ORIGINAL ARTICLE

Potential for Maternally Administered Vaccine for Infant Group B Streptococcus

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ABSTRACT

BACKGROUND

Natural history studies have correlated serotype-specific anti–capsular polysaccharide (CPS) IgG in newborns with a reduced risk of group B streptococcal disease. A hexavalent CPS–cross-reactive material 197 glycoconjugate vaccine (GBS6) is being developed as a maternal vaccine to prevent invasive group B streptococcus in young infants.

METHODS

In an ongoing phase 2, placebo-controlled trial involving pregnant women, we assessed the safety and immunogenicity of a single dose of various GBS6 formulations and analyzed maternally transferred anti-CPS antibodies. In a parallel seroepidemiologic study that was conducted in the same population, we assessed serotype-specific anti-CPS IgG concentrations that were associated with a reduced risk of invasive disease among newborns through 89 days of age to define putative protective thresholds.

RESULTS

Naturally acquired anti-CPS IgG concentrations were associated with a reduced risk of disease among infants in the seroepidemiologic study. IgG thresholds that were determined to be associated with 75 to 95% reductions in the risk of disease were 0.184 to 0.827 μg per milliliter. No GBS6-associated safety signals were observed among the mothers or infants. The incidence of adverse events and of serious adverse events were similar across the trial groups for both mothers and infants; more local reactions were observed in the groups that received GBS6 containing aluminum phosphate. Among the infants, the most common serious adverse events were minor congenital anomalies (umbilical hernia and congenital dermal melanocytosis). GBS6 induced maternal antibody responses to all serotypes, with maternal-to-infant antibody ratios of approximately 0.4 to 1.3, depending on the dose. The percentage of infants with anti-CPS IgG concentrations above 0.184 μg per milliliter varied according to serotype and formulation, with 57 to 97% of the infants having a seroresponse to the most immunogenic formulation.

CONCLUSIONS

GBS6 elicited anti-CPS antibodies against group B streptococcus in pregnant women that were transferred to infants at levels associated with a reduced risk of invasive group B streptococcal disease. (Funded by Pfizer and the Bill and Melinda Gates Foundation; C1091002 ClinicalTrials.gov number, NCT03765073.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Dr. Anderson can be contacted at annaliesa.anderson@pfizer.com or at Vaccine Research and Development, Pfizer, 401 N. Middletown Rd., Pearl River, NY 10965.

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Group B streptococcus is a common cause of sepsis and meningitis in newborns through 89 days of age.[1,2] The primary risk factor for invasive group B streptococcal disease in newborns is exposure to maternal rectovaginal group B streptococcal colonization during delivery. An ascending group B streptococcal infection in the mother can also affect the fetus before delivery, when intraamniotic infection, premature labor, or stillbirth can occur.[3-5] In the United States and many other high-income countries, pregnant women undergo screening for group B streptococcal colonization late in the third trimester, and intrapartum antibiotic prophylaxis is administered to those with positive results.[6,7] Intrapartum antibiotic prophylaxis is more than 80% effective in the prevention of early-onset disease in infants (at 0 to 6 days of age) but has not been effective against late-onset disease (at 7 to 89 days) or prebirth sequelae associated with group B streptococcal infection.[6]

A maternal group B streptococcus vaccine administered during pregnancy to prevent invasive group B streptococcal disease in infants could potentially prevent late-onset disease in addition to early-onset disease and may mitigate the need for intrapartum antibiotic prophylaxis in otherwise healthy women. Such a vaccine could be beneficial because intrapartum antibiotic prophylaxis may contribute to antimicrobial resistance and disrupt development of the infant microbiome.[8-10] It is important to note that this vaccine would provide a much-needed measure in the prevention of group B streptococcal infection worldwide and to a substantial percentage of pregnant women living in resource-limited community settings where microbiologic screening and intrapartum antibiotic prophylaxis are unavailable.

Group B streptococcus expresses capsular polysaccharides (CPSs), which are important virulence factors. Ten CPS serotypes of group B streptococcus have been defined (Ia, Ib, and II through IX), six of which (Ia, Ib, and II through V) are associated with strains that cause approximately 98% of cases of invasive disease in infants worldwide.[11,12] Multiple seroepidemiologic studies have shown an association between acquisition of a sufficient concentration of transplacentally transferred, serotype-specific anti-CPS IgG and a reduced risk of invasive group B streptococcal disease caused by the homotypic serotype in early life. However, differences in study designs and serologic assays of binding antibodies have precluded agreement on a broadly accepted protective concentration.[13-18] The determination of a generally accepted immunologic surrogate of protection is advantageous for vaccine development because clinical efficacy studies would be prohibitively large (≥80,000 maternal participants), given the relatively low incidence of invasive disease in most countries where such studies could be conducted.[2,19]

We previously reported safety and immunogenicity results for a hexavalent CPS–cross-reactive material 197 glycoconjugate vaccine (GBS6) administered to men and nonpregnant women; GBS6 is currently being developed as a maternal vaccine to prevent invasive group B streptococcal disease in infants.[20] Here, we report the results of an assessment of GBS6 in pregnant women and examination of the transfer of anti-CPS antibodies to their newborn infants. To relate the induced antibody concentrations to potential protection, a parallel seroepidemiologic study was conducted with the use of the same validated and standardized GBS6 clinical serologic assay[20] to determine IgG thresholds that are associated with a reduced risk of invasive group B streptococcal disease among infants through 89 days of age.

METHODS

DESIGN AND OVERSIGHT

The seroepidemiologic study was designed in collaboration with the Wits Vaccines and Infectious Diseases Analytics Research Unit and Pfizer. The final protocol, available with the full text of this article at NEJM.org, and informed-consent document were approved by the institutional review board at each of the investigational centers participating in this study. Written informed consent for the seroepidemiologic study was obtained from all the maternal participants. The study principal investigators vouch for the accuracy and completeness of the data and for the fidelity of the study to the protocol. This article includes seroepidemiologic data that Pfizer was not responsible for validating or storing.

