# Review of serocorrelates of protection against infant Group B Streptococcus disease from infection studies

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**Abstract**

Group B Streptococcus (GBS) is a leading cause of young infant mortality and morbidity globally, with vaccines in development for over four decades but none licensed to date. A serocorrelate of protection against invasive disease in young infants is being considered to facilitate vaccine early licensure, followed by demonstration of efficacy, assessed post-licensure. Here we synthesise the scientific evidence currently available to define an immune correlate associated with GBS disease risk reduction based on studies of natural infection. We summarise studies that have investigated serum anti-capsular or anti-GBS protein antibodies, and studies measuring the association between antibody function and disease risk reduction. We highlight how knowledge in the development of correlates of protection from existing vaccines could be harnessed to facilitate GBS vaccine development. These include aggregation of serocorrelates of protection for individual serotypes, understanding the relationship between immunity derived from natural exposure of adults and vaccine-induced immunity, or using extrapolation of protection from in vitro immunoassay results. We also highlight key considerations for the assessment of the role of antibodies to derive a serocorrelate of risk reduction in future seroepidemiological studies of GBS disease.

**Panel 1 - Search Strategy and selection criteria**

We searched PubMed for “*"Group B Streptococcus" OR "Streptococcus agalactiae" (MeSH) AND "antibody" OR "vaccination" OR "immunization" OR "vaccine" OR "pregnancy"* up to 5th May 2018*.* Multiple spellings, truncated nomenclature, and abbreviations were also used as search terms. Articles and their references lists were reviewed without language restriction. Articles were included where they reported:

1. antibodies against Group B Streptococcus (GBS) in studies comparing infants with GBS disease to infants without disease;
2. antibodies against GBS in vaccine trials comparing vaccinated and unvaccinated populations;
3. antibodies measured in studies where the primary end point was either protection against invasive GBS disease or protection against infant or maternal carriage.

**Panel 2 - Lessons learned from other vaccines**

* Mechanisms of natural immune protection are similar to vaccine-derived protection (e.g. serum bactericidal antibody, *Haemophilus influenzae, Neisseria meningitidis*; opsonophagocytosis, pneumococcus)
* Protection depends on functional antibodies (bactericidal antibodies from *Haemophilus influenzae* and meningococcus, opsonophagocytic antibodies for pneumococcus).
* Thresholds defined for one sub/serotype have been used as evidence to inform a putative threshold for rarer serotypes (e.g. pneumococcal 13-valent vaccines that were licenced without efficacy data for serotypes in addition to the 7-valent vaccines). Aggregate thresholds were used temporarily for rarer pneumococcal serotypes before it was possible to establish individual serotype-specific thresholds.

**Panel 3 - Next Steps/Future Research for seroepidemiological studies of GBS disease**

1) Standardise the assays to be used to assess antibody quantity and function against GBS and establish the correlation between them;

2) Conduct large prospective case/control studies in diverse populations, in order to use the immunity derived from natural exposure to GBS, as measured in both maternal and cord/infant serum, to predict serocorrelates of disease risk reduction;

3) Study factors influencing antibody transfer from mother to infant, including malaria, HIV and gestation, on antibody transfer and antibody decline between birth and 3 months of life;

**Introduction**

Group B Streptococcus (GBS) remains a leading cause of neonatal and infant sepsis globally1-3. One in five GBS meningitis survivors will suffer long-term adverse neurodevelopmental outcomes.4,5

Several strategies exist to reduce the early-onset GBS disease burden (first 6 days of life). In the USA, universal screening of pregnant women between 35-37 weeks gestation for GBS rectovaginal carriage has been in place since 20026, and for women with GBS carriage, intrapartum antibiotics (IAP) are offered. In contrast other countries offer IAP to pregnant women only if specific risk factors are present. Such countries have not seen the decline in early-onset disease experienced by the USA. For example, in the UK cases have increased by 19% over the last 15 years (0.48/1000 in 2000/1 vs. 0.57/1000 live births in 2014/15) and are now double the rate found in the USA (0.21/1000 live births in 2015).1,7 In South Africa, incidence rates have remained consistently over 1.4/1000 live births since 2005, despite risk-factor based IAP strategies.8 Importantly, although international and local guidelines exist for the management of neonatal and infant infection risk, these policies and guidelines are poorly implemented, making IAP administration sub-optimal in most low- and middle-income settings.9

Despite the reduction of early-onset disease rates in countries implementing universal screening7, GBS late-onset disease (day 7-90 of life) has remained static over the past 10 years1,7,8,10. GBS is now the most important cause of bacterial meningitis in infants under 3 months of age in countries reporting late-onset disease incidence.11,12 A GBS vaccine could be a cost-effective method of reducing the burden of all forms of infant disease worldwide13.

