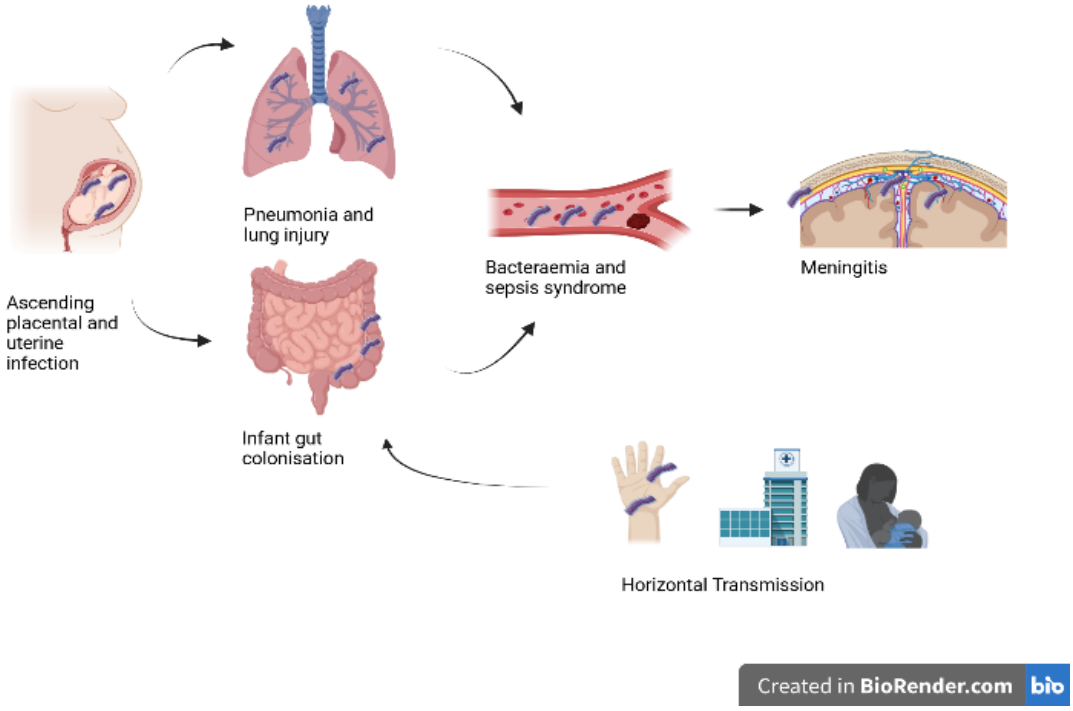


Figure 1. iGBS disease transmission routes

Ascending colonisation of the amniotic cavity leads to colonisation of the foetus or aspiration of infected amniotic fluid. From there, GBS can invade the respiratory epithelium and gains entry to the bloodstream, causing bacteraemia, sepsis and meningitis. Alternatively, intrapartum vertical or horizontal transmission can result in persistent GBS adhesion to newborn mucosal surfaces, followed by an invasion of the bloodstream many days after birth.

Specific clinical characteristics associated with disease pathogenesis are well-recognised risk factors of EOGBS.(51–55) Maternal antenatal colonisation is recognised as the strongest risk factor. It has been hypothesised that GBS primarily colonises the gastrointestinal tract and that the vagina is likely secondarily colonised, given its proximity to the rectum. This was suggested for the first time in a study of 789 pregnant women published in 1977 that found rectal cultures were positive more often than vaginal cultures (17.9% vs 10.2%). (56) Similarly, subsequent studies showed that colonisation of rectal samples is higher than that of vaginal samples,

whereas culturing both the lower vagina and rectum enhanced the yield of GBS compared to rectal or vaginal sampling alone. (57–60) This specimen collection technique has been suggested by all iterations of the IAP guidelines published in the USA since 1996. (61)

High carriage density, irrespective of how it is defined and measured, is consistently associated with a higher risk of GBS vertical transmission and EOGBS. (62) For example, GBS isolated in clean-catch urine specimens is considered a surrogate for heavy maternal colonisation and is associated with a higher risk for EOGBS. (55,63) Also, pregnant women colonised in two or more sites (throat, nose, vagina, cervix, rectum, midstream urine) compared with only one site were two and a half times more likely to have a neonate colonised with GBS in one study (Risk Ratio 2.53; 95% CI: 1.93–3.31). (64) However, it is unclear whether intrapartum vaginal colonisation is associated with a higher risk of iGBS disease compared to intrapartum rectal colonisation. A direct comparison between 140 women colonised with GBS on vaginal swabs in labour and 141 women colonised with GBS on rectal swabs in labour showed no difference in rates of total neonatal colonisation within 24 hours of birth and/or on discharge from hospital (38/140 vs 39/141), although the heaviness of vaginal, but not of rectal GBS carriage, was associated with a higher transmission rate. (65) From a clinical standpoint, an approach that seeks to optimise recognition of maternal carriage so that not to miss any preventable cases of EOGBS is sensible. However, there is a need for better-quality studies to further investigate bacterial load in conjunction with the site of colonisation to guide better-targeted prevention and therapy.

Prolonged rupture of membranes (PROM) contributes to GBS ascension into the uterus. Preterm delivery is associated with less effective neonatal immune responses and lower levels of transplacental antibodies. (66) The birth of a previous infant with iGBS disease may be related to suboptimal maternal antibody responses or other strain-specific virulence factors. Maternal chorioamnionitis, with intrapartum fever used as a clinical indication in most settings, reflects an active intraamniotic infection.

1.4.3 Pathogenesis and Risk Factors for LOGBS

In contrast, the mechanisms underlying GBS transmission and pathogenesis in LOGBS have not been fully elucidated (**Table 2**). (67) It has been suggested that intrapartum vertical transmission from colonised mothers can result in persistent GBS adhesion to newborn mucosal surfaces and invasion of the bloodstream many days after birth. (68,69) (**Figure 1**) However, it remains unclear why GBS colonises 10% of infants in the initial weeks of life but only causes LOGBS in less than 0.1%. It has been hypothesised that mucosal immunity and the infant microbiome might play a role in inhibiting the progression to iGBS disease. (70) In addition, postpartum horizontal transmission from the mother, other colonised household contacts or caregivers can occur. (71) Furthermore, the nosocomial transmission of GBS within neonatal intensive care units (NICU) is probably more frequent than previously recognised. (72) Case reports have described the presence of GBS in the breast milk of women whose infants developed LOGBS. Still, it remains controversial whether this was the actual transmission route or merely reflected the infant's oral colonisation status. (73) Compared to EOGBS, risk factors for LOGBS are less well understood. Some of the identified risk factors are similar to those of EOGBS, such as preterm delivery (74) and maternal GBS colonisation, (75) but have not been systematically reviewed.

1.5 Newborn and Young Infant Immune Responses against GBS

1.5.1 Overview of Immunity of the Newborn

The healthy newborn's immune system is skewed towards suppressing inflammation and auto-immunity, as it has evolved to promote the transition from the intrauterine environment to extrauterine life. (76) At the same time, its cellular and molecular components undergo rapid changes during the first weeks of life. (77) In general, over-permeable mucosal barriers, reduced pro-inflammatory/T helper 1 (Th1) cell responses and a limited ability to form antibodies, especially against polysaccharide antigens, lead to a higher risk of infection during the neonatal period. (76) Intestine and choroid plexuses are more permissive in neonates than in adults through disruption of cell-cell junctions, further exacerbated by the immaturity of the

neonatal intestinal microbiota. (78) In addition, defective Th1 responses and reduced T helper 17 (Th17) cells mean reduced production of interferon-gamma (IFN-γ) and, therefore, less efficient GBS clearance. (79,80) Furthermore, while immunoglobulin M (IgM) production begins in utero, endogenous immunoglobulin G (IgG) production, which requires B cell class-switching, is low in the first three months of life. (81) Plasma-cell differentiation in early life is further limited by a number of intrinsic and extrinsic factors. (82) However, the vast majority of infants remain disease free, mainly due to intact innate immunity and maternally-derived, placentally-transferred IgG. This is particularly true against GBS infections. Phagocytosis and intracellular killing after opsonisation are the key steps to GBS clearance. Efficient opsonisation depends on the synergy of serotype-specific antibodies and the complement to enable phagocytes to kill GBS. (83)

1.5.2 Maternally-Derived, Placentally-Transferred IgG

The concentrations of antibodies transferred across the placenta differ between infants and mainly depend on maternal antibody concentrations. (84) IgG transfer starts early in pregnancy (10% of maternal IgG is transferred between 17 and 22 weeks) (85) and reaches higher values towards the end of gestation as the neonatal Fc receptor (FcRn) expression on the syncytiotrophoblast increases. (86,87) Previous studies found that IgG transfer rates vary significantly by IgG subclass, (88) antigen specificity, (89) and antibody glycosylation. (90)

1.5.3 Neonatal Complement

Since complement is crucial for the opsonisation of GBS, the low levels of serum complement components in neonates contribute to their increased susceptibility to iGBS disease. (91) These differences are even more substantial in premature newborns. (92) It is also likely that complement contributes to the suboptimal humoral immune responses in newborns, as shown by the limited IgG and pro-inflammatory responses in studies with C3-deficient mice. (93,94)

1.5.4 Phagocytes

Both quantitative and qualitative differences exist in neonatal neutrophils compared to older age. Newborns have reduced neutrophil storage pools, and their neutrophils

are less responsive to bacterial challenge and chemotaxis. (95) Instead, neonatal monocytes, which mature into macrophages, play a crucial role in protection against disease. Tissue macrophages positioned at the interface between mucous membranes and the vascular system are the first line of defence. (95) The immaturity of the alveolar, lamina propria and microglia macrophages has been hypothesised to play a critical role in uncontrolled GBS infection. (70,96,97) (**Figure 2**)

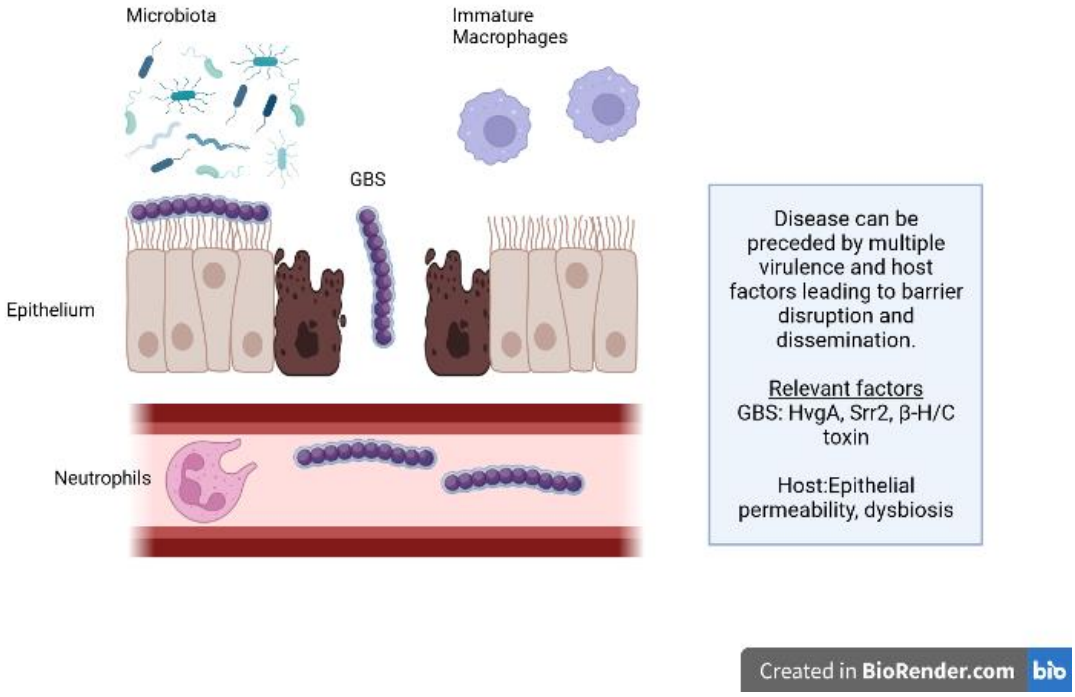


Figure 2. GBS intestinal colonisation and bloodstream invasion

GBS colonises the healthy infants' gut. Immature macrophages and microbiota aberrations can lead to GBS invasion. GBS virulence factors such as HvgA, Srr2 and β-H/C toxin facilitate adhesion to epithelial cells and invasion of the bloodstream. Prematurity is associated with increased epithelial permeability. HvgA: hypervirulent GBS adhesin; Srr2: serine-rich repeat glycoprotein; β-H/C: β-hemolysin/cytolysin toxin

1.6 Virulence Factors

In addition to these newborn host susceptibilities, the pathogenesis of iGBS disease relies on the pathogen's ability to evade clearance from the immune system and

invade vulnerable host niches. CPS is one of the major virulence factors by partially resisting complement-mediated opsonophagocytic killing by human blood leukocytes, in the absence of serotype-specific antibody. (98) The highly virulent sequence type 17 strains express a surface-anchored protein called hypervirulent GBS adhesin (HvgA) and a serine-rich repeat (Srr) glycoprotein Srr2, that confer greater binding affinity to the intestinal epithelium, fibrinogen, plasminogen and the blood-brain barrier cellular components. (99,100) Animal models and human clinical observations showed that β-hemolysin/cytolysin (β-H/C) toxin and hyaluronidase (HylB) promote GBS ascension into the uterus and penetration of chorioamniotic membranes. (101–103) Molecular mimicry is another GBS mechanism that facilitates immune invasion. The same terminal sialic acid in GBS CPS is also present on the host cells. (104) These sialic acids bind to sialic acid-binding immunoglobulin-type lectins (Siglecs) that suppress host immune activation. (105) GBS further counteracts host innate immunity through several proteases, such as C5a peptidase and CspA. (106) Notably, the most recent advances in the field suggest that GBS often utilises the same tightly regulated bacterial factors, such as adhesins and immune modulators both as a commensal and a pathogen. (106,107) Similar to other bacterial pathogens, GBS responds to environmental changes using signal transduction systems, such as the two-component systems (TCS). (108) Among the approximately twenty TCS in GBS, (109) the most studied is control of virulence regulator/sensor (CovR/S), which regulates the expression of over 100 virulence genes. (110) (**Figure 3**)

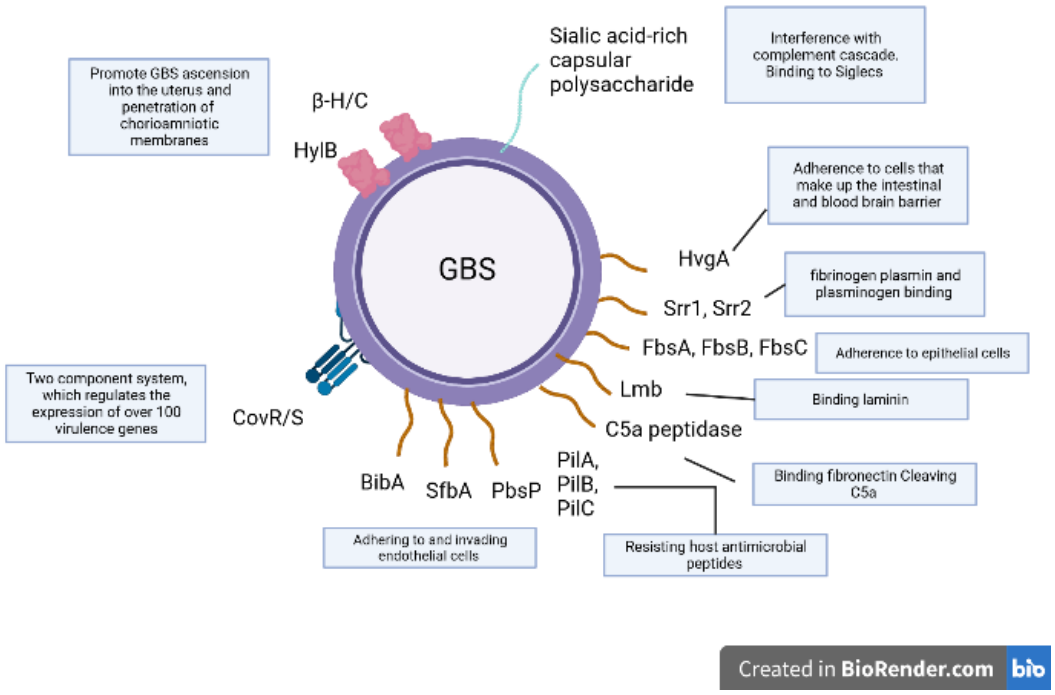


Figure 3. GBS Virulence Factors

HvgA: hypervirulent GBS adhesin; Srr: serine-rich repeat glycoprotein; Fbs: fibrinogen-binding protein; Lmb: ;Pil: Pilus; PbsP: ; SFbA: ; BibA: ; CovR/S: control of virulence regulator/sensor ; β-H/C: β-hemolysin/cytolysin toxin; HylB: hyaluronidase; Siglecs: sialic acid-binding immunoglobulin-type lectins.

1.7 Prevention of iGBS Disease in Neonates and Young Infants

1.7.1 Intrapartum Antibiotic Prophylaxis (IAP) for the Prevention of EOGBS

Most cases of EOGBS present in the first 24 hours after birth, and many newborns are already symptomatic when born, (111) meaning that disease prevention is paramount in reducing neonatal iGBS disease burden. Administration of IAP, usually in the form of an intravenous beta-lactam agent before delivery to colonised women or those with certain risk factors, has significantly reduced the risk of culture-positive EOGBS. (112) A Cochrane review and meta-analysis of three randomised clinical trials including 488 newborns (all assessed to be of high risk of bias) concluded that the incidence of EOGBS was reduced when using IAP for colonised women compared to no IAP (risk ratio 0.17; 95% CI 0.04–0.74). (113) An active surveillance study between 2006 and 2015 from the USA showed a sustained fall in the incidence of EOGBS. (40) It has been proposed that IAP prevents neonatal iGBS disease by temporarily

suppressing the level of maternal colonisation, interrupting the process of neonatal colonisation, and exceeding the minimum inhibitory concentration for killing GBS in the blood of a newborn. (114–116)

GBS remains susceptible to b-lactam antibiotics, and benzylpenicillin (penicillin G) and ampicillin are the antibiotics best studied for prevention of neonatal infection. Benzylpenicillin administered to the mother readily crosses the placenta, reaching levels in cord blood over the minimum inhibitory concentration (MIC) for GBS by one hour and rapidly declining by four hours, reflecting elimination of the antibiotic by the foetal kidney into amniotic fluid. (114) Ampicillin has been detected in cord blood within 30 minutes and in amniotic fluid within 45 minutes of administration to the mother. (117) To optimise the efficacy of IAP, the first dose is recommended as soon as possible after the onset of labour and then four hourly until delivery. Two retrospective observational studies have provided evidence that at least four-hour duration of IAP is associated with optimal effectiveness at reducing the risk of EOGBS compared to shorter duration. (118,119) However, more recent data suggest that effective neonatal concentrations might be independent of the duration of antibiotics prior to delivery. (120–122) Ampicillin concentrations measured in 115 newborn infants at four hours of age were found to be greater than the GBS MIC if maternal IAP dosing occurred at least 15 minutes before delivery. (120) Ampicillin IAP decreases maternal vaginal colonisation and prevents neonatal surface colonisation in 97% of cases if IAP is administered at least two hours before delivery. (117,123) Benzylpenicillin has a narrower antimicrobial spectrum compared to ampicillin and therefore remains the preferred agent, but ampicillin is acceptable. Cefazolin that is recommended for women with a penicillin allergy but at low risk for anaphylaxis has similar pharmacokinetics and mechanisms of action as ampicillin. (124,125)

In 2003, the Royal College of Obstetricians and Gynaecologists (RCOG) published guidelines for a risk-based approach to administering IAP to prevent EOGBS in the UK. An update in 2017 extended the recommendation of IAP to women in preterm labour and women who carried GBS in a previous pregnancy. (126)

1.7.2 IAP and LOGBS

Despite the use of IAP, the incidence of LOGBS has not been reduced (40,113) and is even rising in some countries. (32,127–130) This lack of protective effect could be attributed to the different mechanisms underlying GBS transmission and pathogenesis in LOGBS. Even though IAP might inhibit vertical transmission at birth, this seems to have a temporary effect, as expected by the antibiotics' half-life. Transmission could still occur many days or weeks later. (131) Additionally, IAP does not prevent horizontal transmission of GBS.

1.7.3 Limitations of IAP

IAP against iGBS disease in neonates and young infants has its limitations. First, as discussed above, it has no impact on the incidence of LOGBS. Second, implementation of IAP strategies is not always financially or practically feasible in LMIC, where most births happen in the community or pregnant women only arrive in hospitals at a very late stage; risk factors like prematurity and fever are under-ascertained, and microbiology facilities are lacking. (132) Third, even in HIC, suboptimal compliance with guidelines limits the protective impact of IAP. (133) Fourth, it has been proposed that IAP might induce GBS resistance to beta-lactams or increase the prevalence of other neonatal pathogens, such as *Escherichia coli*. In the era of widespread use of IAP, a 2020 study from the USA showe that *E. coli* infections have increased among very low birth weight (VLBW) indfants and that *E. coli* resistance against ampicillin and gentamicin is on the rise. (134) This warrants continued surveillance as previous studies showed conflicting results on the association of IAP and the odds of early- or late-onset sepsis caused by ampicillin-resistant *E. coli*. (135–137) Fifth, previous studies showed alterations in the intestinal microbiota of neonates and young infants whose mothers received intrapartum antibiotics, (138,139) although it remains unknown whether these changes have any short- or long-term impact on their health. (140) Finally, IAP has no impact on preventing GBS-related stillbirths, preterm births and invasive infections among pregnant and postpartum women. (17)

1.8 GBS vaccine development

1.8.1 Biological Rationale for a Vaccine

Vaccination against GBS could potentially tackle many of these issues. Given that iGBS disease primarily affects infants under three months of age, maternal immunisation is the optimal strategy rather than direct vaccination of newborns. (17,132,141–143) A GBS vaccine offered to pregnant women could significantly reduce the burden of iGBS disease and protect the lives and health of infants around the world. (144) Baker and colleagues first recognised the protective role of placentally transferred anti-GBS CPS IgG in the late 1970s. (145) They showed that women antenatally colonised with GBS serotype III, whose infants subsequently developed iGBS disease, had significantly lower serotype III-specific anti-GBS CPS IgG compared to colonised women whose infants did not develop disease. This finding was later replicated for serotypes Ia and Ib.(146–148) Animal models of passive and active immunisation further corroborated the protective role of anti-GBS CPS IgG. (149,150) A correlation between anti-GBS CPS IgG concentrations and complement-dependent opsonophagocytic bacterial killing by neutrophils was shown in animals and humans, providing evidence of their functional activity. (151,152) Together, these findings established the rationale for using antenatal vaccination to induce anti-GBS CPS IgG to protect infants against iGBS disease. (141)

More recently, some highly conserved surface proteins have been identified as immunogenic antigens and, therefore, potential vaccine targets, including Srr-1, (153) C5a peptidase, (154) pilus islands, (155) and surface immunogenic protein (Sip). (156) The most well-studied surface proteins for the development of vaccines are the alpha-like protein (Alp) family containing Alp1, Alp2, Alp3, Alp4, AlphaC (aC) and Rib (present in serotype III strains). (157) Case-control studies from Sweden and SA have shown that placentally transferred specific antibodies against aC and Rib are associated with a significantly reduced neonatal iGBS disease risk. (158,159)

1.8.2 History and Current Status of GBS Vaccine Development

Currently, there are no licenced GBS vaccines. The first vaccine clinical trials in humans in the late 1970s and 1980s used monovalent polysaccharide unconjugated

vaccines. These first-generation vaccines were tolerated well and, despite their sub-optimal immunogenicity, provided evidence that a maternal vaccination strategy was feasible. (160–162) Second-generation monovalent GBS vaccines were conjugated with tetanus toxoid, which augmented the antibody responses to polysaccharide antigens by inducing a T-cell-dependent response to the protein carrier. (163) They were shown to induce serotype-specific anti-GBS CPS IgG and delay GBS colonisation. (164–169) Subsequently, a trivalent (against serotypes Ia, Ib, and III) CRM_{197} (a non-toxic mutant of diphtheria toxin) conjugate vaccine showed promising safety and immunogenicity results in Phase 1 and 2 clinical trials in non-pregnant and pregnant women with or without Human Immunodeficiency Virus (HIV). (170–174) More recently, a hexavalent polysaccharide CRM_{197} conjugate vaccine against serotypes Ia, Ib, II, III, IV and V, called GBS6 (manufactured by Pfizer), has shown good tolerability and immunogenicity in healthy non-pregnant adults and has progressed to trials in pregnant women. (175,176) At the same time, a protein subunit vaccine consisting of the N-terminal domains of aC, Rib Alp1 and Alp2/3, called GBS-NN/NN2 (manufactured by MinervaX), displayed good safety and elicited IgG responses that mediated opsonophagocytosis of GBS in a Phase 1 study in non-pregnant women. (177,178) This vaccine candidate has also progressed to Phase 2 trials in pregnant women and, most recently, older adults.

Placental antibody transfer rates with CPS vaccines are often lower than that for protein vaccines. (179) These differences might be partly related to the differences in IgG subclass proportions, as protein antigens generally induce IgG1 subclass, whereas polysaccharide antigens induce mainly IgG2 antibodies. (89,180) IgG1 is more efficiently transferred across the placenta than the other three IgG subclasses, with IgG2 displaying the lowest transfer rate. (181)

Investigational CPS-protein conjugate vaccines against GBS, have showed placental transfer ratios of less than one. A trivalent vaccine candidate reported a placental transfer rate of 0.49 to 0.79, whereas the most recent hexavalent vaccine candidate reported ratios of 0.55 to 0.74 against the different serotypes tested. (170,182) In contrast, naturally acquired and vaccine-induced Alp-specific IgG is dominated by the IgG1 subclass with very little contribution of IgG2. (178) Although data have not been

published yet from the Phase 2 trial of GBS-NN/NN2 in pregnant women, the placental antibody transfer rate for naturally acquired total IgG was 1.22 and 1.12 for aC and Rib respectively. (178) Therefore, it is critical to measure antibody levels in cord or infant sera, as fluctuations in placental transfer can impact the concentrations of antibodies utilised for serocorrelate prediction.

1.8.3 GBS Vaccine Development Considerations

Given the low incidence of iGBS disease, any Phase 3 double blind randomised controlled vaccine efficacy trial would need to recruit a very large number of participants (40,000-60,000 pregnant women, assuming a vaccine efficacy of 75% and incidence of 1 per 1000 live births). (183) It was estimated that such a study could last eight years and require a considerable investment that would ultimately impact the vaccine price. (184)

Alternatively, a vaccine could be provisionally licensed based on a substitute immunological endpoint with post-licensure studies needed to demonstrate the impact on reducing disease burden. (132,185–187) In the past, this is how the four-component, protein-based meningococcal serogroup B (4CMenB) vaccine was licenced and implemented in the UK infant immunisation schedule. (188) Such a strategy could accelerate licensure at a more affordable vaccine price, given the smaller size and the significantly lower cost of an immunogenicity study with a follow-up effectiveness study compared to a conventional efficacy trial. (184)

A third option is a hybrid study, including both clinical and immunological endpoints. (189) This approach would also enable earlier licensure based on the immunogenicity assessment, whereas continuing the study to determine the impact on disease burden would provide the clinical data needed for traditional approval. However, such a study will still require a significant investment to enrol and follow up the study population.

The potential of polysaccharide-protein conjugate vaccines to induce mucosal immunity to protect against colonisation has been demonstrated for vaccines against *Haemophilus influenzae* type b, *Streptococcus pneumoniae* and *Neisseria meningitidis* serogroup C. (190–193) A Phase 2 study of an early serotype III

conjugate vaccine candidate tested in non-pregnant women indeed showed reduced rates of acquisition of type-specific GBS colonisation. (168) The possible inclusion of maternal or newborn colonisation as a secondary endpoint in Phase 3 trials is worth considering. Antenatal colonisation is a pre-requisite for EOGBS and to a lesser extent LOGBS, with the risk of mother-to-infant transmission directly associated with the carriage density. (62) However, the perception of colonisation as a possible licensure endpoint is not widely accepted among the GBS research community as it is plausible that a vaccine may prevent iGBS disease yet not impact colonisation.

First, circulating antibodies may prevent invasive disease while not affecting colonisation. The effect of naturally acquired anti-GBS CPS IgG on GBS acquisition and clearance remains an open question. A longitudinal study of pregnant women tested for carriage at six weekly intervals, between 20–25 weeks of pregnancy and delivery, found that higher serotype specific IgG concentrations, including functional antibody measured by Opsonophagocytosis Killing Assay (OPkA) were associated with protection from a new acquisition of serotypes Ia, III and V throughout pregnancy, but not a clearance of existing colonisation. (194) In contrast, a longitudinal carriage study in non-pregnant women over three months showed that serum and mucosal (vaginal) anti-GBS CPS IgG was not associated with changes in colonisation or protection from a new acquisition. (195) Second, whereas the role of maternal GBS carriage as a pre-requisite for EOGBS is well established, horizontal transmission of GBS in hospital or community can also cause LOGBS. Between a fifth and a quarter of infants with LOGBS became colonised after their discharge from the hospital even if they had received appropriate IAP. (21,69,131,196) In addition, nosocomial transmission in NICU is becoming increasingly recognised as a separate route of acquisition. (72,197,198) Also, oropharyngeal GBS colonisation might contribute to the horizontal transmission of GBS in the community. A single postnatal ward study found that an almost equal proportions of mothers, family members and friends who visit the neonate and health care providers were oropharyngeal GBS carriers (26% vs 22% vs 22%). (199) Therefore, reduction of maternal colonisation may not translate to complete LOGBS prevention. Third, infant colonisation does not always lead to iGBS disease, limiting its usefulness as an indirect measurement of

disease endpoint. In the absence of IAP, approximately 50% of newborn infants born to mothers positive for GBS become colonised with GBS, and of those, 1% to 2% will develop EOGBS. (200) Factors such as virulence and other host variables can determine whether colonisation progresses to invasive disease (107). Fourth, there are ethical and standard-of-care considerations related to the use of colonisation as a primary phase 3 endpoint. Knowledge of colonisation status before delivery may necessitate IAP, which might indirectly affect the assessment of the vaccine efficacy against iGBS disease and directly against maternal and/or infant colonisation.

If primary licensure is based on immunological endpoints, the most critical requirement will be to obtain post-licensure evidence for protection against iGBS disease. This could also yield a wide range of potential information about other endpoints of interest like maternal and infant colonisation, GBS-related and all-cause stillbirths and preterm delivery. Ascertaining stillbirth aetiology can be challenging, but methods have been developed and implemented for attributing stillbirths to GBS such as finding GBS from culture or Polymerase Chain Reaction (PCR) in placental tissue or following minimally invasive autopsy. (201) As the mechanisms by which GBS may cause preterm birth are not well documented outside of animal models, the association of GBS with preterm birth would likely be best identified following the introduction of the vaccine, for example, in a vaccine probe study. (202) It will be important that such “real world evidence” is collected using the highest standards of observational studies and in adherence with STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidance. The least costly and most time-efficient studies could use various data sources, including existing obstetric and pregnancy surveillance systems, laboratory reports, vaccine registers, primary care databases, hospital and mortality data, enhanced surveillance via questionnaires to primary care and hospital doctors and case note reviews.

1.8.4 Evidence for Immune Correlates of Protection against iGBS Disease

The terms *correlate* and *surrogate* of protection are used widely but inconsistently or even antithetically in the literature by authors and regulatory agencies. (203–205) The definitions used in this thesis follow the ones proposed by WHO. (205) The term correlate of protection/risk reduction (CoP) is used when referring to a specific

immune response statistically associated with a low risk of disease/infection, even if it is not the actual mechanism of protection. When proven that it directly confers protection, the term surrogate of protection (or mechanistic correlate of protection) is used instead (**Table 1**). (206) The validation of immune markers as CoP depends on meeting certain criteria, such as the Prentice criteria or the Qin framework. (205) WHO published a review of the evidence needed for a GBS CoP (**Table 3**). (186) In this context, naturally acquired placentally transferred anti-GBS CPS IgG is thought to be associated with the protection of infants against iGBS disease; (207,208) and is "reasonably likely to predict clinical benefit" of a vaccine against GBS. (209,210)

Multiple case-control studies over the last 30 years have tried to define antibody thresholds that can be used as a CoP. However, the results from these studies are not fully comparable due to methodological differences in the source of serum (maternal vs infant), the assay used (format and reference serum), and the statistical methodology used. (179,189)

Table 3. Validation of a CoP for approval of a GBS vaccine (Prentice criteria)

Prentice criteria (211)	Evidence base (186)
Protection against the clinical endpoint is significantly related to having received the vaccine (**vaccination affects outcome**)	Post-licensure human studies **Current Status**: Initial discussions with key stakeholders underway.
The substitute endpoint (immune marker) is significantly related to vaccination status (**vaccination affects correlate**)	Vaccine immunogenicity studies (Phase 2) **Current Status**: Hexavalent polysaccharide conjugate vaccine in Phase 2 trials in pregnant women. Four-component protein subunit vaccine in Phase 2 trials in pregnant women and older adults.
The substitute endpoint is significantly related to protections against the clinical endpoint (**correlate affects outcome**)	Seroepidemiological studies **Current Status**: One completed seroepidemiological study in SA. Two seroepidemiological studies in progress in the UK and USA.
The full effect of the vaccine on the frequency of the clinical endpoint is explained by the substitute endpoint, as it lies on the sole causal pathway (**correlate is the full mediator of protection**)	Mechanistic studies to demonstrate that the measured antibody is functional (e.g. through OPkA). **Current Status**: Part of the ongoing seroepidemiological studies.
CoP: Correlate of protection; GBS: Group B *Streptococcus*	

1.8.5 Considerations for the Timing of Maternal Vaccination

Vaccination timing in pregnancy should maximise antibody transfer to the fetus and offspring for protection, including for those born preterm. Vaccination during the first trimester of pregnancy should ideally be avoided, as the first months of pregnancy are associated with an increased risk of spontaneous abortion/miscarriage, which would obscure the vaccination safety assessment. A vaccine that can be delivered over a range of gestational ages, from the second trimester but prior to 36 weeks, minimises the risk of obscuring safety assessment whilst maximising protection to the infant, including those who are born preterm, and maximises opportunities for vaccination in settings with a reduced number of antenatal contact points and reduced opportunities for precise determination of gestational age. The timing of vaccination during pregnancy will be determined in early clinical studies and is anticipated to match Phase 3 trial schedules. The ongoing Phase 2 trials of the two most advanced candidates (discussed in section 1.8.2) GBS6 (NCT03765073) and GBS-NN/NN2 (NCT05154578) include single dose arms at 24 and 22 weeks gestational age, respectively. (212,213) The expectation is that Phase 3 trials will include participants from 20 weeks gestational age. So far, published data from these two candidates showed strong immune responses two weeks after one vaccine dose, associated with high infant-to-maternal transfer ratios. (182,214) Taken together, these preliminary data suggest that a single dose given in the second trimester has the potential to protect a significant proportion of preterm babies, although this will also depend on the time of birth relevant to the time of vaccination.

1.9 Conclusion

In summary, GBS remains among the most common causes of neonatal and young infant sepsis and meningitis in most countries. In the UK, the burden of iGBS disease has increased over the last twenty years. IAP policies have effectively reduced, but not eliminated, EOGBS in many HIC. However, transmission routes and risk factors of LOGBS are not fully understood, and prevention strategies have not yet been identified. Immunisation of pregnant women with a GBS vaccine is a promising

strategy to prevent both EOGBS and LOGBS. Furthermore, vaccine licensure will be significantly accelerated if a CoP is established. The work presented in this thesis aims to characterise the clinical and epidemiological risks of LOGBS and contribute to defining a protective threshold of naturally acquired serotype-specific anti-GBS CPS IgG from a large seroepidemiological study in a UK population.

Chapter 2 – Aims and Research Questions

2.1 Aims

1. To define the clinical and epidemiological risk factors for LOGBS.

2. To contribute to defining a protective threshold of serotype-specific anti-GBS CPS IgG, derived from natural exposure, that may be used as a CoR in the development pathway of GBS vaccines.

2.2 Research Questions

1. Unravelling the risk of LOGBS

a) What are the clinical and epidemiological risk factors for LOGBS?

b) What is the epidemiology of iGBS disease in twins and relapses?

2. Defining a threshold of antibody-mediated immunity resulting from natural exposure

a) What are the serotype-specific anti-GBS CPS IgG concentrations among infants with iGBS disease, healthy infants born to women colonised with GBS and healthy infants born to women not colonised with GBS in a UK population?
b) What are the kinetics of the anti-GBS CPS IgG between birth and three months of age in healthy infants born to women colonised with GBS?
c) Is it possible to reliably calculate both absolute and relative risk reduction at different anti-GBS CPS IgG concentration cut-off values using statistical modelling?

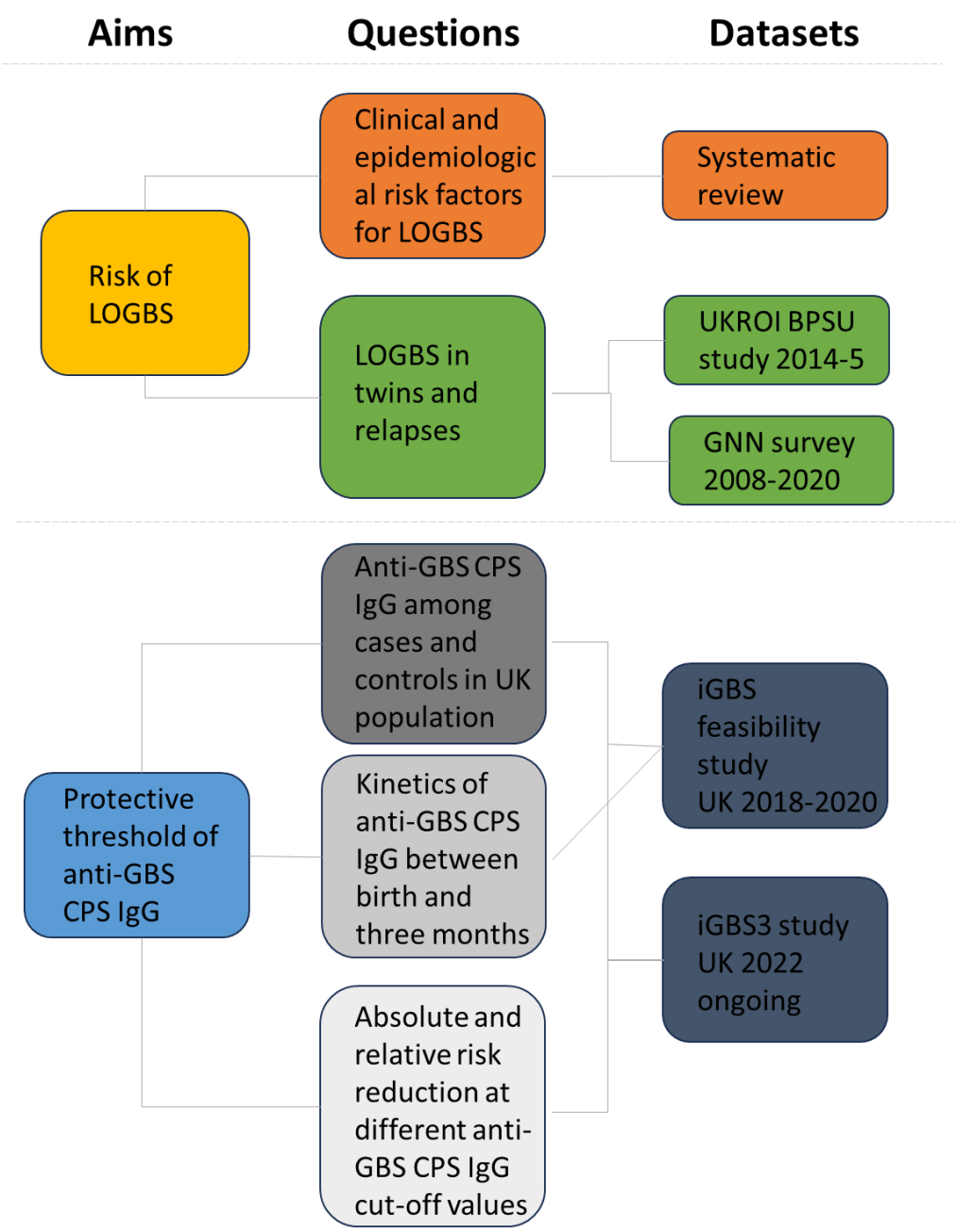


Figure 4. Thesis Datasets Diagram

The diagram shows how all the different datasets link together to underpin the thesis questions and aims.

LOGBS: Late-onset GBS disease; UKROI: United Kingdon and Republic of Ireland; GNN: German Neonatal Network; GBS: Group B streptococcus; CPS: Capsular polysaccharide; IgG: Immunoglobulin G; UK: United Kingdom

Chapter 3 – Methods and Materials

This chapter describes how I addressed the project's specific aims, including study design and the laboratory assays used. The statistical methods used are described separately in each chapter.

3.1 UK Seroepidemiological Studies

The overall aim of the UK iGBS studies (comprising iGBS feasibility and iGBS3 studies) is to provide evidence that the inverse relationship between an immune marker value (anti-GBS CPS IgG) and the probability of iGBS disease is sufficiently strong. Therefore, a vaccine able to induce such an immune response will cause a meaningful decrease in the likelihood of iGBS disease.

