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Methodology for a Correlate of Protection for Group B Streptococcus: Report from the Bill & Melinda Gates Foundation Workshop Held on 10 and 11 February 2021

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Abstract

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Worldwide, childhood mortality has declined significantly, with improvements in hygiene and vaccinations against common childhood illnesses, yet newborn mortality remains high. Group B *Streptococcus* (GBS) disease significantly contributes to newborn mortality and is the leading cause of meningitis in infants. Many years of research have demonstrated the potential for maternal vaccination against GBS to confer protection to the infant, and at least three vaccine candidates are currently undergoing clinical trials. Given the relatively low disease incidence, any clinical vaccine efficacy study would need to include at least 40,000 to 60,000 participants. Therefore, a path to vaccine licensure based on a correlate of protection (CoP) would be the preferred route, with post-approval effectiveness studies demonstrating vaccine impact on reduction of disease burden likely to be required as part of conditional marketing approval. This workshop, hosted by the Bill & Melinda Gates Foundation on 10 and 11 February 2021, discussed considerations and potential statistical methodologies for establishing a CoP for GBS disease. Consensus was reached that an antibody marker with global threshold predictive of a high level of vaccine protection would be most beneficial for licensure assessments. IgG binding antibody in cord blood would likely serve as the CoP, with additional studies needed to confirm a high correlation with functional antibody and to demonstrate comparable kinetics of natural versus vaccine-induced antibody. Common analyses of ongoing seroepidemiological studies include estimation of absolute and relative disease risk as a function of infant antibody concentration, with adjustment for confounders of the impact of antibody concentration on infant GBS disease including gestational age and maternal age. Estimation of an antibody concentration threshold indicative of high protection should build in margin for uncertainties from sources including unmeasured confounders, imperfect causal mediation, and variability in point and confidence interval estimates across regions and/or serotypes.

Keywords

Group B Streptococcus; correlate of protection; statistical analysis; licensure; vaccine development

Introduction to invasive group B *Streptococcus* (GBS) disease and workshop objectives

Despite reductions in childhood mortality in the past 30 years, newborn mortality remains high, with group B *Streptococcus* a significant contributor to morbidity and mortality [1–3].

Invasive GBS disease in newborns occurs from time of birth (early onset disease; EOD [0–6 days after birth]) up to 90 days after birth (late onset disease; LOD [7–90 days after birth]). The majority of GBS disease occurs within the first 72 hours of life and may also be associated with up to 3% of stillbirths. The rapid onset after birth complicates diagnosis and may lead to an underestimation of prevalence, especially in resource-limited settings.

Nearly all GBS disease is caused by six serotypes (Ia, Ib, II, III, IV and V), with serotype III currently the predominant causative serotype for EOD and LOD. Transmission can occur vertically or horizontally, and colonization of women can be up to 40% in some regions [4]. While universal screening and the use of intrapartum antibiotic prophylaxis (IAP) in the United States has reduced the incidence of EOD from 1.7 to 0.22 cases/1000 live births,

there has been no impact on LOD [5]. A risk-based approach to IAP has been associated with increased GBS disease [6]; therefore, a vaccine remains an unmet clinical need. A GBS vaccine would have the benefits of potential higher coverage in challenging settings where antibiotics may not be readily available, as well as reducing antibiotic usage.

Many years of research have demonstrated the potential for maternal vaccination against GBS to confer protection to the infant. Currently three glycoconjugate vaccines and one protein vaccine are in development, with a hexavalent CRM_{197} glycoconjugate vaccine (GBS6) in Phase 2 studies. In a Phase 1 trial in healthy men and non-pregnant women, all tested dosing regimens of the hexavalent vaccine were immunogenic against all six serotypes and the vaccine had an acceptable safety profile [7].

Given the low incidence of EOD, any pivotal clinical study demonstrating vaccine efficacy (VE) would need at least 40,000 to 60,000 participants [8]. Therefore, a regulatory submission for vaccine licensure based on a CoP would be the most feasible route, with commitments for post-licensure studies to demonstrate the impact on reduction of disease burden. Although maternal antibodies against the GBS capsule have been associated with protection of neonates against invasive GBS disease [9, 10] and provides a rationale for a CoP, a clear CoP remains to be established. The aims of this workshop were to evaluate the statistical approaches of ongoing seroepidemiological studies for estimating and defining an antibody marker CoP that may be used to support licensure of a hexavalent GBS glycoconjugate vaccine, and to describe next steps toward defining the evidence package needed for vaccine licensure submission based on a CoP. Summaries of the presentations are provided, together with consensus perspectives from roundtable discussions.

