Landscape analysis of serocorrelates of protection against infant Group B Streptococcus disease

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Abstract

Group B Streptococcus (GBS) is the leading cause of neonatal mortality and morbidity globally against which vaccines have been in development for over four decades. A serocorrelate of protection against invasive disease could be used to facilitate vaccine licensure with effectiveness assessed post-licensure. We undertook a systematic literature review and sought expert opinion from academic, industrial and WHO partners to synthesise the scientific evidence currently available that would facilitate the derivation of a correlate of protection against GBS. We summarise studies that have investigated serum anti-capsule antibody and its association with disease protection, as well as studies investigating putative sero-correlates of protection against neonatal GBS disease. We also discuss anti-protein antibody, issues of placental transfer, antibody decay and functional antibody assessment, all of which are vital in the prediction of a robust serocorrelate. Lessons learned from other pathogens for which vaccines are available can be harnessed for the benefit of the GBS field.

Research in context

Evidence before this study

Group B Streptococcus (GBS) is a leading cause of mortality and morbidity in infants worldwide. A maternal vaccine would be effective in preventing early and late onset GBS disease via placental anti-GBS antibody transfer, as well as protecting the mother against puerperal GBS sepsis. Several capsular polysaccharide (CPS) conjugate vaccine candidates for maternal vaccination are currently in development. However, given the relative rarity of GBS disease in Europe and the USA, the large numbers of pregnant women that would need to be recruited to determine vaccine efficacy (at an incidence rate of 1/1000 live births it is estimated that 60,000 infants would be required to demonstrate vaccine efficacy) has so far prevented phase III studies. We searched PubMed for articles containing the search terms "Group B Streptococcus" OR *Streptococcus agalactiae* (MESH) AND antibody (MESH), with no restrictions on publication date or language. We searched for original articles describing the association between maternally-derived antibody and infant GBS disease.

Added value of this study

In this review we present a comprehensive overview of animal and human studies of natural and vaccine-induced antibody and its association with protection against infant disease. Where previous reviews have focused on IgG antibody against the capsular polysaccharide, we also discuss antibody function, key protein antibody function, placental transfer and antibody decay, all of which are vital in predicting a serocorrelate of protection against infant disease following maternal vaccination.

Implications of all the available evidence

The evidence we present suggests that a serocorrelate of protection could be predicted for the most common serotypes (STIa, STIII and STV) and might be used to help licensure of a multivalent vaccine. Evidence is also emerging that serocorrelates against protein-based vaccines may be predicted if clinical outcomes associated with proteins identified could be elicited from further clinical trials. Lessons learned from existing vaccines against encapsulated bacteria can be harnessed to aid the development of a GBS serocorrelate of protection.

Group B Streptococcus (GBS) remains a leading cause of neonatal sepsis and meningitis in the United Kingdom (UK) [1] and the United States of America (USA), with long-term adverse neurodevelopmental outcomes in up to 50% of survivors of GBS meningitis.[2] Compared to the USA, where universal screening has been in place since 2002, early onset disease (EOD) rates in the UK, where pregnant women are screened only if specific risk factors are present, have increased by 19% over the last 15 years (0.57/1000 live births in 2014/15 vs. 0.48/1000 live births in 2000/1) and are now double the rate found in the USA (0.21/1000 live births in 2015).[1,3]

Despite success in reduction of EOD rates in the USA, late onset disease (LOD) has remained static at between 0.3-0.4/1000 live births in both countries. [1,3]. LOD is not affected by intrapartum antibiotic policies and it is widely recognised that a GBS vaccine would be the most cost-effective method of reducing the burden of all forms of infant disease worldwide.

Clinical evaluation of GBS vaccines using prevention of invasive neonatal disease as a primary endpoint requires large studies, which are therefore best carried out in regions with relatively high prevalence. It is estimated that an efficacy study of approximately 60,000 women in countries with a disease incidence ≥1:1000 live births would be required to detect 75% reduction in EOD and LOD, given that 70-80% of disease is caused by three vaccine serotypes and 15-20% of infants are born before 34 weeks and so may not have optimal placental antibody transfer.[4,5] An alternative approach could be to establish immune correlates of protection based on prior vaccine studies or natural antibody seroepidemiology studies such has been used for meningococcus B and C and higher valency formulations of the pneumococcal vaccines.