Dr Clara Carreras-Abad was the coordinator of the iGBS feasibility study from 1st July to 31st December 2018, after which I took over. I was involved in the study coordination, training of local teams, ethics and regulatory approvals, data curation, receipt and processing of samples, consent taking and infant blood collection for the kinetics sub-study from 1st October 2019 to 30th November 2020. The results from the first phase of the feasibility study have been published by Dr Carreras Abad. (215) The results presented in this thesis were derived from the full iGBS feasibility study dataset and were not published before.

I was the clinical research fellow responsible for coordinating the iGBS3 study from the planning stage until 3rd March 2023. I was responsible for writing and updating the protocol, the lab manual, the standard operating procedures, and the training resources. In addition, my duties included training the local teams, preparing ethics and regulatory submissions, curating the data, and addressing problems raised by the participating sites. Prof Paul Heath has been the Chief Investigator of the iGBS feasibility and iGBS3 studies. Prof Le Doare provided an overview of the laboratory aspect. Dr Abdelmajid Djennad and Prof Nick Andrews contributed to the statistical analysis plan.

3.1.1 iGBS Feasibility Study

3.1.1.1 Study Design

3.1.1.1.1 Main Study

The iGBS feasibility study was a prospective cohort study of pregnant women and their infants. The primary objective was to test the feasibility of collecting and processing rectovaginal swabs, cord blood samples in pregnant women, and blood samples from infants with iGBS disease. (215) In the first phase, pregnant women aged 18 or above were recruited from five maternity units in London and South England (Croydon, East Surrey, Kingston, Poole and St George's hospitals) for six months from 1st July to 31st December 2018. The study was continued for another 13 months, from 1st October 2019 to 30th November 2020, in three maternity units (Northwick Park, Kingston and St George's hospitals). At all participating sites, women consented to have a rectovaginal swab or separate rectal and vaginal swabs taken after 35 weeks of gestation and up to (including) delivery. In addition, cord blood samples were collected at birth at Northwick Park, Kingston and St George's hospitals (**Figure 5**).

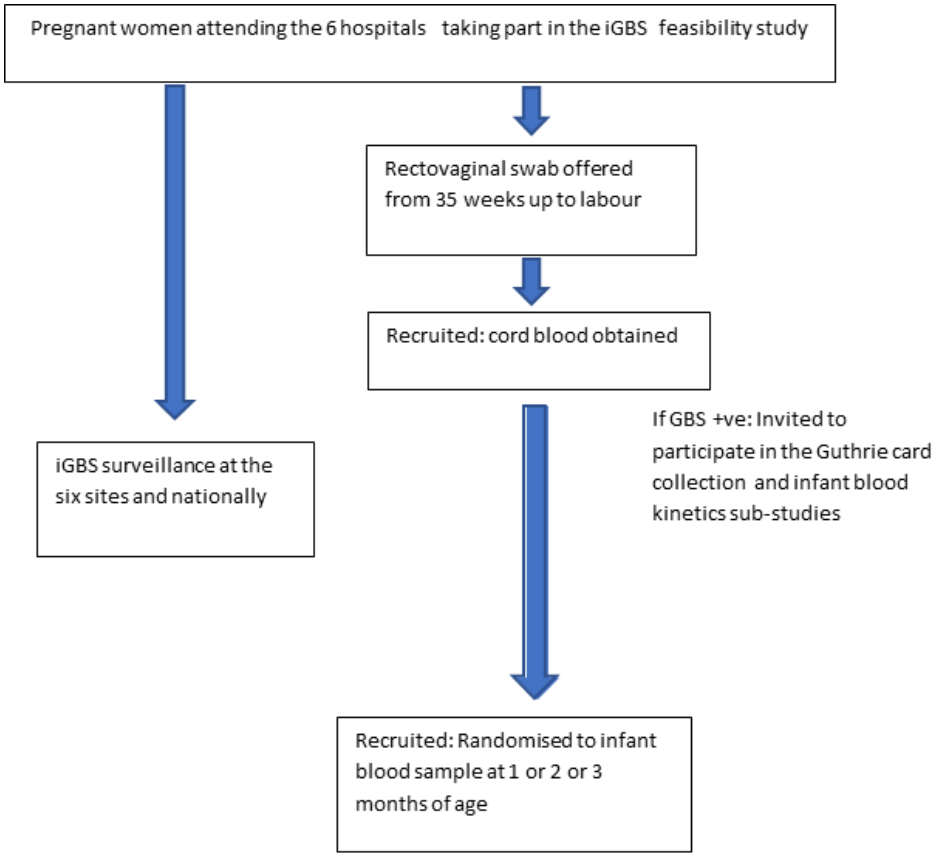


Figure 5. The iGBS feasibility study pathway

3.1.1.1.2 Kinetics Sub-Study

Women antenatally colonised with GBS and whose children did not develop iGBS disease were invited to participate in the kinetics sub-study at Kingston and St George's hospitals. For this sub-study, infants were randomised to have a blood sample collected at birth (cord blood and either one (Group A: day 21-35), two (Group B: day 49-63) or three (Group C: day 77-91) months of age. Simple randomisation was performed using the freely available software RRApp (Icahn School of Medicine at Mount Sinai, US). (216) Group allocations were placed inside sequentially numbered opaque envelopes by a research team member who was not involved in recruitment and blood collection. On recruitment to the sub-study, each participant was allocated, in order of inclusion, the next available envelope after written informed consent was obtained by the parent/legal guardian of the participating infants.

3.1.1.1.3 iGBS Surveillance Subset

National iGBS surveillance to recruit infants less than 90 days old with iGBS disease was also conducted in parallel in selected National Health Service (NHS) Trusts in England. All hospitals in England were invited to participate through established research and clinical networks (The British Paediatric Allergy, Immunity and Infection Group (BPAIIG), Neonatal Infection Surveillance Network (neonIN), and UK Paediatric Vaccine Group (UKPVG)). When a case of iGBS disease occurred in infants under 90 days old, a blood sample was obtained from the mother and her infant and sent to St George's, University of London (SGUL) laboratories at room temperature within 72 hours after collection. The GBS pathogenic strain was retrieved from the local microbiology laboratory and sent to the SGUL laboratories for serotyping (**Figure 6**).

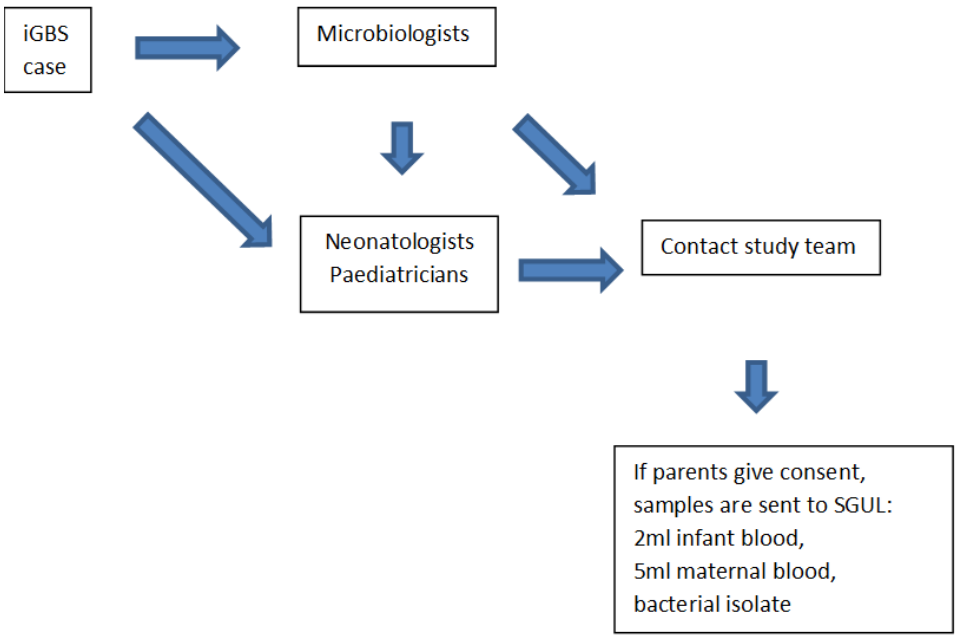


Figure 6. Parallel national surveillance in selected NHS trusts from England and Wales

3.1.1.2 Eligibility Criteria

For the main study, all pregnant women ≥18 years delivering at one of the selected hospitals during the study period were eligible for recruitment, with no other exclusion criteria.

For the kinetics sub-study, the inclusion criteria were a mother with a positive GBS swab taken after 35 weeks gestation and a cord blood sample collected at birth, whose infant did not develop iGBS disease in the first 90 days of life.

For the iGBS surveillance sub-set, only infants less than 90 days old with microbiologically confirmed iGBS disease were eligible for inclusion.

3.1.1.3 Consent

Informed consent from the participant or the parents/guardians/person with legal responsibility for children was obtained following an explanation of the study's aims, methods, benefits and potential hazards and before any study-specific procedures were performed. One of the research midwives/clinical team members gave women information about the study. There was no minimum period between receiving information and providing consent to ensure that participants had sufficient opportunity to consider the information and ask questions. Written consent was taken for participation in the study at enrolment and then reaffirmed verbally before or after delivery. When there was insufficient time to obtain written informed consent from participants presenting in labour, verbal consent was obtained to collect cord blood at delivery, followed by written consent retrospectively and within 24 hours of birth. Participants could have withdrawn at any time during the study period, without having to give a reason. A copy of the signed Informed Consent Form (ICF) along with a copy of the most recent approved Patient Information Sheet (PIS) was given to the study participant. An original signed and dated ICF was retained in the Investigator Site File (ISF) and a copy was placed in the medical notes.

3.1.1.4 Demographic and Clinical Information

Following informed consent, maternal demographics and medical history data were collected and managed using RedCap (Vanderbilt University, USA) electronic data capture tools hosted at SGUL. (217,218) Data were collected on ethnicity (grouped as White British, White Other, Black or Black British, Asian or Asian British, Mixed, Other ethnic group), maternal age, gestation at birth (calculated by ultrasound measurement in the first trimester or measured from the first day of last menstrual period), receipt of IAP (yes/no), what antibiotic, time between first administration of

IAP and delivery (in hours), recent blood transfusion (in last 30 days), parity, gravida, colonisation with GBS in previous pregnancies, history of a previous child with iGBS disease, duration of rupture of membranes, mode of delivery, maternal fever (≥38°C), infant sex, infant birth weight.

3.1.1.5 Sample Collection

Vaginal and rectal swabs: A single rectovaginal swab or two individual swabs (TRANSWAB® Gel Amies with charcoal, Medical Wire & Equipment, UK) from participants' lower vagina and rectum were obtained at any time between 35 weeks gestation and delivery, either by the study midwife or by the participant herself (with appropriate written guidance). Due to the dynamic nature of antenatal GBS colonisation, screening after 35 weeks was chosen because it showed a better positive predictive value of a positive GBS status at birth than screening in earlier pregnancy stages. (219) Similarly, previous studies showed that culturing both the lower vagina and rectum, enhanced the recovery of GBS compared to vaginal sampling alone. (57) After collection, the swab was inserted in Amies Transport Medium (TRANSWAB® Gel Amies with charcoal, Medical Wire & Equipment, UK) and transported to the SGUL research laboratory at room temperature for processing according to the methods described below (section 3.2.2). The result (positive/negative) was then entered into the antenatal notes and the participant was informed by phone (if GBS positive) and by letter (if positive or negative). The obstetric/midwifery team subsequently offered women with a positive swab result IAP according to the relevant RCOG guidelines on GBS. (220)

Cord blood samples: Cord blood was obtained once the placenta had been delivered and when the obstetric/midwifery team deemed it was safe to do so. Research samples were always collected after clinical samples. Blood (0.5-5 ml) was collected into a serum separator tube (BD Vacutainer® SST™, Becton Dickinson, UK). Blood samples were transported at room temperature to the SGUL laboratories within seven days after collection to be spun and the serum was frozen at -80°C for later processing.

Infant blood samples: For the kinetics sub-study, maximum 1 ml infant blood was collected by the study co-ordinator using venepuncture or capillary sampling (BD Microtainer® SST™, Becton Dickinson, UK) and then spun and frozen at -80°C in the SGUL laboratories.

3.1.1.6 Ethics

The West Midlands-Solihull Research Ethics Committee approved the study on 15th June 2018 (reference number 18/WM/0147).

3.1.1.7 Funding

The study was funded by the National Institute for Health Research (NIHR) Health Technology Assessment programme (grant reference number 17/LO/0890).

3.1.2 iGBS3

3.1.2.1 Study Design

The iGBS3 study (ClinicalTrials.gov Identifier: NCT04735419) is an ongoing prospective case-control study designed to compare serotype-specific anti-CPS IgG concentrations in the cord blood of infants who develop iGBS disease and infants of GBS-colonised mothers who do not. The iGBS3 study was designed as an unmatched case-control study. The sample size calculation was based on 100 GBS serotype III cases and 300 controls exposed to the same serotype (1:3 ratio). Prospective cord blood collection from all pregnant women at each participating site was used to capture all infants developing iGBS disease in the first 90 days of life. It was estimated that cord blood would be required from approximately 170,000 women to capture approximately 170 cases of iGBS disease (based on the national incidence of 0.94 per 1000 live births and including the predicted reduction in EOGBS due to increased IAP use), of which 100 will be GBS serotype III. Cord blood collection was embedded in a large cluster-randomised trial called GBS3 (funded by NIHR 17/86/06; trial sponsor University of Nottingham). (221) The GBS3 trial, which started recruiting on 5th November 2021, was designed to assess three different strategies for the prevention of EOGBS disease (enriched culture medium testing of a rectovaginal swab

performed at 35-37 weeks vs PCR testing of a rectovaginal swab taken in labour vs risk factor-based strategy).

3.1.2.1.1 Cases

The iGBS3 study started recruitment on 1st March 2022. It was designed to take place in two phases (**Figure 7**). In Phase 1, prospective cord blood and infant blood at the time of iGBS disease (acute blood) were collected to establish the correlation between anti-GBS CPS IgG in acute and cord blood. Phase 1 is expected to finish recruitment in March 2024. If it demonstrates that the correlation is strong (correlation coefficient ≥0.9), then in Phase 2, only acute blood from cases will be collected (Phase 2a). But if Phase 1 shows a weak correlation, then in Phase 2, the prospective collection of cord blood from all deliveries will continue (Phase 2b).

3.1.2.1.2 Controls

In addition, the study aimed to recruit 300 GBS serotype III controls to achieve a 3:1 ratio of controls to cases (100 cases in total from Phases 1 and 2) to define the CoP with sufficient precision. Based on a GBS colonisation rate of approximately 20% and a serotype III proportion of 30%, it was estimated that 1000 GBS colonised women would be recruited through the GBS3 sites randomised to the routine Enriched Culture Medium (ECM) test, of whom 300 were expected to be colonised with serotype III (**Figure 6)**.

3.1.2.1.3 iGBS Surveillance Subset

For hospitals in England, Scotland and Wales not participating in the GBS3 trial, infants that developed iGBS were recruited and blood samples were collected at the time of disease without cord blood collection. Cases recruited in the iGBS surveillance counted towards the total number of cases.

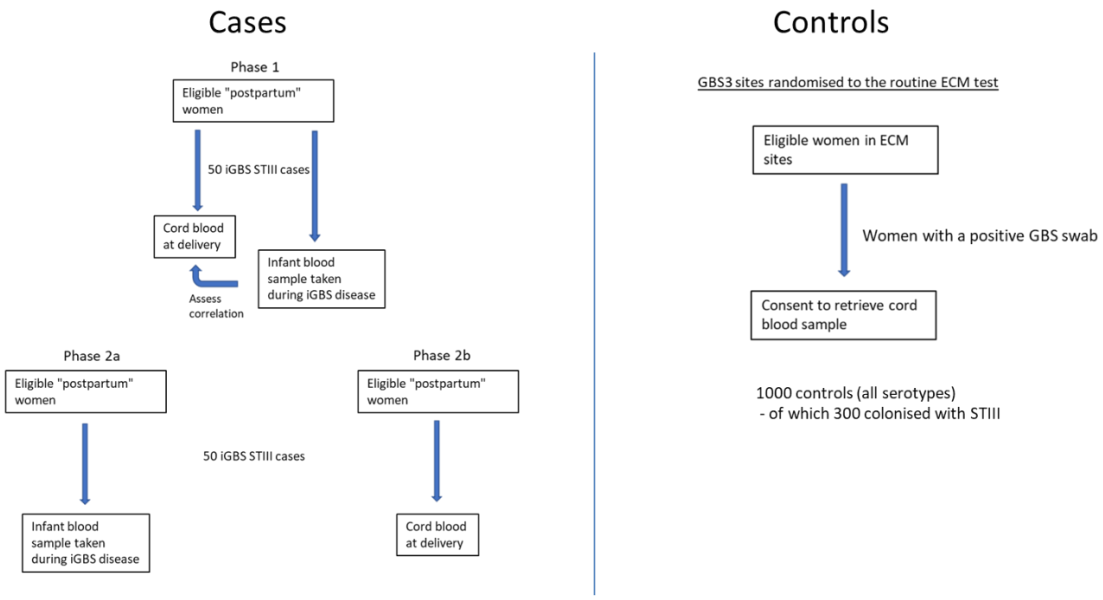


Figure 7. The iGBS3 study design

3.1.2.2 Eligibility criteria

The inclusion criteria for cases were infants who developed iGBS disease (**Table 1**). The inclusion criteria for controls were infants exposed to GBS at birth but did not develop iGBS disease. An infant was not eligible for recruitment to the study as a case or control unless the infant's mother ≥16 years of age gave informed consent.

3.1.2.3 Consent

3.1.2.3.1 Cord Blood

A verbal consent strategy was used for cord blood collection for all pregnant women giving birth in the sites participating in the iGBS3 study. Attending midwives or other health care professionals obtained verbal consent during or after labour using an ethically approved script. Any woman who did not want to give cord blood could decline at any time and samples were not taken or destroyed if already collected.

3.1.2.3.2 Cases/Controls

If a case of iGBS disease occurred, the delegated member of the Primary Investigator's team (nurses, midwives, or doctors who received adequate training before performing any study protocol-related activities as evidenced in the training and delegation logs) sought consent from the infant's mother to recruit the infant to

the study. For women who were identified as being colonised with GBS, a member of the iGBS3 team sought consent to recruit the mother as a control.

3.1.2.4 Demographic and Clinical Information

Following receipt of informed consent, demographic and clinical data were collected and managed using RedCap (Vanderbilt University, USA) electronic data capture tools hosted at SGUL (217,218) were used for the collection of key infant's demographic details (gestation at birth (calculated as described in section 3.1.1.4), sex, birth weight, ethnicity (grouped as defined in section 3.1.1.4)), details of the medical history related to the episode of iGBS disease (age at the onset of symptoms and upon hospital admission, clinical syndrome (septicaemia, meningitis, pneumonia, focal infection) and clinical outcome (full recovery, sequelae, death)), maternal demographics (age at delivery, ethnicity (grouped as defined in section 3.1.1.4)), and details of her pregnancy history (gravida, parity, immunosuppressive medication during pregnancy (systemic glucocorticoids, calcineurin inhibitors, nucleotide synthesis inhibitors, alkylating agents, mammalian target of rapamycin inhibitors, monoclonal antibodies, proteasome inhibitors, tyrosine kinase inhibitors, hydroxychloroquine, methotrexate), GBS colonisation (positive ECM or PCR from 35 weeks gestation up to delivery), GBS bacteriuria, a previous infant with iGBS disease, blood transfusions during pregnancy, single or multiple births, mode of delivery, duration of membrane rupture before birth, Intrapartum fever (≥38°C), confirmed or suspected chorioamnionitis, administration of IAP and interval between IAP and delivery).

3.1.2.5 Sample Collection

3.1.2.5.1 Cord Blood

Cord blood samples (0.5-5 ml) were collected in a serum separator tube (BD Vacutainer® SST™, Becton Dickinson, UK). After collection, the sample was allowed to stay at room temperature for up to seven days before spinning. Spinning took ten minutes at 1300 relative centrifugal force at room temperature. After spinning, only serum (max 1.5 ml) was stored locally in a freezer (-20 or -80°C) for three to six months. If a case of iGBS disease occurred within the first three months of life, the

local research team approached the infant's mother and asked for written consent to retrieve the already collected cord blood and send it to SGUL to measure antibodies against GBS. Unused samples were discarded at the end of the storage period (**Figure 8**).

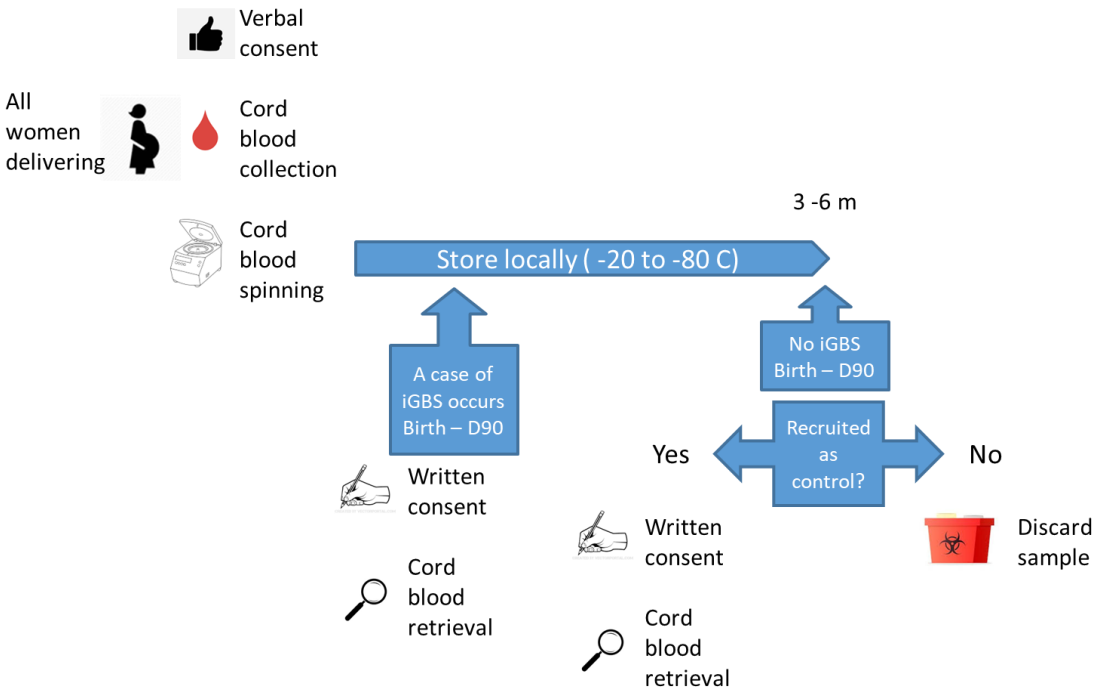


Figure 8. Cord blood pathway for the iGBS3 study

3.1.2.5.2 Cases

The infant blood sample was collected as close as possible to the onset of the acute episode and up to ten days after isolation of GBS. After collection, infant blood was spun and frozen as discussed above for the cord blood (section 3.1.2.5.1). Consent was also sought to track and retrieve the cord blood serum sample using maternal NHS number and the GBS isolate using the infant NHS number (section 3.1.2.3.2). The microbiology services retrieved and prepared GBS isolates at participating sites as a single colony on the surface of a chocolate agar slope. All clinical samples (infant blood sample, cord blood, isolate) were labelled with the unique study number generated for each participant before sending them to SGUL (**Figure 9**).

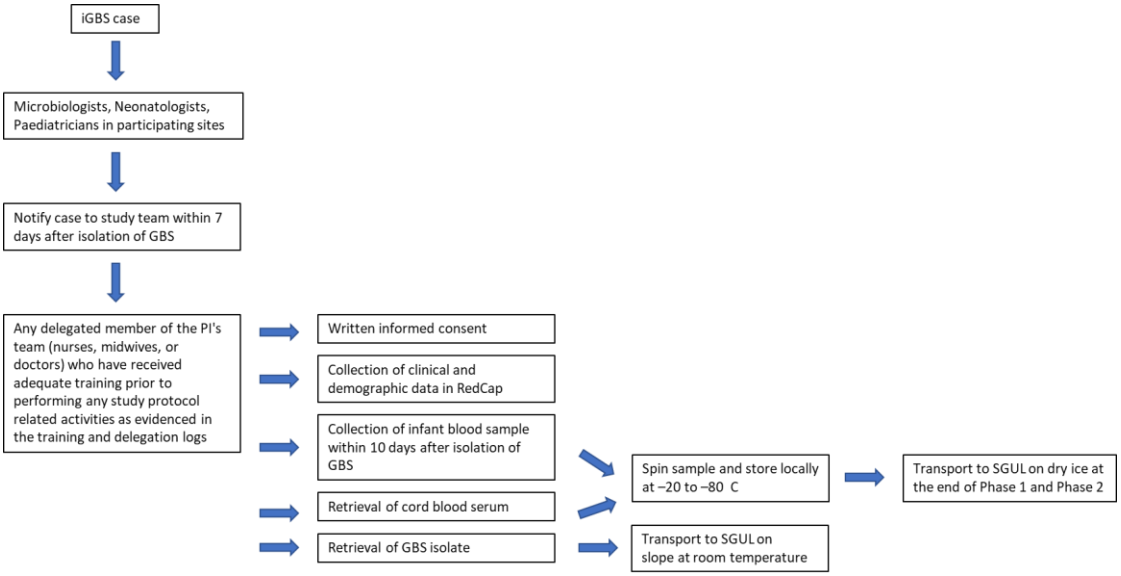


Figure 9. Pathway and study procedures for iGBS3 cases

3.1.2.5.3 Controls

Consent was sought to track and retrieve the cord blood serum and the GBS isolate from the maternal antenatal swab using the maternal NHS number (section 3.1.2.5.1). The microbiology services retrieved and prepared GBS isolates at participating sites as a single colony on the surface of a chocolate agar slope. All clinical samples (cord blood, isolate from the swab) were labelled with the unique study number generated for each participant before sending them to SGUL (**Figure 10**).

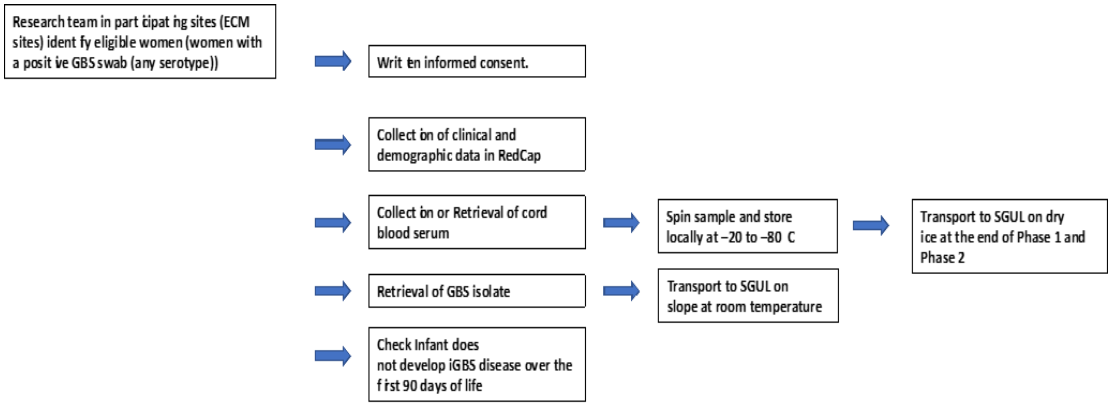


Figure 10. Pathway and study procedures for iGBS3 controls

3.1.2.6 Transport of samples

Cord and infant sera were shipped to SGUL on dry ice. Boxes were labelled clearly with a UN1845 label, dry ice quantity, and a UN3373 label. Bacterial isolates were sent to SGUL at room temperature using a zip-lock bag with absorbent material capable of withstanding an internal pressure of 95kPa, enclosed in a rigid cardboard box with a UN3373 label.

3.1.2.7 Ethics

The East Midlands-Derby Research Ethics Committee approved the study on 4th January 2021 (reference number 20/EM/0260).

3.1.2.8 Funding

This study was funded by the Medical Research Council (grant reference MR/T030925/1) and MinervaX (no external reference).

I prepared the media, was involved in the microbiological identification of GBS from the swabs and ran the Multiplex Immunoassay (MIA) in the blood samples as described below. Matrix Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS) and serotyping PCR were performed by Mr Robert Watts and Mr Simon Beach at the SGUL laboratory for the iGBS feasibility and iGBS3 studies, respectively.

3.2 Microbiological and Molecular Quantification of GBS Colonisation

3.2.1 Media Preparation

3.2.1.1 LIM Broth (Todd Hewitt Broth with Nalidixic Acid and Colistin)

It has been shown that selective enrichment culture media, inhibiting the growth of competing organisms such as Gram-negative enteric bacilli, significantly increases the yield of GBS culture to detect female genital colonisation. (222,223) LIM broth is the most widely recommended selective enrichment culture media. (224,225) Todd Hewitt Broth (Oxoid, UK) provides the growth medium, while Nalidixic Acid (Sigma-Aldrich, USA) and Colistin (Sigma-Aldrich, USA) select for gram-positive bacteria growth by preventing overgrowth of Enterobacteriaceae. To make the LIM broth, 36.4 g/L of Todd Hewitt Broth was dissolved in distilled water. The suspension was autoclaved at 121°C for 15 minutes. The antibiotics were added to the cooled broth to avoid denaturing at a concentration of 0.1% of 10 mg/ml Colistin stock and 0.1% of 15 mg/ml Nalidixic Acid stock. Then LIM broth was aliquoted into 7 ml Greiner tubes (Sigma-Aldrich, USA) for use within three months. Quality control and performance were undertaken weekly using standard culture.

3.2.1.2 Chromogenic Agar

Equivalent or even superior detection of GBS is achieved using selective and chromogenic agar compared to blood agar. (226,227) A commercially available pigment-production-independent chromogenic agar (CAG), CHROMagar StrepB (CHROMagar™ StrepB, France), was used. It allows for more effortless reading thanks to a mauve GBS colony colouration, compared to steel blue colour for *Enterococcus* species. It was prepared according to manufacturer's instructions. (228) Briefly, 44.7 g of powder base was dispersed in 1 L of purified water. Then, 8 mL of supplement S1

was added, and the mix was stirred until the agar was well thickened and autoclaved at 121°C for 15 min. Then, 250 mg of supplement S2 was diluted in 10 mL of purified water, filtered to sterilise and added to the cooled Base + S1 mix at 45/50°C. After gentle stirring to homogenise the mix (Base + S1 + S2), it was poured into sterile Petri dishes to solidify and dry and stored for up to 1 month under refrigeration (2-8°C). Quality control and performance were undertaken weekly using standard culture.

3.2.2 Isolation of GBS from Swabs

The methods used were adapted from the UK Health Security Agency (UKHSA) protocol for pre-incubating swabs in ECM before plating on solid media to enhance the yield of GBS (**Figure 11**). (224)

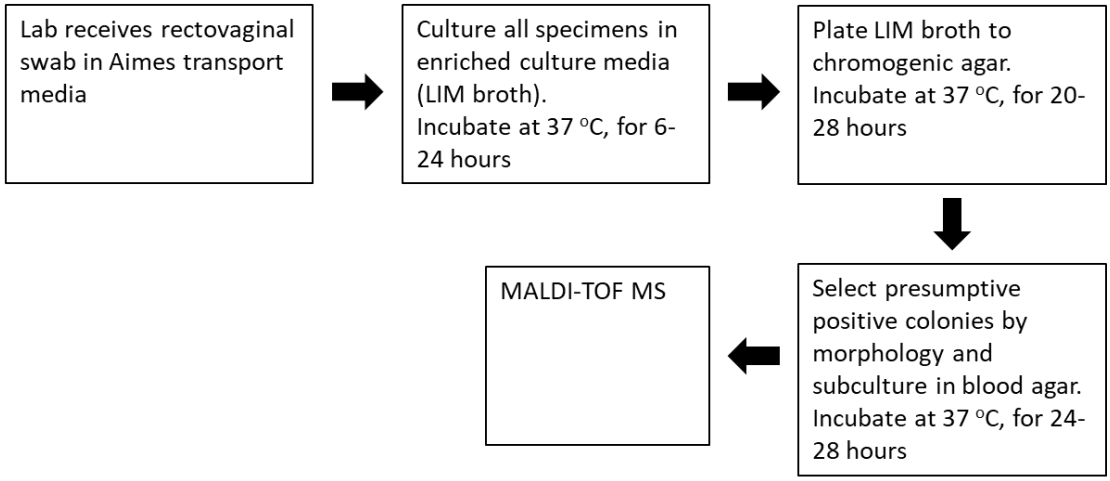


Figure 11. Laboratory workflow for GBS testing in rectovaginal swabs from pregnant women

After collection, the swab was inserted into an Amies transport medium (TRANSWAB® Gel Amies with charcoal, Medical Wire & Equipment, UK), a modification of Stuart's transport medium, that contains an inorganic phosphate buffer and charcoal, which preserves the organism by absorbing inhibitory substances and maintaining the pH. (229) Then, the swab was sent to the SGUL laboratory, where it was stored in a cold room for up to 24 hours. The swab was then brought to room temperature and vortexed thoroughly to resuspend the bacteria, then directly inoculated into a Greiner tube containing 2ml of LIM broth and incubated at 37°C with 5% carbon dioxide (CO_2) for 6-24 hours. Then, 10 µl of the

test sample was inoculated onto the CAG plate and incubated at 37°C with 5% CO2 for 20-28 hours. A mauve-coloured colony was considered a presumptive positive result for GBS. In addition, a known GBS inoculum, a blank LIM bottle, and an E. coli inoculum were also incubated and plated as positive and negative controls weekly.

A maximum of four likely-positive colonies from each CAG plate were picked with a 1 µl inoculation loop and streaked onto a Columbia Agar with Horse Blood (BA) plate (Thermo Scientific, USA), divided into halves/quadrants, and incubated at 37°C with 5% CO2 for 24-48 hours. In a BA plate, GBS colonies look grey, surrounded by a weak zone of haemolysis. When the BA plate's growth was impure, likely-positive colonies were re-streaked.

Chromogenic media are not fully specific for GBS identification and it is recommended that presumptive colonies of GBS are confirmed by a specific antigenic detection test or MALDI-TOF MS. (224) MALDI-TOF MS compares the sample to known mass spectra to verify the identity of the organism and has shown higher sensitivity than antigenic tests. (230) The direct plating method was used in this study, which is equally sensitive and specific as the cell lysis method, but faster and less complex. (231) First, the samples were coated in a-Cyano-4-hydroxycinnamic acid matrix (Sigma-Aldrich, USA). Next, 250 µl of Bruker standard solvent (Sigma-Aldrich, USA) were added to the vial of matrix and vortexed thoroughly. The portioned matrix was kept in an opaque container to protect it from light and was used up to ten days after preparation. Next, each pure GBS colony was evenly spread across a well/spot of the MALDI-TOF MS plate.

3.2.3 GBS Serotyping by PCR

Once GBS colonies were positively identified, confirmed by MALDI-TOF MS and grown pure on BA, the serotype was determined using PCR, which is superior to the latex agglutination method. (232) Genotyping was performed by using a multiplex PCR as previously published. (233) Genomic deoxyribonucleic acid (DNA) was extracted from GBS isolates using the Qiagen DNeasy kit (Qiagen, Germany); GBS was lysed in a lysis buffer containing 20 µl mutanolysin (3000 U/ml) (Sigma-Aldrich, USA), 20 µl lysozyme (100 mg/ml) (Cusabio, USA), and 4 µl RNaseA (100 mg/ml) before

incubation at 37°C for two hours (234) and DNA was quantified using a spectrophotometer (NanoDrop™ Thermo Scientific, USA). PCR was conducted using the primers previously reported by Imperi and colleagues. (233) In brief, PCR reactions were heated at 95°C for 15 minutes, then 40 cycles of denaturing at 95°C for one minute, annealing at 56°C for one minute, extension at 72°C for two minutes and a final cycle of extension at 72°C for ten minutes on a thermocycler (Thermo Scientific, USA). Purified DNA from known UKHSA GBS reference strains was used as a positive control, and sterile water was used as a negative control. The amplified products were visualised by horizontal electrophoresis on 1.5% agarose gel against a purple 100 bp DNA ladder (New England Biolabs, USA), immersed in Tris base, acetic acid and EDTA buffer (Thermo Scientific, USA). The banding pattern illuminated on the agarose gel was compared to the UKHSA GBS reference strains' band pattern to identify the serotype.

3.2.4 Multiplex Immunoassay

Anti-GBS CPS IgG concentrations were measured using the GASTON-adopted MIA in cord and infant sera. (176) The GASTON MIA utilises Luminex technology (Luminex, USA) to simultaneously measure anti-GBS CPS antibodies specific to six GBS serotypes: Ia, Ib, II, III, IV and V.

On the first day, beads coupled to GBS serotype Ia, Ib, II, III, IV, or V polysaccharide poly-L-lysine conjugates (Pfizer Inc, USA) were incubated with appropriately diluted standard reference serum, quality control serum and test serum samples at 4°C for 16-24 hours. Each plate included three quality controls serum samples, serial-diluted standard reference serum, blank wells containing assay buffer and up to eleven test samples. Multivalent vaccinee reference serum (Pfizer Inc, USA) was serially diluted 2.5-fold at each step, with a total of 11 dilutions. Each test sample was analysed in duplicate, at three dilutions: 1:500, 1:5000 and 1:50000.

The next day, the plates were first washed with phosphate-buffered saline (PBS)/Tween (1xPBS/0.05%Tween/0.02%/Sodium Azide, pH 7.2). Next, 50 µL of R-phycoerythrin goat anti-human IgG Fcγ specific secondary antibody (Jackson Laboratories, 109-115-098, Jackson ImmunoResearch, UK) was added to each well

(dilution 1:500), the plates were covered with black light protective lids and incubated for 75-105 minutes at room temperature, while shaking at a speed of 300 rotations per minute. Then, the plates were rewashed. After the final wash, the plate washer (Tecan Hydrospeed, Tecan, UK) automatically dispensed 100 µL of washing buffer to each well. Each assay plate was then read on a Bio-plex 200 (Bio-Rad Laboratories, USA). The signal output expressed as median fluorescent intensities (MFI) was converted to IgG titres (µg/mL) using the standard reference curve and taking into consideration the dilution factor (**Figure 12**).

Assay plates containing sampling errors were re-run. Samples were repeated at the same dilution if the difference between the replicates was too large (replicate ratio >1.4). Samples were repeated 10-fold more diluted if the titre was above the assay range or the MFI was above 20,000. Samples with MFI below the lowest usable point of standard reference curve were repeated if they were above the blank limit of 300 MFI. If they returned the same result, they were assigned a value below the lower limit of quantitation (LLOQ).

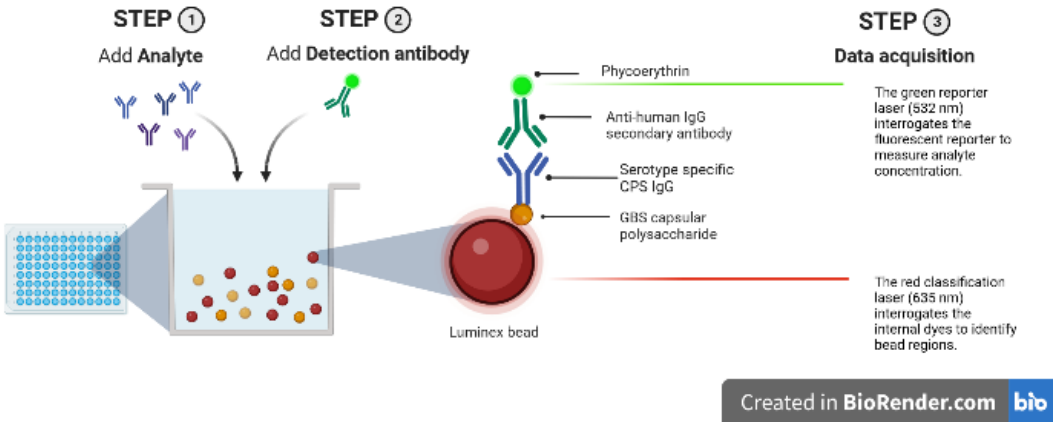


Figure 12. General overview of the multiplex immunoassay detection scheme

Chapter 4 - Clinical Risk Factors Associated with Late-Onset Invasive Group B Streptococcal Disease: Systematic Review and Meta-analyses

The material presented in this chapter has been previously published in a peer-reviewed journal. (235)

4.1 Introduction

Compared to EOGBS, risk factors for LOGBS are less well understood and have not been systematically reviewed. (67) Addressing this gap could help identify vaccine-targetable risk factors and recognise the most vulnerable infants to inform GBS vaccine research priorities and policy decisions.

This chapter provides a comprehensive and systematic literature review and meta-analyses to assess the association between LOGBS and previously reported clinical and epidemiological risk factors.

4.2 Methods

4.2.1 Search strategy

The review protocol was registered with the PROSPERO database (Registration number: CRD42021253749). Medline, Embase and Cochrane Library databases were searched for studies published until 31st December 2022 with no language restrictions (**Table 4**). Additional studies were identified by searching the reference lists of included studies and reviews.

Table 4. Full search strategy for LOGBS risk factors systematic review

Date of search performed: 1st January 2023
Databases searched: Medline, Embase and Cochrane Library databases using the OvidSP interface.
Search terms: "Streptococcus agalactiae" [Mesh] OR "Streptococcus agalactiae" OR "group B adj3 strep" AND "Meningitis" [Mesh] OR "meningit*" OR "Infections" [Mesh] OR "infection*" OR "Pneumonia" [Mesh] OR "pneumonia*" OR "Sepsis" [Mesh] OR "septic?emia" OR "Bacteremia" [Mesh] OR "bacter?emia"
Restrictions: None
Footnotes a: The adj3 operator finds terms in any order with two words (or fewer) between them; b: The question mark (?) inside a word is used to replace one character.