History of CoP for other vaccines

The CoP for the *Haemophilus influenzae* type b (Hib) vaccine was based on data from a large-scale study of the capsular polysaccharide vaccine [11]. The vaccine showed good protective efficacy in children ≥18 months old but was not very immunogenic in younger children. In unvaccinated children, a post-vaccination antibody concentration cutoff of 0.15μg/mL differentiated low and high incidence of disease and was accepted as a CoP for short-term disease [12, 13]. However, while 80% of the vaccinated children achieved this antibody level, this group still had a substantial incidence of breakthrough disease. An antibody concentration >1.0μg/mL was a better predictor of low disease risk most likely because it ensured maintenance of antibody levels >0.15ug/ml during the one year follow-up [13]. In a separate trial of a conjugate vaccine at 7 months of age, only 40% of the infants had an anti-Hib concentration of >1.0μg/mL, yet the estimated VE was 90% [14]. This indicated that a CoP defined based on natural immunity or on polysaccharide vaccines was not entirely representative of protective immunity produced by the conjugate vaccines, possibly due to the memory cells generated by the latter.

For meningococcal serogroup C, studies of natural immunity estimated a CoP threshold from serum bactericidal activity using human complement (hSBA) of ≥1 in 4. These data were used to support the development of polysaccharide vaccines [15]. For conjugate vaccines, a substantial amount of bridging had to be performed, as data were based on SBA

performed with rabbit complement (rSBA), and in infants and toddlers rather than adults. rSBA titers ≥8 or 16 correlated with observed VE based on population-level frequencies [16, 17]. This bridging of complement sources is potentially relevant to GBS, should an opsonophagocytic killing assay (OPKA) be required to assess functional antibody levels.

The CoP for pneumococcal polysaccharide-protein conjugate vaccines (PCV) was based on a very simple model relating VE for invasive disease and the distribution of serum antibody concentrations in vaccinated cohorts (reverse cumulative distribution curves [RCDCs]) [18, 19]. The estimated CoP thresholds above which protection was inferred to be high based on antibody data from the three PCV studies, performed in different geographic regions, varied from 0.2 to 0.99 μg/mL [19]. The data were pooled and weighted, yielding a value of 0.35μg/mL which was set as the CoP [19, 20]. While the estimated threshold of antibody needed varied by serotype (estimated at 0.14 to 2.83 μg/mL) [21], the CoP threshold of 0.35 μg/mL is still used. Because vaccine-induced antibody concentrations have been considerably above this level, approval decisions have not been sensitive to this threshold.

For GBS, a threshold amount of antibody needs to persist through three months of age to confer protection against invasive disease during the first 3 months of life. Given antibody waning and the efficiency of transplacental antibody transfer, the level of antibody that needs to be induced by the vaccine to provide this 3-month protection needs to be established. While binding antibody is the easiest to measure (via ELISA or Luminex), it may not directly relate to functional antibody levels, which can be measured using an OPKA. Measurement of functional antibody activity is more labor intensive and not conducive to high-throughput, and hence is more challenging to measure than binding antibody. Therefore, understanding the relationship between binding and functional antibodies will be critical to establishing a reliable CoP.

Use of biomarkers for regulatory decision-making

Biomarkers are currently used for many regulatory purposes including support of new vaccine submissions, bridging effectiveness, assessment of interference with concomitant vaccine administration, supporting applications to qualify for accelerated approvals, and bridging studies required following significant manufacturing changes. The strength of evidence and data source required varies based on the regulatory objective, with some requiring much stronger evidence than others (e.g., first-in-class vaccines). The regulatory use case scenario most appropriate to GBS vaccines is to support an application for licensure in the United States under their accelerated approval pathway, where approval can be granted based on the demonstration that the biological product “has an effect on a surrogate endpoint that is reasonably likely, based on epidemiologic, therapeutic, pathophysiologic, or other evidence, to predict clinical benefit” [22].