The phase 2 vaccine trial (C1091002) was designed by authors employed by Pfizer, the manufacturer of GBS6; these authors were involved in the collection and analysis of the data. The trial

protocol and informed-consent document were approved by the institutional review board at each of the investigational centers participating in this trial. The trial was conducted in compliance with International Council for Harmonisation Good Clinical Practice guidelines and the ethical principles of the Declaration of Helsinki. Written informed consent for the phase 2 trial was obtained from all the maternal participants. The authors employed by Pfizer and the study investigators vouch for the accuracy and completeness of the data and for the fidelity of the study to the protocol.

Pfizer and the Bill and Melinda Gates Foundation both provided funding for the seroepidemiologic study and phase 2 vaccine trial. Pfizer was the sponsor of record with the regulatory agencies for the phase 2 vaccine trial. The authors reviewed and edited previous versions of the manuscript and approved the final version for submission. Confidentiality agreements were in place between all the authors and Pfizer. A medical writer employed by Pfizer provided editorial and writing assistance with earlier versions of the manuscript under the direction of the authors. Additional details of the conduct of the seroepidemiologic study and phase 2 trial are provided in the Supplementary Appendix, available at NEJM.org.

SEROEPIDEMIOLOGIC STUDY

A case–control, longitudinal, observational cohort study was performed in South Africa. Prospectively enrolled mother–infant pairs were monitored for the development of invasive group B streptococcal disease in the infant through 89 days of age. Anti-CPS IgG concentrations were assessed in cord-blood serum samples obtained from the case patients (the infants with invasive disease) and controls (the infants without invasive disease who were born to mothers with group B streptococcal colonization) in the prospective cohort, and serum samples were obtained at the time of infection from infants who were enrolled retrospectively within 72 hours after laboratory confirmation of invasive group B streptococcal disease at the hospitals participating in surveillance. Vaginal and rectal swab samples were obtained at the time of delivery from a subgroup of maternal participants in the enrolled cohort, and by means of exact matching, case patients were matched to controls with vaginal colonization according to the same serotype of group B streptococcal infection and similar gestational age of the infant at birth (see the Supplementary Appendix).

PHASE 2 VACCINE TRIAL

The clinical vaccine trial is being conducted in three stages; reported here are the results from stage 2. This phase 2, randomized, placebo-controlled trial was conducted at three clinical research centers in South Africa. Healthy pregnant women 18 to 40 years of age were assigned to receive a single dose of 5 μg, 10 μg, or 20 μg per serotype of GBS6 with or without aluminum phosphate ($AlPO_4$) or placebo. Serum samples were obtained from the women before and at various times after maternal immunization, and cord-blood or infant serum samples were obtained at delivery and assessed for anti-CPS IgG (see the protocol and the Supplementary Appendix). The trial staff who dispensed and administered the vaccine or placebo were aware of the trial-group assignments, but the assignments were concealed from all other trial personnel, including the principal investigators, and the participants.

Safety was assessed in both the maternal and infant participants. Maternal participants used an electronic diary to record solicited local and systemic events for 7 days after vaccination and unsolicited adverse events through 1 month after vaccination; serious adverse events, medically attended adverse events, and obstetrical complications were recorded through 12 months after delivery. Among infants, unsolicited adverse events were recorded from birth through 6 weeks of age, and serious adverse events, adverse events of special interest, and medically attended adverse events were recorded through 12 months of age (see the Supplementary Appendix).

SEROLOGIC ASSAY

Anti-CPS IgG concentrations were determined with the use of a quantitative direct immunoassay (Luminex) that measured levels of antibodies binding to CPS serotypes Ia, Ib, and II through V (see the Supplementary Appendix). This assay was used to measure serum anti-CPS IgG concentrations in both the seroepidemiologic study and the phase 2 vaccine trial.

STATISTICAL ANALYSIS

In the seroepidemiologic study, the absolute risk of invasive group B streptococcal disease was esti-