4.2.2 Study Selection and Data Collection

Observational studies were included that reported risk factors for iGBS disease (case-control studies, retrospective and prospective cohort studies). The cohort studies were surveillance studies conducted to estimate the national or regional incidence of iGBS disease. Case reports, case series and reviews were excluded. All previously reported clinical risk factors for LOGBS and EOGBS were included.(200) Studies that reported LOGBS cases were included and studies with a non-representative sample (e.g. studies containing only very high-risk groups like preterm infants) or a non-appropriate comparison group (no denominator data for risk factors) were excluded. Only cases with GBS isolated from a normally sterile site (blood, CSF, joint fluid, peritoneal fluid) were included. The most comprehensive report was included if more than one study was published on the same patients.

As per widely accepted systematic review recommendations, (236) two review authors, myself and Dr Hannah Davies, independently scanned the abstract, title, or both, of every record retrieved to determine which studies should be assessed further. All potentially relevant articles were investigated as full text and any discrepancies were resolved through consensus. For studies that fulfilled eligibility criteria, two review authors, myself and Dr Hannah Davies, independently abstracted key data on maternal colonisation in pregnancy (defined as a positive vaginal, rectal, or rectovaginal swab by culture or PCR on at least one occasion from 35 weeks of gestation until birth), maternal colonisation at the time of LOGBS diagnosis, preterm birth (delivery at <37 weeks of gestation), low birth weight (LBW <2500 g), multiple-gestation pregnancy, maternal age <20 years, infant sex, HIV exposure, GBS detected in mother's breast milk at the time of LOGBS diagnosis, maternal intrapartum fever (temperature ≥38°C during labour), and PROM (≥18 hours before delivery). Published aggregate and not individual participant data were used. When the existing published data included cases isolated from non-sterile sites or with an age of onset ≥90 days, the original researchers were contacted to ask for a summary of cases that met the inclusion criteria. Data on the number of preterm births, LBW, multiple births, and sex ratio in the study population were collected either from the reports included in the articles or from the publicly available national statistics services and previously published systematic reviews that used these datasets.(237–240) For national surveillance studies, the number of live births for the whole population for that period was used as the denominator. For studies reporting cases from multi-site surveillance programmes, regional data were used as the denominator. When population data were not available for the entire duration of the study, a midpoint year was used. Due to a lack of population-wide studies on maternal GBS colonisation, the pooled estimates of GBS colonisation prevalence by country from a systematic review conducted in 2015 were used.(241)

4.2.3 Quality Assessment

Consistent with recommendations to minimise errors and bias in systematic reviews, (242) two review authors, myself and Dr Maren Mynarek, assessed the risk of bias for each included study independently by using a modified Newcastle-Ottawa scale (NOS). Any disagreements were resolved by consensus.

4.2.4 Statistical Analyses

I performed a meta-analysis to calculate weighted odds ratios (OR) with 95% CI across studies and pooled risk of LOGBS for the following parameters: (i) preterm birth, (ii) GBS colonisation in pregnancy, (iii) LBW, (iv) multiple-gestation pregnancy, (v) maternal age <20 years, (vi) intrapartum fever, (vii) PROM, and (viii) infant sex. Data on HIV exposure were collected, but a synthesis of these data has recently been published.(243) Data about the other clinical risk factors (maternal colonisation and isolation of GBS in breast milk at the time of LOGBS diagnosis) were disparate and could not be pooled. Data were summarised with a random-effects model, using the Mantel-Haenszel method and the DerSimonian-Laird approach to estimate the variance of the distribution of true effect sizes ($\tau 2$). Between-study heterogeneity was assessed by using the I2 statistic. Heterogeneity was further explored with subgroup analyses and meta-regression for variables where an association with a higher risk of LOGBS was found. Studies were compared based on the study design, WHO regions, HIC versus LMIC, presence versus absence of IAP policy, length of study, and year of publication. The subgroup pooled estimates were calculated with a mixed-effect model, without a common estimate of $\tau 2$ across subgroups.(244) Meta-regression was performed using a mixed-effect model with continuous and categorical moderators [16] . The R^2 index was used to quantify the percentage of variation explained by the model.(244) Sensitivity analyses were performed to explore the influence of excluding studies that used a different definition of LOGBS (7 to 179 days of age) or studies that only reported sepsis or meningitis cases. Publication bias was assessed by using funnel plots for analyses with more than nine included studies and tested for funnel plot asymmetry using Egger's test. Egger's test *P*-value <0.05 was considered to implicate publication bias.(242) Statistical analyses

were done with R studio (version 3.6.3) using the packages "meta", "metafor", and "dmetar".(244)

4.3 Results

4.3.1 Study Selection

From a total of 14,468 articles identified through the literature search, 104 full manuscripts were assessed and 27 articles that met the inclusion criteria were identified (**Figure 13**).(32,33,40,41,74,75,127,245–264)

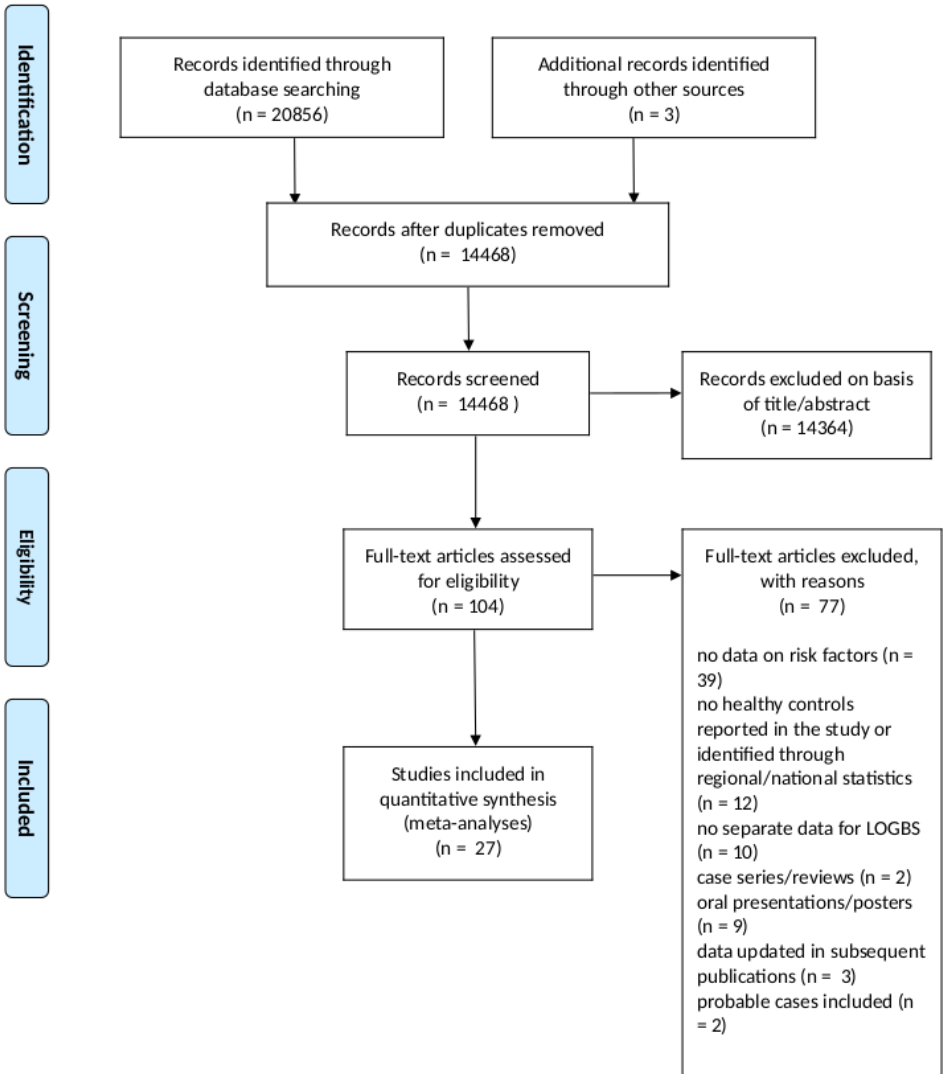


Figure 13. Data search and included studies for risk factor for LOGBS

4.3.2 Study Characteristics

Twenty-seven studies were included with a total of 5,315 cases of LOGBS among 30,487,773 live births. The median incidence of LOGBS was 0.21 cases per 1000 live births (range 0.06-1.18). Of the included studies, 13 (48%) were prospective cohort studies, nine (33%) retrospective cohort studies, and five (19%) case-control studies (**Table 5**). Thirteen (48%) articles were from Europe, six (22%) from the Western Pacific region, five (19%) from North America, and three (11%) from Sub-Saharan Africa. Quality was moderate to high (defined as NOS score >5) for all the included studies (**Table 6, 7**).

Table 5. Characteristics of included studies for risk factor for LOGBS

Reference	Cases	Controls	Preterm births rate population	<34 rate population	LBW rate population	Male ratio population	National GBS colonisation rate	Multiple-gestation pregnancies rate population	Maternal age <20 years rate population
Berardi et al. 2013 (41)	100	Regional Population	7.4% (41)	1.9% (41)	NA	NA	23.2% (241)	NA	NA
Dangor et al. 2015 (245)	46	Study	NA	NA	NA	NA	NA	NA	NA
Dangor et al. 2016 (246)	373	Regional Population	18.0% (265)	NA	18.0% (265)	50.8% (240)	NA	NA	NA
Fluegge et al. 2006 (247)	136	National Population	8.4% (237)	NA	6.5% (239)	NA	18.4% (241)	NA	NA
Frigati et al. 2015 (248)	19	Regional Population	NA	NA	41.3% [a]	50.8% (240)	NA	NA	NA
Giannoni et al. 2016 (249)	46	National Population	7.2% (237)	NA	NA	51.4% (240)	16.00% (266)	NA	NA
Guan et al. 2018 (250)	21	Regional Population	5.7% (267)	NA	5.0% (239)	54.0% (240)	NA	NA	NA
Heath et al. 2004 (33)	191	National Population	NA	NA	6.2% (33)	NA	NA	NA	NA
Ireland et al. 2014 (251)	14	Study	NA	NA	NA	NA	NA	NA	NA

Reference	Cases	Controls	Preterm births rate population	<34 rate population	LBW rate population	Male ratio population	National GBS colonisation rate	Multiple-gestation pregnancies rate population	Maternal age <20 years rate population
Jordan et al. 2008 (252)	468	Regional Population	12.4% (237)	NA	8.1% (268)	51.2% (240)	24.7% (241)	NA	NA
Joubrel et al. 2015 (253)	264	National Population	6.6% (237)	NA	7.4% (239)	51.1% (240)	15.6% (241)	NA	NA
Juncosa-Morros et al. 2014 (254)	143	Regional Population	7.5% (269)	NA	NA	NA	15.5% (241)	1.8% (238)	NA
Ko et al. 2015 (255)	62	National Population	7.6% (237)	NA	6.3% (239)	51.1% (240)	23.8% (241)	1.5% (238)	NA
Lin et al. 2003 (74)	122	Study	NA	NA	NA	NA	NA	NA	NA
Matsubara et al. 2013 (256)	162	National Population	5.8% (237)	1.0% (270)	9.5% (239)	51.4% (240)	NA	1.1% (238)	1.4%
Matsubara et al. 2017 (257)	274	National Population	5.7% (237)	1.1% (270)	9.5% (239)	51.4% (240)	16.2% (241)	1.0% (238)	NA
Mynarek et al. 2020 (258)	199	National Population	6.7% (258)	NA	4.8% (258)	51.3% (240)	NA	3.4% (258)	NA
Nanduri et al. 2019 (40)	1,387	Regional Population	12.0% (237)	NA	NA	51.2% (240)	NA	NA	NA
Neto et al. 2007 (259)	48	National Population	6.0% (259)	NA	NA	NA	NA	NA	NA
O'Sullivan et al. 2019 (32)	339	National Population	6.4-7.4% (237)[b]	NA	7.0% (239)	NA	NA	1.6% (238)	NA
Óladóttir et al. 2011 (260)	34	National Population	5.3% (271)	NA	NA	51.3% (240)	NA	1.0% (238)	NA

Reference	Cases	Controls	Preterm births rate population	<34 rate population	LBW rate population	Male ratio population	National GBS colonisation rate	Multiple-gestation pregnancies rate population	Maternal age <20 years rate population
Pintye et al. 2016 (75)	138	Study	NA	NA	NA	NA	NA	NA	NA
Romain et al. 2018 (127)	597	National Population	6.6% (237)	NA	NA	51.1% (240)	15.6% (241)	NA	NA
Schuchat et al. 1990 (261)	37	Regional Population	10.0% (261)	NA	8.0% (261)	NA	NA	NA	14.0% (261)
Trijbels-Smeulders et al. 2007 (262)	77	National Population	7.6% (262)	NA	NA	51.1% (240)	NA	3.5% (262)	NA
Vergadi et al. 2018 (263)	9	Regional Population	7.4% (272)	NA	7.7% (272)	51.5% (240)	NA	NA	NA
Ying et al. 2019 (264)	9	Study	NA	NA	NA	NA	NA	NA	NA

Abbreviations: LBW, Low-birth weight; GBS, Group G *Streptococcus*; NA, Not Applicable

[a] Personal communication [b] Differs between UK and ROI

Table 6. Newcastle-Ottawa Scale score Cohort Studies

Reference	Selection score	Comparability score	Outcome score	Total score
Berardi et al. 2013 (41)	4	2	3	9
Dangor et al. 2016 (246)	4	0	3	7
Fluegge et al. 2006 (247)	4	0	2	6
Frigati et al. 2015 (248)	4	0	2	6
Giannoni et al. 2016 (249)	4	0	3	7
Guan et al. 2018 (250)	4	0	2	6
Heath et al. 2004 (33)	4	0	3	7
Jordan et al. 2008 (252)	4	0	3	7
Joubrel et al. 2015 (253)	4	0	2	6
Juncosa-Morros et al. 2014 (254)	4	0	2	6
Ko et al. 2015 (255)	4	0	3	7
Matsubara et al. 2013 (256)	4	0	2	6
Matsubara et al. 2017 (257)	4	0	2	6
Mynarek et al. 2020 (258)	4	2	3	9
Nanduri et al. 2019 (40)	4	0	3	7
Neto et al. 2007 (259)	4	0	2	6
O'Sullivan et al. 2019 (32)	4	0	3	7
Óladóttir et al. 2011 (260)	4	0	3	7
Romain et al. 2018 (127)	4	0	2	6
Schuchat et al. 1990 (261)	4	2	2	9
Trijbels-Smeulders et al. 2007 (262)	4	0	3	7
Vergadi et al. 2018 (263)	4	0	3	7

Table 7. Newcastle-Ottawa Scale score Case-Control Studies

Reference	Selection score	Comparability score	Exposure score	Total score
Dangor et al. 2015 (245)	4	2	3	9
Ireland et al. 2014 (251)	3	1	3	7
Lin et al. 2003 (74)	4	2	3	9
Pintye et al. 2016 (75)	3	1	3	7
Ying et al. 2019 (264)	3	1	3	7

4.3.3 Outputs from Meta-analyses

The OR of LOGBS in preterm infants compared to those born ≥37 weeks was 5.66 (95% CI 4.43-7.22), with considerable heterogeneity among the 22 studies included (I2: 92%) (**Figure 14**). An additional analysis was performed using birth <34 weeks as a cut-off when data were available. The OR of LOGBS for infants born <34 weeks compared to those born ≥34 weeks was 19.70 (15.25-25.45) among three studies included (I2: 0%) (**Figure 15**). The OR of LOGBS in all infants with LBW compared to birth weight >2500g was 6.73 (4.68-9.67) with considerable heterogeneity among fourteen studies included (I2: 95%) (**Figure 16**). The OR of LOGBS in all infants born to women colonised with GBS antenatally compared to those born to women not colonised with GBS was 2.67 (2.07-3.45), with substantial heterogeneity among 12 studies included (I2: 66%) (**Figure 17**). Finally, the OR of LOGBS in multiple births compared to singletons was 8.01 (5.19-12.38), with considerable heterogeneity among ten studies included (I2: 72%) (**Figure 18**).

PROM and intrapartum fever were not associated with an increased risk of LOGBS. The OR was 1.49 (0.94-2.36) and 1.06 (0.14-8.18), respectively (**Figures 19, 20**). In addition, there was no difference in risk of LOGBS between male and female infants (OR: 1.02 (0.92-1.13); I2: 20%) (**Figure 21**). Similarly, maternal age <20 years was not associated with an increased risk of LOGBS (OR: 1.86 (0.74-4.68); I2: 78%) (**Figure 22**).

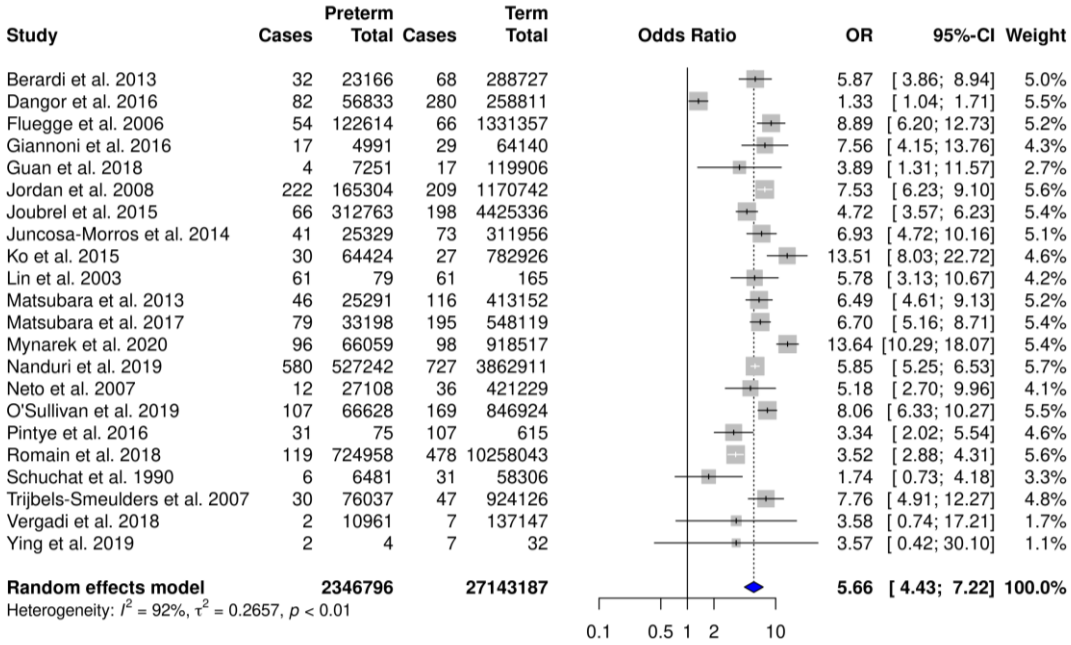


Figure 14. Forest Plot of Meta-analysis of risk of LOGBS for prematurity

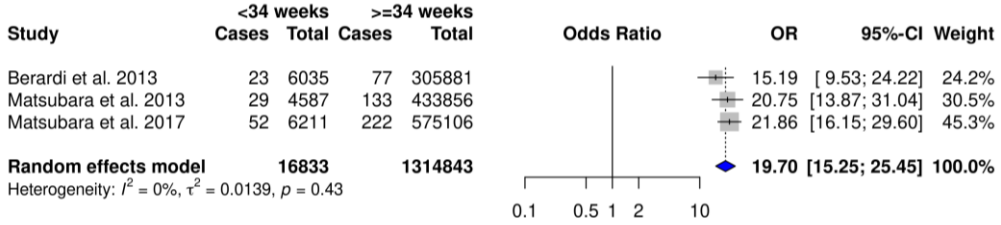


Figure 15. Forest Plot of Meta-analysis of risk of LOGBS for gestation <34 weeks

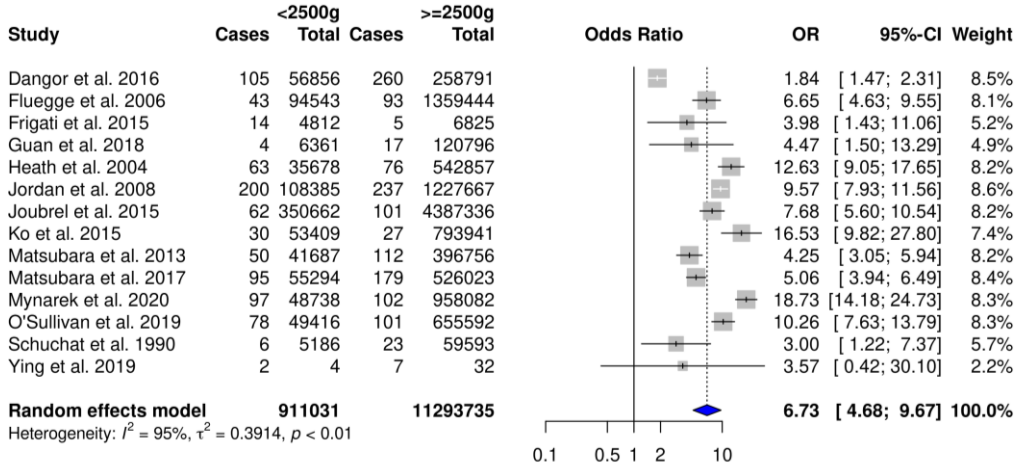


Figure 16. Forest Plot of Meta-analysis of risk of LOGBS for LBW

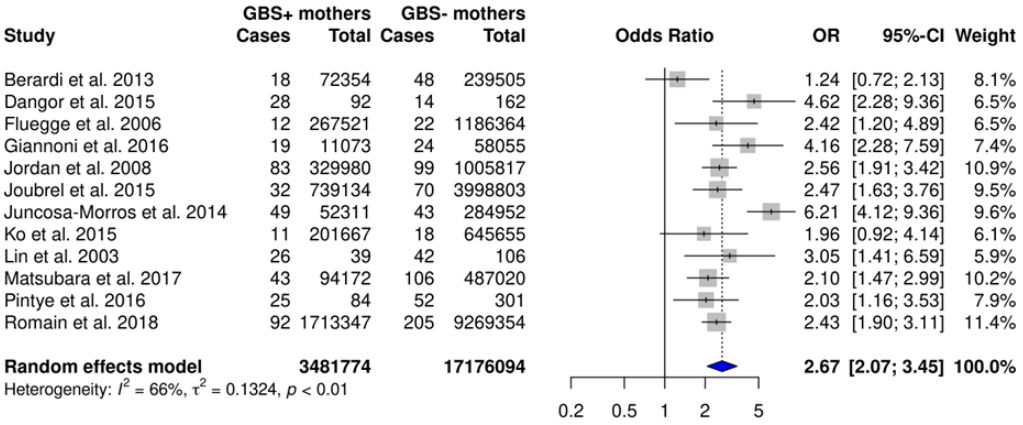


Figure 17. Forest Plot of Meta-analysis of risk of LOGBS for antenatal GBS colonisation

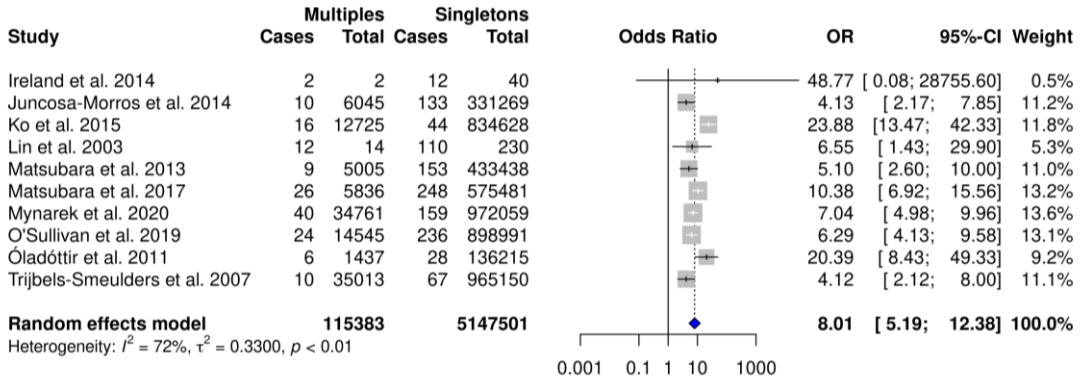


Figure 18. Forest Plot of Meta-analysis of risk of LOGBS for multiple gestation pregnancies

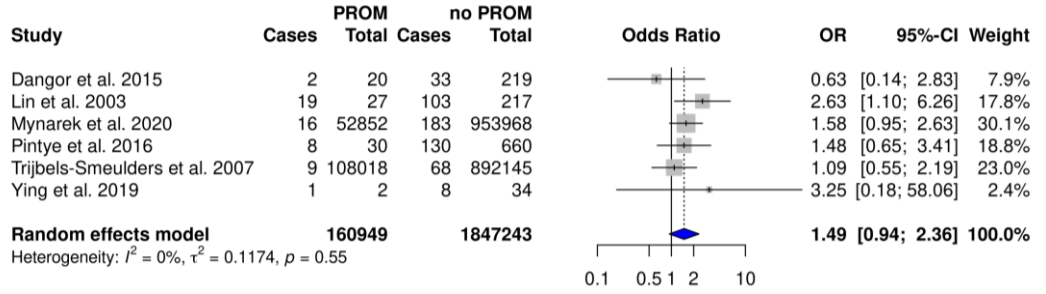


Figure 19. Forest Plot of Meta-analysis of risk of LOGBS for PROM

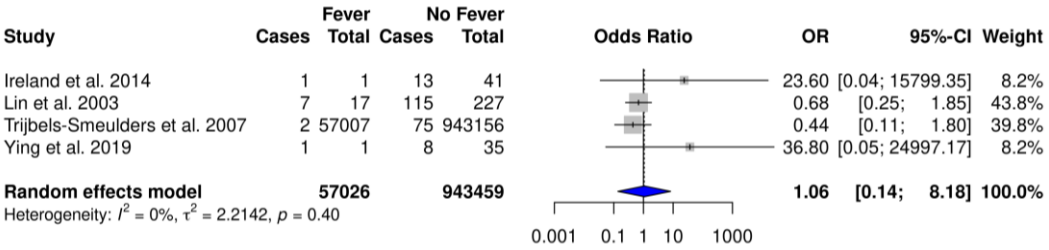


Figure 20. Forest Plot of Meta-analysis of risk of LOGBS for intrapartum fever

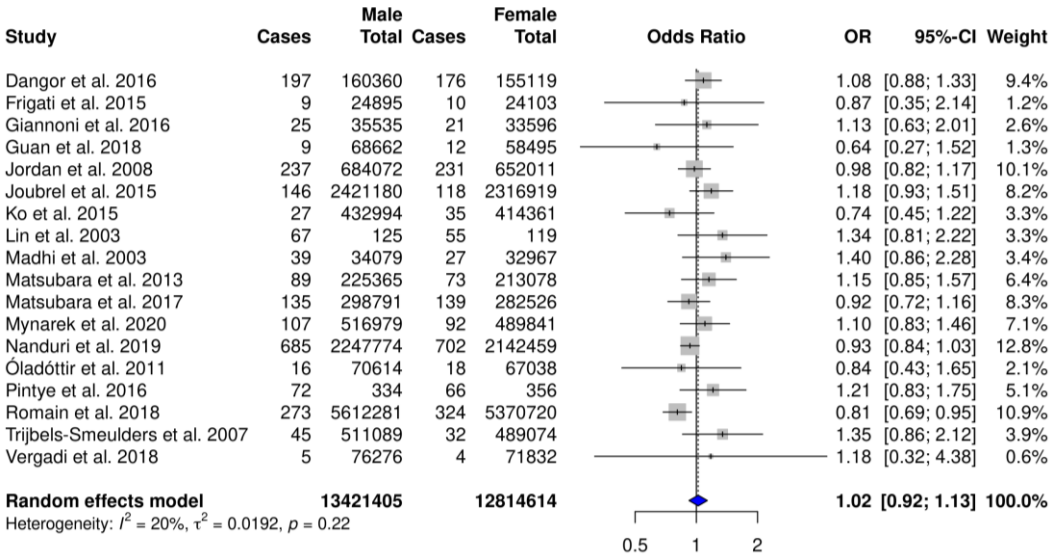


Figure 21. Forest Plot of Meta-analysis of risk of LOGBS for infant sex

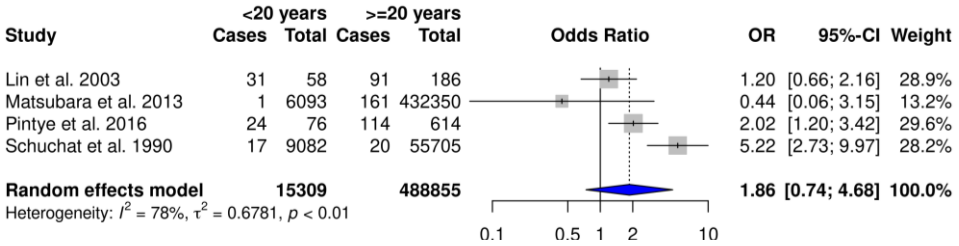


Figure 22. Forest Plot of Meta-analysis of risk of LOGBS for maternal age

4.3.4 Subgroup Analyses and Meta-regression

For prematurity and LBW, studies from Africa/LMIC had lower pooled estimates than the other geographic areas and HIC (**Table 8,9**). Also, single-centre studies had lower pooled estimates than multi-centre or national studies, whereas comparison according to study design and IAP policy showed no significant difference (**Table 8-11**). A meta-regression analysis showed that WHO region, classification of countries based on economic resources and classification of the studies based on the number

of participating sites accounted for a small to moderate proportion of heterogeneity for prematurity (R^2: 46%, 59%, 52%, respectively); and LBW (R^2: 35%, 30%, 64%), but none for colonisation (R^2: 0%, 4%, 0%) and multiple gestations (R^2: 0%). A meta-regression analysis with publication year, duration of the study and NOS score as continuous predictors showed that these factors did not influence the studies' effect sizes for any risk factor (R^2: 0%).

Table 8. Subgroup Analysis of risk of LOGBS - Prematurity

	Number of studies	OR	95% CI	I^2	P subgroup
Region					< 0.0001
Africa	1	1.33	1.04; 1.70		
Americas	5	5.15	3.82; 6.94	79%	
Europe	11	6.65	4.91; 8.99	87%	
Western Pacific	5	7.30	5.25; 10.16	49%	
Resources					< 0.0001
HIC	21	6.30	5.29; 7.50	81%	
LMIC	1	1.33	1.04; 1.70		
Setting					< 0.0001
Single centre	2	1.35	1.05; 1.72	0%	
Multi-centre	12	5.98	5.14; 6.93	49%	
National surveillance	8	7.34	4.96; 10.85	92%	
Design					0.3
Case control studies	3	4.15	2.82; 6.08	0%	
Retrospective cohort studies	7	6.17	4.11; 9.23	81%	
Prospective cohort studies	12	5.77	4.18; 7.95	94%	
IAP Policy					0.2
Yes [a]	18	6.00	4.62; 7.80	93%	
No	4	3.69	1.88; 7.24	53%	

[a] Risk based: 4 studies; Universal screening: 3 studies; Both strategies: 5 studies; Policy changed during study: 6 studies

Table 9. Subgroup Analysis of risk of LOGBS - Low Birth Weight

	Number of studies	OR	95% CI	I^2	P subgroup
Region					< 0.0001
Africa	2	2.28	1.16; 4.49	52%	
Americas	2	5.89	2.01; 17.25	84%	
Europe	5	10.52	7.34; 15.07	85%	
Western Pacific	5	6.18	3.53; 10.79	80%	
Resources					0.001
HIC	12	8.01	5.76; 11.14	88%	
LMIC	2	2.28	1.16; 4.49	52%	
Setting					< 0.0001
Single centre	2	1.89	1.24; 2.86	0%	
Multi-centre	6	5.33	3.78; 7.50	84%	
National surveillance	6	11.19	8.04; 15.56	83%	
Design					0.6
Case control studies	1	3.57	0.42; 30.10	-	
Retrospective cohort studies	6	5.70	3.29; 9.87	93%	
Prospective cohort studies	7	7.80	4.60; 13.21	97%	
IAP Policy					0.7
Yes [b]	10	6.97	4.51; 10.79	96%	
No	4	6.06	2.94; 12.50	74%	

[b] Risk based: 5 studies; Universal screening: 1 study; Both strategies: 2 studies; Policy changed during study: 2 studies

Table 10. Subgroup Analysis of risk of LOGBS - Maternal Colonisation

	Number of studies	OR	95% CI	I^2	P subgroup
Region					0.2
Africa	1	4.63	2.28; 9.36	-	
Americas	3	2.48	1.88; 3.27	0%	
Europe	6	2.80	1.80; 4.36	81%	
Western Pacific	2	2.07	1.50; 2.85	0%	
Resources					0.1
HIC	11	2.57	1.99; 3.33	67%	
LMIC	1	4.63	2.28; 9.36	-	
Setting					0.4
Multi-centre	8	2.86	1.98; 4.14	78%	
National surveillance	4	2.40	1.97; 2.93	0%	
Design					0.6
Case control studies	3	2.95	1.76; 4.92	39%	

	Number of studies	OR	95% CI	I^2	P subgroup
Retrospective cohort studies	2	3.59	1.27; 10.13	94%	
Prospective cohort studies	7	2.36	1.27; 10.13	36%	
IAP Policy					-
Yes [c]	12	2.67	2.07; 3.45	66%	

[c] Risk based: 3 studies; Universal screening: 2 studies; Both strategies: 4 studies; Policy changed during study: 3 studies

Table 11. Subgroup Analysis of risk of LOGBS - Multiple Births

	Number of studies	OR	95% CI	I^2	P subgroup
Region					0.5
Americas	1	6.55	1.43; 29.90	-	
Europe	5	6.60	3.85; 11.30	62%	
Western Pacific	4	11.21	5.16; 24.33	76%	
Resources					-
HIC	10	8.01	5.19; 12.38	72%	
Setting					0.4
Multi-centre	5	6.45	3.72; 11.18	46%	
National surveillance	5	9.47	4.88; 18.40	83%	
Design					1
Case control studies	2	7.64	1.27; 45.98	0%	
Retrospective cohort studies	5	7.70	4.55; 13.04	67%	
Prospective cohort studies	3	8.55	3.10; 23.59	90%	
IAP Policy					-
Yes [d]	10	8.01	5.19; 12.38	72%	

[d] Risk based: 4 studies; Policy changed during study: 6 studies

4.3.5 Sensitivity Analyses

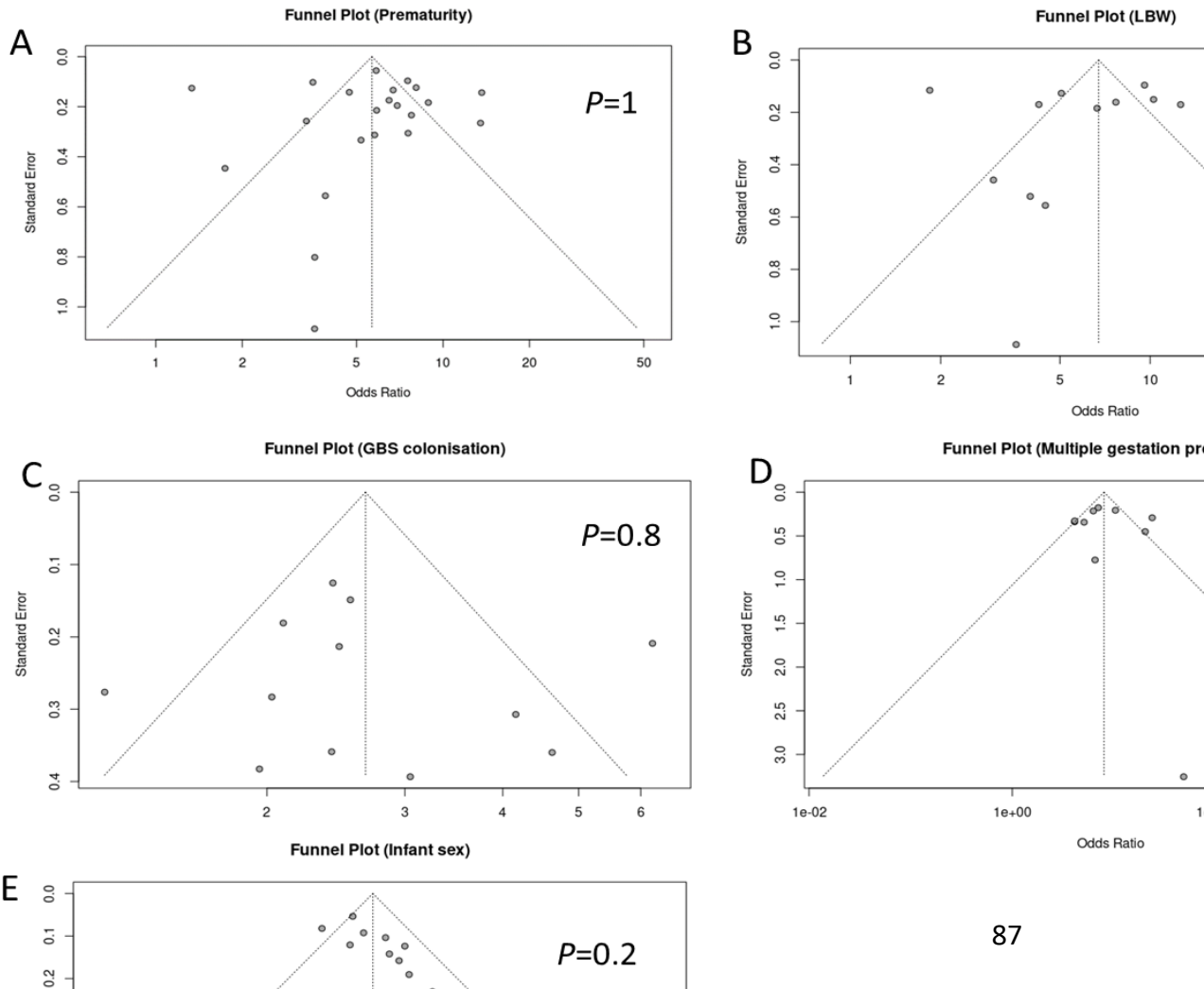
When studies that reported cases up to six months of age (very-late-onset GBS disease) were excluded, the OR was similar to the primary analysis for prematurity (5.90 (4.58-7.60)), LBW (7.07 (4.88-10.24)), antenatal colonisation (2.65 (2.02-3.48)), multiple gestation pregnancies (8.12 (5.07-12.99)), PROM (1.32 (0.82-2.12)), intrapartum fever (1.77 (0.09-34.65)), infant sex (1.01 (0.91-1.12)), and maternal age (1.29 (0.33-4.98)). Similarly, when studies that only reported sepsis or meningitis cases were excluded, the pooled estimate did not differ from the primary analysis for

prematurity (5.74 (4.42-7.45)), maternal colonisation (2.60 (1.94-3.49)), and infant sex (1.05 (0.94-1.17)). For the rest, primary analyses did not include studies only reporting sepsis or meningitis, therefore, sensitivity analyses were not needed.

4.3.6 Assessment of Reporting Biases

Eggers' test did not indicate the presence of funnel plot asymmetry for prematurity, LBW, colonisation, multiple-gestation pregnancies, and infant sex (**Figure 23**).

Figure 23. Funnel Plots and Egger's regression test



Funnel plot of the meta-analysis of included studies for (A) Prematurity (B) LBW (C) Antenatal colonisation (D) Mutiple gestation (E) Infant sex. Each plotted point represents the standard error and odds ratio for a single study. The vertical line represents the average odds ratio found in the meta-analysis. The diagonal lines represent the 95% confidence limits around the odds ratio for each standard error on the vertical axis. The triangle represents the region where 95% of the studies would lie in the absence of heterogeneity or publication bias. P values were estimated using the Egger's test. P value >0.05 suggests that an asymmetrical funnel plot arises merely by chance.

4.4 Discussion

This systematic review and meta-analyses show that the risk of LOGBS was higher in preterm and LBW infants, infants born to women colonised with GBS in pregnancy, and multiple gestation pregnancies. These findings are consistent with previous reviews that identified prematurity and maternal GBS colonisation as risk factors for EOGBS.(54) In contrast, there was no association between LOGBS and other intrapartum risk factors, such as maternal fever and PROM,(54) confirming that intra-amniotic infection has no connection to LOGBS.