In the U.S. Food and Drug Administration (FDA) Vaccines and Related Biological Products Advisory Committee (VRBPAC) Meeting on 17 May 2018, committee members agreed that anti-capsular GBS IgG antibody is reasonably likely to predict clinical benefit [23]. While this is a positive statement for the potential for licensing a GBS vaccine through an immune biomarker, the link between binding and functional antibody, particularly in the context of

vaccine immune response, will also need to be established [24]. The Center for Biologics Evaluation and Research (CBER) accepts the concept of use of an immune biomarker to support accelerated approval of a GBS vaccine. However, further discussion is needed on the details of the supporting data and analyses, and post-licensure real-world evidence studies are required to confirm clinical benefit under the accelerated approval pathway [25].

GBS seroepidemiology studies in process

1. U.S. Study

As the United States has a low incidence of GBS disease due to IAP, this study is a case-control study built onto the CDC's Active Bacterial Core surveillance platform. Cases are infants 0–89 days of age with invasive GBS disease (GBS isolated from a normally sterile site). Controls are mother/infant dyads for which the mother was detected as being colonized during the current pregnancy but their infants did not have invasive GBS disease during the first 90 days of life. The primary objective, revised following FDA feedback, is to describe serotype-specific GBS disease (<90 days of age, and stratified by early and late-onset) probabilities associated with a range of antibody concentrations at birth. The study is using newborn dried blood spot data collected from multiple locations across eight states, with the background of widespread implementation of screening-based IAP. Cases and controls are matched by serotype, and potential confounders include race/ethnicity, gestational age, maternal age, and delivery mode. Study objectives estimate the EOD and LOD probabilities by serotype-specific antibody distributions for the overall study population and by gestational age (<34 and ≥34 weeks). The study uses GBS Assay Standardization Consortium assays and Luminex-based ELISA for the main correlate analyses. OPKA will also be performed on blood samples with sufficient volume. Some of the challenges for this study include the lack of maternal blood samples, limited sample volumes, laboriousness of obtaining consent, laws about accessing infant blood spots vary by state, and recruitment of a control population. The statistical analysis plan (SAP) is yet to be finalized, but at this stage a covariate-adjusted scaled logit model is planned [26].

2. UK study

The iGBS3 study is being performed as part of an existing cluster randomized controlled GBS trial evaluating risk- versus swab-based screening for prevention of EOD. The study collects cord blood from approximately 180,000 women with the aim of capturing 100 cases of serotype III invasive GBS disease. The primary objective is to quantify the relationship between antibody concentration and disease risk by estimating the covariate-adjusted odds ratio of GBS disease for antibody levels above various thresholds. Phase 1 of the study will also assess whether antibody levels obtained in the acute disease sample can be used to predict cord blood antibody levels. Phase 2 of the study will complete data collection for assessment of the primary objective. The study will reflect the diversity of the UK population and obstetric practices. Cases and controls will be matched by serotype. In addition, cases and controls will be matched by the potential confounders maternal age, ethnicity, gestation, and infant sex, which will be adjusted for in the data analyses. Antibody concentrations are measured using multiplex Luminex for IgG and functional responses will be assessed by OPKA. Cases had culture-confirmed GBS identified from a normally sterile

site. Controls include infants exposed to the same GBS serotype at delivery as the case but who do not go on to develop invasive GBS disease. Controls born at gestational age <34 weeks, via caesarean section with intact membranes, following receipt of adequate IAP or of a blood transfusion in the previous month will not be included. Logistic regression will be used to estimate the odds of being a case at different threshold concentrations, starting from a defined lower threshold of a1=0.01 μg/mL, with comparisons of (≥a1 vs. <a1), (≥a2 vs. <a2), etc. Absolute disease reduction will also be assessed through a nonparametric or Bayesian modelling approach. For the kinetics objective, geometric mean slope will be calculated using individual slopes and used to estimate birth concentrations.