Clinical evaluation of GBS vaccines using prevention of invasive neonatal and infant disease as a primary endpoint requires large studies, which are therefore best carried out in regions with relatively high GBS disease prevalence. It is estimated that a vaccine efficacy study of approximately 60,000 pregnant women in countries with a disease incidence ≥1/1000 live births would be required to detect a 75% reduction in early- and late-onset disease assuming the vaccine protects against 90% of circulating serotypes.1413,15

Seroprotective thresholds have been derived for several vaccines and have been useful in regulatory pathways for meningococcal serogroup W and Y polysaccharide vaccines, meningococcal serogroup B vaccines and higher-valency formulations of pneumococcal polysaccharide-conjugate vaccines. Although important differences need to be considered between a GBS vaccine and these vaccines, serocorrelates of protection could play a role in the pathway to GBS vaccine registration, policy decisions and implementation.

Several retrospective case control studies provide data that indicate that serocorrelates of protection against infant GBS disease might be determined16-19. These studies ranged in size from 150 to 140,000 pregnant women and retrospectively captured between 19 and 109 cases of neonatal and infant GBS serotype Ia and III disease. Each study showed that greater maternal anti-capsular antibody concentration was linked to reduced GBS disease risk. However, no study was sufficiently powered to provide a definitive answer, and due to the use of different assays it is not possible to compare and pool these results. Hence the need to establish consensus around the data analyses required to establish serocorrelates of risk reduction in seroepidemiological studies and their role in the regulatory pathway.

In an effort to facilitate more rapid licensure and availability of a GBS vaccine for prevention of early- and late-onset disease, a serocorrelate of risk reduction for neonatal and infant invasive GBS disease may prove useful. However, when applying a serocorrelate of risk reduction with a defined risk period, following maternal vaccination, additional factors including placental IgG transfer, maternal comorbidity and antibody decay must be considered. These factors matter, as a serocorrelate will need to demonstrate that vaccines can generate maternal antibody that can be effectively transferred to her infant, and then persist so that they are protected not only against early- but also late-onset disease.

We undertook a literature search of Medline as outlined in Panel 1 to inform this review. For the purposes of this review, we use the term “serocorrelates of protection” to mean a specific antibody response to a vaccine or natural infection that is closely related to protection against infection, disease, or other defined end point (e.g. prevention of stillbirth or premature birth).20 We define natural antibody as antibody developed from exposure to bacterial challenge, either from invasive disease or carriage. Publications from the non-GBS literature were selected to illustrate key insights from other vaccine-preventable diseases and are not intended to be an exhaustive review, as serocorrelates of protection in vaccination have been described in detail previously.20

 In this review we aim to describe the evidence that antibodies are involved in invasive GBS disease or maternal/infant carriage risk reduction considering all targets that might be relevant for future vaccine evaluation. We also highlight key insights from existing vaccines against other pathogens for which serological correlates have been applied that could be harnessed for the benefit of the GBS field and identify key considerations for the assessment of antibody mediating protection in future seroepidemiological studies.

## The role of serotype-specific anti-capsular antibodies in neonatal and infant disease risk reduction

The importance of maternally-derived anti-GBS-IgG in preventing GBS invasive disease was first demonstrated by Baker and colleagues in the 1970s who showed that infants who developed GBS-serotype III disease had significantly lower serotype III-specific IgG in maternal serum than infants without disease also born to women recto-vaginally colonised by that serotype. 16,21 Subsequent studies have shown similar results for serotypes Ia, Ib and V in maternal serum.22,23

Determining a protective antibody concentration/titre is not easily achieved, as protective antibody may vary by serotype, and the assessment of immunogenicity varies by the assay methods employed. Originally, the radioantigen binding assay (RABA) was seen as the gold standard for the quantification of anti-GBS antibodies as it measures antibody in its native state.16 However, RABA quantifies all isotypes of antibody including IgM and so offers an incomplete picture of placentally-transferred immunity. Subsequently, antibody-binding assays have been used that measure antibody concentrations relative to a standard reference serum. More recently, Luminex® or Bioplex® platforms have been used to improve both sensitivity and throughput of assays by measuring antibody against several GBS serotypes simultaneously.

A standardized immunoassay using standardized reagents will be a vital step forward in the assessment of serocorrelates of protection against invasive GBS disease. Such assays would allow comparison of antibody data from studies in diverse geographies and between different vaccine products. The antigen used to detect the target antibodies needs to be in a native CPS conformation that matches that on the surface of the GBS bacteria. There has been concern regarding the use of conjugated CPS to detect IgG, as conjugation may alter efficiency of surface binding to the CPS and/or the ELISA plate.24 Table 1 highlights the current estimates of serotype-specific serocorrelates of risk reduction for infant GBS disease from published studies.

*Serotype-specific anti-capsular antibodies* ***in maternal serum*** *and neonatal and infant disease risk**reduction*

Several different values of invasive neonatal and infant disease risk reduction have been proposed over the past 20 years, ranging from 0.5-10μg/mL of serotype-specific IgG, depending on GBS serotype and country of study. In total, current estimates for a serocorrelate of protection against serotypes Ia and III GBS infant disease are based on 119 and 179 infant disease cases, respectively. Studies have been too small to determine corresponding information for less prevalent GBS serotypes.