Premature and LBW infants are known to be susceptible to infections due to immature immune responses, low placental antibody transfer, increased gut permeability, and the risk of nosocomial transmission during their prolonged hospitalisation.(66) Prematurity is also characterised by disturbances of microbiome development associated with frequent use of antibiotics, formula feeding and reduced contact with the maternal microbiome that might disturb the adaptation of GBS to its neonatal host environment.(70) The sub-analysis of LOGBS risk in infants <34 weeks showed that very preterm infants are at higher risk. This is in keeping with the previous finding of increasing risk for each week of decreasing gestation.(74)

In addition, the results suggest a strong association between LOGBS and maternal colonisation during pregnancy. Since GBS screening results were only recorded in women who reached 35 weeks of pregnancy, prematurity is unlikely to have accounted for the effect of maternal GBS colonisation on LOGBS risk. The transmission routes underpinning this observation, however, are not fully understood. The age at which infants become colonised with GBS is highly variable. Longitudinal colonisation studies of mother-infant pairs showed that approximately

20-25% of infants born to women colonised with GBS became colonised with the same strain by two months of age, despite adequate IAP and negative GBS screening at birth.(131)GBS seems to persist on mucosal surfaces even after adequate IAP and causes delayed infant acquisition via contaminated hands and possibly breastfeeding, although the latter remains controversial.(73) It is important to note that only a small proportion of colonised infants will develop iGBS disease. There are likely other virulence factors (e.g. adhesins) and host defences (e.g. anti-GBS CPS IgG) that may modify the risk of LOGBS in the presence of maternal GBS colonisation.(107)

In contrast, the association of multiple-gestation pregnancies with increased risk of LOGBS is likely confounded by prematurity and LBW, although this was not adequately tested due to the use of aggregate data for this review. However, a previous review of the clinical risk factors of EOGBS showed that multiple-gestation pregnancies are not an independent risk factor, with LBW accounting for the excess risk in twins.(54) It is important to note that the use of aggregate data did not allow the assessment of the risk of LOGBS for an infant with a twin sibling with iGBS disease (EOGBS or LOGBS), which is known to be significantly raised, since the multiple births have the same mother, thus the same potential exposure to GBS colonisation either vertically or horizontally.(273)

The study findings might have important implications for designing GBS vaccine trials. A vaccine administered during pregnancy could substantially reduce the LOGBS disease burden through passively transferred antibodies.(17) However, to do so would require the persistence of protective concentrations of antibodies in infants until at least two-three months of age. Given that three-quarters of LOGBS cases occur within the first eight weeks (median 34 days, interquartile range: 20–49 days),(40) it is plausible that vaccine-induced antibodies with a half-life between 39 and 46 days would protect most infants.(170) However, transfer (and persistence) of antibodies may be a particular challenge for preterm/LBW infants. This is because the placental transfer of IgG antibody is optimal in the third trimester of pregnancy so that infants born prematurely may not have had a chance to receive protective concentrations. This will, however, be strongly influenced by the timing of vaccination during pregnancy. Several recent studies on maternal vaccination against

pertussis support early vaccination for protecting preterm infants. Kent et al showed that preterm infants whose mothers were immunised from 28 weeks had higher antibody concentrations compared to preterm infants born to unvaccinated women.(274) Eberhardt et al reported higher antibody concentrations in both term and preterm infants when mothers were vaccinated in the second compared to the third trimester.(275) Vaccine effectiveness data from the UK suggests that extending the maternal pertussis vaccination window down to 20 weeks has reduced hospitalised pertussis cases in preterm infants.(276) Finally, a 2023 randomised control trial of 364 pregnant women from the UK who received pertussis vaccination at three different timings (<24 weeks, between 24-27 weeks, or 28–31 weeks) resulted in equivalent concentrations of IgG antibodies in their infants against two of the three pertussis antigens assessed. (277) Therefore, studies seeking to define the optimal window for GBS vaccination, to protect preterm and term infants should be prioritised.

This study has some limitations. First, under-ascertainment of cases is a common problem with invasive infant disease incidence studies.(17) However, many of the included surveillance studies mitigated the problem of under-reporting by using Capture-Recapture methods to ascertain cases, where both reference laboratories and clinical surveillance data were used (**Table 5**). Second, most studies were from HIC and studies from LMIC were all from a single country, South Africa; therefore, the estimated risks might not be generalisable. Subgroup analyses suggested that the OR for prematurity and LBW were lower in LMIC. This difference was driven by higher rates of LOGBS among term infants compared to HIC, whereas the incidence of disease among preterm/LBW infants was similar in LMIC and HIC. This might be explained by comorbidities such as exposure to HIV, or other clinical risk factors in early life that have not been captured in this review (e.g. malnutrition). Third, adjustment for multiple risk factors was not possible because aggregate data were used. Although three case-control studies reported adjusted OR (aOR) for different sets of covariates,(74,75,261) crude OR from these studies were used to compare the effect sizes across all studies. Therefore, the pooled estimates are subject to possible confounding due to other factors influencing LOGBS risk. For the same reasons, it

was not possible to assess the relationship between the risk of LOGBS and gestation or birth weight as continuous variables. Instead, a sub-analysis was performed using a cut-off of 34 weeks that showed a higher risk in more preterm infants. This threshold was chosen because it was the most common subgroup of preterm infants reported in the included studies. Limited availability of national or regional data on the prevalence of very preterm (28-32 weeks) or extremely preterm (<28 weeks) infants did not allow for further comparisons. Similarly, there were insufficient data available to perform a sub-analysis for VLBW (<1500g) or extremely low birth weight (ELBW <1000g) infants. Fourth, subgroup analyses and meta-regression did not provide a convincing explanation for the observed variation between the results of the studies. Finally, there were not enough eligible studies to estimate the risk of horizontal transmission through breast milk or from non-maternal caregivers. Thus, aside from vertical transmission risks, evidence regarding horizontal transmission risk factors that might be used to inform preventive strategies is currently limited.

4.5 Conclusions

Overall, this study shows that prematurity/low birth weight and maternal colonisation with GBS are major risk factors for LOGBS. To fully understand and ultimately prevent LOGBS, there is a need for (i) well-conducted colonisation studies that use genome sequencing and include breast milk samples and specimens from other family members (not restricted to mothers), (ii) mechanistic studies of the role of virulent strains in driving LOGBS, and (iii) globally collaborative seroepidemiological studies of the role of maternally derived antibodies in protecting infants from GBS acquisition and LOGBS. Answering these questions would be key to developing novel strategies to control LOGBS.

Chapter 5 - Invasive Group B Streptococcal Disease: Recurrent Disease and Disease in Multiple Pregnancies

The material presented in this chapter has been previously published in a peer-reviewed journal. (273)

5.1 Introduction

Two particularly instructive entities of iGBS disease occur in infants of multiple births and those with recurrent episodes. The increased risk of multiple pregnancies for preterm delivery and adverse outcomes, in general, is well established,(278) yet uncertainty exists about the specific association with iGBS. In part due to the lack of understanding of disease pathogenesis, there is no consensus about the management of the asymptomatic sibling of an iGBS case from a multiple birth.(279) Additionally, the mechanisms underlying recurrent disease are not fully understood. Prematurity, persistent mucosal colonisation and contaminated breast milk have been proposed as risk factors. (73) Since the shared (multiples) or fixed (recurrence) genetic and environmental conditions may shed some light on iGBS pathogenesis, this chapter focuses on cases in multiples or with recurrence. A model for LOGBS pathogenesis is proposed from this analysis as well as a review of relevant published GBS cases.

5.2 Methods

5.2.1 Study Design

UKROI*:* Enhanced national surveillance of iGBS in infants under three months of age in UKROI was conducted between 1st April 2014, and 30th April 2015. Data were collected through the British Paediatric Surveillance Unit (BPSU) and laboratories in England, Wales, Scotland, Northern Ireland and Ireland. Serotyping using latex agglutination and multilocus sequence typing (MLST) was performed by Public Health England (PHE) and the Irish Meningitis and Sepsis Reference Laboratory. The

South East Coast–Brighton and Sussex Research Ethics Committee approved the study (reference: 13/LO/1912; IRAS Project ID: 137959). A detailed methodology has been previously published.(32)

Germany and Switzerland: Medical centres from the German Neonatal Network (GNN), consisting of 65 sites, were asked for LOGBS cases from 2008-2020 with a recurrent course or with more than one affected multiple. This was complemented by an e-mail request to 120 additional medical centres with NICU in Germany and Switzerland. Data were collected via data entry forms. Data collection was approved by the ethics committee of Freiburg University (Nr. 207/20).

5.2.2 Study definitions

iGBS disease*:* EOGBS (day 0-3), or LOGBS (day 4-89), with GBS isolation from a normally sterile body site. The narrow EOGBS definition was used by the GNN cohort since > 90% of cases in the first week of life occur in this time frame. This definition was retained to harmonise the methods between the two cohorts. One episode of culture-negative sepsis was included because it was highly suggestive of GBS LOGBS (clinical sepsis with consistent laboratory abnormalities, isolation of GBS from infant's oropharynx, a concurrent episode of culture-positive GBS sepsis in the sibling and subsequent culture-positive relapse of iGBS disease).

Index case: The first infant among multiples with iGBS disease.

Recurrent iGBS disease*:* New episode of clinical illness in an infant associated with the isolation of GBS from a sterile site occurring after the completion of the therapy for the first occurrence.

GBS colonisation in infants*:* Positive oropharyngeal, ear or rectal swab or gastric aspirate by culture or PCR.

Interval between two recurrent iGBS episodes: Days between completion of antibiotics and onset of subsequent iGBS episode.

Duration of antibiotic treatment*:* Duration of the antibiotic treatment of iGBS disease (with penicillins or third-generation cephalosporins).

Short course of antibiotic treatment: Treatment duration <10 days.(200)

5.2.3 Statistical Analysis

Continuous variables were presented as median and range, and categorical data as numbers and percentages. Student's t-test or the Mann–Whitney U test and x2 test or Fisher's exact test were used to compare continuous and categorical variables between groups. A univariable regression analysis was used to estimate associations with recurrent GBS infections in the UKROI cohort after removing all the cases that died after the first GBS episode. Missing data were removed from the analysis. *P*-value <0.05 was considered significant. A multivariable model was produced using Akaike's Information Criteria (AIC) for model selection. (280) Analyses were performed using R software.

5.2.4 Literature Review of Cases with iGBS Recurrence

Medline and Embase were searched via Ovid from 1974 - 12/2022 for terms "Streptococcus agalactiae", "group B strep*", "strep* agalact*", "GBS", "double or recur* or episodes or relaps* or consecutive* or twice or two or three or four or five or repet*", "Infant, Newborn/", "newborn*", "neonat*, "infant* adj4 (week* or day* or month* or premature or full term or postmature or preterm). Additional studies were identified in references of articles. Two cases in the current case study were previously reported (duplicate entries) and excluded from the review.(281,282)

5.3 Results

5.3.1 GBS in infants from multiple births

5.3.1.1 UKROI Cohort

A total of 41 iGBS cases in infants from 35 multiple-birth pregnancies were identified, including six infant pairs in which both twins developed iGBS disease (17%) (**Table 12**). The median gestational age for the multiples with two affected infants was 33 (range 30-38) weeks and the median birth weight was 1755 (range 1070-2810) g. The median interval of disease onset between affected siblings was 2.5 (range 0-18) days (**Figure 24**). The median treatment duration was 14 (range 7-32) days for GBS bacteraemia and 15 (range 14-21) days for meningitis. GBS sero- and sequence-

typing revealed serotype III/sequence type 17 in all four tested twin sets, with both infants affected.

Data on managing the asymptomatic twin sibling of an index case were available for 12 twin pairs. Eight infants were clinically evaluated and antibiotics were not started; two of these developed iGBS disease. Antibiotic treatment was preemptively administered to the second twin in four cases and stopped after confirmation of negative cultures.

Table 12. Clinical features of GBS infections in infants from multiple births (UKROI)

		birth							LOGBS episode							
	Patient ID	gestational age (w)	Sex	birth weight (g)	Chorioamnionitis	Delivery mode	GBS maternal swab	ATX labour/birth	age at LOS onset (d)	Signs and symptoms	GBS detection	Serotype	Sequence type	ATX (d)	Management of other twin	recurrence
EOGBS - only one	1	35	M	2650	N	V	neg	N	0	P	B	NA	NA	21	NA	
	2	23	M	610	Y	V	NA	N	1	S	B	NA	NA	NA	NA	
	3	38	M	3425	N	V	neg	N	1	P	B	III	17	14	NA	
	4	36	M	2030	N	V	neg	N	3	M	B/CSF	III	17	14	NA	
	5	39	M	2700	N	V	pos	N	0	S	B	NA	NA	7	NA	
	6	36	F	2110	Y	CS	neg	NA	0	S	B	NA	NA	7	NA	
	7	33	M	1875	Y	CS	neg	N	0	S	B	V	1	NA	pA	
	8	35	F	2272	N	V	neg	N	1	S	B	NA	NA	7	NA	
EOGBS - all siblings with iGBS	9a	36	M	NA	N	V	neg	N	1	S	B	III	17	14	SM	
	9b	36	F	NA	N	V	neg	N	1	M	B/CSF	III	17	21	SM	
	10a	33	F	1760	N	CS	neg	Y	0	S	B	NA	NA	7	SM	
	10b	33	M	1870	N	CS	neg	Y	0	M	B/CSF	NA	NA	14	SM	
LOGBS - only one sibling with iGBS	11	26	M	1060	NA	NA	NA	NA	80	S	B	III	19	10	CE	
	12	37	M	3140	NA	NA	NA	NA	53	S	B	NA	NA	10	CE	
	13	34	M	2040	NA	NA	NA	NA	42	S	B	III	19	NA	CE	
	14	30	F	1440	NA	NA	NA	NA	85	S	B	NA	NA	NA	NA	
	15	32	M	1550	NA	NA	NA	NA	72	S	B	NA	NA	14	NA	
	16	26	M	940	NA	NA	NA	NA	49	S	B	VI	17	NA	NA	
	17	34	F	2150	NA	NA	NA	NA	31	M	B/CSF	III	17	21	NA	
	18	34	F	1690	NA	NA	NA	NA	35	S	B	NA	NA	28	NA	
	19	36	F	2305	NA	NA	NA	NA	7	M	B/CSF	Ia	23	14	NA	
	20	32	M	1150	NA	NA	NA	NA	21	S	B	NA	NA	14	pA	
	21	37	F	2780	NA	NA	NA	NA	52	M	B/CSF	III	17	21	CE	
	22	36	F	1785	NA	NA	NA	NA	86	S	B	NA	NA	14	NA	
	23	25	M	860	NA	NA	NA	NA	46	S	B	NA	NA	15	NA	
	24	38	F	3560	NA	NA	NA	NA	70	S	B	NA	NA	32	NA	
	25	28	F	1115	NA	NA	NA	NA	20	S	B	III	17	14	NA	
	26	32	M	1940	NA	NA	NA	NA	42	S	B	Ia	23	7	NA	
	27	35	F	NA	NA	NA	NA	NA	35	S	B	III	17	21	CE	
	28	34	M	2360	NA	NA	NA	NA	32	M	B/CSF	III	17	15	pA	
	29	27	F	890	NA	NA	NA	NA	66	M	B/CSF	Ia	23	14	NA	
	30	30	F	1450	NA	NA	NA	NA	17	S	B	Ia	23	8	CE	
	31	29	F	1095	NA	NA	NA	NA	25	M	B/CSF	NA	NA	21	pA	
LOGBS - all siblings with iGBS	32a	38	F	2620	N	CS	neg	N	5	S	B	III	17	10	NpA	
	32b	38	M	2810	N	CS	neg	N	4	M	B/CSF	III	17	14	NpA	
	33a	32	M	1750	NA	NA	NA	NA	31	M	B/CSF	III	17	14	NA	
	33b	32	M	2005	NA	NA	NA	NA	13	S	B	III	17	14	NA	
	34a	30	M	1620	NA	NA	NA	NA	50	S	B	III	17	21	NA	
	34b	30	M	1070	NA	NA	NA	NA	54	M	B/CSF	III	17	21	NA	Y
	35a	33	F	1520	NA	NA	NA	NA	30	S	B	NA	NA	14	NpA	
	35b	33	F	1350	NA	NA	NA	NA	24	S	B	NA	NA	14	NpA	

ABX antibiotics, B Blood, CE clinical evaluation, CS caesarian section, CSF cerebrospinal fluid, S sepsis, M meningitis, NpA no prophylactic ABX, P pneumonia, pA prophylactic ABX administered pending the results of bacterial cultures, SM Simultaneous occurrence of GBS infection; V vaginal delivery

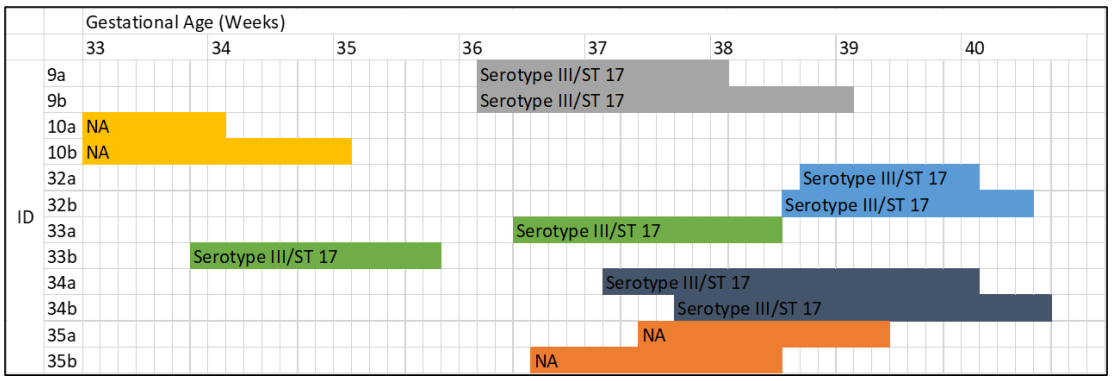


Figure 24. Timeline of iGBS disease in six infant pairs in which both twins developed iGBS disease in the UKROI cohort

Each small box is one day. Horizontal bars represent the duration of antibiotic treatment. The serotype and sequence type of the GBS isolate is reported in each bar. The same colour is used for pair of twins. ST: Sequence type; NA: Not applicable.

5.3.1.2 German/Swiss Cohort

Seven sets of twins and two sets of triplets, a total of 20 infants with iGBS disease were identified (**Table 13**). All infants were born prematurely. The median gestational age at birth was 31 weeks (range 25-36 weeks). The median birth weight was 1220 (range 800-2350) g. Eleven (55%) infants were male. Four pregnancies were di/trizygotic, one monozygotic, two were identified as dichorionic/diamniotic, and the data were unavailable for two pregnancies. Seven mothers had antenatal GBS screening, with negative swabs in all cases, but they received perinatal antibiotics. The median interval of iGBS disease onset between affected siblings was 6 (range 0-18) days. The median duration of antibiotic treatment for GBS bacteraemia was 14 (range 10-19) days, and 20 (range 14-21) days for meningitis. The isolates from twins were all of the same serotype and sequence type. Breast milk was tested in six women; three were positive for GBS by culture, one by PCR only, and two were negative. In five children, transient hypogammaglobulinemia was detected.

Table 13. Clinical features of GBS infections in infants from multiple births (German/Swiss cohort)

	Patient ID[1]	gestational age (w)	zygosity /chorionicity	Sex	birth weight (g)	Chorioamnionitis	Delivery mode	GBS maternal swab	ATX labour/birth	indication for ATX	ATX before LOS (d)	age at LOS onset (d)	onset at HM/HS	Signs and symptoms	GBS detection	breastfeeding/	GBS in breast milk	infant skin/mucosal swabs	Serotype	Sequence type	ATX (d)	outcome	ATB mother	continuation of breastfeeding	recurrence
LOGBS - all children affected	36a	31	NA	F	1820	N	CS	N	Y	Sc	4	28	HS	S	B	Y/NA	neg	NA	NA	NA	10	G	N	N	N
	36b	31	NA	F	1440	N	CS	N	Y	SC	0	16	HS	M	B/CSF	Y/NA	neg	NA	NA	NA	16	NS	N	N	N
	37a	26	DZ	M	890	Y	CS	NA	Y	PR	2	84	HM	AA	B	N/-	NA	NA	NA	NA	NA	L/C	N	F	N
	37b	26	DZ	F	810	Y	CS	NA	Y	PR	2	84	HM	S	-	N/-	pos	NA	NA	5	NA	N	F	Y	
	38a	28	DZ	M	1125	N	CS	N	Y	PR	3	48	HS	Cl	B/CSF	Y/neg	neg	III	17	19	G	N	Y	Y	
	38b	28	DZ	M	1190	N	CS	N	Y	PR	3	48	HS	Cl	B	Y/neg	neg	III	17	19	G	N	Y	N	
	39a	26	DZ	M	800	Y	CS	N	Y	PR	24	117	HM	S	B	Y/neg	neg	V	NA	14	G	R	N	N	
	39b	26	DZ	M	850	Y	CS	N	Y	PR	8	112	HM	M	B/CSF	Y/neg	pos	V	NA	21	G	R	N	N	
	39c	26	DZ	M	890	Y	CS	N	Y	PR	20	118	HM	S	B	Y/neg	neg	V	NA	14	G	R	N	N	
	40a	32	DC	F	1730	N	CS	N	Y	PR	0	35	HS	S	B	Y/pos	NA	NA	NA	14	G	Y	N	Y	
												64	HM	S	B	N/-	NA	NA	NA	14	G	N	F	-	
	40b	32	DC	F	1470	N	CS	N	Y	PR	0	28	HS	M	B/CSF	Y/pos	NA	NA	NA	21	G	Y	N	Y	
												63	HM	M	B	N/-	NA	NA	NA	14	G	N	F	-	
	41a	25	DC	M	950	N	NA	N	Y	NA	5	14	HS	S	B	Y/pos	pos	NA	NA	14	G	N	Y *	N	
	41b	25	DC	M	875	N	NA	N	Y	NA	10	20	HS	M	B/CSF	Y/pos	pos	NA	NA	21	NS	N	Y *	N	
	42a	32	M	F	1600	N	CS	N	N	-	0	69	HM	S	B	Y/neg	neg	NA	NA	16	G	N	Y	N	
	42b	32	M	F	1190	N	CS	N	N	-	0	51	HM	S	B	Y/neg	neg	NA	NA	16	G	N	Y	N	
	42c	32	M	F	1250	N	CS	N	N	-	0	58	HM	S	B	Y/neg	neg	NA	NA	16	G	N	Y	N	
	43a	36	NA	M	2350	N	V	NA	N	-	NA	11	NA	NA	B	NA	NA	III	NA	10	G	NA	NA	NA	
	43b	36	NA	M	2160	N	V	NA	N	-	NA	11	NA	NA	B	NA	NA	III	NA	14	G	NA	NA	NA	
	44a	32	DZ	F	1600	N	CS	N	Y	Sc	NA	NA	NA	NA	B	Y/pos	NA	NA	NA	NA	G	NA	NA	NA	
	44b	32	DZ	M	1860	N	CS	N	Y	Sc	NA	NA	NA	NA	B	Y/pos	NA	NA	NA	NA	G	NA	NA	NA	

AA acute abdomen, ATX antibiotics, B Blood, Cl cellulitis, CS caesarean section, CSF cerebrospinal fluid, DC dichorionic, DZ dizygotic, G good, HM home, HS hospital, L/C laparotomy+colostomy, Me meningitis, NA not available, NS neurologic sequelae, PR PROM, R recommended, SP Section Prophylaxis, S sepsis, V vaginal delivery [1] Infants from the same family are indicated with the same number plus a,b,c * sterilisation

5.3.2 Recurrent iGBS Disease

5.3.2.1 UKROI Cohort

Twelve cases of recurrent iGBS disease were identified, accounting for 1.4% (12/856) of all infants with iGBS reported that year (**Table 10**).(32) Eight (67%) infants were born prematurely, and five (42%) infants were of VLBW. The median gestational age was 31 (range 23-41) weeks, and the median birth weight was 1705 (range 600-3900) g. Ten (83%) infants were male and one (8%) infant was from a twin pregnancy. The median age at the onset of the first iGBS episode was 11 (range 0-73) days. Two (17%) infants had EOGBS and ten (83%) LOGBS as the first iGBS episode. The median duration of antibiotic therapy for the first episode was seven (range 5-14) days. Recurrence of iGBS occurred at a median age of 40 (range 23-84) days. The interval from the completion of initial therapy to the onset of the second episode was 13 (range 5-75) days. GBS capsular serotyping was performed in 17 isolates from 11 (92%) cases. Serotype III was the most commonly isolated serotype (n=7, 64%). MLST was performed in 13 isolates from seven (58%) cases. Sequence type 17 was found in four (57%) cases. Serotypes were identical in seven out of these eight cases (88%).

5.3.2.2 German/Swiss Case Series

Thirteen cases of recurrent iGBS were reported from ten centres in Germany and Switzerland within 12 years (**Table 14**). Nine (69%) infants were born prematurely (median 32, range 23-41 weeks) and six (46%) were VLBW. Nine (69%) infants were female and five (38%) infants were from a twin pregnancy. The median age at the onset of the first episode of iGBS disease was 28 (range 1-150) days. Three (23%) infants had EOGBS. The first episode was treated with antibiotics for a median of 14 (range 7-21) days. Recurrence of iGBS disease occurred at a median age of 44 (range 24-64) days. The median interval from completion of initial therapy to the onset of the second episode was nine (range 3-37) days. Breast milk samples were collected from eight (62%) out of 13 breastfeeding women. A positive result (by culture or PCR) was found in five women during the first or second episode. In six out of 12 cases where data were reported, breastfeeding was stopped after the first or second iGBS

episode and restarted after antibiotics were given for GBS "eradication" in two of these six cases. In one further case, breast milk was pasteurised.

Four infants were treated with antibiotics to decrease GBS colonisation or prevent further bacterial infections until immunological investigations were completed. In nine infants, where immunological tests were done, two were diagnosed with transient hypogammaglobulinemia, one had reduced complement activity, and one had absent memory B cells and neutropaenia.

Table 14. Clinical features of cases of recurrent iGBS disease

						1st episode			2nd episode			3rd episode			Isolates		Colonisation		Breast milk		Outcome	
Country	**ID**	**Sex**	**BW**	**GA**	**DM**	**age**	**GBS**	**Abx**	**age**	**GBS**	**Abx**	**age**	**GBS**	**Abx**	**ST**	**MLST**	**I**	**M**	**GBS**	**C**	**Imm**	**Seq**
Germany	1	F	565	23	CS	1	B	10	48	B	12	124	B	21	NA	NA	pos	pos	neg	Yes	N	no
Germany	2	F	670	25	CS	41	B	14	84	B	21	-	-	-	NA	NA	pos	neg	neg	No*	^	no
Germany	3	F	1230	27	V	46	B	7	62	B	10	-	-	-	NA	NA	neg	neg	pos	Yes**	N	no
Germany	4	F	590	23	NA	150	B	9	166	B/CSF	42	-	-	-	NA	NA	neg	NA	pos	Yes**	^^	DD
Germany	5	F	1732	32	CS	35	B	14	64	B	14	-	-	-	NA	NA	NA	neg	pos	Yes	N	no
Germany	6	F	1470	32	CS	28	B/CSF	21	63	B	14	-	-	-	NA	NA	NA	NA	pos	Yes	N	no
Germany	7	F	2890	39	V	10	B	10	25	B	14	-	-	-	NA	NA	NA	neg	neg	No	NA	no
Germany	8	F	4320	37	V	1	B	15	25	B/CSF	14	-	-	-	NA	NA	neg	pos	NA	No	NA	no
Germany	9	M	3020	37	CS	1	B	14	21	B	14	-	-	-	NA	NA	pos	pos	NA	No	NA	no
Germany	10	M	3020	35	V	20	B/CSF	15	60	B	14	-	-	-	NA	NA	pos	neg	NA	No	NA	no
Germany	11	F	4190	41	V	3	B	7	24	B	14	-	-	-	NA	NA	NA	neg	NA	No	^^^	no
Germany	12	M	1125	28	CS	48	B/CSF	19	74	B/CSF	19	-	-	-	III	17	neg	pos	pos	Yes	N	no
Germany	13	NA	1920	31	CS	47	B	10	60	B	9	-	-	-	NA	NA	NA	NA	NA	NA	^^	no
UKROI	14	F	1082	27	NA	39	B	10	59	B	14	-	-	-	Ia	NA	NA	NA	NA	NA	NA	NA
UKROI	15	M	3770	39	V	0	B	7	23	B	14	-	-	-	III	17	NA	neg	NA	NA	NA	NA
UKROI	16	M	3350	42	NA	11	B	7	25	B	40	-	-	-	III	17	NA	NA	NA	NA	NA	NA
UKROI	17	M	2020	31	NA	73	B	5	84	B	42	-	-	-	NA	NA	NA	NA	NA	NA	NA	NA
UKROI	18	M	770	23	NA	1	B	9	30	B/CSF	21	-	-	-	Ib	new	NA	pos	NA	NA	NA	D
UKROI	19	M	1070	30	V	26	B/CSF	NA	54	B/CSF	21	-	-	-	V/III	17	NA	NA	NA	NA	NA	NA
UKROI	20	M	1490	29	NA	37	B	10	59	B/CSF	21	-	-	-	III	17	NA	NA	NA	NA	NA	NA
UKROI	21	M	3150	40	NA	11	B/CSF	7	38	B/CSF	21	-	-	-	III	17	NA	NA	NA	NA	NA	NA
UKROI	22	M	3900	41	NA	16	B	7	28	B	14	-	-	-	III	NA	NA	NA	NA	NA	NA	NA
UKROI	23	M	600	23	NA	0	B	7	82	B	NA	-	-	-	III	NA	NA	NA	NA	NA	NA	NA
UKROI	24	M	1620	31	NA	8	B	14	41	B	14	69	B	14	III	23	NA	NA	NA	NA	NA	NA
UKROI	25	F	1790	32	CS	0	B	10	23	B/CSF	21	-	-	-	V	NA	NA	NA	NA	NA	NA	NA

BW: Birth weight, GA: Gestational Age, DM: Delivery mode, GBS: Group B *streptococcus*, Abx: Antibiotics, ST: Serotype, MLST: Multilocus sequence typing, I: Infant, M: Mother, C: Cessation of breastfeeding, Imm: Immunology, Seq.: Sequelae, F: Female, M: Male, B: Blood, CSF: Cerebrospinal fluid C: Cellulitis, CS: Caesarean section, D: Death, DD: Developmental delay, NA: Not available, V: Vaginal delivery
* Pasteurised, ** Temporary (restarted after maternal decolonisation)^: lack of Memory B cells and neutropenia, ^^: transient hypogammaglobulinemia, ^^^: reduced CH50 activity (<10%)

5.3.2.3 Literature Review on Recurrent iGBS

Forty-six case reports or case series of GBS recurrence in 86 infants were identified between 1976 and 2022 (**Table 15**). The median age at the onset of the first episode was 14 (range 0-120) days. Of the infants in whom information was available, 43/76 (57%) were preterm, 32/52 (62%) were males, and 28/42 (67%) were born vaginally. Antibiotics were administered for a median of eleven (range 7-28) days for the initial episode. The median interval from completion of antibiotic therapy to the onset of the second episode was eight (range 0-54) days. A third episode of GBS disease occurred in 11 infants (13%) at a median age of 62 (range 32-120) days. Capsular serotyping was performed in 56 isolates (65%), with serotype III in 40 cases (71%). Serotypes were identical in both episodes in all but one case. The hypervirulent sequence type 17 was found in five cases where MLST was performed.

Thirty-one breast milk samples were tested by culture or PCR and were positive for GBS in 25 cases (81%). Mastitis was reported by eight women (32%). GBS serotypes of the 11 serotyped breast milk samples were identical to those found in the infants. In thirteen (52%) cases, breastfeeding ceased. Immunological investigations revealed transient hypogammaglobulinemia in four infants.

Table 15. Literature review of cases of recurrent GBS disease

		Birth				1st episode			2nd episode			3rd episode			GBS	Breast				
Reference	**Year**	**Sex**	**GA (w)**	**Maternal swab**	**BW (g)**	**Age (d)**	**Detection of GBS**	**Abx (d)**	**Age (d)**	**Detection of GBS**	**Abx (d)**	**Age (d)**	**Detection of GBS**	**Abx (d)**	**Serotype**	**GBS**	**Cessation**	**Mother**	**Infant**	**Pathogenesis**
Al Abdali et al (283)	2022	M	T	NA	4120	0	B	14	21	B	28	NA	NA	NA	NA	NA	NA	NA	NA	NA
Moon et al (284)	2021	M	30	NA	1760	0	B	15	28	B	15	NA	NA	NA	NA	NA	NA	NA	NA	5,6
Butler et al (285)	2019	F	37	Pos (pp)	NA	2	B/CSF	14	58	B/CSF	14	NA	NA	NA	III	NA	NA	AM (7)	AM (14)	NA
Berardi et al (286)	2018	NA	27	Pos	947	27	B/CSF	NA	60	B/CSF	NA	NA	NA	NA	III	Yes (STIII)	NA	NA	NA	1
Méndez-Echevarría et al (287)	2018	NA	40	Neg	NA	12	B	10	32	B/CSF	NA	NA	NA	NA	NA	No	NA	NA	NA	NA
		NA	40	Pos (pp)	NA	0	B	10	60	B	NA	NA	NA	NA	NA	No	NA	NA	NA	NA
		NA	31	NA	NA	23	B	10	35	B	NA	NA	NA	NA	NA	No	NA	NA	NA	NA
Ueda et al (288)	2018	M	39	Neg	2858	3	B/CSF	28	34	B	21	NA	NA	NA	III	Yes (STIII)	Yes	NA	NA	2
Buser et al (289)	2017	NA	T	Neg	NA	0	B	11	16	B	14	NA	NA	NA	III	No	NA	NA	NA	3
Thomas et al (290)	2017	F	T	Neg	NA	8	B	10	22	B	10	NA	NA	NA	Ia	Yes (STIa)	Yes	AM (10)	RF (5)	2
Matsubara et al (257)	2017	NA	23	Pos	532	20	B	14	38	B	14	NA	NA	NA	NA	Yes	NA	NA	NA	NA
		NA	24	NA	589	120	B	14	141	B	28	NA	NA	NA	NA	Yes	NA	NA	NA	NA

		NA	30	Neg	925	25	B	15	45	B	28	NA	NA	NA	Ia	NA	NA	NA	NA	NA
		NA	34	Neg	2172	19	B	14	37	B	14	NA	NA	NA	III	Yes (STIII)	NA	NA	NA	NA
		NA	37	NA	2620	46	B	9	76	B	10	NA	NA	NA	NA	NA	NA	NA	NA	NA
		NA	37	Pos	2530	64	CSF	16	109	B	10	NA	NA	NA	NA	NA	NA	NA	NA	NA
		NA	38	NA	2660	8	B	7	22	B	10	NA	NA	NA	NA	Yes	NA	NA	NA	NA
		NA	38	Neg	2914	9	B	14	26	B	21	NA	NA	NA	NA	Yes	NA	NA	NA	NA
		NA	40	Neg	3470	0	B	10	43	B	10	NA	NA	NA	NA	NA	NA	NA	NA	NA
		NA	40	Pos	3860	20	B	10	45	B	10	NA	NA	NA	NA	NA	NA	NA	NA	NA
		NA	40	Neg	2814	25	CSF	17	96	B	14	NA	NA	NA	III	No	NA	NA	NA	NA
		NA	41	NA	2840	21	B	7	35	CSF	28	NA	NA	NA	NA	NA	NA	NA	NA	NA
Suresh et al (291)	2016	M	27	Neg	1050	21	B	10	37	B	42	NA	NA	NA	III	NA	NA	NA	NA	4
Elling et al (281)	2014	M	28	Pos (pp)	1190	48	B	19	74	No	10	NA	NA	NA	III	Yes	Yes	NA	NA	2
Davanzo et al (292)	2013	F	25	NA	719	44	B	10	60	B	10	NA	NA	NA	III	Yes (STIII)	Yes	AM (10)	RF (4)	2
Jawa et al (293)	2013	F	24	NA	640	54	B	10	72	B	14	NA	NA	NA	IV	Yes (STIV)	Yes	AM (10)	NA	2
Pastore et al (294)	2013	M	T	Pos	NA	21	B	10	36	B	10	NA	NA	NA	III	Yes (STIII)	Yes	AM (10)	NA	2
Jones et al (295)	2012	M	29	NA	1465	NA	B	14	NA	B	16	60	B	NA	NA	Yes	Yes	NA	NA	2,5
Lombard et al (296)	2012	M	40	Neg	NA	2	B	10	29	B/CSF	21	NA	NA	NA	III	Yes	NA	NA	NA	2
Shoda et al (297)	2012	M	39	Neg	3112	43	B	10	58	B	14	74	B	14	III	NA	NA	NA	NA	6
Morinis et al (298)	2011	NA	27	NA	1140	25	B	14	42	B	NA	63	B	NA	NA	Yes	Yes	NA	NA	2
		NA	26	NA	1066	40	B	14	NA	B	NA	NA	B	NA	NA	No	NA	NA	NA	1
Poon et al (299)	2010	F	28	NA	730	0	B	14	38	B	28	78	B	21	III	NA	NA	NA	NA	7

Soukka et al (300)	2010	M	38	Pos	NA	0	B	10	14	B	14	32	B	14	NA	Yes	P	AM (5)	RF/C (30)	2,6
Gagneur et al (301)	2009	F	31	Pos	1600	16	B/CSF	14	41	B	21	NA	NA	NA	III	Yes (STIII)	P	AM (10)	AM (21)	2
Gajdos et al (302)	2008	F	38	Pos	NA	15	B	10	30	B/CSF	17	NA	NA	NA	III	Yes (STIII)	Yes	AM (7)	NA	2
Wang et al (303)	2007	F	T	NA	NA	4	B/CSF	10	14	B	21	NA	NA	NA	NA	Yes	Yes	AM (10)	NA	6
Byrne et al (304)	2006	NA	31	NA	1883	10	B	10	25	B	14	NA	NA	NA	NA	Yes	Yes	AM (7)	NA	2
Godambe et al (305)	2005	NA	30	Neg	NA	0	B/CSF	21	30	B	14	NA	NA	NA	NA	Yes	Yes	NA	NA	2
Ekelund and Konradsen (306)	2004	M (3/6)	NA	NA	NA	5 (0-5)*	B	22 (6-45)*	30 (25-99)*	B	24 (7-60)*	NA	NA	NA	Ia	NA	NA	NA	NA	6
			NA	NA	NA		B			B		NA	NA	NA	III	NA	NA	NA	NA	6
			NA	NA	NA		B			B		NA	NA	NA	III	NA	NA	NA	NA	6
			NA	NA	NA		CSF			B		NA	NA	NA	III	NA	NA	NA	NA	6
			NA	NA	NA		B			CSF		NA	NA	NA	III	NA	NA	NA	NA	6
			NA	NA	NA		B/CSF			B/CSF		NA	NA	NA	III	NA	NA	NA	NA	6
Pickett and Gallaher (307)	2004	M	26	NA	799	42	B	10	79	B	14	NA	NA	NA	NA	NA	NA	NA	NA	6
Kotiw et al (308)	2003	M	T	Pos	NA	20	B	14	#REF!	B	21	NA	NA	NA	NA	NA	NA	RF (4)	RF (4)	2
Moylett et al (309)	2000	M	36	Pos (pp)	2523	21	B	10	35	B	10	NA	NA	NA	Ia	NA	NA	NA	RF/CL (11)	6
		M	36	Pos (pp)	2296	20	No	10	34	B	10	NA	NA	NA	Ia	NA	NA	NA	RF/CL (11)	6
		M	30	NA	1260	13	B	13	39	B	10	NA	NA	NA	Ia	NA	NA	NA	NA	6
		M	38	NA	2836	26	B	10	47	B	10	NA	NA	NA	III	NA	NA	NA	NA	6

		M	24	NA	800	59	B/CSF	21	84	B	21	NA	NA	NA	III	NA	NA	NA	NA	6
		M	25	NA	490	112	B	12	130	B	12	NA	NA	NA	V	NA	NA	NA	NA	6
		M	24	NA	783	24	B	14	43	B	21	NA	NA	NA	V	NA	NA	NA	NA	6
		F	25	NA	680	25	B	10	43	B	14	NA	NA	NA	V	NA	NA	NA	NA	6
Olver et al (310)	2000	F	26	Neg	NA	12	B	7	63	B	14	NA	NA	NA	III	Yes (STIII)	Yes	AM (7)	NA	2
Sabui et al (311)	1999	F	27	NA	959	35	B	14	61	B	11	NA	NA	NA	Ia	NA	NA	NA	RF (4)	NA
Atkins et al (312)	1998	F	27	NA	NA	48	B	14	75	B	21	120	B	NA	Ia	Yes (STIa)	NA	RF (7)	RF (7x2)	2
Green et al (313)	1994	NA	28	NA	1800	27	B	13	48	B/CSF	NA	NA	NA	NA	III	NA	NA	NA	NA	NA
		NA	34	NA	1460	1	B	18	23	B	NA	NA	NA	NA	Ib	NA	NA	NA	NA	NA
		NA	30	NA	1130	1	B	10	29	B	NA	NA	NA	NA	III	NA	NA	NA	NA	NA
		NA	28	NA	900	3	B	14	68	B/CSF	NA	NA	NA	NA	III	NA	NA	NA	NA	NA
		NA	27	NA	1070	20	B	10	48	B	NA	NA	NA	NA	III	NA	NA	NA	NA	NA
		NA	25	NA	700	15	B	21	39	B/CSF	NA	NA	NA	NA	III	NA	NA	NA	NA	NA
		NA	25	NA	470	1	B	14	26	B	NA	NA	NA	NA	Ia	NA	NA	NA	NA	NA
		NA	28	NA	1020	25	B	11	50	B	NA	NA	NA	NA	III/V	NA	NA	NA	NA	NA
		NA	37	NA	2350	1	B	14	50	B	NA	NA	NA	NA	Ia	NA	NA	NA	NA	NA
Yagupsky et al (314)	1991	NA	NA	NA	NA	2	B	10	56	B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		NA	NA	NA	NA	1	B	10	47	B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		NA	NA	NA	NA	1	B	21	36	B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Simón et al (315)	1989	F	36	Pos (pp)	2370	0	B	10	33	B	NA	45	B	15	III	NA	NA	NA	NA	5
Denning et al (316)	1988	NA	38	NA	2350	0	No	7	26	B	13	47	B/CSF	18	III	NA	NA	NA	NA	6
Haque et al (317)	1986	M	32	Pos (pp)	1170	0	B	18	62	B	10	NA	NA	NA	NA	NA	NA	NA	NA	NA

Millard et al (318)	1985	M	28	Pos (pp)	920	0	B	10	42	B	14	70	CSF	21	III	NA	NA	NA	RF (4)	6
Barton and Kapoor (319)	1982	M	T	NA	2552	30	B/CSF	14	65	B	21	NA	NA	NA	III	NA	NA	NA	NA	NA
Siegel et al (320)	1981	M	T	NA	3190	14	B/CSF	10	35	AF	28	NA	NA	NA	III	NA	NA	NA	NA	8
		M	35	NA	1,925	3	B	10	24	B	12	NA	NA	NA	III	NA	NA	NA	NA	8
Ruiz-Gomez et al (321)	1979	M	T	Pos (pp)	2600	11	B	10	26	B	12	NA	NA	NA	III	NA	NA	NA	NA	9
		M	38	Pos (pp)	2960	0	B/CSF	20	25	B/CSF	22	NA	NA	NA	III	NA	NA	NA	NA	6
		M	32	NA	1350	47	B/CSF	14	68	CSF	17	NA	NA	NA	NA	NA	NA	NA	NA	9
McCrory et al (322)	1978	F	29	Pos (pp)	1134	7	B	14	35	B/CSF	21	59	B/CSF	21	Ib	NA	NA	NA	NA	5
Kenny and Zedd (323)	1977	M	NA	NA	2690	0	B	10	49	B/CSF	26	NA	NA	NA	NA	Yes	NA	OX	NA	NA
Broughton et al (324)	1976	M	T	Neg	3100	0	B	10	20	B/CSF	28	NA	NA	NA	III	NA	NA	NA	NA	7
Dorand and Adams (325)	1976	F	T	NA	3543	1	B/CSF	8	8	B/CSF	21	NA	NA	NA	III	NA	NA	NA	NA	9
Truog et al (326)	1976	F	37	NA	3200	8	B/CSF	14	26	B/CSF	31	NA	NA	NA	NA	NA	NA	NA	NA	10
Walker et al (327)	1976	F	38	NA	NA	18	B/CSF	10	56	B/CSF	12	NA	NA	NA	III	NA	NA	NA	NA	1

T: term; SN: Single; TW: Twin; TR: Triplet; pp: post=partum; B: Blood; CSF: Cerebrospinal Fluid, A: Abscess Fluid; P: Pasteurisation AM: Amoxicillin, RF: Rifampicin; CL: Clindamycin; C: Cephalexin; PN: Penicillin, OX: Oxacillin NA: Not Available; *: Values expressed as median (range) Pathogenesis: 1=Horizontal transmission; 2=Breast milk; 3=Ingested contaminated placenta capsules containing GBS; 4=Hypervirulence; 5=Host immunity; 6=Persistent mucosal colonisation; 7= Persistent infective focus; 8= Penicillin tolerance; 9=Underdosing; 10=Short course of antibiotics

5.3.2.4 Risk Factors for GBS Recurrence

A univariable logistic regression showed that VLBW (OR 6.8 (1.9-22.0), *P* = 0.001), preterm birth (OR 5.6 (1.7-21.4), *P* = 0.005), a short course of antibiotics (<10 days) (OR 3.2 (1.0-10.9), *P* = 0.04) and male sex (OR 4.4 (1.1-28.7), *P* = 0.05) were associated with increased risk of GBS recurrence (**Table 16**). When the categorical predictor variables were modelled together in a multivariable logistic regression model, the association of GBS recurrence with VLBW (OR 10.0 (2.7-37.4), *P* < 0.001) and a short course of antibiotics (OR 4.5 (1.3-17.5), *P* = 0.02) remained significant (**Table 16**).