3. South Africa study

In a recent seroepidemiology study aimed at establishing a serotype-specific threshold for reduction of risk of invasive GBS disease, blood samples were collected from 38,233 pregnant women and their infants, who were followed up to 90 days post-delivery for assessment of development of EOD and LOD [27]. The primary study objective was to determine the maternal and infant GBS serotype-specific antibody level associated with reduced risk of a composite of EOD and LOD due to serotypes Ia and III in the cohort of infants born at ≥34 weeks gestation. A total of 39,202 live births were enrolled, with 47 eligible GBS cases included in the analysis. A number of statistical approaches were considered for analysis. As published parametric and nonparametric models do not allow for clusters within the same disease status, the suitability of alternative models for analyzing case-control serological studies were explored, focusing on the use of mixture model averaging (MMA) to account for uncertainty in the functional form of IgG distributions. To validate the analysis, 10,000 simulations were performed together with re-analysis of published data from the DEVANI and SA GBS seroepidemiology studies [27, 28]. The MMA included two clusters of cases, to accommodate cases from different distributions (e.g. low vs high IgG concentrations) and was estimated as a weighted sum of different models that used different distributions. In simulation studies, which varied the proportion of cases from the high IgG component, estimates of the relative risk reduction (RRR) were less precise than estimates of the absolute disease risk (ADR); however, in the MMA of data from previous studies, the model performed well for RRR but for ADR there was an estimated rise in risk at higher concentrations (Table 1 defines ADR and RRR). Overall, it was concluded that MMA provides accurate and robust estimates of RRR and ADR, with similar estimates for distributions in the exponential family. While ADR is more sensitive than RRR, this can be improved with prior calibration.

Ultimately, ADR was estimated using Bayesian analysis [29], assuming that antibody concentration followed a Weibull distribution and marginalized risk of disease a β-distribution centered at 0.001. Cord blood IgG concentrations associated with a 90% risk reduction for serotypes Ia and III were ≥1.04 and ≥1.53 μg/mL, respectively, compared to concentrations below these thresholds. Maternal serum IgG concentrations associated with the same degree of risk reduction were ≥2.31 and ≥3.41μg/mL, respectively [27].

Experience with designating CoPs and statistical approaches for the GBS seroepidemiological data

A statistical pathway for the data collected in seroepidemiology studies to a sufficient evidence-basis supporting the use of an immune biomarker for provisional vaccine approval needs to be established (a so-called “non-validated” surrogate, where a “validated surrogate” would go farther to enable traditional approval [30]). While there will be caveats in the statistical results, given these are observational studies susceptible to confounding and selection biases, building conservative margins into threshold estimates together with replication of strong and consistent correlates across the three studies may be sufficient to achieve this goal. The ultimate aim of the analyses is to quantify how well an immune biomarker (IgG concentration from maternal or infant cord blood samples) can be used to predict VE against invasive GBS disease. Approaches to evaluating the validity of a potential surrogate endpoint include seroepidemiological studies, randomized VE trials, and challenge studies, which in different ways assess relationships between vaccination, clinical endpoints, and the potential surrogate endpoint. When considering how to establish that an immune biomarker is reasonably likely to predict VE, it is likely that in the absence of randomized VE data a demonstration of a strong and approximately consistent correlate of risk across different settings is needed, together with knowledge of connection of the surrogate to a biological mechanism of protection. As IgG is an accepted natural immunity mechanism of protection against GBS, arguments based on analogies of vaccines with validated IgG surrogate endpoints are critical.

Potential approaches for estimation of an ADR parameter with no covariate adjustments include Carey et al. [29], Fabbrini et al. [28], and Donovan et al. [31], where an extension of Carey’s Bayesian model or a flexible frequentist targeted maximum likelihood estimation (TMLE) model [32] could be used for covariate adjustment. Potential approaches for estimation of relative association parameters include logistic regression or more robust semiparametric extensions [33], the covariate-adjusted scaled logit model, or TMLE for matched or unmatched studies [34]. Overall, it would be more straightforward to develop a formula for predicting VE based on an absolute risk parameter (i.e., ADR) than on a relative association parameter. Part of codifying evidence for IgG as a reliable CoP is investigation of how well estimated association parameters can be interpreted in terms of the causal effect of IgG on disease risk, which relies on three causal assumptions that should be scrutinized (vaccine immunity is the same as natural immunity, no unmeasured confounders of the effect of the surrogate endpoint on the clinical endpoint, and positivity).

When considering the data from the seroepidemiology studies, relative association parameters such as covariate-adjusted odds ratios could be directly estimated in the case-control studies. Two approaches could be used, either based on relative parameters only, or absolute risk parameters with sensitivity analysis. Given the differing study designs, one potential common method to apply to all three seroepidemiology studies is the TMLE method [34], as this could provide a covariate-adjusted estimate of marginal causal relative risk for both individually-matched and unmatched studies.