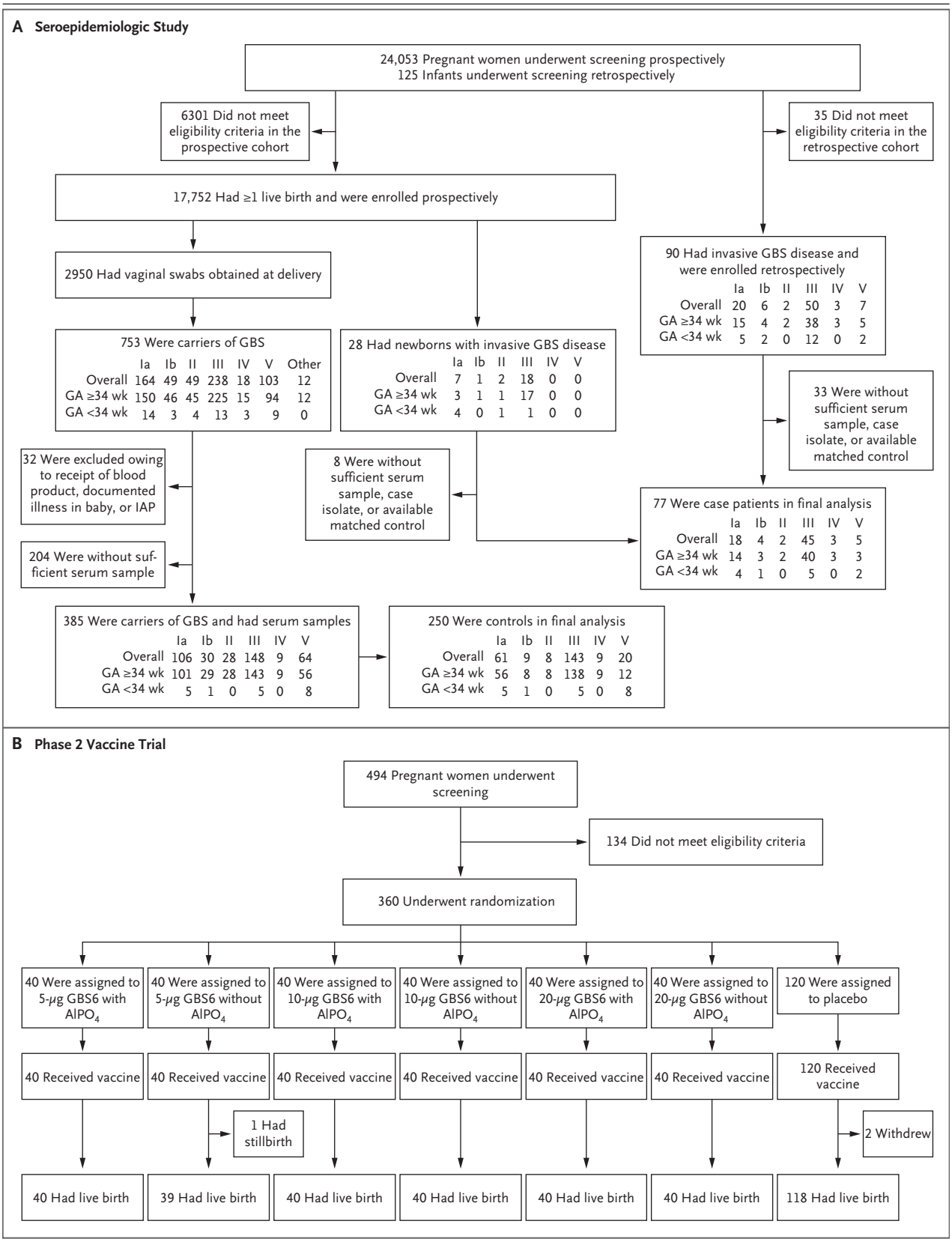

Figure 1 (facing page). Screening, Enrollment, and Analysis in the Seroepidemiologic Study and the Phase 2 Vaccine Trial.

In Panel A, the group of 2950 mothers with vaginal swabs obtained at delivery does not include 4 mothers with infants who were enrolled as case patients in the prospective cohort. Among the 753 carriers of group B streptococcus (GBS), 3 had an unknown gestational age (GA) and 117 had no vaginal isolate. Among the 90 infants with invasive GBS disease who were enrolled retrospectively, the gestational age was unknown for 2, and these infants were not included in the analysis. $AlPO_4$ denotes aluminum phosphate, GBS6 hexavalent capsular polysaccharide–cross-reactive material 197 glycoconjugate vaccine, and IAP intrapartum antibiotic prophylaxis.

mated as a function of distributions of anti-CPS IgG concentrations among case patients and controls by means of a Bayesian method that has been described previously for the estimation of anti-CPS IgG concentration thresholds that are associated with a reduced risk of disease.[21] A posterior predictive analysis showed consistency between the model and the study data (Fig. S1 in the Supplementary Appendix).[2]

The vaccine trial had no formal statistical hypotheses. Sample size was justified by the likelihood of adverse events being observed; safety and immunogenicity analyses were descriptive. Safety was the primary end point and was assessed among all the participants who received GBS6 or placebo (the safety population). Immunogenicity end points were assessed in the evaluable immunogenicity population, which included the participants who were eligible for the trial, received the randomly assigned maternal vaccine or placebo, had at least one valid and determinate assay result that could be included in the analysis, and had no potentially important protocol violations (see the Supplementary Appendix). Point estimates and the exact 2-sided 95% confidence intervals, as calculated with the use of the Clopper and Pearson method, were determined for all the trial groups. Anti-CPS IgG concentrations for the six serotypes were logarithmically transformed for analysis. Geometric mean concentrations and the associated two-sided 95% confidence intervals from each trial group were calculated by back transformation of the mean and the 95% confidence interval of the logarithmically transformed assay results that had been computed with the use of a Student's t distribution. Because this was a phase 2 trial, there was no imputation of missing serologic results. Values below the lower limit of quantification (LLOQ) were analyzed as 0.5 times the LLOQ. No adjustments were made for multiplicity because there were no hypothesis tests; furthermore, all reported confidence intervals should be considered to be descriptive and not be used to assess specific hypotheses.

RESULTS

PARTICIPANTS IN THE SEROEPIDEMIOLOGIC STUDY

From March 2019 to the beginning of June 2020, a total of 24,053 pregnant women at antenatal clinics or in labor underwent screening prospectively for enrollment in the study; 17,752 were included in the prospective cohort (Fig. 1 and Table S1). Rectal and vaginal swabs that had been obtained at the time of delivery from a selected subgroup of 2950 women showed that approximately 26% of those who underwent screening were carriers of group B streptococcus (Fig. 1). Newborns were followed for the development of invasive group B streptococcal disease through 89 days after birth; 28 cases were recorded. The group of case patients in the prospective cohort was supplemented with a separate group of 90 infants with invasive disease who had been enrolled retrospectively within 72 hours after laboratory confirmation of their illness.

The overall incidence per 1000 live births in the prospective cohort was 0.72 for early-onset disease (1.53 at <34 weeks' gestational age and 0.66 at ≥34 weeks' gestational age) and 0.83 for late-onset disease (3.05 at <34 weeks' gestational age and 0.66 at ≥34 weeks' gestational age). Cord-blood or infant serum samples, the group B streptococcal invasive isolate, and matched controls were available for 20 case patients in the prospective cohort and for 57 case patients in the retrospective cohort. Among these case patients, 45 (58%) had serotype III group B streptococcal infection, 18 (23%) had serotype Ia, 4 (5%) had serotype Ib, 2 (3%) had serotype II, 3 (4%) had serotype IV, and 5 (6%) had serotype V (Fig. 1 and Table S2).