Table 16. Univariable and Multivariable Logistic Regression analysis of recurrent iGBS disease (UKROI)

	Outcome		**Univariable**		**Multivariable**	
	Recurrent/ Total cases with characteristic	Recurrent/ Total cases without characteristic	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Antibiotic course <10 d	7/205	5/448	3.2 (1.0-10.9)	0.04	4.5 (1.3-17.5)	0.02
Very Low Birth Weight (<1500 g)	5/67	7/559	6.8 (1.9-22.0)	0.001	10.0 (2.7-37.4)	< 0.001
Preterm Birth (<37 weeks)	8/185	4/488	5.6 (1.7-21.4)	0.005		
Male Sex	10/423	2/357	4.4 (1.1-28.7)	0.05	4.5 (1.1-30.1)	0.06

5.3.3 Summary of Recurrent Cases and iGBS in Multiples

Combining the UKROI and German/Swiss cohorts reveals that infants with recurrent iGBS disease did not differ from multiples, in which more than one sibling developed iGBS disease with respect to gestational age, birth weight and age of LOGBS onset (**Table 17**). However, antibiotic treatment for the first episode of iGBS disease was shorter in UKROI (median 7 vs 14 days in German/Swiss cases, *P* = 0.01; vs 11 days in literature, *P* = 0.001).

Table 17. iGBS disease cases with recurrence and iGBS disease in multiples in the UKROI and Germany/Switzerland and literature

	Gestational age in weeks, median (IQR)	Birth weight g, median (IQR)	Age at onset of 1st iGBS disease in days, median (IQR)[a]	Duration of treatment of 1st iGBS disease in days, median (IQR)	Interval between iGBS episodes in days, median (IQR)[b]
Multiples	32 (5)	1495 (721)	29 (42)	14 (5)	6 (6)
UKROI (n=12)	33 (4)	1755 (426)	9 (29)	14 (2)	2.5 (5)
German/Swiss (n=20)	31 (6)	1220 (743)	48 (58)	16 (5)	7 (3)
Recurrence	31 (10)	1732 (1938)	16 (38)	10 (7)	12.5 (12)
UKROI (n=12)	31 (11)	1705 (2121)	11 (28)	7 (3)	13 (11)
German/Swiss (n=13)	32 (10)	1732 (1895)	28 (43)	14 (5)	9 (8)
Literature Review (n= 86)	31 (11)	1600 (1713)	14 (24)	11 (4)	8 (14)

[a] Multiples: Index case. Recurrence: First iGBS disease episode
[b] Multiples: Interval between siblings developing iGBS. Recurrence: Interval between completion of antibiotics for the first iGBS episode and onset of second iGBS episode for the same infant
[c] Multiple sets >1 sibling affected by iGBS disease

5.4 Discussion

iGBS disease in infants of multiple births and those with recurrence provide intriguing insights as to how a highly virulent bacterial species adapts or fails to do so to its niche. Birth constitutes a major microbial seeding event. Subsequently, a highly dynamic network develops, where microorganisms and immunologically important macromolecules are exchanged between family members. This goes in parallel with neonatal immune development. Typically, GBS reaches its niche in the gut without

health implications for its infant host, and up to 20% of healthy infants are colonised with GBS at two months of age without any consequence.(69)

The work presented here exemplifies what might go wrong in this process. In infants born of a multiple birth, having a sibling with iGBS disease is a significant risk factor, increasing the incidence of iGBS disease in the non-index sibling to 17%, in line with previous reports. (54) The exact mechanisms of GBS transmission in LOGBS are still unclear. There are two main hypotheses. A vertical transmission similar to that involved in EOGBS from genital/gastrointestinal exposure at around the time of birth lowered but not suppressed by IAP can occur, leading to persistent mucosal colonization.(69) Alternatively, a horizontal transmission after delivery can occur through nursing (e.g. from a parent via contaminated hands or other fomites) and possibly through breastfeeding (**Figure 25**). The short interval of onset of iGBS disease in siblings (median of 6 days) occurring many days after birth (median of 29 days) suggests an acute transmission event that infects both siblings at some time after birth. Alternatively, the GBS transmission may have occurred at birth resulting in persistent colonisation of the infant's gastrointestinal tract and later invasion of the bloodstream due to aberrations in this niche (dysbiosis). It is known that GBS can persist at mucosal surfaces even after adequate parenteral antibiotic therapy and cause reinvasion and systemic disease because of biofilm formation.(131,328) The hypothesis of persistent colonisation is supported by the observation that the same serotype typically caused the second episode in recurrent cases in the UKROI cohort and the literature. In keeping with the hypothesis that high GBS virulence promotes recurrence and parallel infections in multiples, sequence type 17 was (when molecular typing performed) the most commonly isolated clone in this study and the literature. However, low numbers preclude any clear association between hypervirulent GBS strains and invasive disease in multiples or recurrence.

More recent attention was given to the role of breast milk in this process. Breast milk is thought to protect against infections through passive transfer of antibodies and promote the development of the infant's immune system.(329) However, it was also hypothesized that it may be the source of infection.(330) The most likely explanation is that breast milk becomes contaminated by GBS through retrograde transmission

from the infant (who is colonised in the nasopharynx) and reinfects the infant in a circular process.(73) Routine screening of breast milk is not recommended, however, and there is no consensus on what to do with high-risk groups (preterm infants, mothers with mastitis) or whether testing is warranted after a first or second episode of GBS.(73) Eradication of GBS colonisation with antibiotics either in the mother or in the infant is recommended by many authors, (294,301,310,312) but there are conflicting reports about its efficacy.(73)

The recurrence rate of iGBS disease is low (1.4% in the UKROI cohort in keeping with previous reports),(257) suggesting that iGBS disease recurrence is usually a sporadic event. Although there is limited evidence in the literature to support a genetic predisposition, a link between GBS recurrence and a primary deficiency of innate immune responses (myeloid differentiation primary response 88 and interleukin 1 receptor-associated kinase 4 deficiency, resulting from single-gene defects) is plausible, at least for a small number of cases where recurrent GBS infection may be part of a broader phenotype that includes susceptibility to multiple pyogenic bacteria.(70,331)

The association between a shorter antibiotic course and increased recurrence risk may relate to a subgroup of insufficiently treated cases rather than being a paradigm for recurrence. The proportion of infants with recurrent iGBS disease that received a short course of antibiotics was disproportionately high in UKROI compared to the German/Swiss case series and the existing literature. Moreover, this result differs from a recent study that did not show any difference in recurrence rate between shortened (≤8 days) and prolonged (>8 days) course of antibiotics among infants with uncomplicated iGBS disease.(332) The recommended ten days of intravenous antibiotics for GBS bacteremia without focus and at least fourteen days for uncomplicated meningitis should be adhered to. (200) Longer therapy might be needed when there is a prolonged or complicated course. Although following the standard practice did not prevent early recurrence in several cases in our study, insufficient duration of antibiotics is a correctable problem and following the recommended guidelines would have reduced the number of recurrent iGBS cases.

(332)

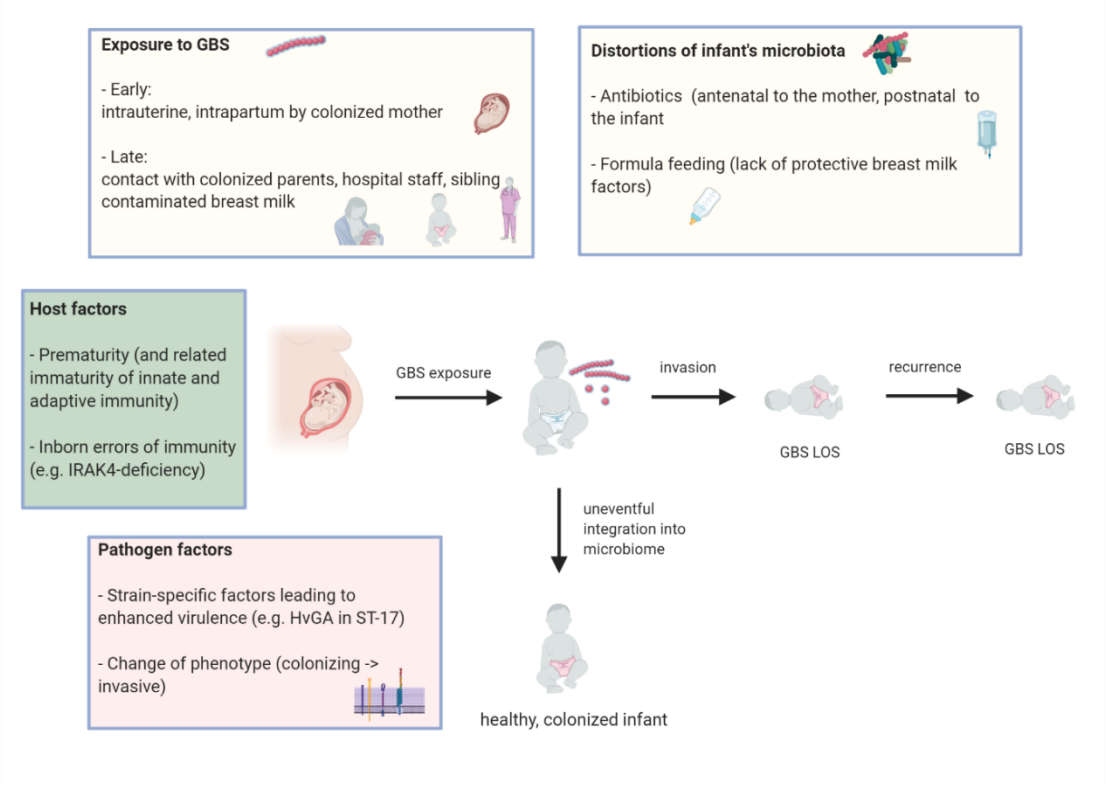


Figure 25. Model of pathogenesis of (recurrent) LOGBS

Several factors (boxes) affect the outcome of postnatal contact of the neonate with GBS: uneventful integration into microbiome vs. invasive GBS infection (Figure was created using Biorender.com).

5.5 Conclusions

The dynamics of recurrent GBS infections or concurrent infections in multiples suggest individual exposure patterns and host immunity fluctuations. As indicated by the short interval between iGBS episodes in affected multiples, GBS can deviate from its usually colonising traits and become highly invasive, spreading across inter-individual boundaries. This occurs with remarkable frequency, e.g. in a sixth of multiple births in which one infant has iGBS disease. Identical GBS sero- and sequence types in recurrent cases and concurrently infected multiples may indicate a "streptococcal niche" at colonising sites, which needs to be demarcated by the immune system and by competing microbes to allow for GBS to become a harmless, metabolically programmed coloniser. Notably, risk factors for recurrence and simultaneous iGBS disease in multiples overlap, and iGBS disease in multiples seems to be a risk factor for recurrence.

Chapter 6 – Antibody Kinetics between Birth and Three Months of Life in Healthy Infants with Natural Exposure to Group B Streptococcus: A UK Cohort Study

6.1 Introduction

Due to the increasing focus on defining a CoP derived from natural exposure to GBS to facilitate vaccine development and licensure, it is important to have accurate estimates of naturally acquired CPS antibody half-lives. (179) It should also take into account any epidemiological and clinical factors that might influence the antibody decay rate over this period. Furthermore, if antibody levels measured in the first weeks of life can be used to accurately back-calculate birth titres, acute sera from LOGBS cases could be used in studies when no birth sera are available.

In this chapter, I analysed data from healthy infants born to women colonised with GBS to estimate the kinetics of anti-GBS CPS IgG between birth and three months of life and to explore factors that might influence the rate of decay, focussing on maternal age, ethnicity, gravida, gestation, and infant sex.

6.2 Methods

6.2.1 Study Population and Design

The samples used in this analysis were collected through the iGBS feasibility study as described in more detail in Chapter 3 (section 3.1.1).

6.2.2 Laboratory methods

6.2.2.1 GBS Identification and Serotyping

As described in Chapter 3 (section 3.2.2 and 3.2.3).

6.2.2.2 Detection of IgG in sera

Serotype-specific anti-GBS CPS IgG concentrations were measured using the GASTON MIA in cord and infant sera, as described in Chapter 3 (section 3.2.4).

6.2.3 Statistical Methods

The serotype-specific antibody decay rate for each infant was calculated as the change in log10 IgG between the infant blood serum and the cord serum, divided by the number of days between the two samples. A mixed effects model with infant-specific random intercepts was used on log10 concentrations at all time points to calculate the mean slope and its 95% CI. The half-life of antibodies with 95% CI was calculated as log10(0.5)/log10 transformed mean slope. I estimated the decay rate of IgG against the serotype that the infants' mother was found to be colonised with (the homologous serotype) as well as IgG against the other five serotypes the mother was not colonised with (non-homologous serotypes). Due to the small number of participants, data from all serotypes were combined to estimate the mean half-life of IgG as the primary analysis, with serotype-specific mean half-lives presented as a supplementary analysis. A value of half the serotype-specific LLOQ was assigned to samples below LLOQ. The LLOQ were 0.004, 0.010, 0.015, 0.012, 0.006 and 0.043 µg/mL for Ia, Ib, II, III, IV and V, respectively.

Some samples were excluded from the primary analysis of half-lives following similar exclusion criteria to those proposed in previous meta-analyses of transplacental antibodies. (333,334) Participants with values below the LLOQ at both time points were excluded since the half-lives cannot be estimated. Participants with increasing antibodies after birth were excluded, as such results give negative half-lives and are likely outliers. Some samples were excluded due to the technical specifications of the assay. Infants born to women colonised with serotype VI, against which the GASTON MIA does not measure IgG, or when the serotype identification failed, were excluded from the analysis of the decay rate against the colonising serotype, but included when titres against non-homologous serotypes were used. To examine the influence of values below LLOQ, a sensitivity analysis of IgG against homologous and non-homologous CPS was performed using only samples with IgG titres >LLOQ at both

time points. The exclusions in this sensitivity analysis also yield a dataset that would best be used to back-calculate birth tires from observed titres above the LLOQ since any results below LLOQ could only be back-calculated to the same level.

To calculate whether decay rates differed by time-invariant covariates such as maternal age, ethnicity, gravida, gestation, and infant sex, I used a mixed effects model, with participant-specific random intercepts, applied to the data from the combined analysis (homologous and non-homologous serotypes) and included the covariate in the model as a fixed effect, along with the covariate-by-day-interaction term. The interaction term *P*-value was used to test if decay rates varied by time-invariant factors.

To evaluate the potential use of titres within 90 days after birth on back-calculating birth titres, a two-step analysis was done based on predicted titres at 30, 60 and 90 days. Predictions were made for all samples with birth titres above LLOQ (homologous and non-homologous serotypes) by using the slope between the observed logged titres for each individual at birth and the time point available and then interpolated or extrapolated this to get the estimated titre at 30, 60 and 90 days. Values below serotype-specific assay LLOQ after birth were given a value of half LLOQ. The first step was to assess the proportion of samples above LLOQ at birth that fell to a predicted level below LLOQ and the proportion of samples below LLOQ that raised above LLOQ at each time point. The same was done using cut-offs of 0.1 µg/mL and 1 µg/mL to evaluate these potential CoP. Where the proportion of samples falling below LLOQ was high (above 10%) at any time point, it was deemed not reliable to back-calculate birth titres from data at this time point. The next step was to estimate the correlations between IgG at birth and the predicted titres from all included samples at each time point. This was done using Spearman's rank correlation coefficient. Pairs of IgG measures with a decline or rise were included as long as at least one time point was above LLOQ as well as restricted to the dataset where predicted levels below LLOQ were excluded. Where a high correlation was achieved and a low proportion of samples falling to below LLOQ and rising from below to above cut-offs, then samples taken at this time point could be used to back-calculate birth titres based on the decay rate.

6.3 Results

The kinetics sub-study of the iGBS feasibility study enrolled 33 healthy infants born to women colonised with GBS who did not develop iGBS disease in the first three months of life (**Figure 26**). Ten infants were enrolled in Group A, ten in Group B, and thirteen in Group C.

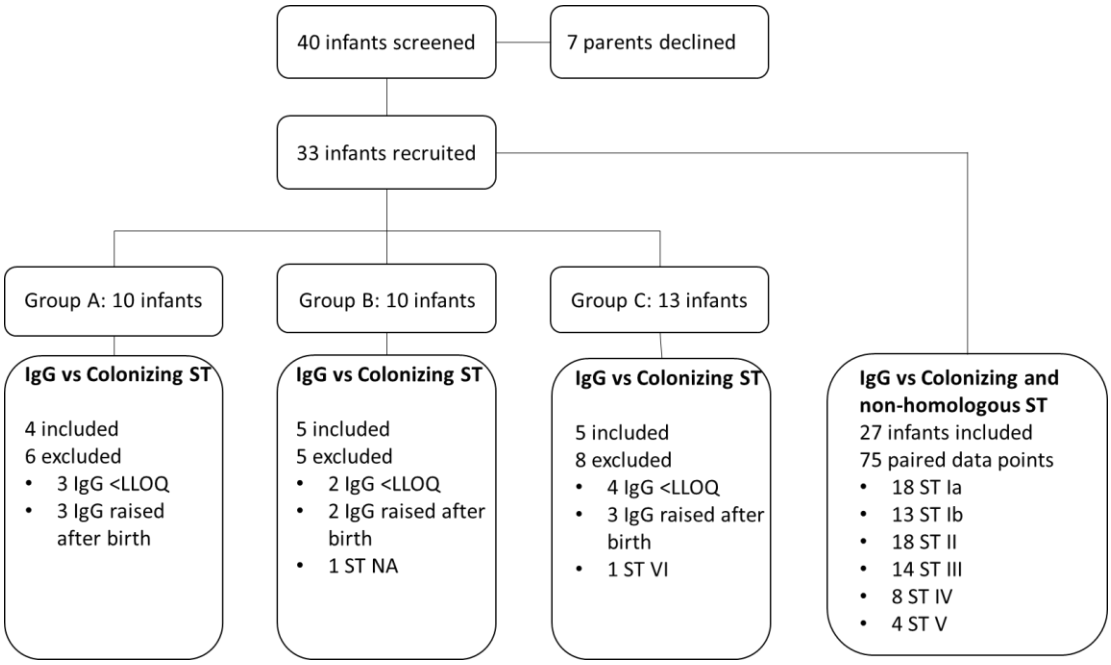


Figure 26. Flow chart of available data and reasons for exclusions from half-life estimations

GBS: Group B *Streptococcus*; ST: Serotype; CPS: capsular polysaccharide; IgG: Immunoglobulin G; LLOQ: lower limit of quantitation; NA: Not available.

Participants' demographic and clinical characteristics are summarised in **Table 18**. The median gestational age was 39 (range 35-42) weeks, with only two infants born <37 weeks. The median maternal age was 34 years, with the youngest aged 22 and the oldest 43 years. Most infants were of white ethnicity (19; 58%). Rectovaginal swabs were collected at a median gestation of 38 (range 35-42) weeks.

Table 18. Demographics and clinical characteristics of participating infants and their mothers

	Group A	Group B	Group C	Total
Maternal age, median (range)	32 (26-43)	37 (22-43)	35 (27-43)	34 (22-43)
Ethnicity, n (%)				
Asian or Asian British	1 (10%)	0 (0%)	3 (23.1%)	4 (12.1%)
Black or Black British	0 (0%)	1 (10%)	2 (15.4%)	3 (9.1%)
White British	5 (50%)	4 (40%)	3 (23.1%)	12 (36.4%)
White Other	2 (20%)	3 (30%)	2 (15.4%)	7 (21.2%)
Other ethnic group	2 (20%)	2 (20%)	3 (23.1%)	7 (21.2%)
Gravida, n (%)				
1	5 (50%)	8 (80%)	6 (46.2%)	19 (57.6%)
2	2 (20%)	0 (0%)	0 (0%)	2 (6.0%)
3	0 (0%)	2 (20%)	3 (23.1%)	5 (15.2%)
4	0 (0%)	0 (0%)	4 (30.8%)	4 (12.1%)
>4	3 (30%)	0 (0%)	0 (0%)	3 (9.1%)
Rupture of membranes, n (%)				
>= 18 hours	3 (30%)	2 (20%)	4 (30.8%)	9 (28.1%)
Delivery type, n (%)				
Vaginal	7 (70%)	2 (20%)	5 (38.5%)	14 (42.4%)
C-Section with rupture of membranes	1 (10%)	4 (40%)	3 (23.1%)	8 (24.2%)
C-Section without rupture of membranes	2 (20%)	4 (40%)	5 (38.5%)	11 (33.3%)
Gestation at birth, median (range)	39 (36-41)	39 (35-41)	39 (38-42)	39 (35-42)
Gestation when swab collected, median (range)	36 (35-39)	37 (35-41)	38 (35-42)	38 (35-42)
Infant sex, n (%)				
Female	5 (50%)	3 (30%)	8 (61.5%)	16 (48.5%)
Intrapartum antibiotics, n (%)				
Yes	6 (60%)	8 (80%)	10 (76.9%)	24 (72.7%)
Serotype Distribution				
Ia	3 (30%)	2 (20%)	4 (30.8%)	9 (28.1%)
Ib	0 (0%)	1 (10%)	3 (23.1%)	4 (12.5%)
II	1 (10%)	2 (20%)	3 (23.1%)	6 (18.8%)
III	5 (50%)	2 (20%)	2 (15.4%)	9 (28.1%)
IV	0 (0%)	1 (10%)	0 (0%)	1 (3.1%)
V	1 (10%)	1 (10%)	0 (0%)	2 (6.2%)
VI	0 (0%)	0 (0%)	1 (7.7%)	1 (3.1%)
NA	0 (0%)	1 (0%)	0 (0%)	1 (3.1%)

Group A: Blood collection day 21-35, Group B: Blood collection day 49-63, Group C: Blood collection day 77-91

The serotype-specific anti-GBS CPS IgG concentrations in cord and infant blood against the colonising serotype were plotted for each infant (**Figure 27A-F**). There were nine (27%) paired samples of cord and infant blood with both IgG values below LLOQ. In eight (24%) participants, IgG values increased between birth and the time of infant blood collection, five of whom had birth levels below the LLOQ. After these data were excluded, 14 paired samples were used to calculate the antibody decay rate against the colonising serotype. Of these 14 samples, six infants were exposed to serotype Ia (43%), four to serotype III (29%), two to serotype II (14%), one to serotype Ib (7%), and one to serotype IV (7%). The half-life of transplacental maternal IgG antibodies was 27.9 (95% CI: 19.9-46.2) days (**Figure 28A**).

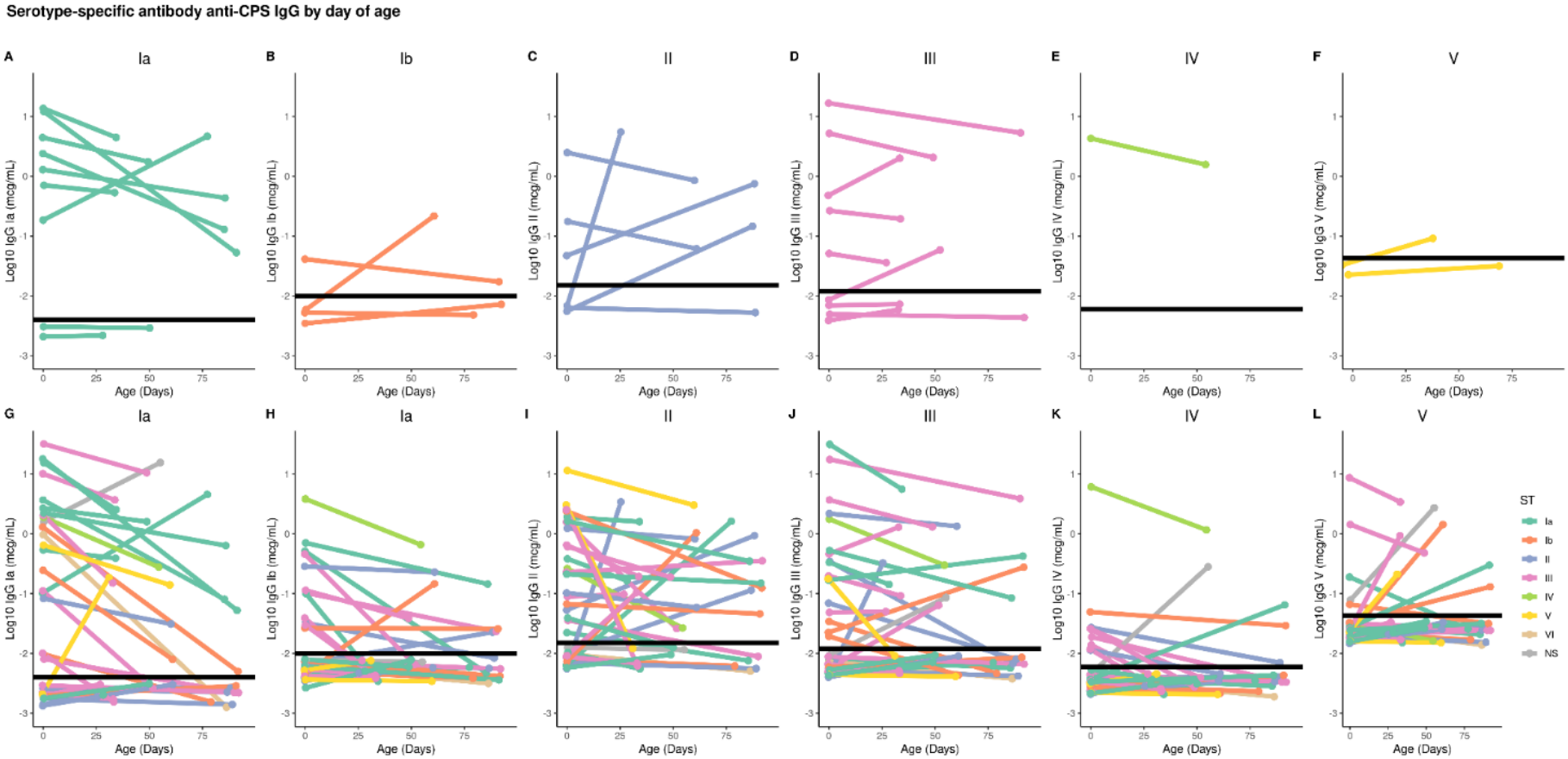


Figure 27. Serotype-specific anti-GBS CPS IgG (expressed as log10) by day of age

(A) Homologous IgG responses to ST Ia. (B) Homologous IgG responses to ST Ib. (C) Homologous IgG responses to ST II. (D) Homologous IgG responses to ST III. (E) Homologous IgG responses to ST IV. (F) Homologous IgG responses to ST V. (G) Homologous and non-homologous IgG responses to ST Ia. (H) Homologous and non-homologous IgG responses to ST Ib. (I) Homologous and non-homologous IgG responses to ST II. (J) Homologous and non-homologous IgG responses to ST III. (K) Homologous and non-homologous IgG responses to ST IV. (L) Homologous and non-homologous IgG responses to ST V. Colours depict the colonising ST.

Black line shows the LLOQ for each serotype. Values below LLOQ were plotted as LLOQ/2. (Ia: 0.002 µg/mL, Ib: 0.010 µg/mL, II: 0.008 µg/mL, III: 0.006 µg/mL, IV: 0.003 µg/mL, V: 0.021 µg/mL). Values below LLOQ were jittered to avoid overplotting. GBS: Group B *Streptococcus*; ST: Serotype; CPS: capsular polysaccharide; IgG: Immunoglobulin G; LLOQ: lower limit of quantitation.

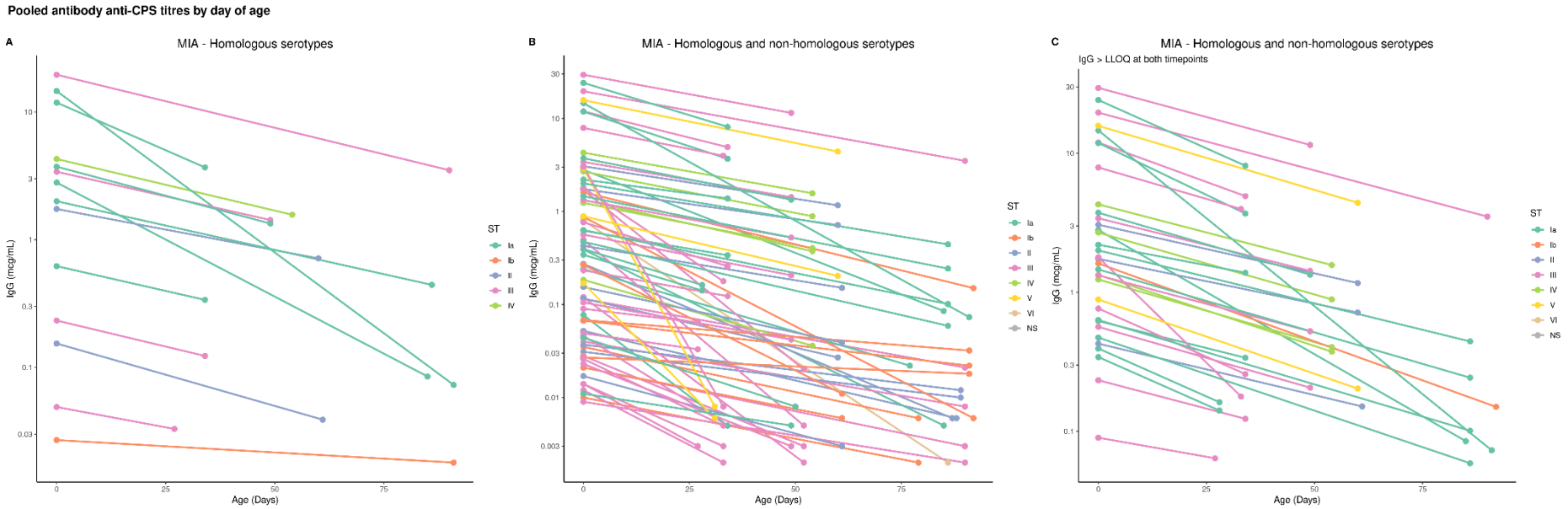


Figure 28. Pooled antibody anti-CPS titres by day of age

(A) Pooled placentally transferred anti-GBS CPS IgG by day of age against colonising serotype for paired samples with decline of antibodies over time. (B) Pooled placentally transferred anti-GBS CPS IgG by day of age for colonising and non-homologous serotypes for paired samples with decline of antibodies over time. (C) Pooled placentally transferred anti-GBS CPS IgG by day of age for colonising and non-homologous serotypes for paired samples with decline of antibodies over time and IgG >LLOQ at both time points. Colours depict the colonising ST. GBS: Group B *Streptococcus*; ST: Serotype; CPS: capsular polysaccharide; IgG: Immunoglobulin G; LLOQ: lower limit of quantitation.

When IgG against non-homologous CPS was included, 76 pairs of IgG measures from 30 infants were used to estimate the half-life of IgG (**Figure 26**). There were 95 (48%) paired cord and infant blood samples with both values below LLOQ (+21% vs colonising serotype analysis; *P* = 0.04), and 27 (14%) pairs with raised IgG measures after birth (-11% vs colonising serotype analysis; *P* = 0.19). The individual slopes for all paired data points are shown in **Figure 27G-L**. Half-lives were 17.7 (14.1-23.6), 27.7 (17.9-61.5), 24.6 (15.3-62.3), 26.4 (20.1-38.4), 27.7 (19.4-48.3) and 34.0 (19.4 - 134.9) days for serotypes Ia, Ib, II, III, IV, V, respectively (**Figure 29**). When all paired results were pooled together, the half-life of IgG was 23.3 (19.8-28.2) days (**Figure 28B**). In the sensitivity analysis, the estimation of IgG half-life was limited to 34 paired data points with both time points >LLOQ and no rise after birth. The half-life was 27.4 (23.5-32.9) days (**Figure 28C**). A Cleveland plot summarising the results of the primary and sensitivity analyses is shown in **Figure 30**. The decay rate of antibodies did not vary by maternal age (*P* = 0.7), ethnicity (*P* = 0.1), gravidity (*P* = 0.1), gestational age (*P* = 0.7) or infant sex (*P* = 0.1).

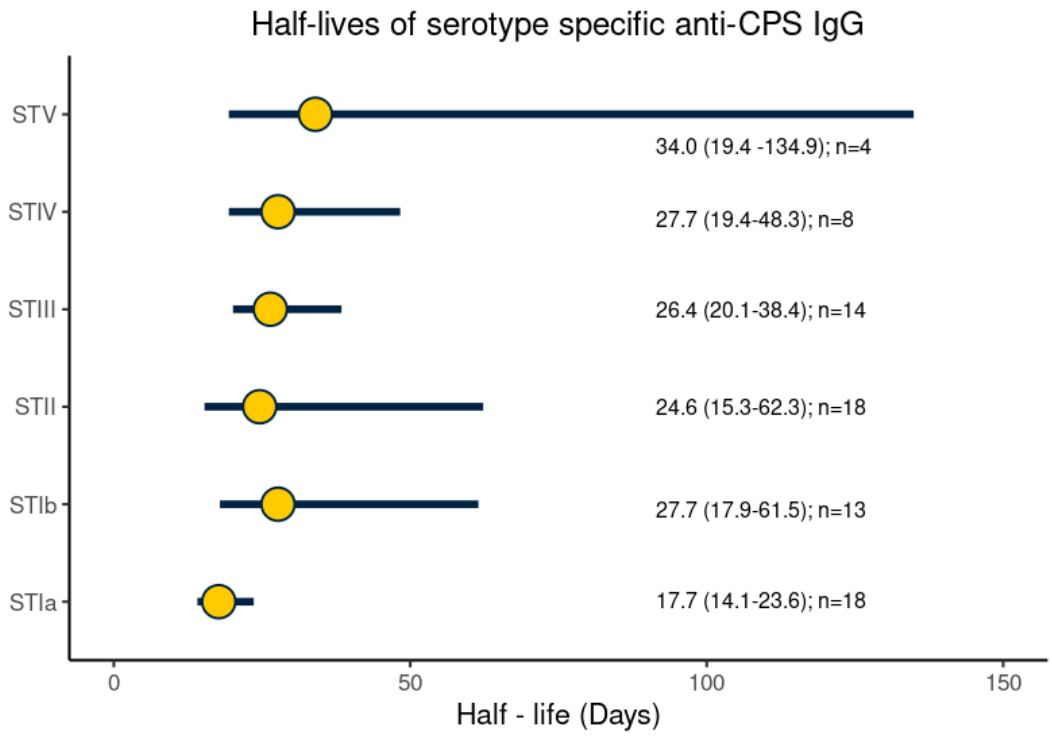


Figure 29. Half-lives of serotype-specific transplacental antibody with point estimate and 95% CI based on IgG against colonising and non-homologous serotypes

GBS: Group B *Streptococcus*; ST: Serotype; CPS: capsular polysaccharide; IgG: Immunoglobulin G; CI: confidence interval.

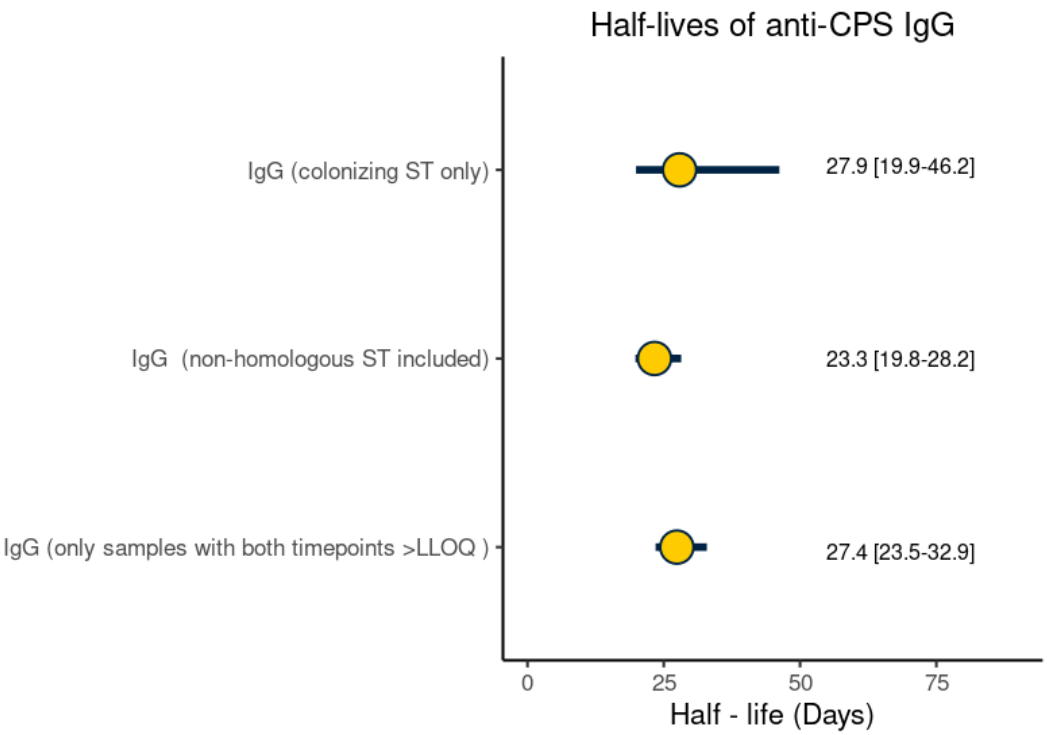


Figure 30. Half-lives of transplacental antibody with point estimate and 95% CI based on IgG against colonising serotype, against colonising and non-homologous serotypes, against colonising and non-homologous serotypes only when IgG >LLOQ at both time points

GBS: Group B *Streptococcus*; ST: Serotype; CPS: capsular polysaccharide; IgG: Immunoglobulin G; CI: confidence interval.

In the correlation analysis, only a small proportion (<10%) of samples with detectable antibodies at birth had predicted titres below LLOQ on day 30. In contrast, approximately a third of samples were predicted to fall below LLOQ on day 60, and two-thirds below LLOQ on day 90 (**Figure 31A**). Similarly, over 95% of samples with birth titres of ≥0.1 µg/ml and ≥1 µg/ml, were predicted to have detectable values on day 30, 75-85% on day 60 and only around 33% on day 90 (**Figure 31A**). Notably, predicted titres raised above the LLOQ in approximately one-sixth of samples with undetectable birth titres, a small but not insignificant proportion (**Figure 31B**). A strong correlation was observed between IgG concentrations at birth and day 30 (r = 0.70 (95% CI: 0.59-0.79)), but not day 60 (r = 0.25 (0.06-0.42)) and day 90 (r = -0.21 (-0.39-0.02)) (**Figure 32A-C**). When the analysis was restricted to day 30 and predicted levels below LLOQ were excluded, the correlation coefficient was 0.71 (0.60-0.80) (**Figure 32D**).