The issue for GBS, however, is a lack of consensus on whether an immune correlate of protection exists and, if so, how it should be measured. There have been several prospective case controlled studies that support evidence for serocorrelates of protection against infant GBS disease[6-8]. However these studies use different immunoassays and thus there is currently no accepted concentration of IgG antibody or standardised assay that could be used to develop and validate a serocorrelate.

Here we describe the collaborative efforts that have been undertaken to establish the correlation between maternally-derived IgG antibody concentrations and protection against infant disease. The aim of this review is to provide the background information to understand how such a correlate of protection could be derived and to summarise the scientific evidence to derive a serocorrelate of protection against GBS serotypes that cause the majority of infant GBS disease globally.

In an effort to facilitate more rapid licensure and availability of a GBS vaccine for prevention of EOD and LOD infant disease, a serocorrelate of protection against neonatal GBS disease will undoubtedly prove useful. However, when determining a serocorrelate of protection against a neonatal disease with a defined risk period, for whom the prevention strategy is vaccinating the pregnant woman, both placental IgG antibody transfer and antibody decay should be considered. These factors matter as a serocorrelate will likely be based on measurements of antibody derived from maternal and cord sera and will need to be high enough to protect the infant throughout the at risk period.

Methods

This article is part of a protocol entitled “Systematic estimates of the global burden of Group B *Streptococcus* in pregnant women, stillbirths and infants”, which was

submitted for ethical approval to the London School of Hygiene & Tropical Medicine (ref 11966) and approved on 30th November 2016.

Data searches and inputs

We identified data through two sources: (1) systematic review of the published literature and (2) expert opinion from academic institutions, industry involved in the development of vaccines against GBS and the World Health Organisation (WHO).

Literature searches

We undertook systematic literature searches of Medline with the last search on 20th April 2017. We used the search terms" Antibody (MESH) and "Group B Streptococcus" and *Streptococcus agalactiae* (MESH).

Expert opinion

We sought expert opinion from academic experts and industry who are currently developing Group B Streptococcal vaccines for insight from other vaccines that might be useful. We also sought the expert opinion of the WHO maternal immmunisation group regarding knowledge gaps pertinent to developing a serocorrelate of protection against GBS disease.

Studies were included if they discussed serotype-specific antibody concentrations measured either by quantitative or functional antibody assay, contained a denominator and compared infants with GBS disease with healthy infants either in natural immunity studies or in vaccine studies.

The role of natural maternal antibody in infant disease protection

IgG antibodies are uniquely transported from the mother to the baby via the placenta.[9] IgG binds to FcRn receptor molecules in the placenta via the Fc portion of the antibody and the receptor molecules can be saturated if maternal concentrations are very high.[10] Although transfer of IgG is thought to occur after 17 weeks of gestation this transfer is not optimal until after approximately 33 weeks gestation. The search for a protective immune correlate has therefore been focused on the maternal IgG concentration at the time of delivery.

The importance of maternally-derived IgG antibody in preventing GBS disease was first demonstrated by Baker and colleagues in the 1970s by demonstrating that infants who developed STIII GBS disease received significantly lower STIII-specific maternal IgG than exposed but uninfected infants. [6,11] Subsequent studies have shown similar results for STIa, STIb and STV.[12,13] Another study demonstrated a similar principle using the Rib protein, the predominant form of the alpha-like proteins (Alp) that cover the surface of GBS. A significant correlation between lower levels of naturally occurring antibodies against Rib was found in neonates developing disease with GBS isolates expressing Rib, compared to controls.[14] Studies have also shown that human sera containing high concentrations of natural CPS-specific antibody are capable of promoting efficient opsonisation and phagocytosis of the bacterium *in-vitro* and affirm the importance of CPS GBS ST-specific antibodies in protection from invasive disease.[12]