SEROCORRELATE ANALYSIS

Data from a sufficient number of case patients were available to determine the risk–concentration relationships for serotypes Ia and III. The unad-

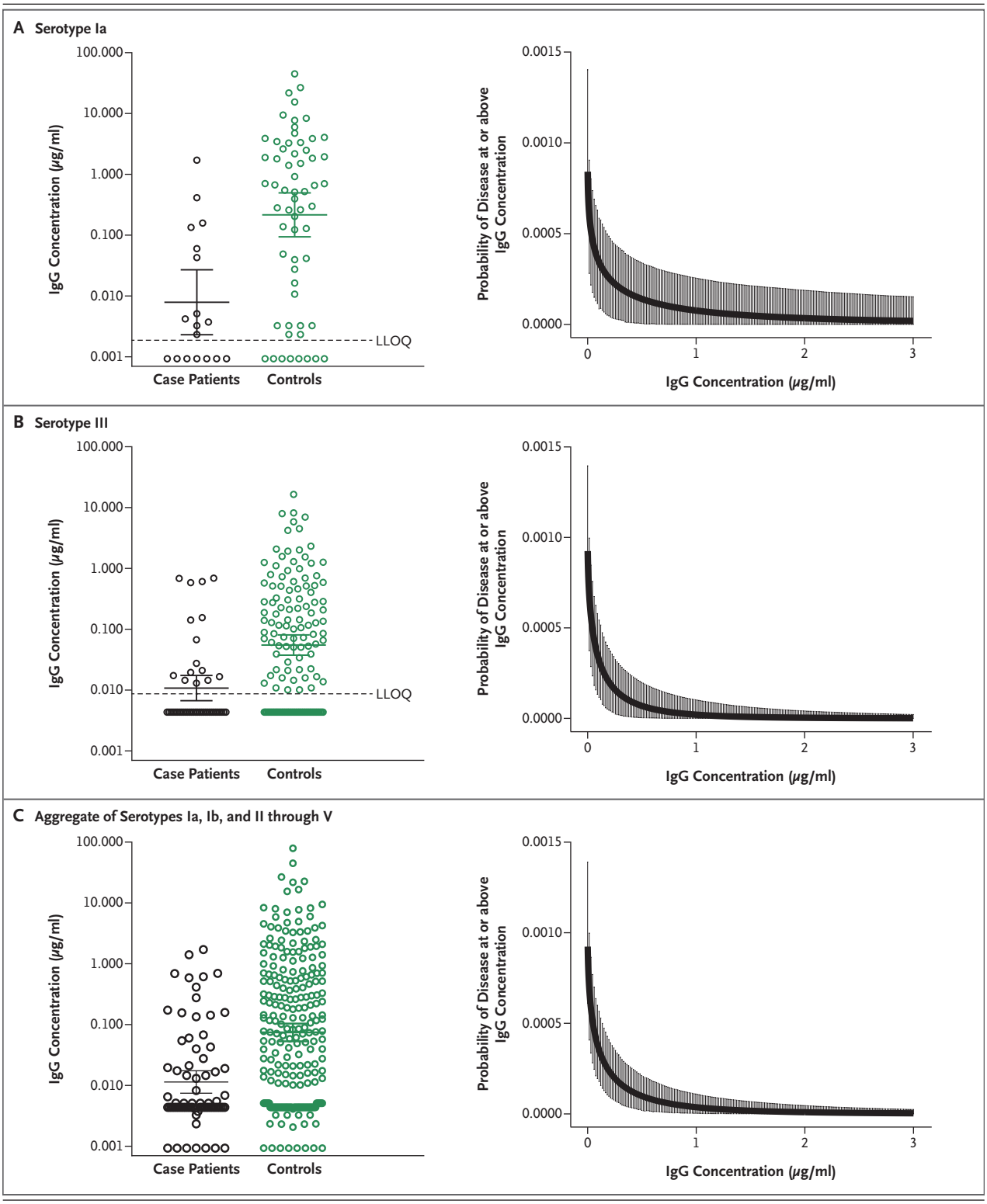

Figure 2 (facing page). Anti-CPS IgG Concentrations among Infant Case Patients and Controls and Risk–Concentration Curves for Major and Aggregated Serotypes in Seroepidemiologic Study.

Shown are the distributions of serotype-specific anti-CPS IgG antibodies among infant case patients and matched controls (Panel A, serotype Ia; Panel B, serotype III; and Panel C, combined serotypes Ia, Ib, and II through V). The horizontal lines and I bars on the left side of each panel indicate geometric mean concentrations and 95% confidence intervals, respectively, and each point represents an individual sample. In each panel, risk–concentration curves on the right side show the probability of invasive GBS disease according to antibody concentration. Vertical lines indicate 95% credible intervals, and the thick line indicates median probability. LLOQ denotes lower limit of quantification.

justed 95% confidence intervals for the geometric mean concentrations overlapped between the prospective cohort and the retrospective cohort for both case patients and controls (Table S3). In addition, for five of the six serotypes, the geometric mean concentrations of anti-CPS IgG against group B streptococcus were higher among the controls than among the case patients, with point estimates of the geometric mean ratios (controls to cases) higher than 1 (Table S4).

The Bayesian model that was fitted to the distributions of concentrations in case patients and controls is shown in Figure 2. Because the serologic assay was cross-standardized and could relate weight-based concentrations among serotypes, risk–concentration curves were compared among case patients and controls with respect to the individual serotypes Ia and III, as well the combination of serotypes Ia, Ib, and II through V. Representative antibody thresholds that are associated with specific risk reductions for invasive disease are provided in Table S5. IgG thresholds that were determined to be associated with 75 to 95% reductions in the risk of disease were 0.184 to 0.827 μg per milliliter.