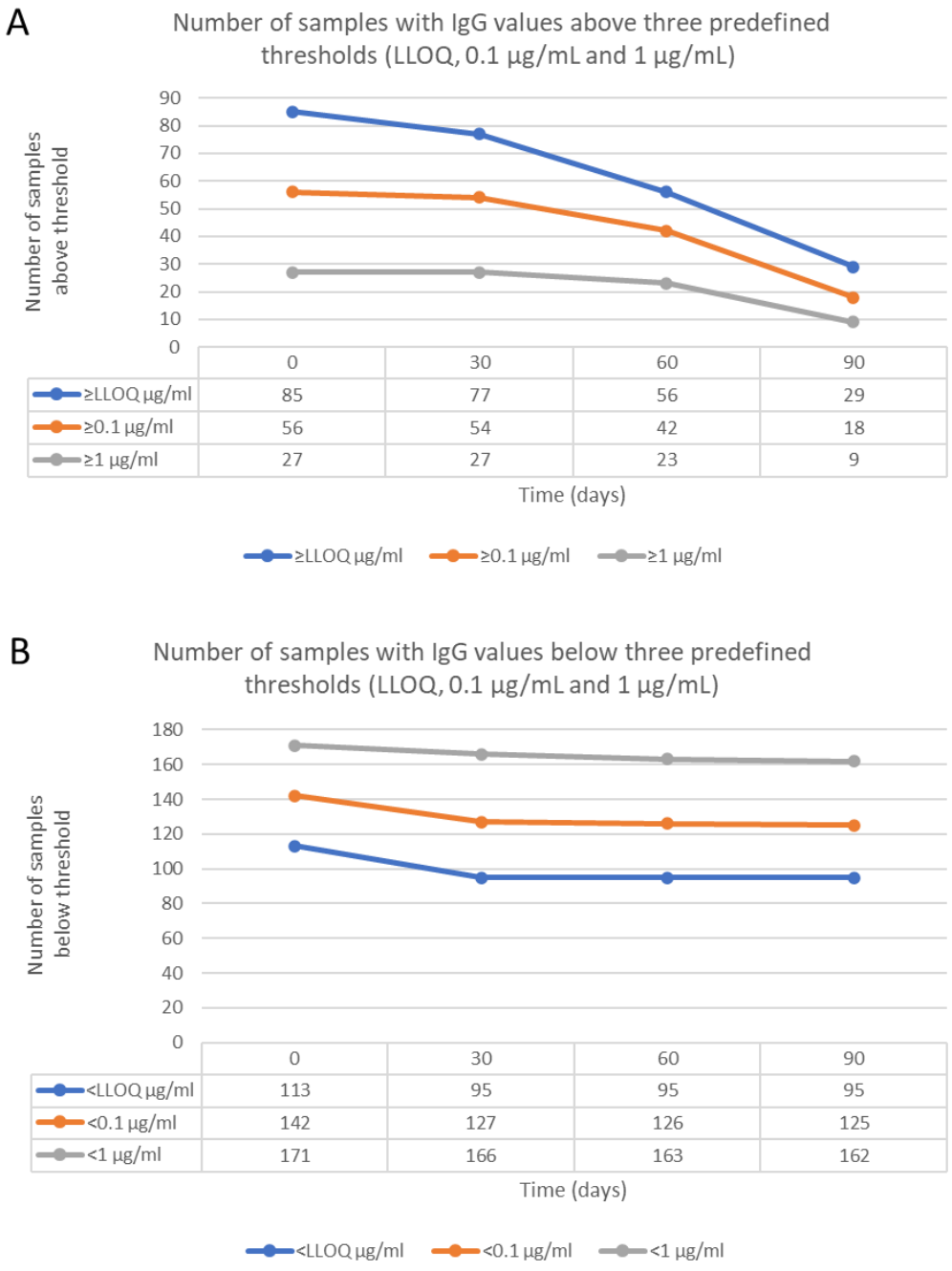


Figure 31. Prediction of samples with IgG values below or above predefined thresholds

(A) Number of samples with IgG values below three predefined thresholds (LLOQ, 0.1 µg/mL and 1 µg/mL) at birth that were predicted to remain below each threshold at three time points (day 30, 60, 90 of age) (B) Number of samples with IgG values above three predefined thresholds (LLOQ, 0.1 µg/mL and 1 µg/mL) at birth that were predicted to remain above each threshold at three time points (day 30, 60, 90 of age)

Serotype specific lower limit of quantitation (LLOQ): Ia: 0.002 µg/mL, Ib: 0.010 µg/mL, II: 0.008 µg/mL, III: 0.006 µg/mL, IV: 0.003 µg/mL, V: 0.021 µg/mL

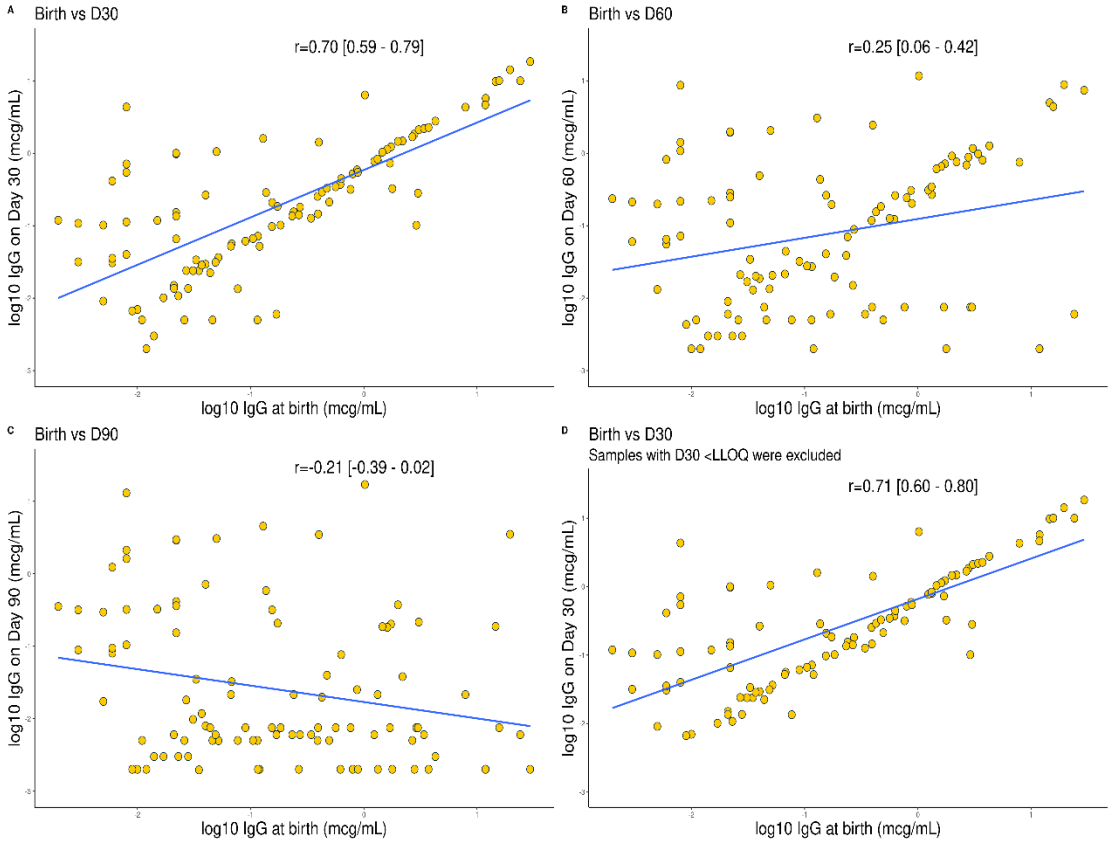


Figure 32. Comparison of log10 placentally transferred anti-GBS CPS IgG at birth and day 30, 60 and 90 of age

(A) Comparison of log10 placentally transferred anti-GBS CPS IgG at birth and day 30 of age for the homologous and non-homologous ST. (B) Comparison of log10 placentally transferred anti-GBS CPS IgG at birth and day 60 of age for the homologous and non-homologous ST. (C) Comparison of log10 placentally transferred anti-GBS CPS IgG at birth and day 90 of age for the homologous and non-homologous ST. (D) Comparison of log10 placentally transferred anti-GBS CPS IgG at birth and day 30 of age for the homologous and non-homologous ST, after exclusion of samples with a value <LLOQ on day 30. Statistical significance assessed by Spearman correlation. X axis scale differs among plots. GBS: Group B *Streptococcus*; CPS: capsular polysaccharide; IgG: Immunoglobulin G; LLOQ: lower limit of quantitation.

6.4 Discussion

This study provides the first estimates of the half-life of placentally transferred anti-GBS CPS IgG concentrations in healthy infants following natural exposure to GBS. The half-life of IgG against the colonising serotype was 27.9 (95% CI: 19.9-46.2) days. When IgG against non-homologous CPS was added, the half-life was 23.3 (19.8-28.2) days. Including IgG against non-homologous serotypes allowed for a larger number of samples to be included and showed equivalent results to the colonising serotype analysis with narrower confidence intervals.

The presence of antibody responses against heterologous GBS serotypes has been previously reported in the literature. A prospective study in the USA of 1787 pregnant women to estimate the maternal prevalence of naturally acquired, serotype-specific anti-GBS CPS IgG using the GASTON MIA found that 11% of the participants had antibodies against all serotypes, 5% had no antibody to any serotype, and the majority had antibodies against more than one serotypes. (336) Similarly, in the Phase 1/2 study of the GBS6 vaccine candidate that used the same assay, most of the 354 participants had pre-vaccination antibody concentrations above the LLOQ against more than one serotypes. (175)

The antibody titres against the heterologous serotypes are unlikely to be explained as measurement errors attributed to sub-optimal specificity of the assay. The GASTON MIA uses six sets of spectrally distinct magnetic polystyrene microspheres coupled to GBS CPS-PLL conjugates and a reference standard that has been cross-standardised and calibrated using serotype-specific monoclonal antibodies. (175,337)During the standardisation process the cross-reactivity index against non-target surface was virtually zero, permitting comparison of IgG levels across serotypes. (337)

It is plausible that many women may have had multiple colonisation events due to different serotypes and this may explain some of the heterologous responses seen. GBS colonisation changes over time, but serotype-specific anti-GBS CPS IgG tend to remain stable. (195) Only a few previous studies were designed to assess the longitudinal patterns of rectovaginal GBS carriage. (195,338–342) A Danish study that followed 77 pregnant women from 16 weeks gestation until one year after delivery

found that 28% of women were persistent carriers over this 2-year period, whereas almost a fifth (19%) were intermittent carriers. (341) A SA study of 507 pregnant women with serial rectovaginal swabs collected at four time points between 20 weeks and 37+ weeks of gestation reported that 15% of participants were persistent carriers, 50% permanent no-carriers and the rest 35% had fluctuating culture positivity. (342) A recent re-analysis of this study using a Bayesian model estimated a higher proportion of persistent carriers. (343) Interestingly, although colonisation appears dynamic over time, serum and mucosal (vaginal) anti-GBS CPS IgG remained stable in a study of 70 non-pregnant women. (195) The question of whether specific patterns of carriage are associated with a higher risk of neonatal disease warrants further prospective studies with more frequent sampling that use both culture and molecular biology techniques and combine longitudinal microbiological and immunological data.

Another possible explanation is co-colonisation with more than one serotype. (344) The prevalence of multiple serotype carriage is probably under-recognised, as it requires laboratory techniques that allow for multiple colony selection. (345)

When the analysis of IgG against homologous and non-homologous serotypes was restricted to paired data points with both time points above LLOQ, the half-life was 27.4 (23.5-32.9) days. Since the exact time IgG fell below LLOQ is unknown, the estimated half-life from paired samples that fall below the LLOQ might be less accurate, introducing a bias to the analysis. In this sense, the estimate of 27.4 days from the sensitivity analysis provides a better estimate of the antibody decay and could be used to back-calculate birth titres from an infant sample that is above the LLOQ. Given that half of LOGBS cases present in the first month of life and three quarters by six weeks of age, (346) it is important that antibodies are still detectable at 30 days.

The point estimates in this naturally-acquired antibody study were comparable but shorter than the previously reported half-life of vaccine-derived anti-GBS CPS IgG by a magnitude of one to two weeks. A double-blind, placebo-controlled trial of a monovalent serotype III CPS-tetanus toxoid conjugate vaccine reported a half-life of serotype III specific IgG of 35 days. (164,346) Half-lives of IgG against serotypes Ia, Ib,

and III following immunisation with a trivalent non-adjuvanted CRM_{197}-conjugated GBS vaccine were 39-46 days, depending on the serotype. (170) However, corresponding confidence intervals were not provided in the GBS vaccine studies above; therefore, it is unknown whether these differences are statistically significant. In addition, the calculations used to derive these estimates are not described in detail; therefore it is unclear whether there are any methodological differences that would account for these differences. Similar estimates to those seen with GBS have been reported for half-lives of other antigen-specific antibodies following maternal vaccination. A recent meta-analysis of placentally transferred antibodies against diphtheria toxoid, tetanus toxoid, fimbriae, pertussis toxoid, filamentous haemagglutinin, and pertactin antigens reported half-lives between 29 and 35 days. (334) A previous study of trivalent inactivated influenza vaccine in pregnancy showed half-lives of 45, 44, and 43 days against H1N1, H3N2, and B/Victoria strains, respectively. (347)

Less is known about the kinetics of passively acquired antibodies in infants secondary to natural exposure. Pawlowski et al. estimated the half-life of naturally acquired IgG against the N-terminal domains of the GBS *α*C and Rib in paired cord and infant blood samples one month after delivery to be 40 and 38 days, respectively, although no confidence intervals were reported. (178) In studies of pneumococcal and meningococcal A and C antibodies, the half-lives were 43, 43, and 40 days. (333) The half-life of neutralising antibodies against respiratory syncytial virus was 38 days in a study of 149 asymptomatic mother-infant pairs in Bangladesh. (348) These differences could be attributed to different target antigens and assays or reflect differences between natural immunity and vaccine-induced immunity, for example in IgG subclass distribution.

In this cohort of healthy infants born >35 weeks gestation, I did not find any factors that affected the decay rate of placentally transferred anti-GBS CPS IgG. Although these results might have been affected by the small sample size, they are in line with those of previous studies. (333,334) Of note, preterm birth is associated with a lower concentration of placentally transferred maternal IgG at birth due to differences in

the expression of the FcRn, (86) but this does not necessarily impact decay rates after birth. (333,334)

Several GBS vaccine candidates are currently in development. (143) Due to the complexities of a vaccine efficacy trial with a clinical primary endpoint, a CoP against iGBS disease could facilitate the licensure of such a vaccine. (179) This would be derived from studies of natural immunity estimating antibody concentrations associated with protection against iGBS disease. The consensus within the scientific community is that infant serum is the preferable source for IgG measurement for this purpose. (349)

As placental antibody transfer of anti-GBS CPS IgG is not 100% and even less in immunosuppressed groups like HIV-infected women, antibody concentration in the cord or infant serum is preferred over maternal serum to predict a CoP against iGBS disease in infants. (84) Given that the CoP should be predictive of infant disease; ideally, cord or infant serum collected at birth should be used to estimate antibody levels in the infant, as IgG levels measured after confirmation of iGBS may be low as a result of "consumption/absorption" or high as a result of an early immune response. It is biologically plausible to assume that IgG at the onset of the EOGBS would reflect the levels at birth as long as the sample was collected shortly after the disease onset. However, the comparability between cord blood and post-disease onset samples in the context of LOGBS warrants more careful consideration. A recent SA seroepidemiological study using the GASTON MIA as iGBS3 found that anti-GBS CPS IgG GMC for EOGBS and LOGBS cases caused by serotype Ia was 0.002 (95% CI 0.001 - 0.006) µg/mL in the cord (n=4) and 0.012 (95% CI 0.003 - 0.056) µg/mL in acute infant sera collected within 72 hours of disease onset (n=14). For serotype III, GMC was 0.012 (95% CI 0.005 - 0.028) µg/mL and 0.010 (95% CI 0.006 - 0.019) µg/mL; for cord (n=15) and acute sera (n=30), respectively. (182) Given that this is the largest seroepidemiological study using infant sera (cord or acute) to date, even in the absence of a formal statistical comparison, it appears that antibody levels in post-disease onset samples are comparable to cord sera for both EOGBS and LOGBS caused by the two most common serotypes.

In the present chapter, the correlation analysis indicated that it is possible to use infant sera with IgG titres over LLOQ that were collected up to 30 days after birth to predict titres at birth using the estimated half-life from the sensitivity analysis. Back-calculating was less accurate beyond day 30, since a significant proportion of samples declined below LLOQ on day 60 and especially day 90, even when started with a fairly high level at birth.

Notably, a significant proportion of participants showed rising IgG levels after birth. A possible explanation is that mother-to-infant transmission of GBS may occur after delivery. Based on studies in Italy, France, the Gambia, and Japan, between a fifth and a quarter of infants become colonised with the same strain as their mothers after hospital discharge, despite adequate IAP and negative GBS screening at birth. (21,69,131,196) Clonal complex CC17 is particularly linked to an enhanced post-delivery mother-to-infant transmission compared to other serotypes (OR, 2.45; 95% CI, 1.02–5.88). (69) In studies with juvenile mice, intestinal colonisation with GBS induces an endogenous IgG response within 20 days of exposure. (350) Ongoing exposure to GBS after birth could potentially induce the production of antibodies in human infants, but the evidence for this is lacking. (351) Although not statistically significant, the lower proportion of samples with an unexpected rise against the non-colonising compared to the colonising serotype in this study supports this hypothesis, as it is biologically plausible that the antibodies produced secondary to postnatal exposure will target the colonising serotype. Another hypothesis that warrants further study is the anti-GBS IgG transfer through breastfeeding. Although colostrum-derived antibodies can pass into serum in rodents and ungulates, the current evidence is that mucosal barriers close at birth in primates, including humans, preventing breast milk antibodies from transferring systemic immunity. (352)

In any case, these findings from healthy infants exposed to GBS need to be validated in actual cases of infant disease. iGBS3 is still underway to determine the correlation between cord and acute phase IgG in infants with iGBS disease. (353) If half-lives in cases and controls look similar, combining these data might increase power for the back-prediction.

This study has some limitations. First, it recruited a relatively small number of participants. Recruitment was affected by the restrictions implemented due to the coronavirus disease (COVID-19) pandemic, which led to the early closure of the study. The imbalance in the size of the three groups reflects the methodological limitations of simple randomisation when the number of participants is small. I partially mitigated the problem of the small number of participants by including IgG against non-homologous CPS that allowed for analysis of a larger dataset. However, I could not estimate a serotype-specific decay rate with meaningful corresponding confidence intervals. It is plausible that differences might exist between serotypes, even if these are only a few days. (333,334) Second, the generalisability of my findings might be limited by the fact that all infants in this study were born after 35 weeks of gestation and the cohort predominantly consisted of white ethnic groups from a single country. However, a previous systematic review found that antibody decay rates did not differ by infant gestational age or World Bank income categories for non-CPS antigens. (334) Third, IgG was measured at two time points for each participant. A third time point might have contributed to a better understanding of the antibody kinetics, although it is unlikely that a linear decline between birth and three months of age would have changed. Fourth, I have not collected infant or maternal colonisation data after birth. Paired maternal and infant swabs and breast milk might have allowed us to understand the dynamics of GBS colonisation and IgG consistency of breast milk in conjunction with the infant antibody kinetics and inform hypotheses about their interplay. Fifth, a high number of samples had IgG values below LLOQ. Starting with lower IgG values at birth meant that many samples were below the LLOQ at the second time point. Since the exact time IgG fell below LLOQ is unknown, the estimated half-life for these paired samples is possibly overestimated. I mitigated this issue by performing a sensitivity analysis, including only samples with both time points above LLOQ. However, many paired samples had both time points below LLOQ and were excluded from the calculations. This problem was exacerbated for certain serotypes (e.g. serotype V) where the assay LLOQ was much higher than other serotypes. The overall issue of defining a negative sample is being addressed within a separate analysis conducted by the GASTON consortium using IgG-free sera.

6.5 Conclusion

These results provide a basis for future investigations into using antibody kinetics in defining a CoP against LOGBS. In combination with clinical trials, such an approach could facilitate the licensure of a GBS vaccine to prevent both EOGBS and LOGBS

Chapter 7 – Quantitative Analysis of Naturally Acquired Immunoglobulin G to Group B Streptococcal Capsular Polysaccharides from UK seroepidemiological studies

7.1 Introduction

In this chapter, I compare the concentrations of serotype-specific anti-GBS CPS IgG in maternal/cord/infant sera among cases of iGBS disease, and healthy infants exposed and non-exposed to GBS at delivery through maternal colonisation recruited in the UK seroepidemiological studies.

7.2 Methods

7.2.1 Study Population and Design

The samples used in this analysis were collected through the iGBS feasibility and iGBS3 studies as described in more detail in Chapter 3 (section 3.1).

The three comparator groups were defined as follows:

Cases: Infants aged 0 to 89 days with iGBS disease (**Table 1**).

Colonised women: Women with a positive GBS rectal, vaginal or rectovaginal swab at any point from 35 weeks gestational age until delivery, whose babies did not develop iGBS disease in the first 89 days of life.

Non-colonised women: Women with a negative GBS rectal, vaginal or rectovaginal swab from 35 weeks gestational age until delivery, whose babies did not develop iGBS disease in the first 89 days of life.

7.2.2 Laboratory Methods

Serotype-specific anti-GBS CPS IgG concentrations were measured using the GASTON MIA in cord and infant sera, as described in Chapter 3 (section 3.2.4). IgG was measured against the invasive serotype for cases and the colonising serotype for controls (the homologous serotype) as well as against the other five serotypes (heterologous serotypes).

7.2.3 Statistical Methods

Descriptive statistics were calculated for all iGBS feasibility study participants with cord blood obtained. The prevalence of GBS colonisation was expressed as a proportion of the total number of participants. Univariable and multivariable logistic regression was performed to assess the association between individual risk factors and maternal colonisation status. A multivariable model was chosen based on AIC. (280)

Demographic and clinical data were summarised across iGBS feasibility and iGBS3 studies for cases, colonised and non-colonised participants and differences were assessed by Fisher exact test or Kruskall Wallis test. Serotype-specific anti-GBS CPS IgG concentrations in infant blood samples with EOGBS or LOGBS collected within ten days of the onset of disease were compared to cord blood samples from healthy infants born to women antenatally colonised with GBS and women non-colonised with GBS. Each colonised woman was matched to two non-colonised women recruited within eight weeks before or after the colonised woman was recruited. IgG geometric means concentrations (GMC) were calculated for cases (against the invasive (homologous) serotype and the other five (non-homologous) serotypes), colonised (against the colonising (homologous) serotype and the other five (non-homologous) serotypes) and non-colonised women (against all six serotypes) and comparisons made by the Wilcoxon rank sum test. Serotype-specific anti-GBS CPS IgG concentrations in paired acute infant/maternal blood samples from EOGBS and LOGBS cases were compared using the Wilcoxon signed-rank test. A P-value ≤ 0.05 was considered to be statistically significant.

To assess whether demographic and clinical factors (maternal age, maternal ethnicity, gestational age, maternal co-morbidities, immunosuppressive medication, GBS colonisation in previous pregnancy) were associated with antibody concentrations, comparisons made by Kruskall Wallis method or Wilcoxon rank sum test. A *P*-value ≤ 0.05 was considered to be statistically significant. Statistical analyses were done with R studio (version 3.6.3).

7.3 Results

7.3.1 iGBS Feasibility Study Population

The total number of participants enrolled to GBS colonisation screening and cord blood collection for the iGBS feasibility study was 1283 across the three sites. The demographic and clinical risk factors of these women and their infants are summarised in **Table 19**.

Table 19. Demographics and clinical risk factors for all study participants in the iGBS feasibility study

	Site A n = 507	**Site B** n = 108	**Site C** n = 668	**Total** n = 1283
Maternal age group, n	507	108	665	1280
<= 25	29 (5.7%)	7 (6.5%)	55 (8.3%)	91 (7.1%)
26-30	87 (17.2%)	36 (33.3%)	117 (17.6%)	240 (18.8%)
31-35	216 (42.6%)	36 (33.3%)	288 (43.3%)	540 (42.2%)
36-40	145 (28.6%)	22 (20.4%)	164 (24.7%)	331 (25.9%)
>40	30 (5.9%)	7 (6.5%)	41 (6.2%)	78 (6.1%)
Ethnicity, n	281	108	440	829
White British	164 (58.4%)	9 (8.3%)	225 (51.1%)	398 (48.0%)
White Other	66 (23.5%)	19 (17.6%)	79 (18.0%)	164 (19.8%)
Black or Black British	7 (2.5%)	8 (7.4%)	27 (6.1%)	42 (5.1%)
Asian or Asian British	27 (9.6%)	60 (55.6%)	51 (11.6%)	138 (16.6%)
Mixed	7 (2.5%)	3 (2.8%)	8 (1.8%)	18 (2.2%)
Other ethnic group	9 (3.2%)	9 (8.3%)	49 (11.1%)	67 (8.1%)
Parity, n	507	108	668	1283
Primiparous	293 (57.8%)	50 (46.3%)	402 (60.2%)	745 (58.1%)
GBS colonisation in previous pregnancy, n	197	70	136	403

Yes	30 (15.2%)	10 (14.3%)	26 (19.1%)	66 (16.4%)
Previous child with iGBS, n	58	89	125	272
Yes	1 (1.1%)	0 (0%)	2 (1.6%)	3 (1.1%)
Positive GBS antenatal swab, n	495	108	658	1261
Yes	88 (17.8%)	9 (8.3%)	122 (18.5%)	219 (17.4%)
Rupture of membranes, n	503	107	633	1243
>= 18 hours	94 (18.7%)	9 (8.4%)	149 (23.5%)	252 (20.3%)
Delivery type, n	503	107	645	1255
Vaginal	312 (62.0%)	58 (54.2%)	460 (71.3%)	830 (66.1%)
C-Section with ROM	86 (17.1%)	29 (27.1%)	88 (13.6%)	203 (16.2%)
C-Section without ROM	105 (20.9%)	20 (18.7%)	97 (15.0%)	222 (17.7%)
Gestation age, n	503	107	644	1254
Median (range)	39 (35-43)	39 (36-42)	40 (35-44)	39 (35-44)
<37 weeks	13 (2.6%)	1 (0.9%)	10 (1.6%)	24 (1.9%)
Infant sex, n	368	107	485	960
Female	177 (48.1%)	51 (47.7%)	239 (49.3%)	467 (48.6%)
Intrapartum antibiotics, n	417	107	440	964
Yes	153 (36.7%)	6 (5.6%)	141 (32%)	300 (31.1%)

7.3.2 GBS Colonisation in Pregnant Women from the iGBS Feasibility Study: Serotype Distribution and Risk Factors

A total of 219 (17%) women were antenatally colonised with GBS. Colonisation rates were similar between Site A (18%) and C (19%), but were significantly lower in Site B (8%; *P* = 0.03) (**Table 19**). 163 (74%) GBS strains were successfully serotyped by PCR: 46 serotype III (21%), 36 serotype Ia (16%), 33 serotype II (15%), 24 serotype V (11%), 11 serotype Ib (5%), ten serotype IV (5%) and three serotype VI (1%). The isolate was missing from 29 (13%) women and 25 (11%) strains were not serotyped because the cord blood was not collected. Eighty-seven serotyped strains were from Site C, 70 from Site A, and six from Site C. Serotype distribution varied by study site (**Figure 33**).

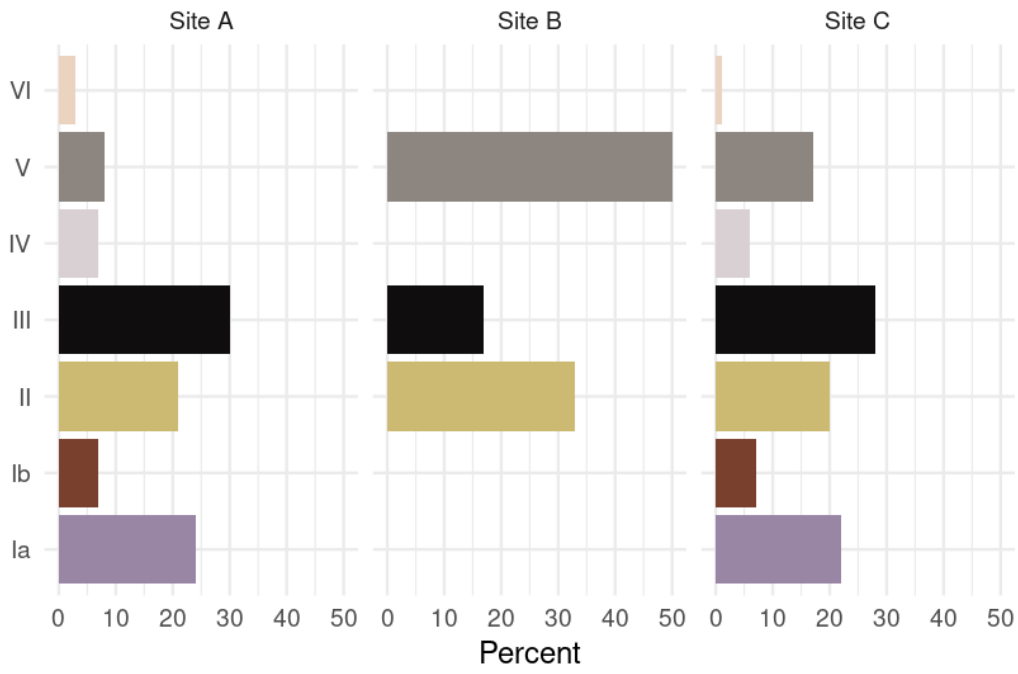


Figure 33. Distribution of GBS serotypes causing maternal colonisation, by study site

Cord blood was collected from 148 women colonised with a serotype GBS strain and 675 women not colonised with GBS. Demographic and clinical characteristics did not significantly differ between these two groups except for maternal age (*P* = 0.05), GBS colonisation in a previous pregnancy (*P* = 0.01) and ethnicity (*P* = 0.002) (**Table 20**).

Table 20. Demographics and clinical characteristics of iGBS feasibility participants by colonisation status

	Colonised n = 148	**Non-Colonised** n = 675	***P*-value** [a]
Maternal age group, n	124	673	0.05
<= 25	7 (4.7%)	49 (7.3%)	
26-30	26 (17.6%)	119 (17.7%)	
31-35	64 (43.2%)	281 (41.8%)	
36-40	35 (23.6%)	191 (28.4%)	
>40	16 (10.8%)	33 (4.9%)	
Ethnicity, n	125	407	0.002
White British	49 (39.2%)	205 (50.4%)	
White Other	34 (27.2%)	77 (18.9%)	
Black or Black British	10 (8.0%)	15 (3.7%)	
Asian or Asian British	15 (12.0%)	73 (17.9%)	
Mixed	1 (0.8%)	13 (3.2%)	
Other ethnic group	16 (12.8%)	23 (5.7%)	
Parity, n	148	675	0.5
Primiparous	81 (54.7%)	375 (55.6%)	
GBS colonisation in a previous pregnancy, n	51	229	0.01
Yes	14 (27.5%)	29 (12.6%)	
Previous child with iGBS, n	31	153	1
Yes	0 (0.0%)	3 (1.9%)	
[a] Fisher's exact test			

A univariable regression showed that women previously colonised with GBS (OR 2.0 (95% CI 1.0-3.6), *P* = 0.04) and of age >40 years old (OR 2.7 (1.2-6.1), *P* = 0.02) were more likely to be colonised with GBS (**Table 21**). Women of "Other ethnic group" and "White Other" were more likely to be colonised with GBS (OR 1.7 (1.0-3.1), *P* = 0.06 and OR 1.5 (1.0-2.3), *P* = 0.07; respectively), whereas Asian or Asian British were less likely to be colonised with GBS compared to White British (OR 0.6 (0.3-1.0), *P* = 0.08). In a multivariable logistic regression analysis, GBS colonisation in a previous

pregnancy remained the most significant predictor of GBS colonisation (OR 2.1 (1.1-3.8), *P* = 0.03) (**Table 21**).

Table 21. Univariable and Multivariable Logistic Regression analysis of maternal GBS colonisation in the iGBS feasibility study

	Univariable		**Multivariable**	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
GBS colonisation in a previous pregnancy	2.0 (1.0-3.6)	0.04	2.1 (1.1-3.8)	0.03
Maternal age >40	2.7 (1.2-6.1)	0.02		

7.3.3 iGBS3 Study Population

As of 20th February 2023, 45 cases and 25 controls were recruited to the iGBS3 study, which continues recruitment for Phase 1 until March 2024. Over 15,000 cord blood samples were collected and stored in the participating sites. The isolates from the first 16 cases were serotyped and the serotype-specific anti-GBS CPS IgG concentrations in the acute infant sera were measured by 31st August 2022. I have included these 16 cases in the combined maternal-infant cohort in which antibody responses were compared.

7.3.4 Combined Mother-Infants Cohort from iGBS Feasibility and iGBS3 Studies

The combined maternal-infants cohort included 22 EOGBS cases (serotype III: 14 (67%), serotype Ia: 4 (19%), serotype II: 1 (5%), serotype IV: 1 (5%), serotype V: 1 (5%)), 12 LOGBS cases (serotype III: 4 (44%), serotype Ia: 3 (33%), serotype II: 2 (22%)), 144 colonised women (serotype III: 30, serotype Ia: 28, serotype II: 22, serotype V: 14, serotype IV: 8, serotype Ib: 6, serotype VI: 3, NA: 33) and 265 non-colonised women with available serology samples (**Figure 34,35**).

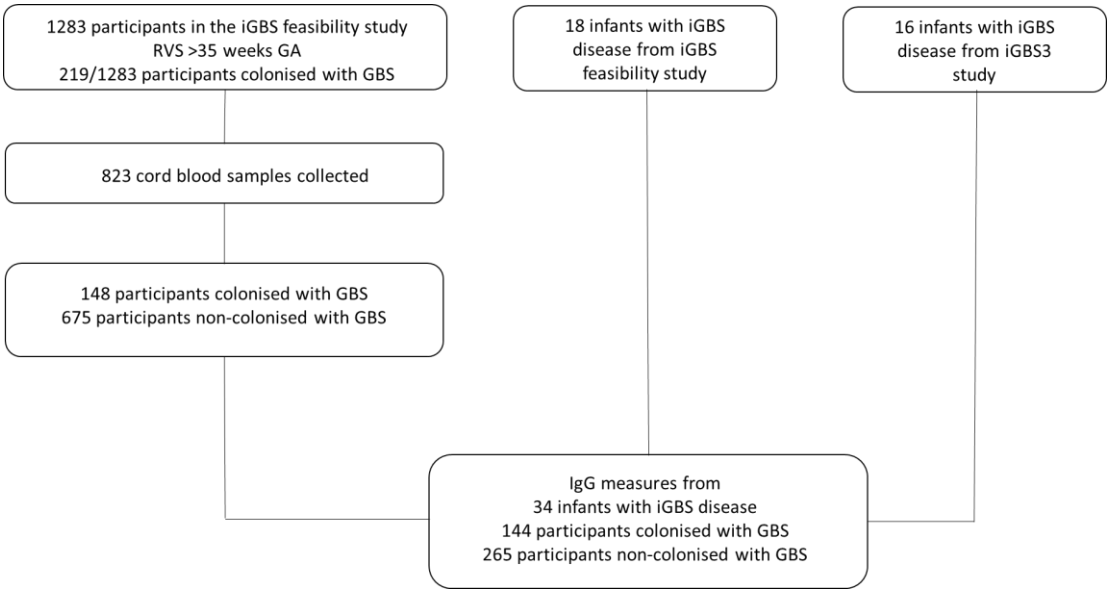


Figure 34. Combined Mother-Infants cohort from iGBS feasibility and iGBS3 studies

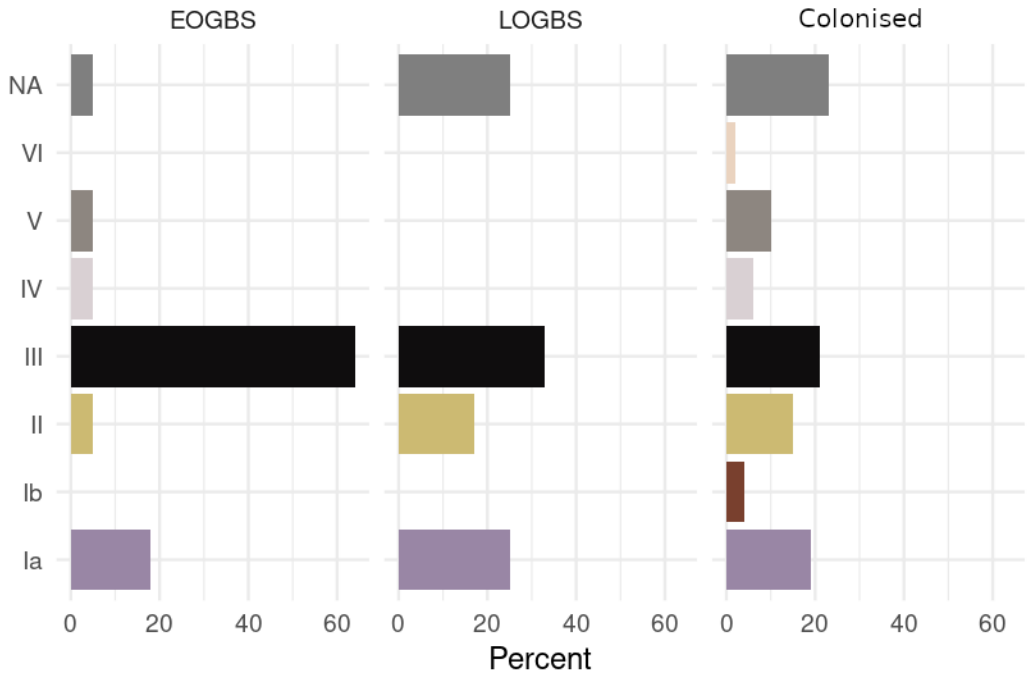


Figure 35. Distribution of GBS serotypes among EOGBS cases, LOGBS cases and colonised women

The demographic and clinical characteristics are shown in **Table 22**. Each of the 34 infants with iGBS disease had bacteraemia, and ten infants also had meningitis (six with EOGBS and four with LOGBS). Of those with available clinical outcome data, 13

fully recovered, two infants had neurological sequelae and one infant with EOGBS died. The median onset of disease was on the first day of life (day 0 range 0-3 days) for EOGBS and 21 days of life (range 7-53 days) for LOGBS. The median interval between the onset of disease and the blood collection was six (range 0-10) and five (range 1-10) days for EOGBS and LOGBS respectively.

Asian or Asian British and Black or Black British ethnicities were over-represented in the cases group and “Other White” ethnicity was over-represented in the other two groups. Nine (27%) infants with iGBS disease were born <35 weeks gestational age, of which seven presented with LOGBS, whereas all infants in the other two groups were born ≥35 weeks. A higher proportion of elective caesarean sections occurred in colonised and non-colonised women compared to cases, whereas the opposite was noticed for emergency caesarean sections. Women colonised with GBS were significantly more likely than non-colonised women or women whose babies developed iGBS disease to receive any intrapartum antibiotics (70% vs 21% vs 30%; $P < 0.001$).