Placental IgG antibody transfer

Studies comparing maternal and neonatal antibody concentrations demonstrate that naturally occurring anti-GBS CPS antibody is transferred with a high degree of

efficiency after 30 weeks gestation.[15,16] Several phase II trials have now taken place for monovalent (STIII) tetanus-toxoid (NIH) and multivalent CRM197 (STIa, STIb and STIII)-conjugate GBS vaccines in pregnant women (Novartis/GSK). The first small study of 30 pregnant women demonstrated a median placental transfer ratio of 0.77 with post-vaccination maternal serotype IgG GMC of 10μg/mL. Functional activity using an opsonophagocytosis killing assay (OPkA) was observed in infant sera, when containing >0.5 ug/mL serotype-specific IgG, up to two months post-partum.[17] Several studies have investigated immunogenicity of the Novartis/GSK trivalent CRM197 (STIa, STIb and STIII) conjugate vaccine. A small study from Belgium and Canada enrolled 51 pregnant women and demonstrated GMC placental antibody transfer of 0.68-0.81 dependent on serotype.[18] A large study from South Africa in 320 healthy HIV-negative South African women and 317 infants demonstrated geometric mean concentration (GMC) placental antibody transfer of 0.58 for STIa, 0.65 for STIb and 0.72 for STIII.[19] A second multi-centre study from South Africa and Malawi of 270 HIV-infected and uninfected pregnant women and 263 infants demonstrated comparable GMC of placental transfer between HIV-infection with low and high CD4 counts and HIV-uninfected women (STIa 0.58, 0.6, 0.72; STIb 0.51, 0.64, 0.49; STIII 0.6, 0.51, 0.56 respectively).[20]

Antibody persistence following vaccination

Post-vaccination, antibody responses to GBS STIa, STIb, STII, STIII and STV peaked at 4-8 weeks with antibody persistence of between 26-52 weeks. [21-24] The South African healthy pregnant woman study reported maternal antibody at day 91 that remained >1μl/mL for STIa and STIb and >0.5μl/mL for STIII.[19] In HIV-infected pregnant women lower post-vaccination antibody concentrations were noted

compared to HIV-uninfected women but remained higher than in the placebo group with fewer women above the presumed threshold of 0. 5µl/mL [20]

Results from phase I/II trials in pregnant women show that conjugated-CPS GBS vaccines induce IgG antibody that decreases by 75% from maternal concentrations by three months of age in the infant [17,18,20]. In a small study of women vaccinated in pregnancy with a GBS ST III conjugate vaccine, their infants were found to have lower GMC of antibody at one month and two months of age than at birth (50% and 30% respectively), although opsonophagocytosis was observed up to two months of age. Antibody levels remained >1µg/mL in 95% of infant sera at two months and were well above those of infants born to unvaccinated women.[17] A second small study reported antibody decay to 22-25% of infant birth levels at day 91 of life but remained at least 5-fold higher than in the unvaccinated control group.[18]

Most recently, the Novartis/GSK DEVANI study investigated anti-pilus protein BP-1, API-2a and BP-2b antibodies against isolates from EOD expressing these proteins. This study found that sera of mothers delivering infants with EOD had significantly lower antibody titres against BP-1 and API-2a, but not BP-2b, compared to infants who remained healthy.[25] These results suggest that it may be possible to estimate a serocorrelate of protection against protein antibodies that might be of use in protein-based vaccines if clinical endpoints associated with identified proteins can be determined.

Serocorrelates of protection

Determining a protective antibody concentration is not easily achieved, as protective antibody concentrations vary by serotype [7,26], and the assessment of immunogenicity varies by the assay methods employed.[12,26] One of the points of

debate concerns the discussion around use of conjugated or unconjugated CPS to detect IgG antibodies, as conjugation may alter efficiency of surface binding to the CPS and/or the ELISA plate.[27] There also appears to be some variation in specific antibody concentration thresholds of protection found in different studies for individual strains, as well as among different GBS STs (Table 1).

Prediction of protective immunity to GBS disease using IgG antibody

Originally, the radioantigen binding assay (RABA) was seen as the gold standard for the quantification of anti-GBS antibody as it measures antibody in its native state.[6] However, RABA is unable to identify different subclasses of antibody and so offers an incomplete picture of immunity. Subsequently, antibody binding assays, for example by enzyme-linked immunoabsorbent assays (ELISA), have been used that measure antibody concentrations relative to a standard reference serum (provided by Carol Baker, NABI Pharmaceuticals or human pooled immunoglobulin). More recently, Luminex® or Bioplex® platforms have been used to improve both sensitivity and throughput of assays by measuring antibodies against several serotypes in the same sample simultaneously.