The first studies to describe a correlation between GBS disease risk reduction and anti-CPs IgG were undertaken by Lin et al17,25. These case-control studies conducted in the USA, measured GBS CPS-specific IgG by ELISA from maternal and cord serum samples in infants born to GBS-colonised women who went on to develop early-onset disease compared to infants who remained healthy but were GBS-colonised at birth. Logistic regression analysis was used to compare the relative risk of disease at different antibody thresholds in 1 μg/mL increments to a reference value of <0.5 μg/mL for serotype Ia and <2 μg/mL for serotype III. Using these reference values, they identified that thresholds of ≥5μg/mL for serotype Ia and ≥10μg/mL for serotype III in maternal serum resulted in a 90% relative risk reduction of disease caused by these serotypes.17,25

An alternative modeling approach in three more recent studies involved Bayesian data analysis to estimate both absolute and relative risk reduction at different anti-GBS CPS IgG concentration cut-off values in case-control studies18,22,26. The Bayesian model uses data on the background disease risk in the population and the antibody distribution in the cases and controls to calculate the probability of disease above different antibody thresholds. The antibody distributions in cases and controls can either be modeled using parametric functions27 or based on the empirical distributions.26 The relative reduction in risk when antibody concentrations are above a threshold compared to the overall population risk can then be calculated with the advantage of not needing to define a reference comparator antibody level as was necessary in the Lin studies.

Baker and colleagues compared maternal serum from colonised women who had healthy infants to women who had infants with early-onset disease in a case control study using Bayesian modeling. They found a 90% relative risk reduction of early-onset disease for serotypes Ia (95% CI 26-99) and III (28-100) with antibody concentrations ≥0.5 μg/mL in maternal serum compared to a reference value of <0.1 μg/mL.22 Combining data for serotypes Ia, III and V provided an estimated 70% risk reduction for disease due to these three serotypes with antibody concentrations in maternal serum > 1 μg/mL.22

Antibody thresholds were similar for serotypes Ia and III in the Fabbrini study, based on cases and controls of European maternal sera from GBS colonised and non-colonised women. This study used positive reference serum developed by Carol Baker in a multiplex ELISA assay and Bayesian modelling26. Fabbrini and colleagues predicted a 75% absolute early-onset disease risk reduction with anti-CPS IgG concentrations ≥1μg/mL for serotypes Ia and III.26 They also predicted a 76% risk reduction (95% prediction interval 21-100) in late-onset disease caused by serotype III with antibody concentrations ≥1μg/mL26.

Dangor et al used the Bayesian model in South Africa to determine the relative and absolute risk of a mother giving birth to an infant with GBS disease (early or late-onset) at different antibody thresholds using both colonised and non-colonised mothers with healthy infants as controls18. In this analysis maternal anti-CPS IgG concentrations of >6μg/mL for GBS serotype Ia and >3μg/mL for GBS serotype III were associated with < 10% relative risk of combined GBS disease in the infant (6.5% (50% credible interval 1.4-21.9%) and for serotype Ia; 1.3% (50% credible interval 0.1-9.9%) 18.

Comparison between these four studies is difficult for several reasons. It is not possible to compare results between the Lin studies and any of the Bayesian modeling studies because of differences in data analysis methods and the selected end points (relative risk reduction at threshold cutoffs vs. absolute or relative risk reduction using continuous antibody thresholds). Even for those studies using the Bayesian model direct comparison is problematic. Baker22 and Dangor18 used the same disease risk assumption (1% risk) whereas the Fabbrini study26 uses a 3% background risk. Additional issues surrounding the different reference sera, assay parameters and CPS used, leading to the appearance of variation in specific antibody concentration thresholds of protection for individual strains, as well as for different GBS serotypes. The Lin studies used vaccinee sera from Nabi pharmaceuticals for an adult vaccinated with a quadravalent vaccine and commercially available CPS (Dynatech Laboratories)17,25 whilst the Baker and Fabbrini studies used reference serum from monovalent vaccine studies carried out in the USA with CPS from in house (Baker)22 or commercially developed (GSK)26 stocks and the Dangor study used human gammaglobulin standards (calibrated to Carol Baker reference standards) and CPS supplied by GSK18. Assay methodology also differs in each study. The Baker and Lin studies used different monoplex ELISAs and CPS-conjugation methods, the Fabbrini study used a multiplex ELISA and the Dangor study used the Luminex® platform all with different data analysis. Finally, control populations varied between studies, with the Baker and Lin studies using colonised women with healthy infants as controls and the Fabbrini and Dangor studies selecting both colonised and non-colonised women controls (but with considerable variations in control selection in both studies).