Table 22. Demographics of cases/colonised/non-colonised participants with anti-GBS CPS IgG concentrations

	STIa			STIII			STII	STIb	STIV	STV	Total				
Characteristic	**EOGBS N=4**	**LOGBS N=3**	**Colonised N=28**	**EOGBS N=14**	**LOGBS N=4**	**Colonised N=30**	**Colonised N=22**	**Colonised N=6**	**Colonised N=8**	**Colonised N=14**	**EOGBS N=22**	**LOGBS N=12**	**Colonised N=144**	**Non-colonised N=265**	**P value***
Maternal age, median (range)	33 (31-34)	37 (37-37)	34 (25-45)	28 (22-31)	29 (20-38)	33 (26-43)	34 (22-43)	35 (32-40)	31 (21-36)	35 (27-46)	30 (22-36)	37 (20-42)	33 (21-46)	34 (21-46)	0.05
Ethnicity, n (%)	4	3	28	14	4	30	21	6	8	14	22	12	142	131 (	0.005
Asian or Asian British	2 (50.0%)	0 (0.0%)	1 (3.6%)	4 (28.6%)	1 (25.0%)	4 (13.3%)	1 (4.8%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	6 (27.3%)	1 (8.3%)	15 (10.6%)	22 (16.8%)	
Black or Black British	0 (0.0%)	0 (0.0%)	1 (3.6%)	2 (14.3%)	0 (0.0%)	1 (3.3%)	1 (4.8%)	1 (16.7%)	1 (12.5%)	1 (7.1%)	2 (9.1%)	1 (8.3%)	7 (4.9%)	4 (3.1%)	
Mixed	1 (25.0%)	1 (33.3%)	0 (0.0%)	0 (0.0%)	1 (25.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (12.5%)	0 (0.0%)	2 (9.1%)	1 (10.0%)	2 (1.4%)	4 (3.1%)	
Other ethnic group Other	0 (0.0%)	0 (0.0%)	4 (14.3%)	1 (7.1%)	1 (25.0%)	6 (20.0%)	4 (19.0%)	2 (33.3%)	0 (0.0%)	1 (7.1%)	2 (9.1%)	2 (16.7%)	20 (14.1%)	6 (4.6%)	

White British	1 (25.0%)	2 (66.7%)	18 (64.3%)	5 (35.7%)	1 (25.0%)	11 (36.7%)	9 (42.9%)	2 (33.3%)	4 (50.0%)	8 (57.1%)	8 (36.4%)	5 (41.7%)	64 (45.1%)	69 (52.7%)	
White Other	0 (0.0%)	0 (0.0%)	4 (14.3%)	2 (14.3%)	0 (0.0%)	8 (26.7%)	6 (28.6%)	0 (0.0%)	2 (25.0%)	4 (28.6%)	2 (9.1%)	1 (8.3%)	34 (23.9%)	26 (19.8%)	
Rupture of membranes , n (%)	4	2	27	14	3	30	22	6	8	13	22 (	10	142	260	<0.001
>= 18 hours	1 (25.0%)	0 (0.0%)	8 (29.6%)	1 (7.1%)	1 (33.3%)	9 (30%)	4(18.2%)	0 (0.0%)	2 (25.0%)	3 (23.1%)	2 (9.1%)	2 (20.0%)	31 (21.8%)	41 (15.8%)	
Delivery type, n (%)	4	3	28	14	4	30	22	6	8	13	22	12	143	263	0.001
Vaginal	3 (75.0%)	1 (33.3%)	6 (21.4%)	11 (78.6%)	2 (50.0%)	20 (66.7%)	5 (22.7%)	2 (33.3%)	0 (0.0%)	1 (7.7%)	16 (72.7%)	6 (50.0%)	84 (58.7%)	159 (60.5%)	
C-Section with rupture of membranes	1 (25.0%)	2 (66.7%)	6 (21.4%)	3 (21.4%)	2 (50.0%)	5 (16.7%)	6(27.3%)	2 (33.3%)	3 (37.5%)	4 (30.8%)	6 (27.3%)	6 (50.0%)	23 (16.1%)	34 (12.9%)	
C-Section without rupture of membranes	0 (0.0%)	0 (0.0%)	16 (57.1%)	0 (0.0%)	0 (0.0%)	5 (16.7%)	11(50.0%)	2 (33.3%)	5(62.5%)	8 (61.5%)	0 (0.0%)	0 (0.0%)	36 (25.2%)	70 (26.6%)	
Gestation at birth,	40 (38-42)	36 (33-39)	39 (35-41)	39 (27-40)	37 (30-39)	39 (36-42)	40 (36-44)	40 (36-41)	39 (38-41)	39 (37-43)	39 (27-42)	34 (23-40)	39 (35-44)	39 (36-43)	<0.001

median (range)															
Infant sex, n (%)	4	3	25	14	4	25	20	3	6	12	22	12	123	170	0.3
Female	1 (25.0%)	0 (0.0%)	11 (44.0%)	6 (42.9%)	2 (50.0%)	10 (40.0%)	10 (50.0%)	2 (66.7%)	1 (16.7%)	5 (41.7%)	9 (40.9%)	13 (33.3%)	55 (44.7%)	87 (51.2%)	
Intrapartum antibiotics, n (%)	4	2	22	14	4	27	17	4	7	9 (	22	11	118	173	<0.001
Yes	2 (50.0%)	1 (50.0%)	16 (72.7%)	2 (14.3%)	2 (50.0%)	19 (70.4%)	12 (70.6%)	2 (50.0%)	4 (57.1%)	6 (66.7%)	5 (22.7%)	5 (45.5%)	82 (69.5%)	37 (21.4%)	

*cases vs colonised vs non-colonised using Fisher exact test or Kruskall Wallis test

ST: Serotype; EOGBS: Early-onset disease; LOGBS: Late-onset disease; C-Section: Caesarean section

7.3.5 Antibody Responses in iGBS Cases, GBS Colonised, and Non-Colonised Pregnant Women from iGBS Feasibility and iGBS3 Studies

Serotype III specific anti-GBS CPS IgG GMC were significantly lower in serotype III EOGBS cases compared to women colonised with GBS serotype III (0.01 µg/mL vs 0.1 µg/mL; *P* = 0.002) (**Figure 36A**). The same was found when serotype III LOGBS cases were included (0.01 µg/mL vs 0.1 µg/mL; *P* < 0.001) (**Figure 36B**). The majority of EOGBS (12/14, 82%) and LOGBS (3/4, 75%) cases had undetectable IgG titres compared to less than half of controls (8/30, 27%; *P* <0.001). Similar differences were shown when serotype III specific anti-GBS CPS IgG GMC from all cases and colonised women (homologous and heterologous serotypes) were compared allowing for a larger dataset; both for EOGBS cases (0.01 µg/mL vs 0.1 µg/mL; *P* = 0.004) (**Figure 36C**) and EOGBS/LOGBS combined (0.01 µg/mL vs 0.1 µg/mL; *P* = 0.001) (**Figure 36D**).

The trend was similar for serotype Ia specific anti-GBS CPS IgG GMC, but the difference was not statistically significant (EOGBS vs colonised: 0.18 µg/mL vs 0.39 µg/mL, *P* = 0.8; EOGBS/LOGBS vs colonised: 0.08 µg/mL vs 0.39 µg/mL, *P* = 0.3) (**Figure 37A-B**). The differences were diluted when samples from non-homologous cases and colonised women were included (EOGBS vs colonised: 0.09 µg/mL vs 0.12 µg/mL, *P* = 0.9; EOGBS/LOGBS vs colonised: 0.05 µg/mL vs 0.12 µg/mL, *P* = 0.2) (**Figure 37C-D**).

Anti-GBS CPS IgG GMC were higher in colonised than non-colonised women for serotypes III (0.1 vs 0.03 µg/mL; *P* = 0.003) (**Figure 36A-B**), Ia (0.39 vs 0.05 µg/mL; *P* = 0.002) (**Figure 37A-B**), and IV (0.5 vs 0.009 µg/mL; *P* < 0.001) (**Figure 38C**). No significant differences were found between colonised and non-colonised women for serotypes Ib (0.017 vs 0.014 µg/mL; *P* = 0.74), II (0.17 vs 0.09 µg/mL; *P* = 0.15) and V (0.06 vs 0.06 µg/mL; *P* = 0.98) (**Figure 38A, B, D**).

Serotype III

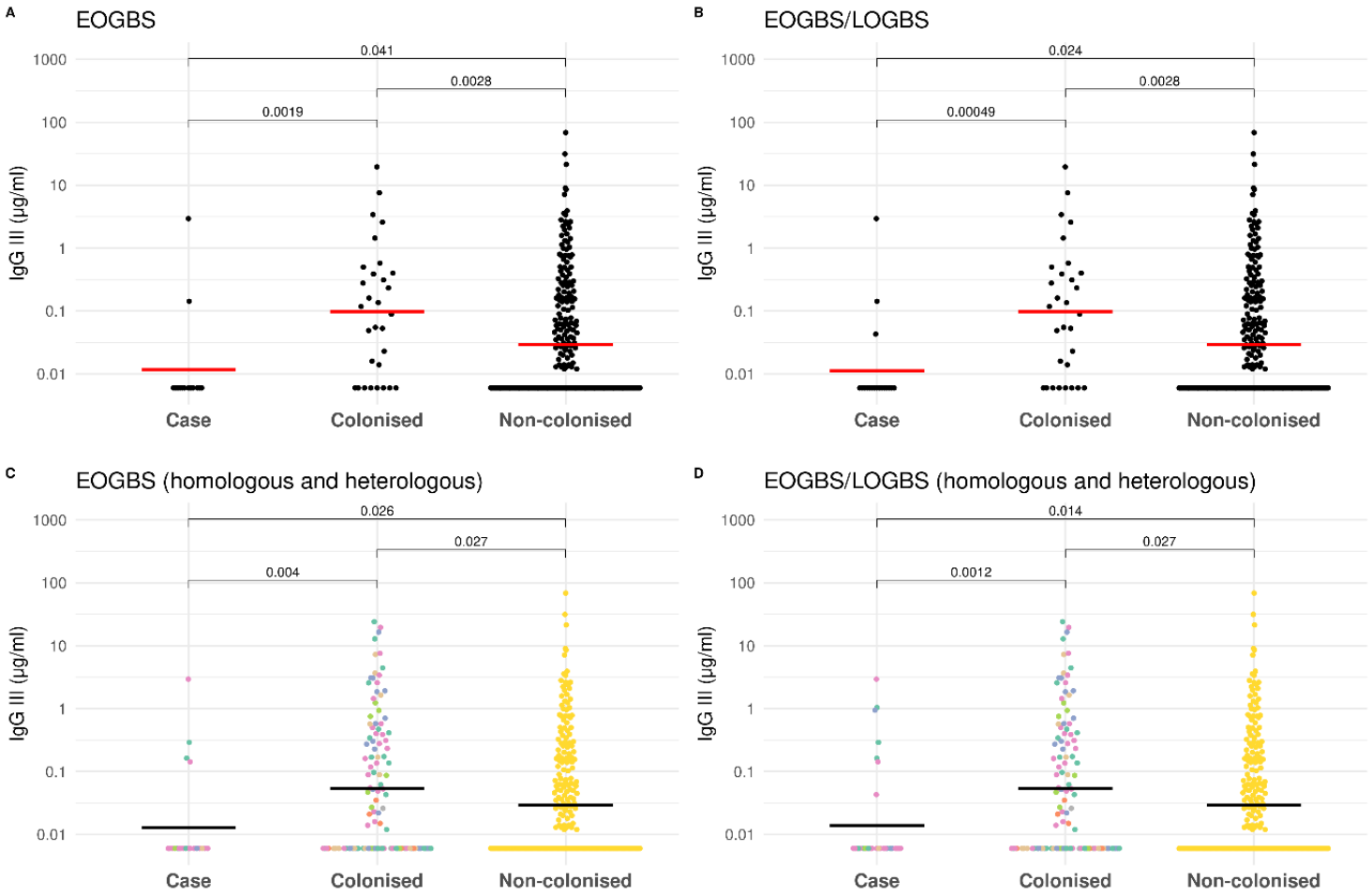


Figure 36. Comparison of STIII IgG concentrations in cases, colonised and non-colonised women

Dot plots comparing serotype III specific anti-GBS CPS IgG

(A) measured in individual post-disease sera (within ten days of onset) from infants with serotype III associated EOGBS; in cord sera (day 0) from healthy infants born to mothers colonised by serotype III; and in cord sera (day 0) from healthy infants born to non-GBS colonised women.

(B) measured in individual post-disease sera (within ten days of onset) from infants with serotype III associated EOGBS or LOGBS; in cord sera (day 0) from healthy infants born to mothers colonised by serotype III; and in cord sera (day 0) from healthy infants born to non-GBS colonised women.

(C) measured in individual post-disease sera (within ten days of onset) from infants with EOGBS caused by serotype Ia (cyan), II (navy), III (magenta), IV (green), or V (brown); in cord sera (day 0) from healthy infants born to mothers colonised by serotype Ia (cyan), Ib (orange), II (navy), III (magenta), IV (green), V (brown) or VI (grey); and in cord sera (day 0) from healthy infants born to non-GBS colonised women (yellow).

(D) measured in individual post-disease sera (within ten days of onset) from infants with EOGBS or LOGBS caused by serotype Ia (cyan), II (navy), III (magenta), IV (green), or V (brown); in cord sera (day 0) from healthy infants born to mothers colonised by serotype Ia (cyan), Ib (orange), II (navy), III (magenta), IV (green), V (brown) or VI (grey); and in cord sera (day 0) from healthy infants born to non-GBS colonised women (yellow).

Horizontal bars indicate the mean IgG concentrations. Statistical comparisons done with Wilcoxon rank-sum test and p values reported for each pair comparison.

GBS: group B Streptococcus; CPS: capsular polysaccharide; IgG: immunoglobulin G; EOGBS: Early-onset GBS disease (0-6 days); LOGBS: Late-onset GBS disease (7-89 days)

Serotype Ia

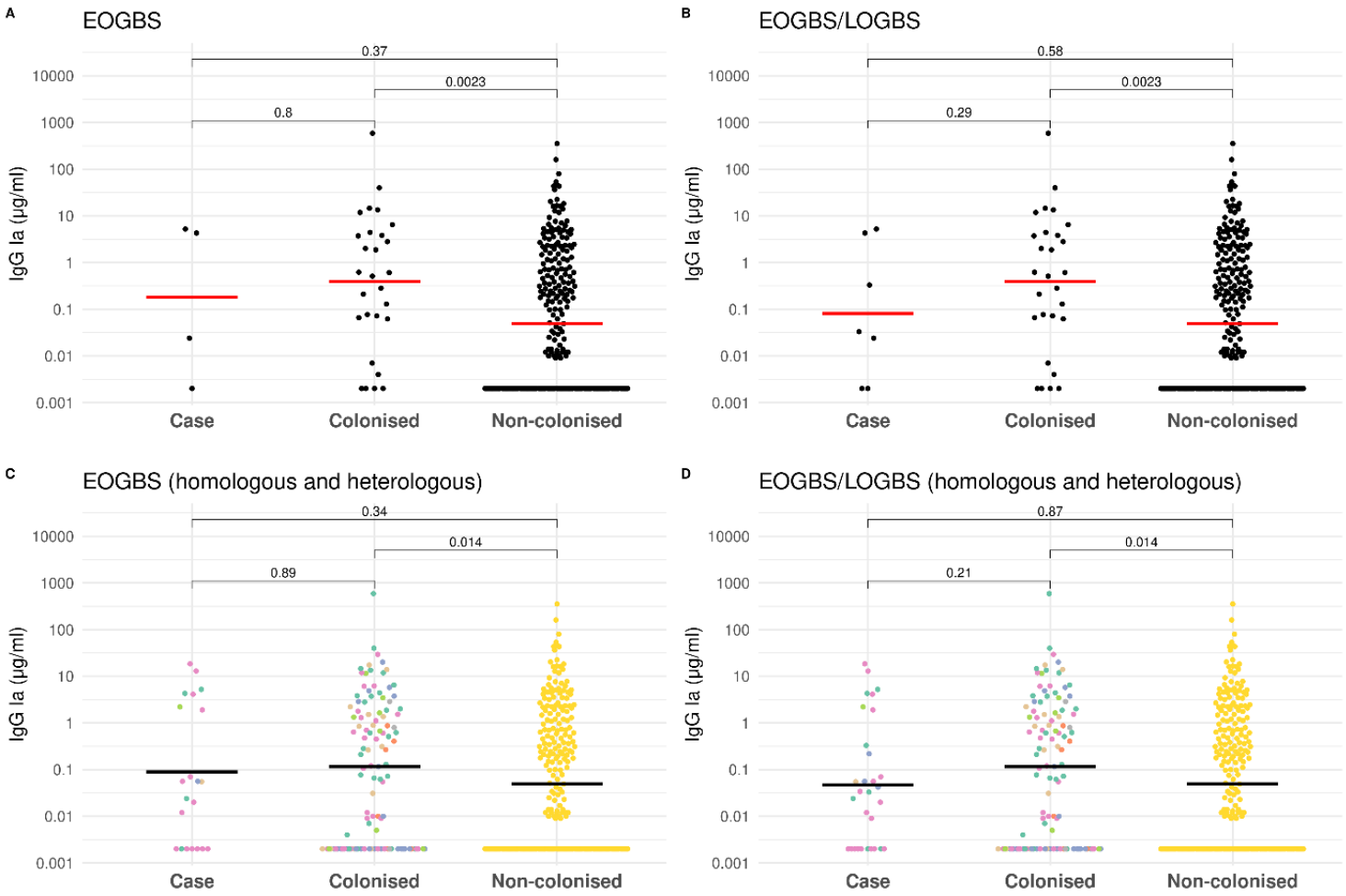

A
EOGBS
0.37
0.0023
0.8
IgG Ia (µg/ml)
10000
1000
100
10
1
0.1
0.01
0.001
Case
Colonised
Non-colonised
B
EOGBS/LOGBS
0.58
0.0023
0.29
C
EOGBS (homologous and heterologous)
0.34
0.014
0.89
D
EOGBS/LOGBS (homologous and heterologous)
0.87
0.014
0.21

Figure 37. Comparison of STIa IgG concentrations in cases, colonised and non-colonised women

Dot plots comparing serotype Ia specific anti-GBS CPS IgG

(A) measured in individual post-disease sera (within ten days of onset) from infants with serotype Ia associated EOGBS; in cord sera (day 0) from healthy infants born to mothers colonised by serotype Ia; and in cord sera (day 0) from healthy infants born to non-GBS colonised women.

(B) measured in individual post-disease sera (within ten days of onset) from infants with serotype Ia associated EOGBS or LOGBS; in cord sera (day 0) from healthy infants born to mothers colonised by serotype Ia; and in cord sera (day 0) from healthy infants born to non-GBS colonised women.

(C) measured in individual post-disease sera (within ten days of onset) from infants with EOGBS caused by serotype Ia (cyan), II (navy), III (magenta), IV (green), or V (brown); in cord sera (day 0) from healthy infants born to mothers colonised by serotype Ia (cyan), Ib (orange), II (navy), III (magenta), IV (green), V (brown) or VI (grey); and in cord sera (day 0) from healthy infants born to non-GBS colonised women (yellow).

(D) measured in individual post-disease sera (within ten days of onset) from infants with EOGBS or LOGBS caused by serotype Ia (cyan), II (navy), III (magenta), IV (green), or V (brown); in cord sera (day 0) from healthy infants born to mothers colonised by serotype Ia (cyan), Ib (orange), II (navy), III (magenta), IV (green), V (brown) or VI (grey); and in cord sera (day 0) from healthy infants born to non-GBS colonised women (yellow).

Horizontal bars indicate the mean IgG concentrations. Statistical comparisons done with Wilcoxon rank-sum test and p values reported for each pair comparison.

GBS: group B Streptococcus; CPS: capsular polysaccharide; IgG: immunoglobulin G; EOGBS: Early-onset GBS disease (0-6 days); LOGBS: Late-onset GBS disease (7-89 days)

Other Serotypes

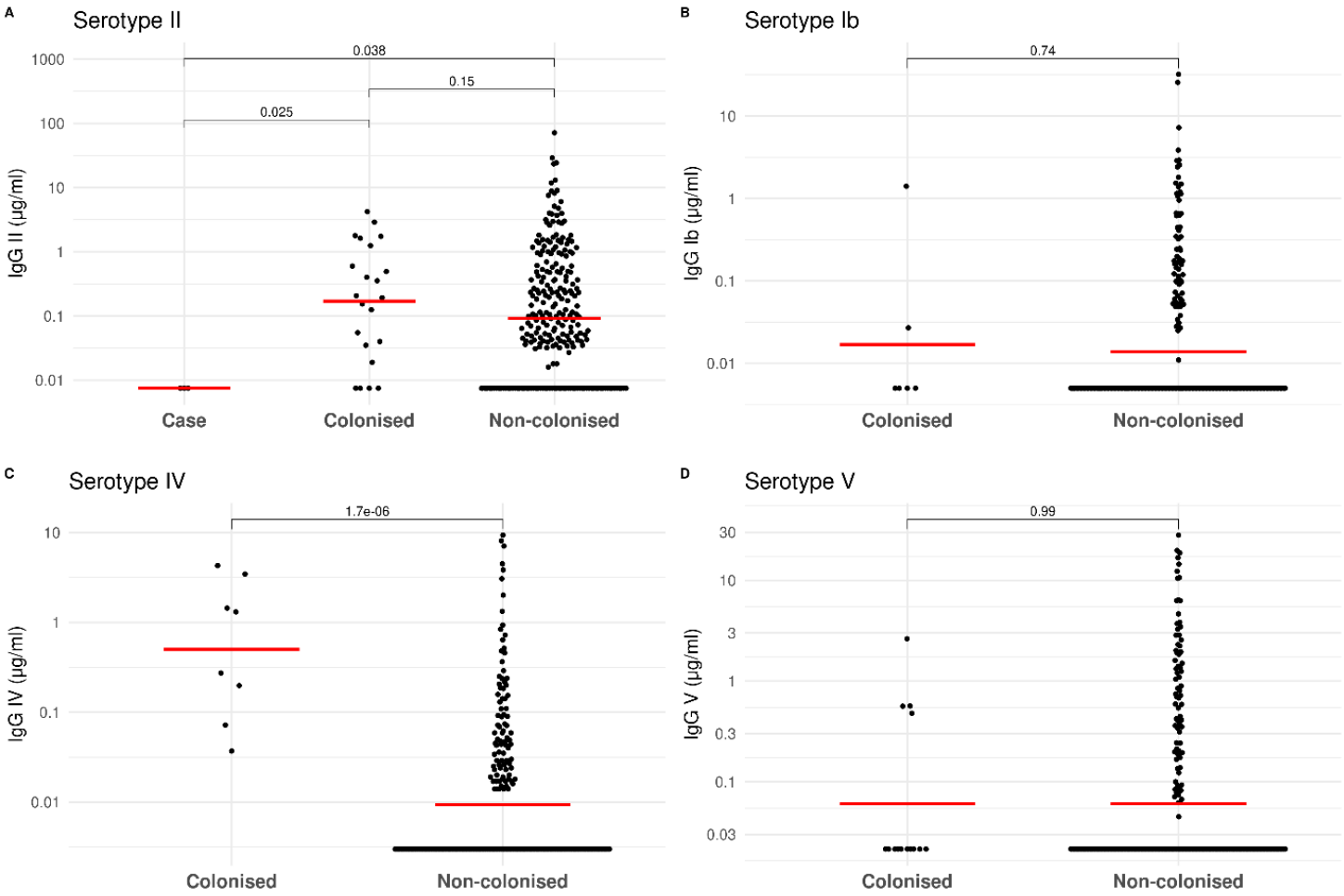


Figure 38. Comparison of serotype specific IgG concentrations in colonised and non-colonised women

(A) Dot plots comparing serotype II specific anti-GBS CPS measured in individual post-disease sera (within ten days of onset) from infants with serotype II associated EOGBS or LOGBS; in cord sera (day 0) from healthy infants born to mothers colonised by serotype II; and in cord sera (day 0) from healthy infants born to non-GBS colonised women.

(B) Dot plots comparing serotype Ib specific anti-GBS CPS measured in cord sera (day 0) from healthy infants born to mothers colonised by serotype Ib; and in cord sera (day 0) from healthy infants born to non-GBS colonised women.

(C) Dot plots comparing serotype IV specific anti-GBS CPS measured in cord sera (day 0) from healthy infants born to mothers colonised by serotype IV; and in cord sera (day 0) from healthy infants born to non-GBS colonised women.

(D) Dot plots comparing serotype V specific anti-GBS CPS measured in cord sera (day 0) from healthy infants born to mothers colonised by serotype V; and in cord sera (day 0) from healthy infants born to non-GBS colonised women.

Horizontal bars indicate the mean IgG concentrations. Statistical comparisons done with Wilcoxon rank-sum test and p values reported for each pair comparison.

GBS: group B Streptococcus; CPS: capsular polysaccharide; IgG: immunoglobulin G; EOGBS: Early-onset GBS disease (0-6 days); LOGBS: Late-onset GBS disease (7-89 days)

7.3.6 Antibody Responses in Maternal and Infant Serum from iGBS Cases

For ten EOGBS cases (serotype III: eight, serotype Ia: two) and three LOGBS cases (serotype III: two, serotype Ia: one), both maternal and infant acute sera were available from the same case. Eleven (85%) infant sera had undetectable IgG titres compared to seven (54%) maternal sera (*P* = 0.2). The very small number of samples with IgG levels above LLOQ precluded any further meaningful comparisons of GMC (**Figure 39**).

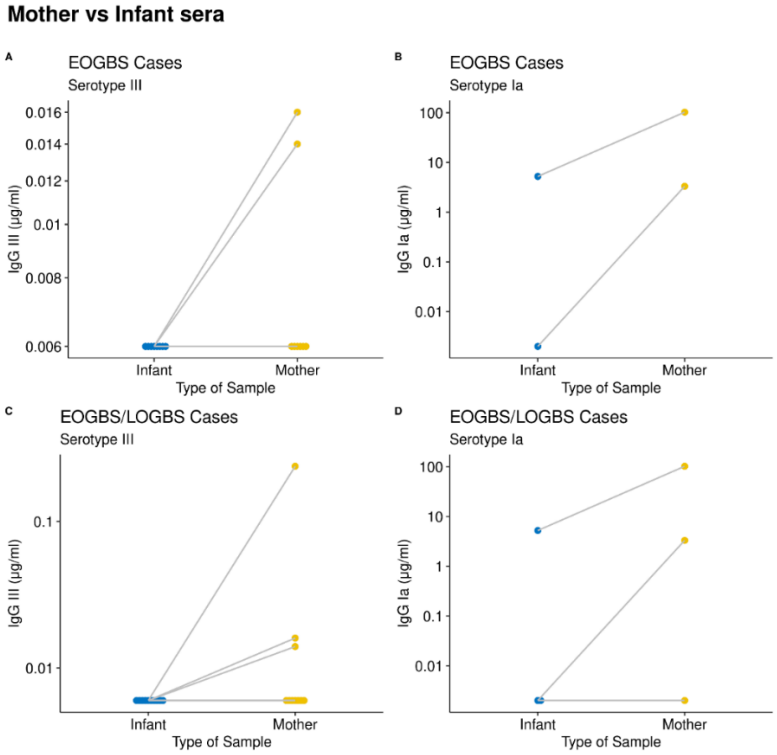


Figure 39. Comparison of serotype specific IgG concentrations for serotypes Ia and III in paired mother/infant samples from cases

(A) Infant vs Maternal acute sera in STIII EOGBS cases (B) Infant vs Maternal acute sera in STIa EOGBS cases (C) Infant vs Maternal acute sera in STIII EOGBS/LOGBS cases (D) Infant vs Maternal acute sera in STIa EOGBS/LOGBS cases. Blue dots represent infant sera and yellow dots represent maternal sera. Lines join paired samples from the same case. Statistical comparisons done with Wilcoxon signed-rank test. ST: serotype; IgG: immunoglobulin

7.3.7 Demographic and Clinical Factors Associated with Antibody Concentrations

Serotype-specific IgG concentrations in colonised women appeared unaffected by maternal age, maternal ethnicity, gestational age, co-morbidities, immunosuppressive medication, or GBS colonisation in previous pregnancy for all serotypes (**Table 23**).

Table 23. Comparison of serotype specific anti-GBS CPS IgG concentrations in colonised women according to demographic and clinical factors

	Anti-STIa IgG GMC (95% CI) µg/ml	*P* value	Anti-STIb IgG GMC (95% CI) µg/ml	*P* value	Anti-STII IgG GMC (95% CI) µg/ml	*P* value	Anti-STIII IgG GMC (95% CI) µg/ml	*P* value	Anti-STIV IgG GMC (95% CI) µg/ml	*P* value	Anti-STV IgG GMC (95% CI) µg/ml	*P* value
Ethnicity		0.6		0.08		0.1		0.3		0.7		0.1
White British	0.11 (0.05-0.26)		0.02 (0.009-0.03)		0.15 (0.08- 0.27)		0.04 (0.02- 0.08)		0.007 (0.005-0.01)		0.05 (0.03- 0.07)	
Asian	0.25 (0.03- 1.99)		0.05 (0.01- 0.19)		0.06 (0.01-0.25)		0.02 (0.006- 0.07)		0.005 (0.002-0.01)		0.04 (0.02- 0.09)	
Black	0.33 (0.02-5.84)		0.02 (0.003-0.10)		1.05 (0.17- 6.61)		0.16 (0.02- 1.20)		0.008 (0.002-0.04)		0.07 (0.01-0.34)	
Mixed	0.04 (NA)		0.005 (NA)		0.22 (NA)		0.01 (NA)		0.10 (NA)		0.07 (0.001-3.52)	
Other	0.05 (0.01- 0.24)		0.02 (0.006-0.06)		0.08 (0.03- 0.21)		0.07 (0.02- 0.22)		0.005 (0.003-0.01)		0.04 (0.02-0.09)	
White Other	0.12 (0.04-0.42)		0.06 (0.02-0.14)		0.14 (0.06- 0.33)		0.10 (0.04- 0.27)		0.01 (0.007- 0.03)		0.07 (0.04-0.14)	
Maternal Age		0.3		0.5		0.2		0.5		0.8		0.2
18-25	0.40 (0.009-17.1)		0.08 (0.006-0.95)		0.25 (0.04- 1.62)		0.02 (0.004- 0.09)		0.02 (0.001- 0.33)		0.04 (0.01-0.11)	
26-30	0.14 (0.03- 0.62)		0.01 (0.005-0.03)		0.27 (0.09- 0.81)		0.05 (0.01- 0.14)		0.01 (0.004-0.03)		0.04 (0.02-0.07)	
31-35	0.16 (0.07-0.38)		0.03 (0.01-0.05)		0.09 (0.05-0.16)		0.05 (0.02- 0.08)		0.006 (0.004-0.01)		0.04 (0.03- 0.07)	
36-40	0.06 (0.02- 0.16)		0.02 (0.01- 0.05)		0.13 (0.05- 0.31)		0.07 (0.03- 0.17)		0.008 (0.004-0.02)		0.06 (0.03- 0.13)	
>40	0.06 (0.007- 0.40)		0.03 (0.01- 0.10)		0.22 (0.06- 0.78)		0.12 (0.04- 0.43)		0.009 (0.003-0.02)		0.10 (0.03- 0.32)	

Gestation		1		0.6		0.7		0.5		0.8		0.8
35-37	0.36 (0.04- 3.25)		0.02 (0.006-0.08)		0.11 (0.03-0.41)		0.08 (0.01- 0.50)		0.004 (0.002-0.006)		0.05 (0.02- 0.11)	
>37	0.10 (0.06-0.18)		0.03 (0.02- 0.04)		0.14 (0.09- 0.21)		0.05 (0.03- 0.08)		0.009 (0.006-0.01)		0.05 (0.04- 0.07)	
Comorbidities		0.3		0.6		0.7		0.2		0.9		0.7
No	0.11 (0.06- 0.23)		0.03 (0.02- 0.05)		0.13 (0.08- 0.21)		0.04 (0.03- 0.08)		0.009 (0.006-0.01)		0.06 (0.04- 0.09)	
Yes	0.12 (0.04-0.33)		0.02 (0.01- 0.04)		0.15 (0.07- 0.29)		0.08 (0.04- 0.15)		0.007 (0.004-0.01)		0.04 (0.03- 0.05)	
Immunosuppression		0.7		0.1		0.09		0.9		0.3		0.6
No	0.12 (0.07- 0.21)		0.03 (0.02- 0.04)		0.14 (0.09- 0.20)		0.05 (0.04- 0.08)		0.008 (0.006-0.01)		0.05 (0.04- 0.07)	
Yes	0.05 (NA)		0.04 (NA)		0.07 (NA)		0.04 (0.001- 1.93)		0.005 (0.001-0.07)		0.06 (0.001-6.07)	
GBS in previous pregnancy		1		0.8		0.9		0.7		0.5		0.5
No	0.19 (0.06- 0.56)		0.02 (0.01- 0.05)		0.12 (0.05- 0.27)		0.07 (0.03- 0.18)		0.009 (0.005-0.02)		0.05 (0.03- 0.07)	
Yes	0.04 (0.007-0.20)		0.03 (0.007-0.09)		0.12 (0.02- 0.59)		0.02 (0.008-0.05)		0.005 (0.002-0.01)		0.10 (0.03- 0.37)	

Statistical comparisons done with Wilcoxon rank-sum test when comparing two groups and Kruskal-Wallis for more than 2 groups.
ST: Serotype; GBS: Group B *Streptococcus*; GMC: Geometric Mean Concentration; CI: Confidence interval

7.4 Discussion

This was the first case-control study to compare serum anti-GBS CPS IgG in a UK population. Several important observations were made in this respect. First, serotype III specific anti-GBS CPS IgG GMC in samples from colonised women with healthy infants were higher than those of EOGBS and LOGBS cases for the same serotype. Second, this difference remained statistically significant when non-homologous cases and controls were included. Third, for most serotypes, GBS colonisation was associated with increased anti-GBS CPS IgG compared to non-colonised. Fourth, common maternal demographic and clinical factors did not affect the antibody distribution in colonised women with healthy infants.

The overall prevalence of GBS colonisation was 17.4% (95% CI 15.3-19.5%), lower compared to the pooled estimate of 22.8% (95% CI 18.8-26.9%) reported in previous UK studies done many years ago. (241) Seventeen participants had a positive swab done through a private service without having a study swab. When these participants were included, the prevalence increased to 18.4%. The very low prevalence of GBS colonisation in site B could be partially explained by suboptimal participant compliance with self-swabbing instructions. When the local team changed their practice from a single rectovaginal swab to two separate vaginal and rectal swabs, 2/7 (29%) participants had a positive result. The prevalence of GBS colonisation in the other two sites combined with the positive swabs done privately was 19.7%. Accounting for these factors, the prevalence of GBS colonisation in the iGBS feasibility cohort was probably closer to the previous estimates, although a real decrease in the GBS colonisation rate cannot be ruled out. The serotype distribution among colonising isolates was similar to the previous UK report, with serotype III being the most prevalent. (354) Due to the very small number (n=6) of positive swabs, it is difficult to ascertain whether the higher prevalence of serotype V noted in site B reflects a real trend.

In the iGBS feasibility population, women colonised with GBS in a previous pregnancy were more likely to be colonised with GBS in the current pregnancy. This finding is consistent with a recent systematic review that showed approximately 50% of

women colonised with GBS would also be colonised in a subsequent pregnancy. (355) As a result, for this group of pregnant women, the RCOG guidelines recommend either IAP or bacteriological testing in late pregnancy, followed by the offer of IAP if still positive. (126)

There was a higher prevalence of GBS colonisation in some ethnic minorities (Other ethnic group, White Other and Black or Black British), but lower prevalence in Asian or Asian British women, in accordance with a recent hospital study in the UK. (356) At the same time, Asian or Asian British and Black or Black British infants were over-represented among cases, in keeping with a recent population-wide UK study that showed higher rates of iGBS disease in these ethnic groups. (357) Taken together these findings suggest that factors other than GBS colonisation, like higher preterm births in ethnic minorities might have accounted for some of the observed differences. (357)

Serotype III specific anti-GBS CPS IgG were higher in the cord blood of colonised women with healthy babies compared to EOGBS and LOGBS cases for the same serotype. While the difference for serotype Ia was not significant, most likely because of the very small number of cases, the trend was similar. These findings are in line with previous research in this area. (155,207,208,358,359) However, antibody concentrations were generally very low across all groups (cases, colonised and non-colonised women) compared to previous studies (**Table 24**). These differences are likely due to the different assays and standard sera used, (179) although a real difference driven by the different epidemiological characteristics of the study populations cannot be ruled out. Second, this difference remained statistically significant when non-homologous cases and non-homologous controls were included. As with women colonised with serotype III, women colonised with other serotypes had higher serotype III specific anti-GBS CPS IgG compared to cases. This observation is in accordance with Dangor and colleagues (**Table 25**). (208) These findings could be explained by the induction of antibodies against different antigens due to intermittent co-colonisation with more than one serotypes. (345)

Table 24. Studies reporting serotype III specific anti-GBS CPS IgG in cases and controls

	Anti-STIII IgG (µg/ml)			
Study (Year)	**STIII Cases**	**Women Colonised with STIII**	**Women Colonised with serotypes other than STIII**	**Non-Colonised Women**
Lin et al (2004) (358)	2.73 [a]	4.27 [a]	NA	NA
Baker et al (2014) (207)	0.060 [b]	0.64 [b]	NA	NA
Dangor et al (2015) (208)	0.04 [b]	0.15 [b]	0.12 [b]	NA
Fabbrini et al (2016) (155)	0.2 [b]	0.9 [b]	0.19 [b]	0.18 [b]
Madhi et al (2021) (359)	0.20 [a]	0.38 [a]	NA	NA
Present study	0.01 [a]	0.1 [a]	0.1 [a]	0.03 [a]
[a] Geometric Mean Concentration [b] Median; ST: serotype; IgG: immunoglobulin G; NA: Not available				

The demographics of the recruited women differed across the participating sites. However, maternal age or ethnicity did not affect anti-GBS CPS IgG concentrations in colonised women with healthy infants. At the same time, clinical factors that could change the probability of developing iGBS or the antibody distribution, such as prematurity, mode of delivery and IAP differed significantly between iGBS cases and healthy infants born to colonised and non-colonised women. The impact of these factors is explored further in Chapter 8.

The iGBS feasibility study had some limitations. First, the results from the three centres that collected rectovaginal swabs and cord blood may not be representative of the catchment population they serve or generalisable to other maternity units across the UK. Women who delivered through a caesarean section were over-represented, and preterm infants were under-represented. All the healthy infants of colonised women were born at ≥35 weeks gestational age; therefore, the impact of

prematurity on antibody concentrations was not fully assessed. Although maternal co-morbidities and immunosuppressive medications (in most cases, a short course of steroids) did not affect cord blood IgG concentrations, no participants with positive HIV status or long-term immunosuppressive medications were enrolled. These issues were taken into account when designing the iGBS3 study, which aims to collect cord blood from all pregnant women giving birth in the participating sites. Second, important clinical and demographic factors such as birth weight, the presence of chorioamnionitis or intrapartum fever and GBS positivity in urine culture were not recorded in the iGBS feasibility study. These data were collected as part of the iGBS3 study. Third, no difference in the antibody concentrations between maternal and infant acute sera from the same case was found. However, this analysis was significantly restricted by the very small number of paired maternal/infant samples from iGBS cases. The most recent literature suggests that maternal sera concentrations tend to be higher and less reliable than infant sera for EOGBS and LOGBS due to differences in the transplacental IgG transfer ratios. (359) Fourth, in the absence of cord blood samples from cases, IgG concentrations were measured in acute disease sera. This is likely to be a reliable surrogate for EOGBS but not for LOGBS due to the antibody decline after birth. This issue was further explored in Chapter 6 but it should be acknowledged that the number of iGBS cases with cord blood samples recruited during Phase 1 of the iGBS3 study has been less than anticipated. The iGBS3 study aimed to capture a cord blood sample from all pregnant women giving birth at each participating site, including the full spectrum of gestational age, time of delivery (day and night) and mode of delivery (vaginal delivery, elective and emergency caesarean sections). This was considered the ideal approach to capture prospectively all the iGBS cases from this cohort, including those infants (particularly LOGBS) with no clinical risk factors at delivery. However, it would not have been practically or financially feasible for a dedicated research midwifery team to conduct such a large-scale study at each participating site. Instead, the iGBS3 study had to rely on the attending clinical midwives to ask for verbal consent and collect this sample for research purposes. This can be particularly challenging in a busy labour and delivery setting, where cord blood collection is not routine. Although an “opt-out” approach might have been better integrated into the standard practice,

it was deemed ethically controversial. In addition, even if the sample volume required was small, recent guidance for umbilical cord milking added an extra layer of complexity. In reality, this meant that a disproportionate number of samples were collected from term babies born via elective caesarean section, as these deliveries were well attended and scheduled in advance, allowing sufficient time for consenting and cord blood collection.

7.5 Conclusions

In conclusion, the results from this study conducted in a high-resource setting with a relatively high prevalence of antenatal GBS colonisation accumulate more evidence to support the role of naturally acquired humoral immunity against serotype III-associated iGBS disease. Using samples from women colonised with the homologous and the heterologous serotypes seems an acceptable strategy when formulating a control group to compare with cases. On the contrary, antibody distributions were different in the non-colonised women and, therefore would be less suitable for this purpose. Finally, commonly recorded demographic factors such as maternal ethnicity and age did not affect the antibody distribution, so adjusting for these covariates might not be needed when establishing a CoP for iGBS disease from observational data.

Chapter 8 – Estimation of iGBS Disease Risk from UK Seroepidemiological Studies

8.1 Introduction

A GBS vaccine offered to pregnant women could significantly reduce the burden of iGBS disease and protect the lives and health of infants around the world. (144) GBS vaccine candidates have shown promising safety and immunogenicity results in Phase 1 and 2 studies. (175,178) However, given the low incidence of iGBS disease, any vaccine efficacy trial would need to recruit a very large number of participants. (183) Alternatively, a vaccine could be provisionally licensed based on a CoP, with post-licensure studies needed to demonstrate the impact on disease burden, or via a hybrid study that includes both clinical and immunological endpoints. (189) Placentally transferred anti-GBS CPS IgG is associated with the protection of infants against iGBS disease; (207,208) and is considered an accepted surrogate for vaccine efficacy, (210) that is "reasonably likely to predict clinical benefit". (209) Multiple case-control studies over the last 30 years tried to define antibody protection thresholds that can be used as CoP (**Table 25**). The results from these studies are not fully comparable due to methodological differences in the source of serum (maternal vs infant), the assay used (format and reference serum), and the statistical methodology used. (179,189) The key statistical concept is to convert IgG titres for cases and controls into a risk-titre curve and estimate the probability of iGBS disease using relative risk reduction (RRR) or absolute disease risk (ADR) approaches. A case-control study can directly estimate RRR parameters such as odds ratios and adjust for covariates. The ADR methods combine the frequency distributions from cases and controls with a population-derived disease incidence to predict the probability of disease along the IgG range. (360) The advantages and limitations of proposed statistical methodologies when adjusting for confounders in observational studies have been recently assessed. (349) In this chapter, I use a variety of statistical methods to define the concentrations of CPS-specific IgG in cord/infant sera that conferred protection against EOGBS and LOGBS caused by serotype III.