The studies by Lin et al measured antibody by ELISA from maternal and cord serum samples collected from multiple sites across the USA. The study used a case-control design and measured antibody concentrations in infants born to GBS-colonised women who went on to develop infant disease compared to infants who remained healthy. These studies identified thresholds for STIa of ≥5μg/mL and for STIII of ≥10μg/mL, to be associated with an approximately 90% disease risk reduction.[7,26]

Subsequent studies using different statistical methods and assays have

proposed alternative antibody concentrations associated with protection from disease. In a meta-analysis undertaken to compare the proportions of cases and controls with antibody levels ≥2 μg/mL, the odds of invasive GBS disease was 6.56 (95% CI: 2.10–20.55) and 2.38 (95% CI: 1.20–4.70) times greater in infants whose mothers had antibody levels <2 μg/mL for GBS STIII and STIa, respectively.[28] Case control studies by Baker and colleagues using Bayesian modeling proposed a threshold of 1 μg/mL as a correlate of protection against disease caused by GBS STIa and STIII.[12] Thresholds were similar for STIa and STIII in the Novartis/GSK DEVANI study, based on European sera, which predicted 75% EOD risk reduction with antibody concentrations ≥1μg/mL for STIa and STIII.[25] However, a recent study by Dangor in South Africa using the Luminex® platform and human gammaglobulin standards (calibrated to Carol Baker SHRS) suggested that maternal concentrations of >6μg/mL for GBS STIa and >3μg/mL for GBS STIII were associated with a less than 10% risk of early and late onset disease in the infant [8](Table 1).

Both the Baker and Dangor studies used Bayesian modeling to determine thresholds for protection and can be considered the most robust estimates of serocorrelates of protection against STIa, STIII and STV to date. [28,29] In total, current estimates of protection against STIa and STIII are based on 119 and 179 neonatal disease cases, respectively, which could provide robust serocorrelate data if assays were standardized. To date, study sizes have been too small to determine corresponding data for less prevalent serotypes.

A standardized immunoassay using standardized reagents would be a great step forward in the assessment of serocorrelates of protection against invasive GBS

disease. Such assays would allow the translation of antibody data from studies in diverse geographies and between vaccine products.

Functional immunity against GBS disease

In the case of GBS, antibody function has been evaluated through the opsonophagocytosis killing assay (OPkA) [30,31], which mimics the *in vivo* process of the killing of the bacterium by host effector cells following opsonisation by specific antibodies and complement. Currently there are two different in vitro OPkAs using two different approaches. One approach, which is used for pneumococcal vaccine studies and its licensure, determines the dilution of antiserum that kills half of the target bacteria in the presence of exogenous complement. The other approach, which was used in almost all GBS studies, determines the degree of bacterial killing over a fixed incubation period by a human serum sample with endogenous complement. Passive protection of mice by sera from adults immunised with GBS CPS-based vaccines has been shown to correlate with high functional antibody titres measured by OPkA.[12,32,33] Studies in both animals and humans indicate that functional activity appears to correlate well with antibody concentration.[31,34] It appears that infant serum containing ≥2μg/mL anti-STIII CPS IgG antibody is able to promote 90% *in-vitro* opsonophagocytosis and killing of STIII bacteria. Further studies in non-pregnant adults indicate that higher vaccine-induced antibody concentrations promoted greater opsonophagocytic killing against STIa, STIb[21], STII[22] and STV.[35]

In the largest study to date to measure natural maternally-derived anti-GBS antibody and protection against infant GBS disease, the GSK DEVANI study of 1610 pregnant women and their infants investigated natural antibody concentrations in colonised women with healthy infants, colonised women with diseased infants and non-

colonised women from eight European countries. This study found significant correlations between maternal IgG against STIa, STIb and STIII (as measured by ELISA) with opsonophagocytosis, when IgG antibody concentrations were >1μg/mL (STIa R=0.8, STIb R=0.8, STIII R=0.85). These estimates suggest that doubling the IgG concentration will increase the OPkA titre by 70-80%. Extrapolation of these data may add weight to the proposed serocorrelate of protection of around 1μg/mL for STIa and STIII with OPkA titres of between 64-128.