PARTICIPANTS IN THE PHASE 2 VACCINE TRIAL

Between July 8, 2019, and August 31, 2020, a total of 494 pregnant women underwent screening, and 360 were enrolled and randomly assigned to receive one of six different vaccine formulations (40 participants per group) or placebo (120 participants) (Fig. 1 and Table S6). Live births were recorded for all the participants except for a single stillbirth that occurred in a woman who received 5μg GBS6 without $AlPO_4$. Two participants in the placebo group withdrew from the trial before delivery. Data from 360 maternal vaccine or placebo recipients and 357 infants were included in the safety analyses.

VACCINE SAFETY

Local reactions were generally mild or moderate, and pain at the injection site was reported more frequently among the participants who received GBS6 with $AlPO_4$ than among those who received GBS6 without $AlPO_4$ (Fig. 3). Solicited systemic events were similar among the GBS6 groups and the placebo group (Fig. 3), with most events being mild or moderate. Severe systemic events were reported in four GBS6 recipients and four placebo recipients. Two GBS6 recipients reported severe fever (39.0°C and 40.0°C) lasting 1 day; one fever occurred on day 3 after vaccination and the other on day 7 after vaccination. Among the maternal participants, unsolicited adverse events occurred in 45 to 70% of the participants in the GBS6 groups and in 61% of those in the placebo group (Table 1). The most common adverse events and serious adverse events were in the system organ class of pregnancy, puerperium, and perinatal conditions; the most common event was fetal distress syndrome.

A single stillbirth that occurred in a GBS6 recipient was deemed by the investigator to be unrelated to the vaccine. One maternal participant and her infant were involved in a fatal motor vehicle accident that was deemed by the investigator to be unrelated to the trial vaccine. Few participants in the GBS6 groups and the placebo group reported adverse events that were deemed by the investigator to be related to the trial vaccine or placebo; the most common such events were headache and vomiting.

Among the infants, adverse events occurred in 62 to 75% of the participants in the GBS6 groups and in 74% of those in the placebo group; the most common event was upper respiratory tract infection (Table 1). There were three infant deaths (one in the group that received 5-μg GBS6 without $AlPO_4$ and two in the placebo group), which were all deemed by the investiga-

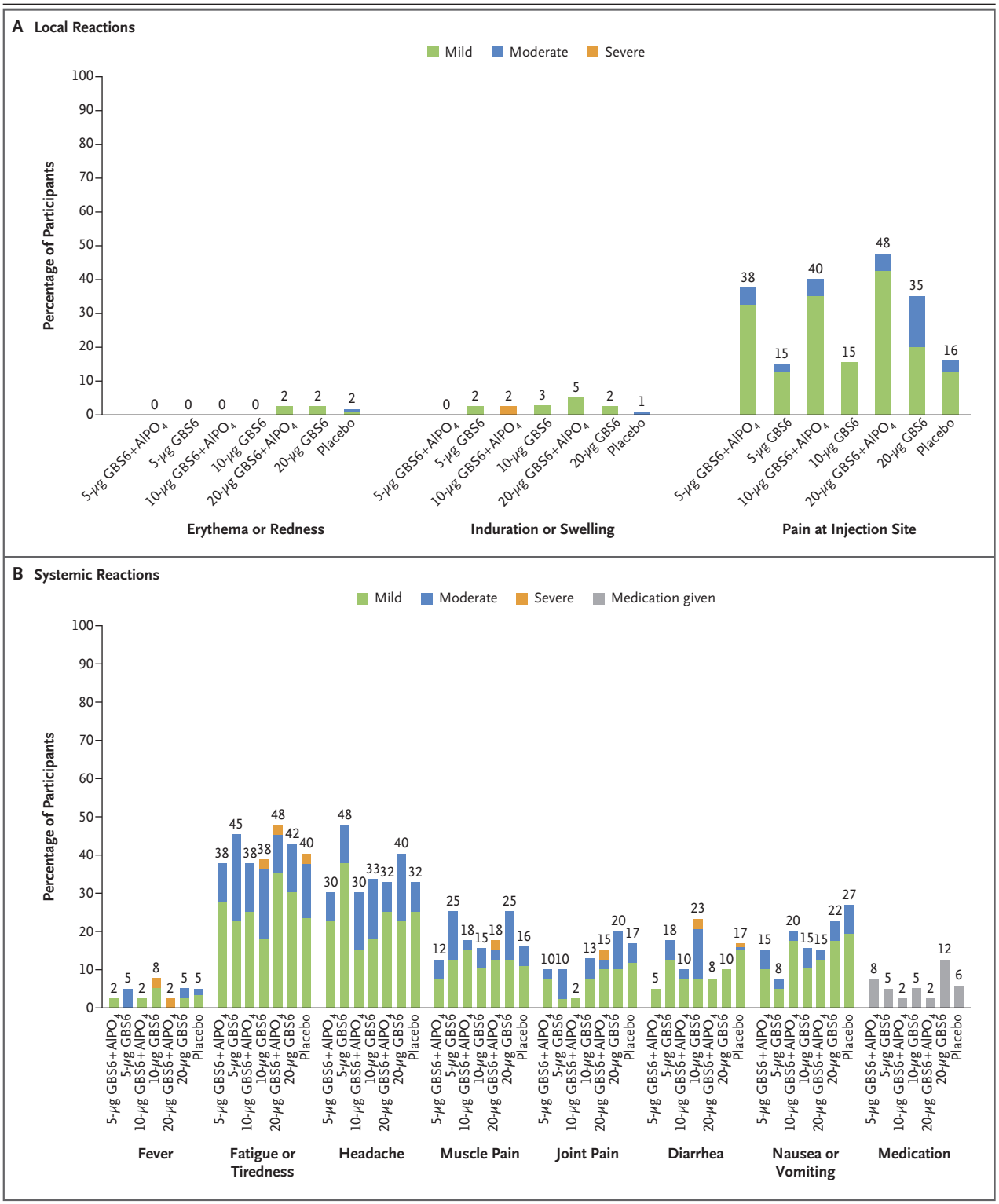

Figure 3 (facing page). Local and Systemic Reactogenicity Within 7 Days After Study Vaccination in Phase 2 Vaccine Trial.