Table 25. Summary of previous seroepidemiological studies of iGBS disease

Study (Year)	Design	Cases (ST)	Controls	EOGBS/LOGBS	Statistical methods	Protective threshold (serum source)
Lin et al (2001) (361)	Case-control	50 (Ia)	336 healthy colonised infants	EOGBS	RRR: Logistic regression with reference <0.5 µg/mL	Ia: 5 µg/ml (maternal) 4 µg/ml (cord/infant)
Lin et al (2004) (362)	Case-control	26 (III)	143 healthy colonised infants	EOGBS	RRR: Logistic regression with reference <2 µg/mL	III: 10 µg/ml (maternal) 7 µg/ml (cord/infant)
Baker et al (2014) (207)	Case-control	17 (Ia) 9 (III) 7 (V)	99 healthy infants born to colonised women	EOGBS	RRR: Logistic regression with reference <0.1 µg/mL ADR: Bayesian model with 1% background risk	Ia: 0.5 µg/ml (maternal) III: 0.5 µg/ml (maternal)
Dangor et al (2015) (208)	Matched Case-control	27 (Ia) 29 (III)	74 healthy infants born to colonised women	EOGBS + LOGBS	RRR: Logistic regression with reference <0.1 µg/mL ADR: Bayesian model with 0.4% background risk	Ia: 6 µg/ml (maternal) III: 3 µg/ml (maternal)
Fabbrini et al (2016) (155)	Case-control	8 (Ia) 23 (III)	280 healthy infants born to colonised women	EOGBS	RRR: Logistic regression with reference <0.1 µg/mL ADR: Bayesian model with 1% background risk among colonised women	Ia: 1 µg/ml (maternal) III: 1 µg/ml (maternal)
Madhi et al (2021) (359)	Case-control Cohort	14 (Ia) 23 (III)	128 healthy infants born to colonised women	EOGBS + LOGBS	ADR: Bayesian model with 0.1% background risk	Ia: 2.31 µg/ml (maternal) 1.04 µg/ml (cord/infant) III: 3.41 µg/ml (maternal) 1.53 µg/ml (cord/infant)
EOGBS: Early-onset disease; LOGBS: Late-onset disease; RRR: Relative risk reduction; ADR: Absolute disease risk						

8.2 Methods

8.2.1 Study Population and Design

The samples used in this analysis were collected through the iGBS feasibility and iGBS3 studies, as described in Chapter 3 (section 3.1).

The two comparator groups were defined as follows:

Cases: Infants aged 0 to 89 days with iGBS disease (**Table 1**).

Controls: Infants born to women with a positive GBS rectal, vaginal or rectovaginal swab at any point from 35 weeks gestational age until delivery, who did not develop iGBS disease in the first 89 days of life.

8.2.2 Laboratory Methods

Serotype-specific anti-GBS CPS IgG concentrations were measured using the GASTON-adopted MIA in cord and infant sera, as described in Chapter 3. IgG were measured against the invasive serotype for cases and the colonising serotype for controls (the homologous serotype) as well as against the other five serotypes (non-homologous serotypes).

8.2.3 Statistical Methods

Serotype III-specific anti-GBS CPS IgG concentrations in infant blood samples with EOGBS or LOGBS collected within ten days of the onset of disease were compared to cord blood samples from infants born to women colonised with serotype III. The small number of cases did not allow meaningful comparisons for the other serotypes. Demographic differences between cases and controls were assessed by Fisher exact test and Mann–Whitney U test. Infant IgG concentrations in case and control sera were summarised using non-parametric Kaplan–Meier estimates of their reverse cumulative distributions. A *P*-value ≤ 0.05 was considered to be statistically significant.

Three methods were used to estimate protective antibody thresholds. First, a univariable logistic regression model was used to estimate OR, using Methods 1 and 2 below. Multivariable logistic regression was then used to adjust for the covariate

effects of gestation at birth, prolonged rupture of membranes and administration of intrapartum antibiotics (IAP targeted against GBS, antibiotic treatment for chorioamnionitis, or prophylactic antibiotics for caesarean section).

Method 1: OR for fixed antibody thresholds. This method calculates an OR at break points in antibody concentrations that correspond to potentially clinically meaningful differences of risk starting with the assay's LLOQ and increasing by one log10 (0.01 µg/ml, 0.1 µg/ml and 1 µg/ml). At each breakpoint, it calculates the OR by comparing the odds of disease in participants with concentration values above vs below the breakpoint: <0.01 vs ≥0.01 µg/ml, <0.1 vs ≥0.1 µg/ml, <1 vs ≥1 µg/ml.

$$OR = [P(D+|A \geq a0)/P(D-|A >= a0)]/[P(D+|A < a0)/P(D-|A < a0)]$$

Method 2: OR against a reference value of <0.1 µg/mL. This method divides antibody values into small groups, starting with the LLOQ for the assay and increasing by one log10 (<0.01 µg/ml, 0.01-0.1 µg/ml, 0.1-1 µg/ml and ≥1 µg/ml) and compares disease rates between each to the reference group of those with undetectable antibody (Method 2): <0.01 vs 0.01-0.1 µg/ml, <0.01 vs 0.1-1 µg/ml, <0.01 vs ≥1 µg/ml.

$$OR = [P(D+|A = a0-a1)/P(D-|A = a0-a1)]/[P(D+|A < a0)/P(D-|A < a0)]$$

$$OR = [P(D+|A = a1-a2)/P(D-|A = a1-a2)]/[P(D+|A < a0)/P(D-|A < a0)]$$

$$OR = [P(D+|A = a2-a3)/P(D-|A = a2-a3)]/[P(D+|A < a0)/P(D-|A < a0)]$$

ADR was estimated using Method 3.

Method 3: Reverse Cumulative Disease (RCD) Probability Curve based on the ADR. This method estimates the ADR assuming that antibody concentrations among cases and controls (all serotypes) followed a parametric Weibull distribution as previously described. (360) The antibody level distributions for cases and controls were estimated separately. This was combined with unconditional population risk of 0.4

cases per 1000 live births for EOGBS and 0.9 cases per 1000 live births for EOGBS/LOGBS combined as per the most recent UK enhanced surveillance study (32) to derive the probability of disease conditional on the titre exceeding a given value.

$$P(D+ \mid A > a) = P(A > a \mid D+)P(D+)/P(A > a) = P(A > a \mid D+)P(D+)/$$
$$P(A > a \mid D+)P(D+) + P(A > a|D-)(1 - P(D+))$$

Further sensitivity analyses were done to test the impact of the interval between the onset of disease and the sera collection, restricting the analysis to only samples taken within eight days of birth and using a longer cut-off of <14 days. Acute IgG titres from LOGBS cases might not be directly comparable to controls' cord IgG titres due to a presumed antibody decay after birth. To account for this, a sensitivity analysis of ADR was done using back-predicted cord titres, as described in Chapter 6. For LOGBS cases with acute titres below LLOQ, cord titres were also assumed to be below LLOQ. When titres over LLOQ were detected during the acute LOGBS episode, cord titres were back-calculated using a half-life estimate of 27 days (Chapter 6).

Prof Merryn Voysey wrote the Weibull model R code and kindly shared it with me. The Weibull model was coded using the survreg function from the survival package. (363) The plots were produced using the ggplot2 package with the geom_step function that creates a stairstep plot, highlighting exactly when changes occur. (364)

8.3 Results

8.3.1 Participant Characteristics

Thirty-four infants with iGBS disease were identified. Eighteen infections were caused by serotype III (14 EOGBS and four LOGBS). Each of the 18 infants had bacteraemia, and six also had meningitis (five EOGBS and one LOGBS). The demographic characteristics of cases and controls are shown in **Table 26**. A higher number of emergency caesarean sections occurred in cases, especially those with LOGBS, compared to controls (32% vs 59%; *P* <0.001). Nine (26.5%) infants with iGBS disease were born <35 weeks gestational age, whereas all controls were born ≥35 weeks. Controls were significantly more likely than cases to receive any intrapartum antibiotics (70% vs 30%; *P* <0.001).

Table 26. Demographics of case-control participants and summary statistics of anti-GBS-CPS IgG concentrations

Characteristic	ST III				Total			
	EOGBS N = 14	LOGBS N = 4	Controls N = 30	*P value	EOGBS N = 22	LOGBS N = 12	Controls N = 144	*P value
Maternal age, median (range)	28 (22-31)	29 (20-38)	33 (26-43)	0.02	30 (22-36)	37 (20-42)	33 (21-46)	0.1
Ethnicity, n (%)								
Asian or Asian British	4 (28.6%)	1 (25.0%)	4 (13.3%)	0.3	6 (27.3%)	1 (8.3%)	15 (10.6%)	0.01
Black or Black British	2 (14.3%)	0 (0.0%)	1 (3.3%)		2 (9.1%)	1 (8.3%)	7 (4.9%)	
Mixed	0 (0.0%)	1 (25.0%)	0 (0.0%)		2 (9.1%)	2 (16.7%)	2 (1.4%)	
Other ethnic group Other	1 (7.1%)	1 (25.0%)	6 (20.0%)		2 (9.1%)	2 (16.7%)	20 (14.1%)	
White British	5 (35.7%)	1 (25.0%)	11 (36.7%)		8 (36.4%)	5 (41.7%)	64 (45.1%)	
White Other	2 (14.3%)	0 (0.0%)	8 (26.7%)		2 (9.1%)	1 (8.3%)	34 (23.9%)	
Rupture of membranes >= 18 hours, n (%)	1 (7.1%)	1 (33.3%)	9 (30%)	0.04	2 (9.1%)	2 (20.0%)	31 (21.8%)	0.001
Delivery type, n (%)								
Vaginal	11 (78.6%)	2 (50.0%)	20 (66.7%)	0.2	16 (72.7%)	6 (50.0%)	84 (58.7%)	<0.001
C-Section with rupture of membranes	3 (21.4%)	2 (50.0%)	5 (16.7%)		6 (27.3%)	6 (50.0%)	23 (16.1%)	
C-Section without rupture of membranes	0 (0.0%)	0 (0.0%)	5 (16.7%)		0 (0.0%)	0 (0.0%)	36 (25.2%)	
Gestation at birth, median (range)	39 (27-40)	37 (30-39)	39 (36-42)	0.06	39 (27-42)	34 (23-40)	39 (35-44)	0.004
Infant sex, n (%) Female	6 (42.9%)	2 (50.0%)	10 (40.0%)	1	9 (40.9%)	13 (33.3%)	55 (44.7%)	0.6
Intrapartum antibiotics, n (%) Yes	2 (14.3%)	2 (50.0%)	19 (70.4%)	0.002	5 (22.7%)	5 (45.5%)	82 (69.5%)	<0.001
Samples with anti-STIII IgG < LLOQ	12 (85.7%)	3 (75.0%)	8 (26.7%)	<0.001	18 (81.8%)	9 (75.0%)	66 (45.8%)	<0.001
Anti-STIII IgG (µg/ml), median (range)	0.006 (0.006-2.94)	0.006 (0.006-0.04)	0.1 (0.006-19.6)	<0.001	0.006 (0.006-2.94)	0.006 (0.006-1.04)	0.02 (0.006-24.1)	<0.001

*Cases vs controls ST: Serotype; EOGBS: Early-onset disease; LOGBS: Late-onset disease; C-Section: Caesarean section; CPS: Capsular polysaccharide; IgG: Immunoglobulin G

8.3.2 Antibody Responses in Cases and Controls

Anti-GBS-CPS IgG concentrations in cases and controls were analysed in Chapter 7. In summary, serotype III-specific anti-GBS-CPS IgG concentrations were significantly lower in cases compared to controls (0.006 µg/ml vs 0.02 µg/ml ; *P* <0.001) (**Table 26**). The majority of EOGBS (82%) and LOGBS (75%) cases had undetectable IgG titres compared to less than half of controls (46%; *P* < 0.001).

8.3.3 RRR and ADR of iGBS Disease Predicted by Infant Serotype III CPS IgG Concentration in Serum

Non-parametric RCD of serotype III-specific anti-GBS-CPS IgG concentrations in sera of infants who developed serotype III-associated EOGBS and EOGBS/LOGBS combined and cord sera of those infants who remained healthy despite exposure to the same serotype are shown in **Figure 40A-B**. Distributions of serotype III-specific anti-GBS-CPS IgG concentrations in all cases and controls are shown in **Figure 40C-D**. Overall, the distribution of IgG serum titres for cases was shifted toward lower values driven by the samples with titres below LLOQ and remained below the RCD curve for controls along the full range of IgG values.

Relative risks for EOGBS and EOGBS/LOGBS combined expressed as odds ratios for fixed antibody thresholds (Methods 1) are shown in **Table 27**. Serotype III-specific anti-GBS-CPS IgG concentrations of at least 0.01 µg/mL were associated with a 95% (68%-99%) reduction in relative risk (*P* < 0.001). Similarly, the relative risk of EOGBS alone was reduced by 95% (66%-99%).

OR calculated using Method 2 are shown in **Table 28**. Intermediate serotype III-specific anti-GBS CPS IgG concentrations (0.1 to 0.9 µg/mL) were associated with a 96% (61%-99%) reduction in relative risk of iGBS disease compared to those with concentrations of < 0.01 µg/mL. Similarly, for EOGBS alone, the relative risk was reduced by 96% (60%-99%).

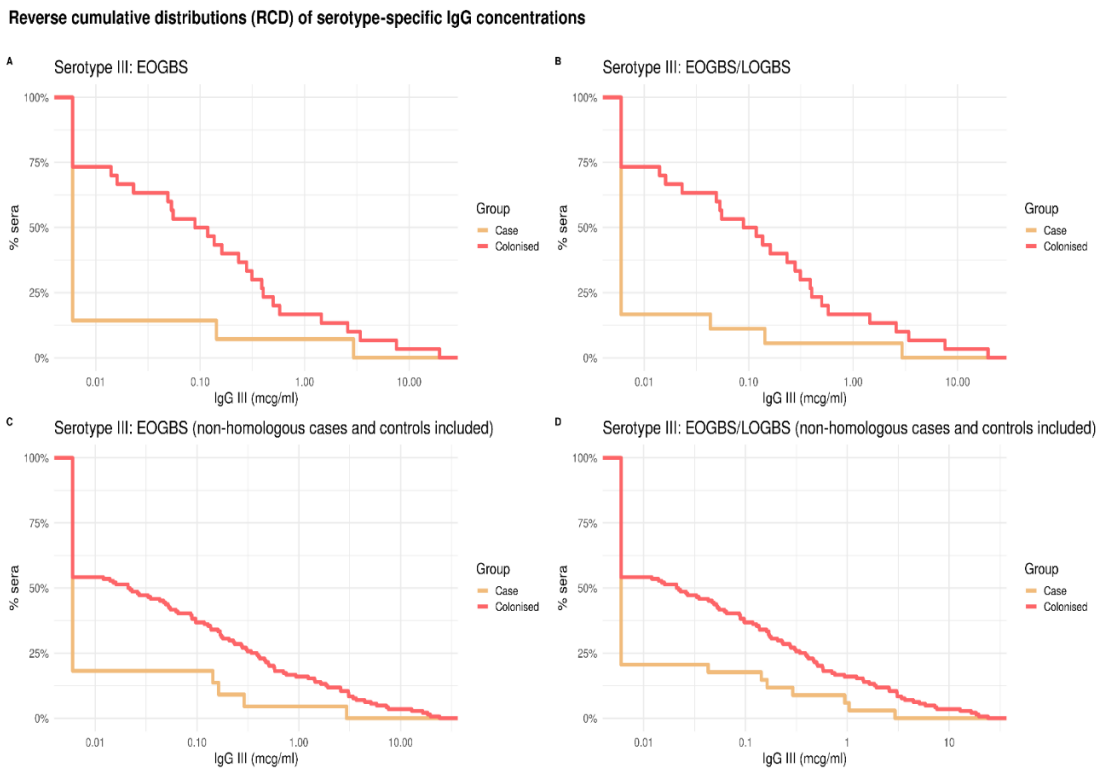


Figure 40. Non-parametric RCD of IgG concentrations against CPS III in case and control sera

Numbers on y axis represent the percentage of samples with measured anti-CPS IgG concentrations larger or equal to each value shown on the x axis. (A)Anti-STIII IgG in STIII-associated EOGBS and control samples. (B) Anti-STIII IgG in STIII-associated EOGBS/LOGBS and control samples. (C) Anti-STIII IgG in all EOGBS cases and control samples. (D) Anti-STIII IgG in all EOGBS/LOGBS cases and control samples. RCD: reverse cumulative distributions; IgG: immonoglobulin G; CPS: capsular polysaccharide; ST: serotype; EOGBS: early-onset invasive Group B streptococcal disease; LOGBS: late-onset invasive Group B streptococcal disease

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Table 27. Odds ratios of iGBS disease for fixed antibody thresholds (Method 1)

CPS-Specific IgG (µg/ml)	Controls (%) N=30	EOGBS (%) N=14	OR (95% CI)	*P* value	aOR (95% CI)	*P* value	EOGBS/ LOGBS (%) N=18	OR (95% CI)	*P* value	aOR (95% CI)	*P* value
<0.01	8 (26.7%)	12 (85.7%)	0.06 (0.008-0.28)	0.001	0.05 (0.004-0.34)	0.006	15 (83.3%)	0.07 (0.01-0.28)	<0.001	0.05 (0.003-0.32)	0.006
<0.1	15 (50.0%)	12 (85.7%)	0.17 (0.02-0.75)	0.03	0.12 (0.01-0.78)	0.04	16 (88.9%)	0.13 (0.02-0.54)	0.01	0.12 (0.01-0.80)	0.04
<1	25 (83.3%)	13 (92.9%)	0.38 (0.02-2.72)	0.4	1.01 (0.03-21.9)	0.99	17 (94.4%)	0.29 (0.015-2.04)	0.3	0.88 (0.02-20.1)	0.9
CPS: Capsular polysaccharide; IgG: Immunoglobulin; EOGBS: Early-onset Disease; LOGBS: Late-onset Disease; OR: Odds ratio; CI: Confidence interval; aOR: adjusted odds ratio											

Table 28. Odds ratios of iGBS disease against lowest antibody level (Method 2)

CPS-Specific IgG (µg/ml)	Controls (%) N=30	EOGBS (%) N=14	OR (95% CI)	*P* value	aOR (95% CI)	*P* value	EOGBS/ LOGBS (%) N=18	OR (95% CI)	*P* value	aOR (95% CI)	*P* value
<0.01	8 (26.7%)	12 (85.7%)	Ref		Ref		15 (83.3%)	Ref		Ref	
0.01-0.09	7 (23.3%)	0 (0.0%)	NA		NA		1 (5.6%)	0.08 (0.004-0.5)	0.03	NA	
0.1-0.9	10 (33.3%)	1 (7.1%)	0.07 (0.003-0.4)	0.02	0.04 (0.001-0.4)	0.02	1 (5.6%)	0.05 (0.003-0.35)	0.01	0.04 (0.001-0.39)	0.02
≥1	5 (16.7%)	1 (7.1%)	0.13 (0.006-1.03)	0.09	0.3 (0.006-7.2)	0.5	1 (5.6%)	0.11 (0.005-0.81)	0.06	0.25 (0.004-7.3)	0.5
CPS: Capsular polysaccharide; IgG: Immunoglobulin; EOGBS: Early-onset Disease; LOGBS: Late-onset Disease; OR: Odds ratio; CI: Confidence interval; aOR: adjusted odds ratio; Ref: reference group; NA: Non calculatable											

This study estimated ADR for undetectable IgG titres as 0.42% and 0.69% for EOGBS and EOGBS/LOGBS, respectively. Serotype III-specific anti-GBS CPS IgG concentrations >1.84 µg/mL (95% CI 0.98 -10.20) and >2.28 1.16-7.03) µg/mL were associated with a 90% reduction of the predicted absolute risks of serotype III-associated EOGBS, and EOGBS/LOGBS combined, respectively (**Figure 41**).

When a more stringent cut-off of <8 days between the onset of disease and blood collection was applied, 24 cases were included in the model, and the antibody threshold associated with a 90% reduction of the ADR for EOGBS/LOGBS was 2.33 (1.17-14.16) µg/mL (**Figure 42A**). When a longer cut-off of <14 days was applied, the antibody threshold derived by 39 cases was 2.72 (1.34-14.40) µg/mL (**Figure 42B**).

The predicted cord titres for LOGBS cases based on acute titres are shown in **Table 29**. When predicted cord titres rather than acute sera titres were used, the protective antibody threshold for EOGBS/LOGBS combined was estimated as 2.31 (1.18-7.16) µg/mL (**Figure 42C**).

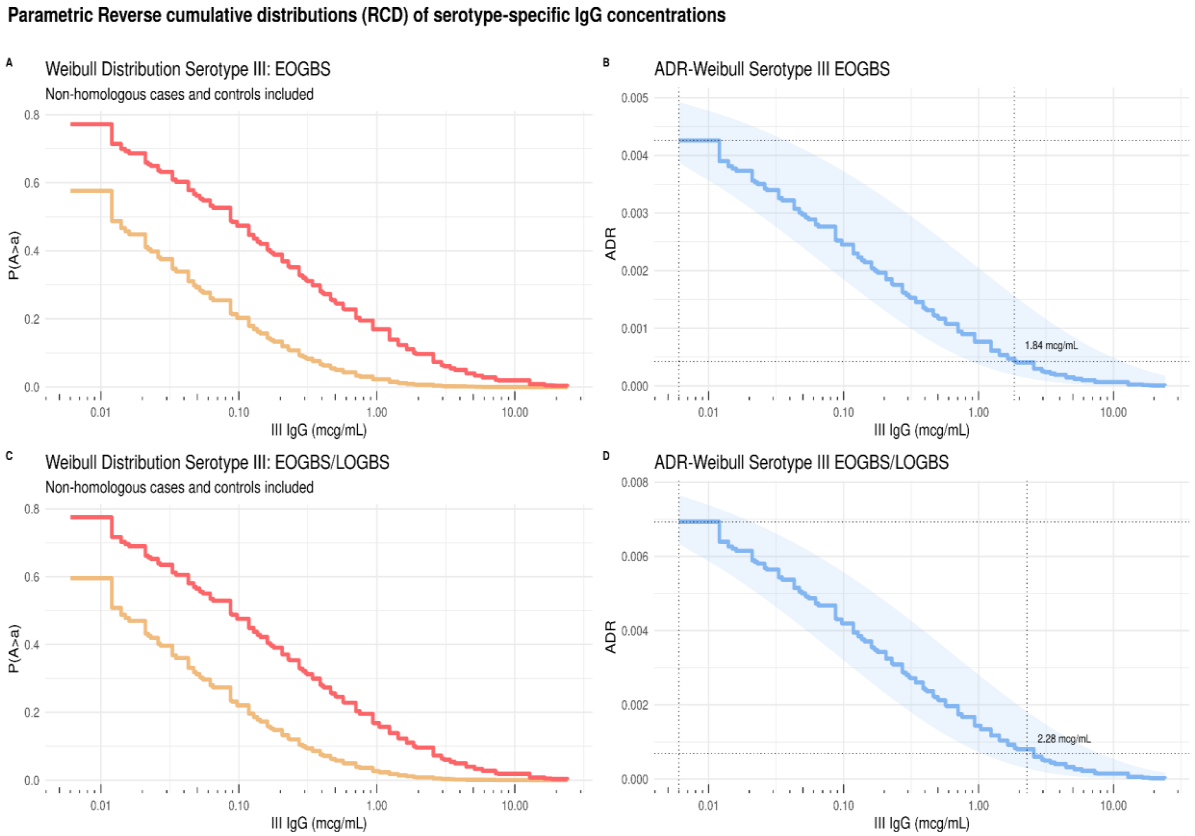

Figure 41. RCD of anti-STIII-IgG concentrations for STIII cases and control sera and estimates of ADR for GBS STIII across the range of infant IgG concentrations determined using Weibull regression

(A) Anti-STIII IgG concentrations in all EOGBS cases and control samples. (B) ADR curve with 95% CI for STIII EOGBS (C) RCD curve for all EOGBS/LOGBS cases and control samples. (D) ADR curve with 95% CI for STIII EOGBS/LOGBS. Red lines depict controls and orange lines depict cases. RCD: reverse cumulative distributions; IgG: immonoglobulin G; ST: serotype; ADR: absolute disease risk; EOGBS: early-onset invasive Group B streptococcal disease; LOGBS: late-onset invasive Group B streptococcal disease; CI:confidence interval.

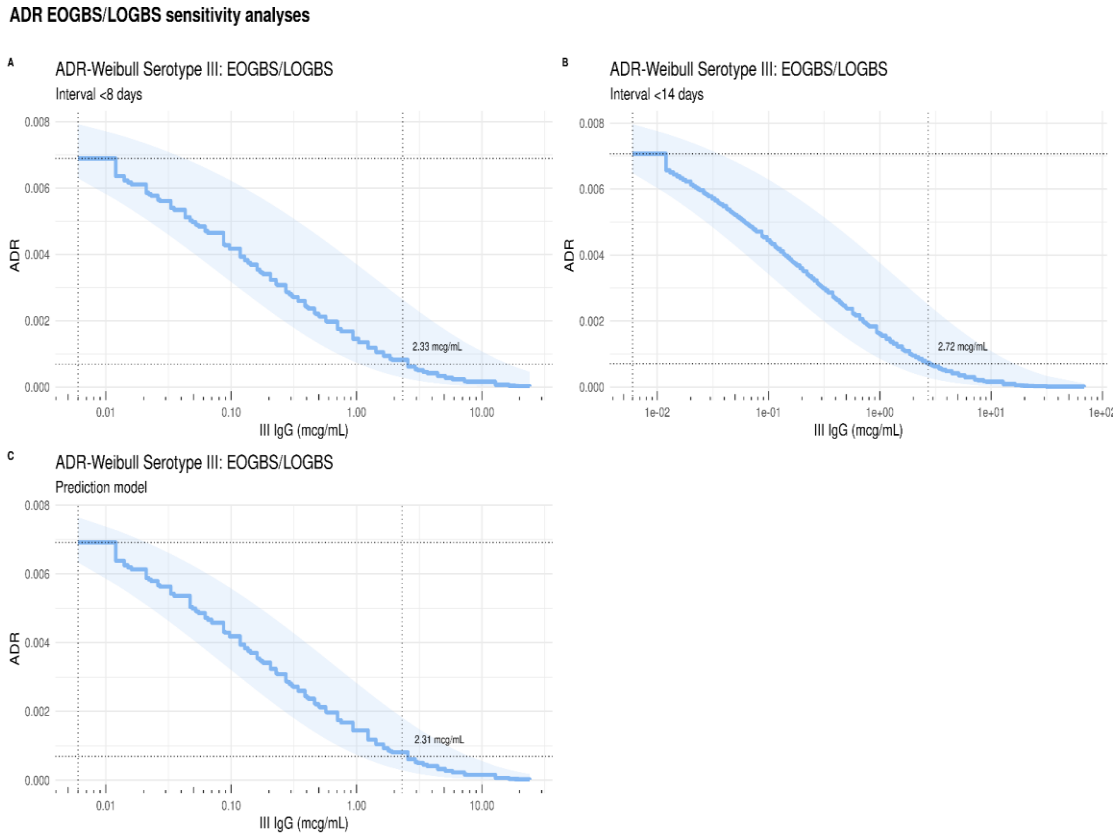

Figure 42. Estimates of ADR for GBS STIII across the range of infant IgG concentrations determined using Weibull regression (sensitivity analyses)

(A) ADR curve with 95% CI for STIII EOGBS/LOGBS when interval <8 days between onset of disease and collection of infant serum. (B) ADR curve with 95% CI for STIII EOGBS/LOGBS when interval <14 days between onset of disease and collection of infant serum (C) ADR curve with 95% CI for STIII EOGBS/LOGBS, when IgG titres at birth were back-calculated from acute LOGBS infant sera (onset of disease <30 days) CI: confidence interval; IgG: immonoglobulin G; ST: serotype; ADR: absolute disease risk; EOGBS: early-onset invasive Group B streptococcal disease; LOGBS: late-onset invasive Group B streptococcal disease.

Table 29. Predicted cord anti-serotype III IgG titres based on the anti-serotype III IgG titres measured in acute sera of LOGBS cases

ID	Serotype	Age at onset of disease, days	Age at blood collection, days	Acute anti-STIII IgG, µg/ml	Cord anti-STIII IgG, µg/ml
1	Ia	7	15	0.006	0.006
2	III	9	19	0.04	0.07
3	III	13	19	0.006	0.006
4	NA	17	20	0.006	0.006
5	Ia	18	21	1.04	1.78
6	Ia	20	30	0.006	0.006
7	NA	22	32	0.006	0.006
8	III	26	30	0.006	0.006
9	II	40	48	0.94	*
10	III	42	43	0.006	*
11	NA	43	46	0.006	*
12	II	53	56	0.006	*

*Excluded since day of onset >30 days

IgG: Immonoglobulin G; ST: Serotype; NA: Not available

8.4 Discussion

The results of this study confirm that a sufficient concentration of naturally acquired anti-GBS CPS IgG at delivery confers substantial protection to infants against EOGBS and LOGBS caused by serotype III. Consistent with prior literature, this study estimated iGBS disease risk using both RRR and ADR methods. The relative (logistic regression) and absolute (Weibull model) risk analyses provided complementary information about the role of placentally transferred anti-GBS CPS IgG in the protection of infants, but also showed notable inconsistencies.

Logistic regression analysis results affirmed the inverse relationship between antibody concentration at birth and disease risk in neonates exposed to GBS serotype III through their colonised women. Based on the logistic regression, a cut-off at 0.01 μg/mL appeared to be the most informative threshold associated with a reduction in disease since ≥0.01 μg/mL vs <0.01 μg/mL had aOR 0.05, but the 95% CI was very wide (0.003-0.32). The main limitations were the relatively small sample sizes of cases and controls and the lack of variability in IgG titres among serotype III cases. Over 80% of serotype III cases had undetectable IgG titres, and over 80% of serotype III controls had antibody titres <1 μg/ml. Furthermore, as the RCD of the controls decreased more rapidly than cases, the reduction of relative risk above a cut-off >1 μg/ml was not statistically significant. Although it was theoretically expected the aOR to get smaller as the cut-offs increased, in practice, this did not happen, most likely because of the small numbers, as shown by the 95% CI.

The Weibull point estimates of ADR suggested that serotype III antibody concentrations of >1.84 (0.98-10.20) μg/mL and >2.28 (1.16-7.03) μg/mL were associated with a 90% reduction of predicted risks of serotype III-associated EOGBS and EOGBS/LOGBS combined, respectively. Applying a parametric model that used the full range of values from all cases and controls and not only serotype III, allowed for smoother RCD curves for cases and controls when compared to the non-parametric RCD curves. Notably, whereas Weibull and non-parametric RCD curves showed similar data distribution for IgG concentrations >0.1 μg/mL, Weibull regression shifted up the curves for values between 0.01 and 0.1 μg/mL. Since only a

very small proportion of cases presented with high IgG titres in this population, the ADR decreased monotonically as anti-serotype III antibody concentrations increased, in line with previous models. (365) Also, the 95% CIs of Weibull ADR estimates were quite narrow, in keeping with those reported by Izu et al., who tested the same method to re-analyse the SA seroepidemiological study. (365) Therefore, when compared to the aOR approach, the Weibull model of ADR provided a solution to the small sample size and the limited IgG variability of the cases group.

However, this solution has its own limitations and some notable inconsistencies. First, the Weibull model fits a parametric curve to the data using the same shape distribution in cases and controls. It is very likely that a non-parametric ADR model would not have performed the same with such small numbers of cases and controls. In their study, Izu et al. showed substantially different ADR curves but little difference between non-parametric and parametric ADR estimates for cases with low IgG titres. (365) I have not applied a non-parametric ADR model to my data to address this question, but this is worth exploring further when the complete dataset from the iGBS3 study is available. Another important inconsistency between aOR and ADR was that Weibull regression did not predict any risk reductions for values <0.01 μg/mL, due to the truncation of the data at the lower limit.

The predicted protective serotype III-specific IgG concentrations against EOGBS in this study differed from previous early studies, (362) but were comparable with more recent estimates (**Table 26**). (155,207,359) However, substantial differences in assays, standard sera, statistical analyses and sera sources do not allow for a meaningful comparison. (179) When considering protection against both EOGBS and LOGBS, predicted antibody titres were higher than EOGBS alone, in keeping with the DEVANI and the SA seroepidemiological studies. (155,359,365)

Adjustment for covariates is a challenge for observational studies. Covariates could change the probability of developing disease or affect the distribution of antibody levels. Administration of intrapartum antibiotics with activity against GBS belongs to the first category, whereas gestation at birth could have affected the antibody distribution since preterm birth, which was more common among cases, is associated with lower placental antibody transfer. (366) Exclusion of controls and

cases who received antibiotics as well as cases born before 35 weeks gestation, would have substantially reduced the number of participants. Instead, these participants were included in the analysis, and a logistic regression was used to obtain aOR, which were found to be similar to the crude OR. Adjustment for confounders was not performed in the ADR model. A recent review has concluded that adjustment for this statistical method is complex with no clear solution. (349)

A limitation of this study is that only controls' sera were collected at the time of birth, whereas cases' sera were collected after the onset of the disease. It is biologically plausible that IgG titres from EOGBS cases accurately reflected those transferred to neonates at birth and could be directly compared to the cord samples. On the contrary, IgG titres in LOGBS cases likely decayed after birth. In accordance with this, previous data from the SA seroepidemiological cohort study showed lower anti-serotype III IgG titres when acute and cord sera from cases were combined compared to cord sera alone. (359) To account for the decay of antibodies, the prediction model discussed in Chapter 6 was applied to back-calculate birth titres. When these predictions were applied, the 90% ADR reduction threshold estimates did not differ from the main analysis. However, the applicability of this model was quite limited in this dataset. Eight out of twelve LOGBS cases presented within the first 30 days of life, and only two had IgG values above LLOQ. In addition, average antibody half-lives used in this model were derived from healthy controls and might not be representative of kinetics in cases. The ongoing iGBS3 study aims to address this question fully. Another potential limitation is the time of blood collection relative to the onset of the disease. It is biologically plausible that IgG titres measured after confirmation of iGBS disease may be low due to consumption or high due to an early immune response. Sensitivity analyses of different intervals between the onset of disease and collection of infant sera showed a trend towards higher IgG titres in cases with longer intervals. However, this was not statistically significant and was likely affected by the small numbers included.

8.5 Conclusions

In summary, these findings indicate that relatively low levels of naturally acquired anti- serotype III CPS IgG protect most infants against iGBS disease caused by GBS serotype III. Using at least two statistical methods can contribute to the robustness of results. A parametric Weibull model of ADR is an effective method to overcome issues with small-size groups of cases and controls in settings with low levels of anti-GBS antibodies. Supplementarily, confounder-adjusted OR against a baseline disease probability can derive a confounder-adjusted CoP threshold estimate. These results provide a preliminary framework to estimate the antibody concentrations that candidate polysaccharide-protein conjugate vaccines should aim for to induce high levels of protection. The analyses presented here are just a demonstration of what can be done and not an attempt to actually identify a CoP, which given the inconsistency in results of the approaches presented here clearly has not been done yet. Further work is needed to explore the protective antibody thresholds against the less prevalent serotypes and to develop immunobridging formulas for predicting vaccine efficacy based on absolute risk parameters derived from natural immunity studies.

Chapter 9 – General Discussion / Conclusions

In this chapter, I briefly summarise my thesis contributions by combining the results and conclusions from the previous chapters. Then I discuss the limitations of this work as a whole and explore future research questions that arise from my work.

9.1 Summary of Findings

My thesis's two broad overarching objectives were first to further the understanding of the clinical and epidemiological risks of LOGBS and second to contribute to defining a protective threshold of naturally acquired serotype-specific anti-GBS CPS IgG for both EOGBS and LOGBS. I addressed the first objective in Chapters 4 and 5 and the second in Chapters 6-8.

In contrast to EOGBS, the risk factors for LOGBS were not previously systematically reviewed. In Chapter 4, I undertook a systematic review and meta-analyses of clinical and epidemiological risk factors of LOGBS to address this gap in the literature. I showed that antenatal maternal colonisation and prematurity/low birth weight are major risk factors for LOGBS and calculated the pooled risk estimates. These findings have important implications for supporting maternal vaccination as a strategy to prevent LOGBS. Many pregnant women colonised with GBS do not have sufficient levels of anti-CPS IgG at delivery to provide passive protection from iGBS disease for the entire at-risk period (Chapter 7). Therefore, a vaccine offered to pregnant women could confer protection against LOGBS through the passive transfer of protective antibodies, as long as these antibodies can persist until three months of age, since most cases occur in the first six weeks of life and cases after three months are very rare (Chapter 6). A vaccination strategy alone is unlikely to prevent all LOGBS in very preterm neonates due to the sub-optimal placental transfer of maternal IgG. However, this is not yet clear, and it is still possible that a significant proportion of preterm infants will benefit from a GBS vaccine given early in pregnancy.

In Chapter 5, I reviewed the literature on cases of recurrent iGBS disease and analysed cases with recurrence and in multiples from UKROI (national surveillance study 2014/15) and Germany and Switzerland (retrospective case collection 2008-

2020). I found that having a sibling with iGBS disease is a significant risk factor in infants born of multiple gestation pregnancies. I also showed that prematurity/very low birth weight and insufficient (<10 days) antibiotic treatment are important contributing factors associated with the recurrence of iGBS disease. These findings were used to inform recommendations for investigating and managing the asymptomatic twin in iGBS disease in multiple births or recurrent cases. In addition to the clinical perspective, the association between prematurity/very low birth weight (therefore likely immature immune responses) and iGBS recurrence, the short interval of onset of iGBS disease in twin siblings (median of 6 days) occurring many days after birth (median of 29 days), and the observation that the same serotype (in many cases hypervirulent sequence type 17) typically causes the second episode in recurrent cases provide valuable insights into understanding recurrent iGBS disease and LOGBS in general. Together, they support persistent mucosal colonisation and later invasion of the bloodstream or acute horizontal transmission as the two most likely hypotheses.

In Chapters 6-8, I explored some of the unanswered questions about the CoP for neonatal and infant iGBS disease. I coordinated a pilot cohort study conducted between 2018 and 2020 followed by a large-scale UK seroepidemiological case-control study (iGBS3) in 2022 (still underway). Cord blood samples were collected from colonised and non-colonised women with healthy infants, as well as acute phase sera from infants with iGBS disease. I used sera from these two studies to measure serotype-specific anti-GBS CPS IgG with a standardised multiplex immunoassay developed by the GASTON consortium.

In Chapter 6, I investigated the kinetics of antibody decay in healthy infants in the first three months of life and estimated the half-life of naturally acquired antibodies. If these findings are validated in cases of iGBS disease when the first phase of the iGBS3 study is concluded, they could be used to back-estimate cord blood concentrations from acute sera concentrations in LOGBS cases. Subsequently, this will facilitate seroepidemiological studies, since it would mean that cord blood collection is not necessary, thus avoiding the cost and effort associated with cord blood collection.

Next, I compared geometric mean concentrations in cases and controls for EOGBS and LOGBS (Chapter 7). The results from this analysis conducted in a high-resource setting with a relatively high prevalence of antenatal GBS colonisation confirmed the role of naturally acquired humoral immunity against iGBS disease. In addition, commonly recorded demographic factors such as maternal ethnicity and age did not affect the antibody distribution, so adjusting for these covariates might not be needed when estimating the CoP.

Finally, I estimated the relative risk reduction and the absolute disease risk at different anti-GBS CPS IgG cut-off values (Chapter 8). To this purpose, I calculated odds ratios (with and without covariate adjustment) of iGBS disease for antibody concentrations above various thresholds for serotype III. I also used a Weibull parametric model that enabled inclusion of all cases and controls and not only serotype III, which provided a solution to the small sample size and the limited IgG variability of the cases group. These methods can be further tested and refined as more data from the iGBS3 study become available to form a robust body of evidence for a CoP against iGBS disease which will subsequently facilitate the development, licensure and implementation of GBS vaccines.

9.2 Limitations

The limitations of each included study are discussed in the relevant chapter. In this section, I aim to discuss the limitations of this thesis as a whole. First, my initial plan was to use the entire dataset from the iGBS3 study to answer the research questions. Due to the unprecedented circumstances associated with the COVID-19 pandemic, the start of the study was significantly delayed. Therefore, by the time I wrote this thesis, only a small proportion of samples from the iGBS3 study were available for analysis. However, the same laboratory and statistical methods will be used upon completing the full iGBS3 study as presented here. Second, MIA does not provide information on the functional ability of antibody to mediate complement-dependent opsonophagocytosis by neutrophils. I intended to measure antibody functionality using a standardised OPkA, miming the in *vivo* process of killing GBS as introduced above. (367,368) Due to technical issues with the assay (especially the effect of

antibiotic treatment during the acute phase of iGBS disease) and time restrictions, I did not succeed in obtaining good-quality data from the OPkA analysis to include in this thesis. This additional analysis is still ongoing.

9.3 Future work

Our understanding of the pathogenesis of LOGBS is still incomplete. To fully understand and ultimately prevent LOGBS, there is a need for large-scale, prospective, longitudinal (from pregnancy until the time of LOGBS diagnosis) case-control studies that integrate genome sequencing and serology from infant sera, breast milk samples and specimens from other family members (not restricted to mothers) and healthcare staff (where relevant). Although not directly addressed in this thesis, the interplay between host innate immunity, microbiota development and the successful incorporation of GBS into its niche emerged as a priority for future research in this field.

The next steps in the accelerated approval pathway for a vaccine against GBS involve (i) completion of the ongoing seroepidemiological studies in UK, SA and USA, (ii) pooling data from all seroepidemiological studies, (iii) agreeing an immunobridging formula for predicting vaccine efficacy based on an absolute risk and a relative association parameters and (iv) designing Phase 4 post-approval studies to establish the vaccine effectiveness. In parallel, there is a need to focus on the "how"-oriented research questions about vaccine acceptancy among pregnant women and equity of vaccine distribution.

9.4 Concluding Remark

I hope the data in this thesis will help understand the risk factors of LOGBS better and stimulate further work to unravel its pathophysiological mechanisms fully. As this thesis comes to an end, the prospect of the first licensed GBS vaccines comes closer to fruition. Hopefully, the work in this thesis will contribute to this collective effort to enable the prevention of this devastating disease through. equitable access to an effective and safe vaccine worldwide

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