Other non-killing functional assays, including an antibody-mediated complement C3b/iC3b deposition assay,[36] have been used to measure functional antibody activity and to demonstrate a threshold above which no colonisation was found in infants born to colonised mothers.[37] At present, a functional IgG antibody titre is based on limited data from STIa, STIb and STIII in colonised women, with no infants with disease in the study.[25]

However, whilst bactericidal activity is important in studies of infant vaccination, in the case of protection against invasive neonatal GBS disease where a maternal vaccine producing IgG antibody that crosses the placenta is the sole mechanism of protection, it might suffice to measure IgG antibody that correlates with bactericidal activity.[9] If the clinical endpoints for vaccine efficacy include maternal colonisation or puerperal sepsis, where IgM may also contribute to protection, determination of functional activity may be required.[38] There are currently no standardized OPkA assays for the assessment of functional antibody against GBS.

Antibody protection against maternal GBS colonisation

As maternal colonisation is a pre-requisite for EOD, several studies of vaccine and natural immune sera have investigated whether GBS colonisation 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,[15] longitudinal studies assessing concentration and function indicate that increased IgG antibody is associated with delayed or absent acquisition of colonisation.[38] In a study of 661 pregnant women, Kwatra and colleagues found that IgG antibody above ≥1μg/mL for serotype V and ≥3μg/mL for STIa and STIII were associated with absence of colonisation by these serotypes during pregnancy.[38] Further, absent colonisation was associated with OPkA titres >1:14 STIa and 1:132 STIII.[38] A recent study of 750 pregnant women from the Gambia also indicated that high functional antibody(>upper 95% confidence interval of the geometric mean, defined by antibody-mediated complement deposition onto the surface of whole bacteria) measured by flow cytometry and equivalent to an OPkA titre 1:3000 was associated with absent neonatal colonisation for STV.[37]

Whereas the role of maternal GBS colonisation in developing EOD is well established, transfer of GBS from a source other than the mother can also cause LOD. Using reduction of maternal carriage as a clinical endpoint would therefore be less predictive of prevention against LOD than against EOD. This consideration is particularly salient given that an important differentiator of the vaccine versus intrapartum antibiotic prophylaxis is its expected protection against LOD, which is not achievable by the latter.

Lessons learned from other vaccines against encapsulated bacteria

Experience from *Haemophilus influenzae* type b (Hib) may provide important lessons for the GBS correlates of protection effort. As early as 1933, an inverse relationship between age and serum bactericidal activity was noted.[39] However, it was the recognition that bactericidal activity reflected the concentration of IgG antibody to

the CPS that aided development of the first generation vaccine.[39,40] The correlate of protection against Hib invasive disease was first determined by RABA (against all antibody isotypes) in adult sera based on the premise that adult disease is rare and therefore antibody concentrations in adult sera are protective.[41,42]. However, as protection is needed against infant disease (meningitis) where antibody responses required for protection might differ, Smith et al. determined IgG concentrations >0.2μg/mL to be protective.[43] Further evidence for protection against invasive disease came from phase II/III vaccine trials in over 30,000 children in Finland. Following introduction of the vaccine during this trial, age-specific Hib disease declined significantly when antibody concentrations were above 0.15 μg/ml, with long term protection associated with antibody >1μg/mL.[44] Although there was some debate about the correct cutoff [42] and assay to use[45], the consensus of the scientific community was that an antibody level of 0.15 μg/ml was likely to be protective against infant bacteremia.[46]

Based on this example, prediction of adequate protection from invasive GBS disease might therefore be possible, using IgG antibody as a serocorrelate of protection. This correlate however may vary by population and by serotype (as it may in an efficacy study).

For meningococcus C, early discussions between manufacturers and the UK Medicines Control Agency indicated that licensure of meningococcal C conjugate vaccines on the basis of immunogenicity data alone, without direct evidence of protective efficacy, would be considered.[47] The basis of this decision was the existing licensure of plain serogroup C polysaccharide vaccines for children aged 2 years and above for whom there was direct evidence of efficacy, and serum bactericidal activity was an accepted serological correlate of protection.[48] Extrapolation of these correlates

to infants, in whom the unconjugated Meningococcus C polysaccharide is neither immunogenic nor efficacious, was the basis for licensure of the meningococcal C conjugate vaccines in the UK and has established an important precedent for other meningococcal conjugate polysaccharide vaccines.[47] For meningococcus B, where the rarity of disease precluded a large efficacy trial, bactericidal titres were evaluated following vaccination and 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.[49] As with the meningococcocal vaccines, if a laboratory serocorrelate of protection could be determined for GBS (a rare disease), then efficacy could be demonstrated in post-implementation surveillance.