Review of existing methods for estimating a CoP threshold

The designs of the three seroepidemiology observational studies pose a challenge for the ultimate goal of establishing a common CoP for infant GBS disease. In observational studies, collected samples are not usually representative of the broader study population, in particular with regards to the distributions of relevant covariates such as gestational age, race/ethnicity, maternal age, and maternal immunocompromised state. Different study designs can also introduce discrepancies between the samples and population from which they were drawn. If these covariates are correlated with both antibody concentration and incident disease, then the distributions of antibody concentration and disease will also differ among the samples (as well as differing from the distributions in the population). Methods for establishing a CoP should take such discrepancies into account as otherwise different CoPs will be generated from different study designs and different study populations. Hence, confounder adjustment is probably the most important and challenging issue for identification of a CoP from observational data, relevant both at the study design and analysis phases. Confounders in this context would be covariates that correlate with both antibody concentration and incident disease but are not part of the biological mechanism by which antibody concentration impacts disease. While logistic regression is a common approach to adjust for confounding of an odds ratio that contrasts two exposure (concentration) levels, adjusting for confounding is more challenging when estimating odds ratios that quantify how disease risk varies across the whole spectrum of antibody concentration. We therefore evaluated a range of methods for their ability to adjust for confounders and consider the need to develop innovative approaches.

The primary methods under consideration for establishing a CoP threshold for GBS include:

- Method 1: Reverse Cumulative Disease Probability Curve (RCDPC) based on the ADR
- Method 2: Continuous odds ratio curve for all antibody levels
- Method 3: ADR based on estimating antibody distributions separately for cases and controls
- Method 4: ADR based on logistic regression
- Method 5: Local odds ratio curves

Method 1 estimates the RCDPC – the probability of experiencing disease and having antibody concentration above a given threshold – with key step estimation of the ADR curve, and then derives the CoP threshold based on a specified protection probability threshold. It can be constructed either parametrically or nonparametrically with good accuracy and robustness [31] especially if the disease rate is a decreasing function of antibody concentration. The method is well-suited for simple random samples and can be easily adapted for any other probability sampling scheme with characterized sampling weights. From prospective studies confounders can be adjusted for in many ways, but in case-control studies with unknown population-level disease probability confounding adjustment is more challenging. Also, Method 1 does not lend itself naturally to pooling data or comparing results across studies with different collected potential confounding variables.

In contrast to Method 1, Method 2 employs a relative association parameter approach with the aim of obtaining a curve that does not rely on particular samples (i.e., specific confounding variables with a specific distribution). This method calculates an odds ratio at each concentration value (a0), by comparing disease odds of participants with concentration values larger than a0 vs. below a0, hence generating an odds ratio curve. This method has the advantage of being applicable to both cohort and case-control studies, and under simplifying assumptions provides a connection to VE [19]. Covariates can be adjusted for by logistic regression to obtain an adjusted odds ratio for each concentration value a0, which estimates a conditional causal odds ratio under successful confounding control. However, simulation experiments suggest that this proposal is not generally successful, showing that different CoP threshold estimates would be generated from different underlying antibody distributions. In other words, Method 2 does not lend itself to a truly confounder-adjusted CoP. Alternative versions of Method 2 would estimate the marginal causal odds ratio or marginal causal relative risk, which may provide more interpretable results with better confounding control [35].

Method 3 is based on constructing separate ADR curves for cases and controls. Hence, it is appealing for case-control studies, especially if the controls are a random sample from the study population and if the overall disease probability is known. The method can be implemented either parametrically [29] or nonparametrically [28] and can be applied to both cohort and case-control designs. However, its modeling structure makes the adjustment for confounders complex with no clear solution. Analogous to matching in matched case-control studies, matching of cases and controls on confounding covariates at the study design has been explored. However, no statistical method of this kind has yet been demonstrated to effectively adjust for confounders.

Method 4, a logistic regression approach, is commonly used to obtain confounder-adjusted odds ratios [26]. However, while logistic regression can succeed at adjusting the effect of confounders on the slope of the ADR curve, it does not account for effects of confounders on the intercept. Since both the slope and the intercept characterize the antibody-disease relationship curve (ADR), this approach does not generate a confounder-adjusted ADR-based CoP threshold estimate.

Method 5, estimation of a local odds ratio curve, divides antibody values into small bins and compares disease rates between each bin to the bin with the lowest antibody value. It has the advantage that baseline disease probability for the lowest bin cancels out in the calculation. Hence, confounder effects on the baseline disease probability will be removed if assuming a common local odds ratio curve for all participants. Logistic regression or a more robust regression approach can be used to adjust for covariates. Preliminary simulation experiments suggest that this approach holds promise to yield a confounder-adjusted CoP that is not affected by distributions of disease probabilities and antibody levels in the study sample, although more theoretical exploration and simulation experiments are needed.