*Serotype-specific anti-capsular antibodies* ***in infant serum*** *and neonatal and infant disease risk reduction*

All studies have measured maternal17,18,22,25,26 serum to estimate protective antibody concentrations against early-onset disease. However, as placental antibody transfer of anti-CPS antibodies is less than 100%28, it is important to also consider antibody concentration in cord/infant sera. Lin et al also measured antibodies from cord serum, and found thresholds ≥4μg/mL for serotype Ia and ≥7μg/mL for serotype III associated with a 91% (28-99) relative risk reduction for serotype Ia and 85% (29-97) for serotype III disease.17,25 Dangor et al also collected paired mother and infant sera and determined adjusted odds ratios for disease with antibody concentrations >0.5 μg/mL in infant serum for serotype Ia were 0.18 (0.04-0.85) and for serotype III 0.14 (0.02-1.38)18. However, these data were not used in the Bayesian model.18 At present, it is not possible to estimate serocorrelates of GBS disease risk reduction from studies reporting antibody concentrations in infant sera.

### *Anti-protein IgG antibodies and neonatal and infant disease risk reduction*

Several case-control studies have demonstrated an association between high anti-Rib protein antibodies and GBS disease risk reduction. A significant association was noted between high concentrations of naturally occurring antibody against Rib and reduced invasive GBS disease risk (adjusted odds ratio 0.002 (0.00 to 0.57) p=0.03).29 It should be noted that this was a small study (n=30 infants with disease and 60 healthy controls) and thus was unable to fully assess a correlation between anti-Rib protein antibody and disease. In addition to anti-CPS IgG, the Fabbrini study used the Bayesian model to investigate anti-pilus protein BP-1, API-2a and BP-2b antibodies against early-onset disease isolates expressing these proteins26. They found that sera of mothers delivering infants who developed early-onset disease had significantly lower antibody titres against BP-1 (cases 13.5EU/mL (<LLOQ-555 EU/mL); controls 21.3 EU/mL (<LLOQ-1083 EU/mL; 37% lower in disease) and API-2a (cases 28.8 EU/mL (<LLOQ-160 EU/mL); controls 37.3 EU/mL (<LLOQ to 523 EU/mL; 23% lower in disease)), but not BP-2b, compared to infants who remained healthy during the 7-day follow up period.26 However, no association between fibrinogen-binding protein A and pilus-island (PI) PI-1, PI-2a, PI-2 antibodies and disease was observed in a similar study of all-cause GBS invasive disease in South Africa.30 This study used Bayesian modeling to predict antibody thresholds associated with a relative risk of less than 10% for invasive disease but was unable to predict anti-protein antibody concentrations associated with protection for any of the proteins tested.

These conflicting data highlight some of the outstanding issues in determining the role of protein targets and serological protection against invasive disease. As with anti-GBS-CPS IgG studies, difficulties in comparing these three studies also arise from variations in assay methodology, source of proteins and lack of a common reference serum, in addition to study design differences described above. Table 2 outlines the current evidence.

## The role of antibody function (opsonophagocytosis) in neonatal and infant disease risk reduction

Fewer studies have considered the role of antibody and GBS disease risk reduction using opsonophagocytosis assays (OPkA). 26,31-33 Currently there are two *in vitro* GBS OPkAs that use different approaches. One approach, which was also used for pneumococcal vaccine studies and licensure, determines the dilution of antiserum that kills at least half of the target bacteria in the presence of exogenous complement26. The other approach, which has been used in the majority of GBS studies, determines the degree of bacterial killing over a fixed incubation period by a human serum sample with endogenous complement32.

Studies in both animals and humans indicate that functional activity appears to correlate well with GBS-serotype specific anti-CPS antibody concentration.26,32 Baker et al have demonstrated that adult sera containing high concentrations of anti-GBS CPS antibodies are capable of promoting efficient opsonisation and phagocytosis of GBS in-vitro33. Studies in non-pregnant adults also indicate that higher vaccine-induced antibody concentrations generate greater opsonophagocytic killing against serotypes Ia, Ib34, II35 and V.36

 *Opsonophagocytosis mediated by antibodies* ***in maternal serum*** *and neonatal and infant disease risk reduction*

Only one study has compared maternal antibody concentration with opsonophagocytosis from natural antibody26. This study found significant correlations between maternal IgG against serotypes Ia, Ib and III (measured by multiplex ELISA) and opsonophagocytosis (% killing, exogenous complement), when IgG antibody concentrations were >1μg/mL (serotype Ia R=0.8, serotype Ib R=0.8, serotype III R=0.85).26 Extrapolation using the correlation coefficients from this study suggests that doubling the IgG concentration may increase the OPkA titre by 70-80% and also adds evidence that a serocorrelate of protection might be established at around 1μg/mL and OPkA titres of between 1:64-128 for serotypes Ia and III.26 (Table 1).

*Opsonophagocytosis mediated by antibodies* ***in infant serum*** *and neonatal and infant disease risk reduction*

It may be important to consider the effect of antibody function in neonatal/infant serum, as differences in their immune function may alter the response to GBS challenge compared to adult sera. No studies have investigated the association between antibody function in neonatal/infant serum at time of disease and disease risk reduction. There has been only one small study of antibody function in convalescent sera of ten infants with GBS disease and their mothers using endogenous complement33. This study demonstrated that when antibody concentrations measured by RABA were >2μg/mL, serum killing was uniformly >1 log reduction in CFU (n=5 infants) whilst bactericidal activity in those with antibody concentrations below this level were highly variable.33

Whilst opsonophagocytic killing is important to measure in studies of infant vaccination, in the case of protection against invasive neonatal and infant GBS disease where a maternal vaccine producing IgG antibody that crosses the placenta is the sole mechanism of protection, it might suffice to measure non-functional IgG antibody that correlates with opsonophagocytic killing activity. If the clinical endpoints for vaccine efficacy include endpoints other than invasive GBS disease such as maternal carriage (see below) or puerperal sepsis, where IgM could also contribute to protection37, determination of functional activity may become more important.