Initial pneumococcal IgG antibody serocorrelates of protection against invasive disease were developed based on an aggregate value of three main serotypes across three clinical trials by consensus opinion agreed at 0.35 μg/mL. With more information from subsequent trials more data became available to identify serocorrelates of protection against other (but not all) serotypes. Serocorrelates of protection for individual serotypes range widely for pneumococcus and the same may be true of GBS. As with Hib, Goldblatt and colleagues reported a good correlation between postvaccination opsonophagocytic antibody titer and IgG measured by ELISA (0.2 μg/ml IgG equates to an opsonophagocytic killing titre of 1:8).[50] Again, it is important to note that in higher burden settings, correlates of protection may need to be higher because of coinfection and higher pre-existing antibody, and this should be considered when extrapolating efficacy data between populations.[51] As for both Hib and meningococcus, correlates of protection against mucosal colonisation are likely to be much higher than for invasive disease.[52] Building on this experience from pneumococcus, it may be possible to use the same approach in the development of a

multivalent GBS vaccine by identifying an aggregate correlate in initial seroepidemiological and vaccine studies. Phase IV studies could then be conducted to define the serocorrelates of protection for each individual serotype.

Extensive consultation was undertaken to standardize both binding and functional assays for the assessment of pneumococcal antibodies to aid prediction of serocorrelates, facilitated by the WHO, which have paved the way for collaborative working for other pathogens such as GBS. Current efforts are underway within a large scientific, industrial and governmental consortium to focus on standardizing assays in order to define serocorrelates of protection against neonatal GBS disease.

Conclusion

Overall, these data provide a rationale for the development of serocorrelates of protection for the most prevalent serotypes measured by ELISA-type IgG binding assays and functional antibody titres. However, consensus needs to be built regarding choice of clinical endpoints, serological assay type and standardization, and an agreement to license GBS vaccines on the basis of serological correlates. 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

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BC is an employee of GSK. AA is an employee of Pfizer Inc. PF is an employee of Minervax.

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Authors' contributions

KLD devised and prepared the manuscript and performed the literature search. BK, PTH, CB, ME, GK, SM, AStM, AA, BC, PF and JV all provided expert input into the preparation of this manuscript and comments on the final draft. DG, MN and AG provided expert opinion on serocorrelates of protection for inclusion in this manuscript and contributed to the editing of the manuscript.

Table 1 – Studies proposing protective antibody concentrations for GBS STIa, STIII and STV

Author	Country	Year of Study	Study design	Method of IgG determination	Proposed protective IgG concentration	OR/RR (95% CI) of disease protection
Mouse studies						
Baltimore [32]	USA	1981	Clinical trial of STIII monovalent unconjugated vaccine. Protection evaluated in a mouse passive protection model	RABA	STIII ≥2μg/mL	All vaccinated pups survived
Gotoff[53]	USA	1986	Murine protection model and comparison to pregnant woman natural antibody (n=102; 25 GBS colonised) and 42 EOD STIII	ELISA using KR human serum	STIII >1.3ug/mL in healthy adults <0.3ug/mL in EOD	GMC of STIII lower in GBS disease cases
Klegerman[54]	USA	1983	Murine protection model and comparison to case control of 50 pregnant women and 11 EOD STIa.	ELISA using KR human serum	STIa ≥1μg/mL	Mouse protection if Ab >0.5ug/mL. No infants with EOD had detectable Ab. 36% of colonised women had Ab >1ug/mL
Laboratory studies						
Edwards et al[31]	USA	1979	Laboratory study of 45 naturally immune and vaccine immune sera against STIII tested against 22 strains of STIII	OPkA using CB standard reference sera, human PMN and endogenous complement	STIII ≥10μg/mL promoted >2.0 $\log_{10}$ reduction in CFU/mL compared to antibody deficient sera	No disease in this cohort
Baker et al [21]	USA	1999	Clinical trial of STIa and Ib monovalent conjugate vaccines in healthy adults (n=165)	RABA ELISA using CB standard reference sera OPkA with fresh human PMNs and endogenous sample complement and CB standard reference sera	post-vaccinee sera >1ug /mL promoted >1.0 $\log_{10}$ reduction in CFU/mL in OPkA for STIa and Ib. Good correlation between concentration of STIa and STIb antibody and OPkA (r=0.65 STIa; r=0.8 STIb)	No disease in this cohort
Baker et al[23]	USA	2005	Clinical trial of STV monovalent conjugate vaccine in healthy adults (n=30)	RABA ELISA using CB standard reference sera OPkA with fresh human PMNs and endogenous sample complement and CB standard reference sera	post-vaccinee sera >1ug/mL promoted >0.8 $\log_{10}$ reduction in CFU/mL in OPkA for STV.	No disease in this cohort
Baker et al [24]	USA	2003	Clinical trial of STII and III monovalent conjugate vaccines in healthy adults	RABA ELISA using CB standard reference sera OPkA with fresh human PMNs and endogenous sample complement and CB standard reference sera	post-vaccinee sera showed 4-fold antibody increase (n=17) and >1.0 $\log_{10}$ reduction in CFU/mL in OPkA for STII and III	No disease in this cohort