Local (Panel A) and systemic (Panel B) reactogenicity was recorded for maternal participants in study stage 2 who were enrolled in the phase 2 vaccine trial. In Panel B, mild fever was defined as a body temperature ranging from 38.0 to 38.4°C, moderate fever as a body temperature ranging from 38.5 to 38.9°C, and severe fever as a body temperature ranging from 39.0 to 40.0°C; no participant had a body temperature higher than 40.0°C.

tor to be unrelated to the trial vaccine or placebo. The most common serious adverse events were minor congenital anomalies (umbilical hernia and congenital dermal melanocytosis). No adverse event that occurred among the infants was deemed by the investigator to be related to the trial vaccine.

IMMUNOGENICITY

Across all the groups, 11 maternal participants (3%) and 60 infant participants (17%) were excluded from the evaluable immunogenicity population (Tables S7 and S8). Induction of maternal anti-CPS IgG antibodies against all GBS6 serotypes was seen after immunization with any of the vaccine formulations, as compared with placebo, with geometric mean factor increases in vaccinated maternal participants at delivery ranging from 11.6 to 383.1 (Table 2 and Table S9). Antibody concentrations varied among serotypes, with the highest geometric mean concentrations recorded for serotype Ia and the lowest for serotypes Ib and V (Table 2 and Figs. S2 and S3). The highest anti-CPS IgG geometric mean concentrations were generally observed with the 20-μg GBS6 dose, formulated without $AlPO_4$. Because this was a phase 2 trial, no formal comparisons between the trial groups were performed. Anti-CPS IgG concentrations remained elevated through delivery (Fig. S4), and infant–maternal transfer ratios of approximately 0.4 to 1.3 were recorded across the different serotypes for the six vaccine formulations (Table 2). Because vaccine-induced and naturally acquired anti-CPS IgG concentrations were measured with the same standardized assay, anti-CPS IgG could be compared with the calculated protective thresholds from the seroepidemiologic study. For the most immunogenic formulation, the percentage of infants with anti-CPS IgG concentrations that reached the threshold associated with a 75% risk reduction at birth (0.184 μg per milliliter) ranged by serotype from 57 to 97% (Table 2).

DISCUSSION

In this initial assessment of the GBS6 vaccine in pregnant women, no safety concerns associated with GBS6 were identified, and IgG responses to all six serotypes were elicited, which were sustained through delivery and transferred to infants. Immunogenicity did not improve with $AlPO_4$ coformulation, a finding that was also observed in the previous phase 1 study involving nonpregnant adults.[20] Higher reactogenicity was noted with $AlPO_4$-containing formulations than in those that did not contain $AlPO_4$, which is generally consistent with aluminum-adjuvanted vaccines.[22] The kinetics of antibody induction and persistence were similar to that observed in the previous study involving nonpregnant adults, in which concentrations remained elevated through 6 months of follow-up.[20] Durable concentrations of anti-CPS IgG against group B streptococcus may provide additional benefit for the prevention of sepsis that can occur directly post partum in the mother.[23]

The distribution of cases in the seroepidemiologic study followed the global distribution of serotypes causing invasive group B streptococcal disease in infants.[2,19] Strengths of the study included the use of infant controls born to mothers with colonization by the homologous case serotype, as well as the determination of IgG concentrations from serum samples approximating directly or indirectly the circulating antibody level in the infant. This latter aspect is of particular relevance because maternal IgG transfer ratios can vary among persons, and anti–group B streptococcus CPS IgG concentrations in infants generally do not reach maternal concentrations[24,25]; moreover, it is biologically significant because the disease occurs in the infant. Previous serocorrelate studies that included both maternal and infant estimates showed higher calculated thresholds on the basis of maternal serum samples.[12] Because South Africa represents

Table 1. Adverse Events Reported in Maternal and Infant Participants during the Phase 2 Vaccine Study According to Vaccine Group.*

Variable	5-μg GBS6 with $AlPO_4$	5-μg GBS6	10-μg GBS6 with $AlPO_4$	10-μg GBS6	20-μg GBS6 with $AlPO_4$	20-μg GBS6	Placebo
Maternal participants							
No. of participants	40	40	40	40	40	40	120
Adverse event — no. (%)							
Any	19 (48)	28 (70)	20 (50)	18 (45)	19 (48)	22 (55)	73 (61)
Serious†	7 (18)	18 (45)	9 (22)	11 (28)	8 (20)	14 (35)	30 (25)
Immediate‡	0	0	0	0	0	0	0
Severe§	4 (10)	15 (38)	8 (20)	6 (15)	6 (15)	7 (18)	20 (17)
Treatment-related	1 (2)	1 (2)	2 (5)	0	0	0	3 (2)
Medically attended	11 (28)	17 (42)	12 (30)	11 (28)	9 (22)	11 (28)	47 (39)
Led to withdrawal	0	0	0	0	0	0	1 (1)
Infant participants							
No. of participants	40	39	40	40	40	40	118
Adverse event — no. (%)							
Any	29 (72)	27 (69)	30 (75)	28 (70)	28 (70)	25 (62)	87 (74)
Serious	17 (42)	19 (49)	15 (38)	12 (30)	19 (48)	14 (35)	59 (50)
Serious, excluding minor congenital anomalies¶	7 (18)	8 (21)	8 (20)	2 (5)	8 (20)	5 (12)	24 (20)
Severe	2 (5)	7 (18)	4 (10)	1 (2)	5 (12)	2 (5)	11 (9)
Treatment-related	0	0	0	0	0	0	0
Medically attended‖	22 (55)	14 (36)	19 (48)	21 (52)	17 (42)	18 (45)	56 (47)
Led to withdrawal	0	1 (3)	0	0	0	0	2 (2)