While Method 5 was conceived to be implemented nonparametrically, a parametric approach can be developed to help elucidate the underlying logic and to smooth out random fluctuations due to partitioning the antibody levels into small bins. This proposed approach,

based on the covariate-adjusted scaled logit model [26], introduces a baseline disease probability for participants with zero or very low antibody levels. While this baseline disease probability might vary by study design and the associated study samples, the relative reduction in disease risk compared to the baseline disease risk as antibody level increases is assumed to be free of confounders, and hence can be used to derive a confounder-adjusted CoP threshold estimate. Analytic approaches can be developed to estimate this relative relationship curve under different study designs, including cohort, case-control and matched case-control studies.

Considerations when assessing methodologies

Workshop participants had consensus about a set of considerations that should be taken into account when assessing methodologies used for estimating a CoP threshold. First, there should be data for at least one serotype (probably serotype III) demonstrating that in infants vaccine-induced antibody is at least as high as naturally-induced antibody, and does not decay faster [36]. There would need to be data from several studies in different populations, and at least two statistical methods used to analyze the data. Consistency of results across studies and methods is important, and separate analysis of studies, rather than pooling or a meta-analysis, is preferred given the many differences among studies, with appropriate bridging analysis. However, data analyses pooling across studies are also useful for addressing questions that are underpowered based on a single study (e.g., subgroup analyses such as pre-term births). Analyses that include a relatively small number of covariates across the three data sets from the seroepidemiology studies would be desirable. The design of these studies are such that only GBS prognostic factors clearly related to antibody distribution need be considered for adjustment. Regarding the CoP, there ideally should be a high probability of clinically important risk reduction for vaccine recipients with antibody level above an estimated threshold. Finally, a suitable package of post-approval research for clinical benefit verification should already be planned or recruiting.

Harmonization of data and pooling across studies

In addition to individual analyses, potential benefits of pooling data across studies include making the CoP more generalizable across different populations, adding precision where there are small sample sizes, and providing greater insight into potential confounders and effect modifiers. Additionally, pooling across studies would be useful if a CoP is required based on a different assay than that used in the studies (e.g. OPKA).

Three different approaches to pooling could be applied. First, results from individual studies can be assessed, and if they give the same broad interpretation, can support an overall conclusion. If studies have differences in populations, endpoints, antibody assays, or sampling designs/frames for measuring antibody levels or endpoints, then it may not be possible to pool data for a cross-protocol meta-analysis. Secondly, different studies may provide estimation of a similarly interpretable target parameter, although potentially with different designs, making it possible to conduct a meta-analysis to produce more precise results. For this purpose, the data would need to be reported with the same antibody cut-offs and participant stratifications, and populations should have similar inclusion/exclusion

criteria and adjustments for confounders. Finally, the different studies may provide estimates of the same target parameter and use similar designs and covariates, such that individual level data can be combined to obtain pooled results. The latter is more difficult in practice, as it will require agreements to share data, very similar methodologies, and the same covariates collected in the same way, so may not be possible with the three studies discussed. For any pooling, the studies would all have to use assays that have the same interpretation across the quantitative range, standardization of storage, transport and processing, and the same sample type (e.g., cord blood).

Toward a single CoP for GBS: Statistical considerations

A number of decisions were made regarding the best course of action for establishing a CoP for GBS (Table 2). As it may not be possible or necessary to estimate threshold values for each serotype individually, particularly for the rarer serotypes, it may be that one CoP can be applied to all serotypes, or there is a CoP for the most common serotype and a different CoP from pooled data across the other serotypes. There was consensus to leave this issue open until data from the seroepidemiology studies have been fully analyzed. A single CoP should be established across geographies with statistical analysis to build in some margin for uncertainties, including unmeasured confounding, imperfect causal mediation, and sampling variability.

Individual analysis of studies was preferred, given the differences in study design, with common analysis methods to be established but including an ADR-based method and a relative association-based method, with preference for simple threshold and covariate-adjusted methods. The statistical analysis should include the minimum number of covariates that are expected to predict both disease and antibody concentration, rather than trying to control for all possible confounders. A strong case for using IgG for provisional approval would include robust results from the three studies, showing strong and consistent inverse correlations of infant cord blood IgG concentration with invasive GBS disease across all studies and by at least two statistical methods (for individual major serotypes and pooled over serotypes).