## The role of antibody in reduction of maternal and neonate/infant carriage risk

As maternal carriage is a pre-requisite for early-onset disease, several studies of vaccine and natural immune sera have investigated whether GBS carriage could be used as a clinical endpoint in trials of vaccine efficacy. Whilst cross-sectional studies show higher concentrations of naturally acquired GBS IgG antibody in colonised compared with non-colonised women,38 in a longitudinal study of pregnant women tested for carriage at 6 weekly intervals between 20-25 weeks of pregnancy and delivery, Kwatra and colleagues found that IgG antibody above ≥1μg/mL for serotype V (OR 0.23 (95% CI 0.05-1.02) and ≥3μg/mL for serotypes Ia (OR 0.37 (95% CI 0.14-0.98) and III (OR 0.11 (95% CI 0.01-1.75) were associated with absence of carriage by these serotypes throughout pregnancy.39 Further, absent carriage was associated with OPkA titres >1:14 (1:14 vs. 1:5 p<0.001) for serotype Ia and >1:132 (132 vs. 20 p<0.001) for serotype III.39

A study of 750 pregnant women from the Gambia also indicated that antibody-mediated complement deposition onto the surface of whole GBS bacteria measured by flow cytometry above the 95% centile of the GMC (equivalent to an OPkA titre 1:3000), was associated with absent neonatal and infant carriage for serotype V at birth and on day 60-89 of life (P<0.001).40

It is not possible to compare these results directly because of major methodological differences in study design and antibody analysis. In addition, whereas the role of maternal GBS carriage in developing early-onset disease is well established, transmission of GBS from a source other than the mother can also cause late-onset disease. Using reduction of maternal carriage as a clinical endpoint may therefore be less predictive of prevention against late- than against early-onset disease. This consideration is particularly important given that an advantage of vaccination over IAP as a strategy is its expected protection against late-onset disease.

## Lessons learned from other vaccines against bacterial pathogens

The experience gained from defining serocorrelates of protection against the encapsulated bacterium *Haemophilus influenzae* type b (Hib) may provide important lessons in defining serocorrelates of risk reduction from seroepidemiological studies. As early as 1933, an inverse relationship between disease occurrence and serum Hib bactericidal activity was noted.41 The same inverse relationship was later demonstrated for specific anti-capsular antibodies.42 However, as protection was required against infant Hib disease (especially meningitis), where antibody levels required for protection might differ, Smith et al. determined that IgG concentrations >0.2μg/mL were protective.43 Further evidence for antibody-mediated protection against invasive Hib disease came from phase II/III vaccine trials in over 30,000 children in Finland. As the Hib vaccine was rolled out during this trial, age-specific Hib disease incidence declined significantly when antibody concentrations were >0.15 μg/ml, with long term protection associated with concentrations >1μg/mL.44 Although there was some debate about the correct threshold 45 and assay to use46, the consensus of the scientific community was that an anti-Hib antibody concentration of 0.15 μg/ml was likely to be associated with protection against infant bacteremia.42

In addition to Hib, seroepidemiology studies were used to demonstrate the importance of complement-dependent, antibody‑mediated bactericidal activity for protection from invasive meningococcal disease in two seminal seroepidemiological studies47,48. The first was in infants through to young adults (up to 26 years of age) and demonstrated an inverse relationship between the age-specific prevalence of serum bactericidal activity and the incidence of meningococcal disease caused by serogroups A, B, and C. In infants these antibodies were maternally-derived and declined after birth. The second study, conducted in military recruits, was able to define the serocorrelate of protection based on serum bactericidal titre. This work was validated by the demonstration of efficacy of serogroup C polysaccharide vaccines49 and led to the licensure of subsequent meningococcal vaccines based on serocorrelates of protection. For example, for the meningococcus C conjugate vaccine, early discussions between manufacturers and the UK Medicines Control Agency indicated that licensure on the basis of immunogenicity data alone, without direct evidence of protective efficacy, might be considered.50 The basis for this decision in the UK was firstly that plain serogroup C polysaccharide vaccines were already licensed for children aged 2 years and above and there was direct evidence of efficacy from earlier trials50. Secondly, serum bactericidal activity was accepted as a serological correlate of protection and this could be extrapolated to infants in whom the unconjugated meningococcus C polysaccharide was neither immunogenic nor efficacious.51 This approach established an important precedent for other meningococcal conjugate polysaccharide vaccines.50