* Participants with multiple occurrences of the same type of adverse event or any adverse event were counted only once. Adverse events that occurred before vaccination are not included in the table. The total dose of hexavalent CPS–cross-reactive material 197 glycoconjugate vaccine (GBS6) in the 5-μg GBS6 groups was 30 μg (5-μg capsular polysaccharide [CPS] per serotype); in the 10-μg GBS6 groups, 60 μg (10-μg CPS per serotype); and in the 20-μg GBS6 groups, 120 μg (20-μg CPS per serotype). $AlPO_4$ denotes aluminum phosphate.

† A serious adverse event was defined as any untoward medical occurrence at any dose that resulted in death, a life-threatening condition, hospitalization or prolonged existing hospitalization, congenital anomaly or birth defect, or a medical event that was considered to be important.

‡ An immediate adverse event was defined as an adverse event occurring within the first 30 minutes after administration of the vaccine or placebo.

§ A severe adverse event was defined as an event that substantially interfered with a participant's usual function.

¶ A minor congenital anomaly was defined as a congenital anomaly that did not require medical or surgical treatment, did not seriously affect health and development, and did not have substantial cosmetic effect (e.g., congenital dermal melanocytosis).

‖ A medically attended adverse event was defined as a nonserious adverse event that resulted in an evaluation at a medical facility.

Table 2. Maternal and Infant Anti-CPS IgG Concentrations for Different GBS6 Formulations (Evaluable Immunogenicity Population).*

Variable	5-μg GBS6 with $AlPO_4$ (N=34–37)	5-μg GBS6 (N=29–36)	10-μg GBS6 with $AlPO_4$ (N=29–37)	10-μg GBS6 (N=29–34)	20-μg GBS6 with $AlPO_4$ (N=35–38)	20-μg GBS6 (N=34–40)	Placebo (N=91–108)
Maternal GMC at delivery — μg/ml (95% CI)							
Serotype Ia	11.94 (5.57–25.61)	14.71 (6.16–35.11)	14.26 (6.57–30.96)	18.40 (8.18–41.35)	21.99 (8.81–54.88)	40.34 (23.87–68.18)	0.11 (0.06–0.19)
Serotype Ib	0.45 (0.16–1.33)	0.28 (0.10–0.76)	0.53 (0.18–1.56)	0.89 (0.34–2.31)	0.84 (0.39–1.84)	1.28 (0.56–2.94)	0.01 (0.01–0.02)
Serotype II	8.68 (4.46–16.87)	3.26 (1.60–6.65)	9.91 (5.41–18.15)	8.38 (4.81–14.61)	15.54 (7.82–30.91)	27.64 (15.63–48.88)	0.14 (0.10–0.20)
Serotype III	2.52 (0.99–6.38)	1.67 (0.64–4.34)	3.57 (1.49–8.56)	3.77 (1.75–8.13)	2.59 (1.16–5.81)	6.38 (2.83–14.38)	0.02 (0.01–0.03)
Serotype IV	1.69 (0.92–3.12)	0.54 (0.25–1.14)	1.41 (0.79–2.52)	1.29 (0.68–2.42)	1.82 (1.70–3.10)	2.48 (1.49–4.15)	0.01 (0.10–0.02)
Serotype V	0.19 (0.10–0.36)	0.24 (0.09–0.66)	0.68 (0.31–1.52)	1.40 (0.54–3.59)	0.85 (0.41–1.76)	0.87 (0.38–1.98)	0.02 (0.01–0.02)
Infant GMC at birth — μg/ml (95% CI)							
Serotype Ia	6.56 (2.61–16.51)	15.06 (7.26–31.28)	11.89 (5.46–25.85)	12.30 (4.88–31.04)	8.26 (2.84–24.00)	29.56 (16.96–51.51)	0.08 (0.04–0.14)
Serotype Ib	0.26 (0.08–0.84)	0.27 (0.08–0.90)	0.32 (0.09–1.18)	0.45 (0.15–1.39)	0.32 (0.14–0.75)	0.71 (0.27–1.82)	0.01 (0.01–0.02)
Serotype II	6.61 (3.62–12.06)	4.37 (2.40–7.94)	7.44 (3.81–14.53)	6.95 (3.19–15.12)	7.95 (3.47–18.20)	20.77 (10.66–40.45)	0.10 (0.07–0.14)
Serotype III	1.21 (0.45–3.23)	1.41 (0.52–3.86)	2.04 (0.82–5.10)	2.26 (0.84–6.04)	1.01 (0.36–2.83)	3.15 (1.29–7.69)	0.02 (0.01–0.02)
Serotype IV	1.42 (0.74–2.74)	0.81 (0.35–1.91)	1.07 (0.64–1.82)	0.68 (0.33–1.37)	1.02 (0.55–1.90)	2.09 (1.18–3.72)	0.01 (0.01–0.01)
Serotype V	0.11 (0.05–0.24)	0.20 (0.06–0.62)	0.42 (0.16–1.09)	0.78 (0.26–2.30)	0.36 (0.15–0.87)	0.58 (0.24–1.43)	0.01 (0.01–0.02)
Infant-to-maternal GMR (95% CI)							
Serotype Ia	0.53 (0.35–0.81)	1.07(0.45–2.53)	0.64 (0.51–0.81)	0.66 (0.52–0.83)	0.44 (0.27–0.69)	0.70 (0.57–0.86)	0.76 (0.62–0.93)
Serotype Ib	0.52 (0.36–0.75)	1.09 (0.52–2.32)	0.57 (0.41–0.80)	0.46 (0.26–0.83)	0.41 (0.32–0.54)	0.66 (0.48–0.93)	0.92 (0.69–1.22)
Serotype II	0.72 (0.52–1.00)	1.12 (0.61–2.04)	0.78 (0.60–1.03)	0.70 (0.47–1.05)	0.51 (0.34–0.76)	0.74 (0.60–0.92)	0.67 (0.54–0.83)
Serotype III	0.50 (0.36–0.69)	0.84 (0.54–1.28)	0.58 (0.44–0.77)	0.56 (0.38–0.84)	0.36 (0.25–0.50)	0.55 (0.41–0.74)	0.81 (0.69–0.95)
Serotype IV	0.81 (0.59–1.11)	1.30 (0.68–2.50)	0.85 (0.57–1.26)	0.67 (0.50–0.88)	0.50 (0.37–0.70)	0.71 (0.55–0.92)	0.66 (0.52–0.83)
Serotype V	0.58 (0.42–0.81)	0.78 (0.42–1.44)	0.52 (0.38–0.71)	0.44 (0.24–0.83)	0.40 (0.29–0.53)	0.65 (0.52–0.82)	0.28 (0.62–0.83)
Infants reaching IgG threshold — % (95% CI)							
Serotype Ia	89 (74–97)	100 (88–100)	97 (82–>99)	93 (78–99)	83 (66–93)	97 (85–>99)	40 (29–50)
Serotype Ib	49 (31–66)	62 (42–79)	57 (37–74)	57(37–74)	63 (45–78)	71 (52–85)	14 (8–23)
Serotype II	100 (90–100)	97 (82–>99)	97 (83–>99)	97 (83–>99)	94 (81–99)	97 (85–>99)	35 (25–45)
Serotype III	72 (55–86)	77 (58–90)	77 (58–90)	83 (65–94)	69 (52–84)	83 (66–93)	13 (7–21)
Serotype IV	85 (69–95)	70 (51–85)	87 (69–96)	73 (54–88)	80 (63–92)	97 (85–>99)	4 (1–11)
Serotype V	36 (21–54)	43 (26–63)	57 (37–74)	70 (51–85)	53 (36–70)	57 (39–74)	9 (4–16)

