GBS carriage in pregnant women

Part 2 of the Netherlands observational study on group B streptococcal disease, bacterial virulence and protective serology

(NO GBS)

(June 2019)

- Version 1.4

PROTOCOL TITLE 'GBS carriage in pregnant women in the Netherlands. Part 2 of the Netherlands observational study on group B streptococcal disease, bacterial virulence and protective serology (NO GBS)'

Protocol ID	NO GBS (NL-63124.018.17)
Short title	GBS carriage in Dutch pregnant women
EudraCT number	Not Applicable
Version	1.4
Date	June 2019
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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
EOD	**Early Onset Disease**
GCP	**Good Clinical Practice**
GBS	**Group B Streptococcus**
IC	**Informed Consent**
LOD	**Late Onset Disease**
METC	**Medical research ethics committee (MREC); in Dutch: Medisch Ethische Toetsing Commissie (METC)**
NRLBM	**Netherlands Reference Laboratory for Bacterial Meningitis**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**
WGS	**Whole Genome Sequencing**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale:

Streptococcus agalactiae (Group B Streptococcus, GBS) and *Escherichia coli* are the leading cause of neonatal sepsis and meningitis.[1] One out of five pregnant women is asymptomatically colonized by GBS.[2] Transmission of GBS bacteria to the neonate can result in invasive disease, which has been associated with a case fatality rate of 7%.[3]

Dutch GBS prevention guidelines recommend intrapartum antibiotic prophylaxis for pregnant women with risk factors for GBS disease. We have shown that the incidence of neonatal GBS disease is increasing, despite guideline implementation in 1999.[1] In addition, current guidelines recommend bacterial prophylaxis and treatment for mothers and their children based on a risk-calculation. With this strategy a relatively large group of children is exposed to antibiotics. Another shortcoming of these guidelines is the focus on early onset disease. Late onset disease occurring after 7 days of age is an important problem. The incidence of late onset disease has not changed in the western world over the past decades. Improved risk assessment, a better understanding of GBS pathophysiology and new prevention strategies are needed.

An important future option to reduce invasive disease in neonates is GBS vaccination of mothers during pregnancy. GBS vaccines were shown to be safe and immunogenic in pregnant woman. However, further evaluation of these vaccines is hampered because of the high costs of a phase 3 RCT with clinical endpoints. Therefore, immune correlates of protection are needed to evaluate potential effectiveness of these vaccines.

In this observational cohort study we will determine the prevalence and genetic profile of colonizing GBS isolates in pregnant women in the Netherlands. We will collect serum from pregnant women and their newborns to determine specific IgG concentrations and functionality against vaccine targets that protect against GBS colonization.
These results will be combined with results from the other parts of the "*Netherlands observational study on group b streptococcal disease, bacterial virulence and protective serology (NO GBS)*" to discover GBS bacterial virulence genes and determine specific antibody concentrations that protect neonates against invasive GBS disease.

Objective:
The primary objectives of the *NO GBS* study part 2 are to determine the prevalence and genetic profile of colonizing GBS bacteria, and to determine IgG antibody concentrations and functionality against GBS vaccine targets in Dutch pregnant woman that are associated with protection against GBS colonization.
The secondary objectives are to determine genetic determinants of GBS for invasive disease, and to determine immunological parameters associated with protection against invasive GBS disease.

Study design: We will conduct a prospective, observational, multi-center cohort study on GBS carriage in Dutch pregnant women. We will collect the medical correspondence about the obstetric history and outcome of the current pregnancy, GBS isolates, and serum from mothers and their newborns.

Study population: All pregnant women who are expecting to give birth in a participating hospital in the Netherlands are eligible for this study.

Main study parameters/endpoints:
- Prevalence of GBS colonization in pregnant women in the Netherlands
- Genetic profile of colonizing GBS isolates
- Specific IgG concentrations and functionality against vaccine targets in pregnant women and their newborns and its association with concurrent GBS colonization.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:
In this observational study patients will be treated according to national and local guidelines. We will ask for blood from the mother to be collected on admission at delivery. Blood from the newborn will be collected from the umbilical cord from the placenta after the cord is cut at delivery. Maternal GBS colonization cultures will be obtained concurrently to blood collection. GBS culture results and GBS isolates obtained in routine obstetric care will be collected. Left-over serum and blood spots from routine neonatal and maternal screening programs will be collected. Screening for GBS colonization is part of standard obstetric care in many developed countries. In the Netherlands only women with identified risk factors for disease transmission are screened.
Study participants will be screened for GBS colonization when they are admitted for the delivery. If GBS colonization is detected, we will inform the midwife or gynecologist responsible for her obstetric care. Dutch guidelines recommend discussing the possibility of antibiotic prophylaxis during delivery for pregnant women with GBS colonization without

other risk factors and screening in a subsequent pregnancy.[4]

1. INTRODUCTION AND RATIONALE

Streptococcus agalactiae or group B *Streptococcus* (GBS) and *Escherichia coli* are the most common causes of neonatal infections.[5] GBS colonizes the human genital and gastro-intestinal tracts, which usually results in asymptomatic carriage. One in five pregnant women is a GBS carrier.[2] GBS transmission to a susceptible newborn can result in devastating disease, with a case fatality rate of 7%.[3] Early-onset disease, occurring in the first week of life, results from aspiration of amniotic fluid infected with bacteria that have ascended from the colonised maternal genital tract.[6-8] Transmission of GBS in late-onset disease (day 8 to 90 after birth) and in *E. coli* disease is poorly understood.