Representativeness is a key issue that needs resolution. Many statistical analyses are designed to make inference for a study population based on a direct or biased sample from a study population. These seroepidemiology studies themselves are reflecting populations of interest, including broader sets of individuals than would be included in randomized trials. The studies have the advantage of minimizing two major concerns of case-control studies (differential ascertainment of exposure cases vs. controls and systematic missing diagnoses of cases), given that almost all invasive GBS cases are captured and antibody measurement is well controlled. Thus, it may be worth considering defining IgG thresholds and prediction of VE for the seroepidemiology study participants themselves. Moreover, these studies have designs that are able to develop observations consistent with the existing model of IgG as a mechanism of protection, without representativeness [37].

Conclusions: Immunological considerations and key knowledge gaps

This workshop identified a number of knowledge gaps that would need to be addressed for achieving a robust CoP of clinical benefit for invasive GBS disease (Table 3).

Bridging analysis should be performed to assess the correlations between binding and functional antibody levels. Currently, correlations vary by assay. There is also a serotype-dependent element as serotypes Ia and Ib, for example, are structurally very similar meaning that antibody binds to both serotypes but functional capacity varies. Functional assays should also be performed to compare natural vs vaccine-induced immunity, including the potential effects of naturally acquired antibody on estimates. Animal models may be useful for this purpose, and should assess the affinity and IgG isotype mix, as well as the kinetics of antibody transfer (e.g., differences in glycosylation) and antibody persistence, as these may differ between natural and vaccine-induced antibodies.

For vaccine development, maternal antibody concentration post-vaccination would be the ideal CoP as infant antibody concentration cannot be controlled by vaccine design and is dependent on multiple factors such as gestational age, interval between vaccination and birth, time of colonization of the mother, and how much maternal antibody is transferred. However, given that the CoP should be predictive of infant disease, cord blood should be used to estimate antibody levels in the infant as blood samples after disease onset may already have been influenced by infection. As these samples may no longer be available for LOD cases, assays comparing kinetics in cord blood and post-disease onset samples are needed and can be performed on data from the ongoing seroepidemiology studies. Collecting data that enable comparing the quality of IgG from maternal sera vs. cord blood as a CoP would potentially allow a shift to a maternal sera CoP that would simplify vaccine development.

Major questions that need to be addressed include the optimal timing of vaccination and number of doses during pregnancy to obtain the highest antibody levels at birth; antibody profiles in the infant in the first three months after birth, and the level of cord blood antibodies achieved by maternal immunization. Additionally, data need to be collected on potential confounders such as IAP, chorioamnionitis, maternal HIV, maternal age, prior blood transfusion and gestational age. While it would be preferable to have as few covariates as possible in the analysis, these will need to be identified and standardized across studies. Finally, post-licensure considerations should include effectiveness studies and validation of the CoP as a high-protection threshold in real-world situations.

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Table 1.

Parameters that are estimated in seroepidemiological studies in the pursuit of defining an antibody concentration correlate of protection*

Parameter	Definition	Notation: **D = Indicator of invasive GBS disease occurrence** **A = IgG antibody concentration in cord blood** **W = Potential confounder variables** **P(a) = Distribution of A**
Reverse cumulative distribution curve (RCDC)	Frequency of the cohort with antibody concentration above a0, varying across thresholds a0	P(A >= a0)
Absolute disease risk (ADR) curve	Probability of disease at a given antibody concentration level a0, varying across thresholds a0	P(D=1|A=a0)
Relative risk reduction (RRR)	Ratio of disease probabilities at two given antibody concentration levels a1 < a2	P(D=1|A=a2) / P(D=1|A=a1)
Reverse cumulative disease probability curve (RCDPC) of ADR	Probability of disease and antibody concentration above a0, varying across thresholds a0	P(D=1 and A >= a0) = $\int_{a0}$ ADR(t)P(t)dt
Odds ratio curve	Odds of disease at antibody concentration above a0 divided by odds of disease at antibody concentration below a0, varying across thresholds a0	[P(D=1|A>a0)/P(D=0|A>a0)]/[P(D=1|A<=a0)/ P(D=0|A<=a0)]

*Parameters unadjusted for potential confounders are shown. The parameters have covariate-adjusted counterparts, for example the W-marginalized ADR parameter with direct-standardization adjustment for W is E[P(D=1|A=a0,W)] (expectation relative to the distribution of W) and the conditional odds ratio curve is [P(D=1|A>a0,W=w)/P(D=0|A>a0,W=w)]/[P(D=1|A<=a0,W=w)/P(D=0|A<=a0,W=w)]. a0, a1, and a2 are specified IgG concentration values (a0 < a1 < a2) from a cord blood sample.