Efficacy of meningococcus B outer membrane vesicle vaccines was demonstrated in Cuba52 and Norway53 and was associated with induction of serum bactericidal activity54. For new protein-based meningococcus B vaccines, protection against disease was extrapolated from the *in-vitro* ability of vaccinee sera to kill bacteria with vaccine-matched antigens in the presence of human complement.55 The efficacy of 4CMenB vaccine is being evaluated following implementation in the UK infant immunization schedule.56

Initial pneumococcal IgG antibody serocorrelates of protection against invasive disease were developed after vaccines had been licensed using efficacy studies, based on an aggregate IgG value of three common serotypes from three clinical trials and agreed by consensus opinion to be >0.35 μg/mL.57 As more data became available from subsequent trials, serocorrelates of protection against other (but not all) serotypes were identified. A UK post licensure study indicated that serotype-specific correlates of protection were higher than 0.35 μg/mL for serotypes 1, 3, 7F, 19A, 19F and lower for 6A, 6B, 18C and 23F.58 Correlation between post-vaccination opsonophagocytic pneumococcal antibody titre and IgG as measured by ELISA has also been reported (0.2 μg/ml IgG corresponds to an opsonophagocytic killing titre of 1:8).59

Extensive consultations, facilitated by WHO, were undertaken to standardize both binding and functional assays for the assessment of pneumococcal antibody to aid prediction of serocorrelates. This approach has paved the way for collaborative working for other pathogens such as GBS. Current efforts are underway within a large scientific, industrial and technical consortium to standardise assays in order to define serocorrelates of risk reduction for neonatal and infant GBS disease. Panel 2 outlines the lessons learned from other vaccines that might be useful in the development of serocorrelates of reduction of risk for neonatal and infant GBS disease.

**Further considerations for prediction of serocorrelates of risk reduction for invasive neonatal/infant GBS disease**

In considering a serocorrelate of risk reduction for invasive neonatal/infant disease provided by maternal anti-GBS CPS IgG, either from immunity derived from natural exposure or from a vaccine study, additional consideration should be given to the site of measurement (maternal or infant serum), the passage of the IgG across the placenta at different gestations and thus the effect of prematurity or maternal comorbidity or infection on the correlate, and the longevity of the antibody to protect for the duration of the at risk period for GBS.

### *Antibody persistence following vaccination in pregnancy*

Both IgG concentration and opsonophagocytosis have been measured in a small study of 30 vaccinated women and their infants up to day 90 postpartum with rates of decline of between 50-75% in the infants.60

In pregnant women, post-vaccination IgG responses to GBS serotypes Ia, Ib, II, III and V peak at 4-8 weeks and then remain detectable for between 26-52 weeks post delivery. 34,35,61,62 Healthy South African vaccinated pregnant woman showed maternal antibody post vaccination at day 91 post partum that remained well over 1μg/mL for serotypes Ia, Ib and III.63 The most recent multicentre study of a variety of doses of a trivalent vaccine reported measurable antibody concentrations post-vaccination at 361 days postpartum in South African, Belgian and Canadian women (1.97 to 2.78 μg/mL for serotype Ia, 0.51 to 0.69 μg/mL for STIII),63 with an antibody half life of 42 days in HIV-uninfected infants.64

In infants, results from phase I/II trials of GBS-CPS conjugate vaccines show that IgG antibody falls to around 25% of maternal concentrations by three months of age 60,64,65. For example, in a small study of women vaccinated in pregnancy with a GBS serotype III conjugate vaccine, the infants were found to have 50% of birth anti-GBS-CPS antibody concentrations at one and 30% at two months of age, although opsonophagocytosis was observed up to two months of age in all infants with detectable anti-CPS antibody60. In this vaccine study, antibody levels remained >1μg/mL in 95% of infant sera at two months of age, well above those of infants born to unvaccinated women.60 A second small study reported antibody decay to 22-25% of birth concentrations by day 91 of life in vaccinated infants, but with antibody concentrations at least 5-fold higher than in the unvaccinated control group.65

*Placental antibody transfer and the effect of comorbidities*

Placental antibody transfer with CPS-vaccines is often less than that for protein vaccines due to the antibody isotype generated. In the case of the investigational GBS CPS-protein conjugate vaccines, placental transfer ratios of <1 have been reported for all serotypes tested, despite conjugation to tetanus toxoid or CRM197. Thus measuring antibody in both mother and cord/infant is important, as variations in placental transfer will affect antibody concentrations that are used to predict any serocorrelate.

The first small study of 30 pregnant women vaccinated with a CPS-tetanus-toxoid conjugate vaccine demonstrated a post-vaccination serotype-specific IgG concentration of 10μg/mL and a median placental transfer ratio of 0.77.60 Several studies have investigated the immunogenicity of the Novartis/GSK trivalent CRM197 (serotypes Ia, Ib and III) conjugate vaccine. A small study from Belgium and Canada enrolled 86 pregnant women and demonstrated geometric mean placental antibody transfer ratio of 0.68-0.81, depending on serotype.65 A large vaccine study from South Africa of 320 healthy HIV-negative women and 317 infants demonstrated placental antibody transfer ratios of 0.58 for serotype Ia, 0.65 for serotype Ib and 0.72 for serotype III.63 A second multi-centre vaccine study from South Africa and Malawi of 270 HIV-infected and uninfected pregnant women and 266 infants demonstrated comparable placental transfer between HIV-infected women with low and high CD4 counts and HIV-uninfected women but reduced immunogenicity associated with HIV infection.64 To date, there have been no studies to assess other conditions that may affect placental transfer of anti-CPS GBS antibody, such as prematurity or malaria. Similarly, there are no studies assessing placental transfer of protein-based vaccines.