* The numbers of participants in each group are presented as ranges because of occasional missing values in assays for a particular serotype. The total GBS6 dose in the 5-μg GBS6 groups was 30 μg (5-μg CPS per serotype); in the 10-μg GBS6 groups, 60 μg (10-μg CPS per serotype); and in the 20-μg GBS6 groups, 120 μg (20-μg CPS per serotype). The standardized lower limit of quantitation (LLOQ) values for IgG are 0.002 μg per milliliter for serotype Ia, 0.005 μg per milliliter for serotype Ib, 0.022 μg per milliliter for serotype II, 0.009 μg per milliliter for serotype III, 0.004 μg per milliliter for serotype IV, and 0.01 μg per milliliter for serotype V. Assay results below the LLOQ were set to 0.5×LLOQ. The IgG threshold that was determined to be associated with a 75% reduction in the risk of disease was 0.184 μg per milliliter, as derived from a universal Bayesian model. CI denotes confidence interval, GMC geometric mean concentration, and GMR geometric mean ratio.

a location with high group B streptococcal disease endemicity where microbiologic screening with intrapartum antibiotic prophylaxis is not the standard of care, the risk–concentration relationship may be more accurately assessed without the confounding effect of routine use of antibiotics.

The use of an assay that is cross-standardized enabled evaluation of potential protective thresholds among serotypes. Our finding that the potential protective thresholds for serotypes Ia and III are similar is consistent with findings in other studies that examined protective thresholds for these two major serotypes, including in a population similar to that in the current study.[12,26] We attempted to derive a risk–concentration curve that may be applicable to all serotypes of group B streptococcus. The biologic plausibility for the notion that equivalent functional activity is achieved by similar unit amounts of anti-CPS antibodies may be underpinned by the common role provided by the group B streptococcus CPS for evasion of the alternative complement pathway and the likely uniform mechanism of protection of anti-CPS antibodies.[19] The application of a single set of protective concentrations across serotypes has precedent. A single anti-CPS IgG concentration adequately describes the protective threshold against invasive *Streptococcus pneumoniae* disease imparted by pneumococcal glycoconjugate vaccines with few exceptions.[27] A single protective threshold, however, may not capture protective capacity because concentrations above and below the threshold are not given appropriate weight with respect to risk reduction.

The risk–concentration curve that was established in our seroepidemiologic study is from a low- and middle-income region with a high incidence of disease. Effectiveness studies for pneumococcal vaccines have shown that estimates derived from low- and middle-income regions can overestimate the protective thresholds for invasive pneumococcal disease in high-income countries.[28] The protective antibody concentrations that were derived from this study warrant correlative data with respect to high-income countries to better define proper thresholds.[29] Concurrent infections during pregnancy, such as malaria or human immunodeficiency virus infection, can additionally affect placental integrity and antibody transfer.[29] Consequently, levels of transferred anti-CPS antibodies may be different in this context. This possibility should be further studied.

GBS6 elicited anti-CPS antibodies against group B streptococcus in pregnant women that were transferred to infants at levels associated with a reduced risk of invasive group B streptococcal disease. The use of seroepidemiologic studies to identify quantitative immune correlates for invasive bacterial pathogens has in many cases correctly identified both the protective nature of the target vaccine antigen and the associated protective antibody level.[30-34] The potential protective concentrations that are reported herein are based on a single seroepidemiologic study. Future studies are needed to better define these immunologic relationships.

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APPENDIX

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