Table 2.

Statistical considerations and ongoing data needs for establishing a CoP for GBS: Workshop consensus findings

Topic	Consensus
Analysis of seroepidemiology studies	Data should be analyzed separately for the three seroepidemiology studies given the differences in the study designs and study cohorts Pooled exploratory analysis across studies/regions could be performed for generating hypotheses for rare subgroups such as pre-term babies Common analysis methods should be applied, as applicable, across the three studies. A preference was stated for the simple threshold and covariate-adjusted scaled logit model (CALM) methods. These analyses should be pre-specified rather than post-hoc. A few targeted sensitivity analyses should be pre-specified as part of the common set of methods that are selected Validation subset data from the UK and South Africa studies can be used to predict cord blood IgG from disease onset IgG. Approaches to estimating geometric mean cord blood IgG accounting for use of a validation subset include augmented inverse probability weighting, targeted minimum loss-based estimation, or the less robust but easy to implement multiple imputation.
CoP threshold estimation	Planned analyses will estimate a single threshold across all serotypes or separate CoP thresholds for the most common serotype and for all other serotypes. Comparisons and modelling should be performed to inform if a single threshold is reasonable. Single threshold across geographic regions
Statistical methodology	Statistical analyses for CoP threshold estimation should build in some margin for uncertainty, including robustness to: 1. Imperfect causal mediation (vaccine immunity ≠ natural immunity) 2. Potential unmeasured confounding 4. Selection bias in transporting results to populations of interest 3. Minimum level of predicted vaccine efficacy 5. Variability in point and confidence interval estimates across studies/regions and serotypes (sampling variability) For example, one technique would estimate a CoP threshold as the lower limit of a 90% credible interval from a covariate-adjusted ADR curve Include both an ADR-based method and a relative association-based method in the small common set of methods. In addition to thresholds, the relationship of the entire IgG concentration distribution to GBS disease risk should be assessed. A simple analysis could compare RCDC curves for non-cases vs. cases vs. ~peak antibody time point for vaccine recipients in a phase 1 or 2 trial. Via direct standardization/G-computation or TMLE, these RCDC curves could be estimated for a given reference cohort such as the phase 1 or 2 vaccine trial cohort for any one of the seroepidemiology study cohorts (creating standardization on the distribution of baseline prognostic factors). The Siber model may be applied to estimate a CoP threshold [19].
Covariates	Preference for simplicity given advantages of the seroepidemiological case-control studies (high percentage of GBS cases captured and common ascertainment of IgG in cases and controls) and avoidance of trying to control for a large number of covariates Results to be presented with no covariate adjustment and using one or two ways to adjust for covariates, focusing on a small number of variables that provide the most information No adjustment for variables that are expected to be in the causal pathway between vaccination and GBS disease. A set of known GBS prognostic factors could be studied for their correlation with infant cord blood IgG concentration, and only those variables with correlation would then be included.

Table 3.

Knowledge gaps and focus areas for research

Topic	Gap
Immunological	Antibody kinetics studies should be performed to establish the relationship between vaccine-induced and natural immunity and between binding and functional antibody. Knowledge from antibody kinetic studies can be inputted into models that predict vaccine efficacy from an IgG distribution in vaccine recipients and from data results in the seroepidemiology studies.
	Characterization of neonatal antibody response (functionality, affinity, isotype mix, kinetics, glycosylation pattern, persistence)
	Relationship between maternal antibody at time of delivery and protection against maternal colonization, compared to the relationship between cord blood antibody and protection against LOD
	Mucosal immunity: characterization of memory B cell response in gut and genital tract (and in other relevant mucosal immune system compartments e.g. nasal cavity)
	Role of differences in pathogenesis between EOD and LOD regarding amount of antibody needed to confer protection
	Evaluation of timing and characteristics of protective antibody transfer to pre-term infants
Methodological	Identification of optimal functional antibody assay to be used
	Harmonization of statistical analysis methods that can be applied to each of the ongoing seroepidemiological studies
Post-licensure	Post-approval effectiveness studies – large scale, simple trial designs are preferred
	Validation of protective antibody threshold via post-approval real-world evidence