## Conclusion

 These data provide background evidence about serocorrelates of GBS disease risk reduction for the most prevalent GBS serotypes through the use of anti-GBS-CPS IgG binding and opsonophagocytosis assays. Data points to anti-GBS-CPS IgG concentrations between 1-10 μg/mL being protective for serotypes Ia and III. These concentrations would be well within responses seen in the Phase I/II vaccine studies indicating that vaccination may provide protective antibody concentrations.34,60,63,64 The most likely mechanism of action for a GBS-CPS protein conjugate vaccine is to induce antibodies that can facilitate GBS killing via opsonophagocytosis. Currently there is no indicated protective opsonophagocytosis titre. Studies where antibody is measured in maternal serum are complicated by the presence of IgM that, unlike IgG, is not transferred to the baby, thus it remains important to measure maternal and cord/infant serum antibodies.

It is important to note that in higher disease-burden settings, antibody thresholds required for protection may need to be higher because of co-infection leading to impaired GBS-specific placental antibody transfer, and this should be considered when extrapolating efficacy data between populations.66 A further consideration is that there may be higher antibody concentrations because of higher background GBS carriage and this may mean that thresholds for protection would need to be higher in these populations.

Sponsors will need to agree on the approach and the assay to be used for serocorrelates to be the basis for licensure. In addition, for a vaccine to be implemented, work on consensus building with public health bodies is needed to demonstrate that the vaccine adds benefit above existing IAP strategies. As vaccine development progresses these decisions become imperative if the vaccine is to reach the people that need it most as quickly as possible.

**Declaration of interests**

KLD has received an honorarium to attend a meeting at Pfizer Inc.. BK directs IMmunising PRegnant women and INfants neTwork (IMPRINT), funded by the GCRF Networks in Vaccines Research and Development that was co-funded by the MRC and BBSRC and the National Vaccine Program Office (NVPO). BK is theme lead at The MRC Unit The Gambia that has previously received funding for vaccine trials, including vaccines produced by Pfizer and GlaxoSmithKline (GSK). PTH is an investigator for clinical trials done on behalf of St George’s, University of London, UK, sponsored by various vaccine manufacturers, including Novartis, Pfizer, and GSK, and has been a consultant to Novartis and Pfizer on GBS vaccines, but received no personal funding for these activities. DG is a UK National Institute of Health Research Senior Investigator. He participates in occasional advisory boards and consultancies for vaccine manufacturers including Merck, Sanofi Pasteur and GSK. CJB has been an advisor to GSK and Pfizer Inc. MSE has received a research grant from Pfizer, Inc. GK has received funds from Pfizer Inc. to attend a meeting.

ASTM is an employee of the Bill & Melinda Gates Foundation. ASA is employed by Pfizer and as such may own stock in the company. BC is employed by the GSK group of companies and owns stock of the GSK group of companies. The phase II studies investigating the trivalent CRM197 conjugate GBS vaccine were initially supported by Novartis Vaccines and Diagnostics, Inc., prior to the divestiture of its non-influenza vaccine business, which was acquired by GlaxoSmithKline Biologicals SA in March 2015. PBF is an employee of Minervax and may own stock in the company. AG has received a grant for meeting travel from Pfizer Inc.

SM, JV, MN and NA declare no conflict of interests.

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**Authors’ contributions**

KLD devised and prepared the manuscript and performed the literature search; BK, JV, PTH provided expert input into the preparation of this manuscript and revisions to the final draft; DG, MN, AG provided expert opinion on serocorrelates of protection for inclusion in this manuscript and contributed to the editing of the manuscript; CJB, provided expert opinion on the preparation of and content of this manuscript and revisions to the final draft and provided expert opinion on serocorrelates of protection for inclusion in this manuscript; MSE, GK, NA, SM, AStM, ASA, BC and PF provided expert input into the preparation of this manuscript and comments on the final draft;

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Table 1 – Studies proposing protective antibody concentrations for GBS Serotype Ia and III