Baker et al[17]	USA	2003	Clinical trial of STIII monovalent conjugate vaccine in pregnant women (n=30)	ELISA using CB standard reference sera OPkA with fresh human PMNs and endogenous sample complement and CB standard reference sera	STIII ≥0.5µg/mL promoted >1.0 log_{10} reduction in CFU/mL in OPkA	No disease in this cohort
Human case control studies						
Baker et al [12]	USA	1998-1999	33 mothers delivering infants with EOGBS and 99 controls (healthy infants) STIa=17 STIII=9 STV=7	ELISA using CB standard reference sera	STIa, STIII, and STV ≥1µg/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)
Feldman [55]	UK	1990	19 EOGBS and 104 controls (pregnant and non-pregnant women and their infants)	ELISA using CB standard reference sera	STIII ≥2µg/mL	GMC of STIII lower in GBS disease cases
Lin [7]	USA	2001	Case control study of 50 STIa EOD and 336 controls (colonised infants without disease)	ELISA using NABI reference sera with known quantity of IgG (66ug/mL STIa)	STIa ≥5 µg/mL	OR: 0.12 (0.02–0.93)
Lin [26]	USA	2004	Case control study of 26 EOD and 143 controls (colonised infants without disease)	ELISA using NABI reference sera with known quantity of IgG (66ug/mL STIa)	STIII ≥10 µg/mL	OR: 0.09 (0.01– 0.78)
DEVANI study[56]	Europe	2014	Case control study of 984 GBS colonised pregnant women, 473 uncolonised pregnant women, 82 EOD, 71LOD). Natural Ab EOD: STIa=14; STIb=1 STII=4; STIII=41; STV=17 LOD: STIa=11; STII=2; STIII=55; STV=2	ELISA using CB standard reference sera and OPkA using HL60 cells and rabbit complement	If Ab >1ug/mL OPkA titres 1:64-128 (STIa, Ib and III). No measurable OPkA titre in disease STIa and STIII ≥1 µg/mL	OR: STIa: 0.19 (0.0-0.6) STIII: 0.22 (0.0-0.55)
Matsubara[57]	Japan	2002	583 women and 5 EOGBS (not matched)	ELISA using human immunoglobulin standard curve	STVIII ≥1 µg/mL	Infants with disease had Ab <0.5ug/mL
Dangor[29]	South Africa	2015	27 STIa GBS disease (15 EOGBS, 12 LOGBS) 29 STIII GBS disease (7 EOGBS, 22 LOGBS) 400 controls	Fluorescence based micro-bead immunosorbent assay using human immunoglobulin standard curve	STIa ≥6 µg/mL STIII ≥3 µg/mL	Used Bayesian modelling to impute the degree of protection based on specific threshold

TOTAL CASES	**STIa=119** **STIII=179** **STV=26** **(STIb=1; STII=6; STVII=4)**
TOTAL CONTROLS	**3522 (colonised women and healthy infants)**

GBS=Group B Streptococcus; ELISA=enzyme-linked immunoabsorbent assay; OPkA=opsonophagocytosis killing assay; RABA=radioantigen binding assay; DEVANI=design of a vaccine to immunise neonates against GBS through a durable maternal immune response; ST=serotype; OR=odds ratio; RR=risk ratio; CFU/mL=colony-forming units per millilitre; GMC=geometric mean concentration.

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