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Author** | **Year**  | **Cases** | **Controls** | **Sampling methods** | **Method of IgG determination** | **Data analysis method** | **Proposed protective IgG concentration**  | **OR/RR (95% CI) of disease protection** |
| Baker et al 22 | 1998-1999 | EOD:STIa=17STIII=9 | 99 healthy infants born to colonised mothers | Maternal serum | Monoplex ELISA using CB in-house CPS and CB reference sera | Bayesian model1% background risk, antibody compared to reference value of 0.1μg/mL | STIa, STIII≥0.5 μg/mL Combined > 1 μg/mL  | OR: if Antibody ≥0.5μg/mL: STIa: 0.11 (0.01-0.74) III: 0.09 (0.00-0.72)V: 0.29 (0.01-3.10) |
| Lin 25 | 2001 | EOD: STIa=50 | 336 colonised infants without disease | Maternal and cord serum | ELISA using NABI reference sera, with known quantity of IgG (66ug/mL STIa); commercially available CPS | Logistical regression at fixed 1 μg/mL compared to a reference of <0.5 μg/mL | Maternal:STIa≥5 μg/mLCord: STIa ≥4μg/mL  | Maternal serum: OR:0.12 (0.02–0.93)Cord serum: relative risk reduction 91% (28-99) |
| Lin 17 | 2004 | EOD: STIII=26 | 143 colonised infants without disease | Maternal and cord serum | ELISA using NABI reference sera, with known quantity of IgG (66ug/mL STIa); commercially available CPS | Logistical regression at fixed 1 μg/mL compared to a reference of <2 μg/mL | Maternal: STIII≥10 μg/mLCord STIII ≥7μg/mL for serotype | OR:0.09 (0.01– 0.78)Cord serum: relative risk reduction 85% (29-97) |
| Fabbrini 26 | 2014 | EOD: STIa=14; STIII=41; LOD: STIa=11; STIII=55;  | 984 GBS colonised pregnant women, 473 non-colonised pregnant women,  | Maternal serum | ELISA using CB standard reference sera and OPkA using HL60 cells and rabbit complement; Commercially developed CPS | Bayesian modeling (3% background risk) | >1ug/mL for STIa and STIII  | OR:STIa: 0.19 (0.0-0.6)STIII: 0.22 (0.0-0.55)  |
| Dangor 18 | 2015 | EOD: STIa= 15STIII=7LOD:STIa=12 STIII=22 | 135 colonised women and 352 non -colonised women | Maternal and infant serum | Fluorescence based micro-bead immunosorbent assay using human immunoglobulin standard curve and commercially available CPS. | Bayesian modeling (1% background risk) | Maternal: STIa ≥6 μg/mLSTIII ≥3 μg/mLCord: ≥0.5 μg/mL STIa and III  | Maternal serum RR:STIa 6.5% (50% credible interval 1.4-21.9)STIII 1.3% (50% credible interval 0.1-9.9)Infant serum RR: STIa 0.18 (0.04-0.85) STIII 0.14 (0.02-1.38). |
| Total cases | EOD: STIa = 96; STIII = 83LOD: STIa = 23; STIII=77 |
| Total Controls | Healthy, GBS-colonised infants = 479Healthy infants born to GBS-colonised women = 1218Healthy infants born to non-colonised women = 825 |

 *GBS=Group B Streptococcus; EOD = Early onset GBS disease; LOD = Late onset GBS disease; CPS = capsular polysaccharide; ELISA=enzyme-linked immunoabsorbent assay; OPkA=opsonophagocytosis killing assay; ST=serotype*

Table 2 – Studies proposing protective antibody concentrations for GBS surface proteins

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Author** | **Year**  | **Cases** | **Controls** | **Sampling methods** | **Method of IgG determination** | **Data analysis method** | **Proposed protective IgG concentration**  | **OR/RR (95% CI) of disease protection** |
| Larsson29 | 2006 | Rib = 30 | Infants admitted to the neonatal unit without signs of infection | Maternal and infant serum | ELISA against Rib α proteins using in-house protein preparations and γ globulin reference sera | Logistical regression | Not stated | AOR: Rib 0.002 (0.00 to 0.57)  |
| Fabbrini 26 | 2014 | Pili 1 = 35Pili 2a = 25Pili 3 = 18 | 428 Pili 1 GBS colonised pregnant women, 568 Pili 2a GBS colonised pregnant women,107 Pili 2b GBS colonised pregnant women, | Maternal serum | ELISA against Pili 1, 2a and 2b proteins using in-house protein preparations and high protein titre reference sera | Logistical regression | Not stated | BP-1 (cases 13.5EU/mL (<LLOQ-555 EU/mL) vs. controls 21.3 EU/mL (<LLOQ-1083 EU/mL; 37% lower in disease) and API-2a (cases 28.8 EU/mL (<LLOQ-160 EU/mL) vs. controls 37.3 EU/mL (<LLOQ to 523 EU/mL; 23% lower in disease))No association between antibody against 2b and disease |
| Dangor30 | 2015 | FbSA/BiBAEOD = 34LOD = 35 | Healthy infants without diseaseUnder 6 days old = 757 -89 days – 53 | Maternal and infant serum | ELISA against BibA, FbsA, PI-1, PI-2a, and PI-2b proteins using in-house protein preparations and high protein titre reference sera | Bayesian modeling | None found | No associations found |

*GBS=Group B Streptococcus; EOD = Early onset GBS disease; LOD = Late onset GBS disease; ELISA=enzyme-linked immunoabsorbent assay; Pili = pilus protein.*