Many countries have implemented GBS prevention programmes with antibiotic prophylaxis at time of delivery. Two major strategies have been adopted; GBS screening and intrapartum antibiotic prophylaxis for women with risk factors for perinatal disease, antibiotic prophylaxis for all GBS carriers identified through screening of all pregnant women. Universal GBS screening in the United States resulted in a modest further decline of early-onset GBS cases, but no effect was found on the occurrence of late-onset GBS and *E. coli* disease.[9-11] Universal screening results in antibiotic treatment for up to a third of healthy pregnant women and their newborns.[12,13]

The impact of prevention guidelines are limited because neonates with GBS disease are frequently born to mothers who tested negative for GBS during pregnancy and who have no other identified risk factors.[13] Suboptimal adherence to guideline recommendations might be another limiting factor.[13] In the Netherlands, GBS prevention guidelines recommend intrapartum antibiotic prophylaxis for pregnant women with obstetric risk factors for GBS disease; GBS carriage with prelabor rupture of membranes or preterm labor, intrapartum fever, GBS bacteriuria or a previous child with GBS disease.[4,14] We have previously shown that the incidence of neonatal GBS disease is *increasing*, despite guideline implementation in 1999.[1]

Improved understanding of GBS and *E.coli* transmission and invasion, and new prevention strategies are necessary. Specific genetic GBS lineages have disproportionately high invasion rates. These hyperinvasive lineages have virulence factors that enhance

penetration of epithelial or blood–brain barrier, and resistance to immune clearance.[6-8] We have shown that the increase in the occurrence of GBS disease was associated with one emerging bacterial genotype (ST-17)[1], whole genome sequencing (GWAS) of carrier and invasive isolates might identify bacterial virulence factors, elucidating GBS pathophysiology.

Differences in host susceptibility are another important determinant of the host-pathogen interaction. GBS disease occurring in otherwise healthy infants could reflect an immunodeficiency caused by rare genetic defects.[15] Protective IgG antibodies are actively transported over the placenta and can circulate in the newborn's bloodstream, reducing susceptibility to invasive disease.[16]

Vaccination against GBS during pregnancy may reduce invasive disease in neonates.[17] GBS vaccines were shown to be safe and immunogenic in pregnant woman in phase 1 and 2 randomized controlled trials (RCTs). A phase 3 RCT using invasive disease as outcome is thought to be unfeasible because of high costs of such a study. Immune correlates of protection are needed to evaluate potential effectiveness of these GBS vaccines.[17]

In this observational cohort study we will determine the prevalence and genetic profile of colonizing GBS isolates and determine IgG concentrations and functionality against vaccine targets in pregnant women and their newborns that protect against colonization. These results will be combined with the findings from the other parts of the *NO GBS* study to discover new GBS virulence genes and establish immune correlates of protection against neonatal GBS disease.

The *GBS carriage study in pregnant women* is part of the *Netherlands observational study on group B streptococcal disease, bacterial virulence and protective serology (NO GBS)* study.

The *NO GBS* study has three complementary parts:
Part 1: Prospective multicentre observational cohort study on perinatal bacterial infections.
Part 2: GBS carriage study in pregnant women.
Part 3: Surveillance study on bacterial genetic epidemiology and virulence.

Key objectives of the NO GBS study are to:
1. study GBS disease outcome, bacterial genetics and human serology;
2. determine the potential long-term coverage of all GBS vaccines that have been tested in phase 1 or phase 2 studies;

3. develop a methodology to measure IgG antibody concentrations and functionality against bacterial antigens in dried blood spots obtained in routine perinatal care;
4. establish immune correlates of protection against maternal colonization and neonatal invasion by GBS;
5. discover bacterial virulence factors associated with invasive disease using whole genome sequencing.

2. OBJECTIVES

The primary objectives of the *NO GBS* study part 2 are to:

- determine the prevalence and genetic profile of colonizing GBS isolates in pregnant women in the Netherlands;
- determine specific IgG antibody concentrations and functionality against vaccine targets in pregnant women (serum) and their newborns (cord blood and/or blood spots from routine neonatal screening) that are correlated with concurrent GBS colonization.

The secondary objectives are to:

- discover genetic GBS virulence factors for invasive disease;
- develop a methodology to measure IgG antibody concentrations and functionality against bacterial antigens in dried blood spots;
- determine antibody concentrations and functionality against GBS vaccine targets that are correlated with protection against invasive GBS disease.

To accomplish these secondary objectives, the results will be combined with the findings from the other parts of the *NO GBS* study.

3. STUDY DESIGN

We will conduct a prospective, observational, multi-centre cohort study on GBS carriage in Dutch pregnant women. Informed consent will be asked by a treating physician or midwife at routine control in outpatient clinic or on admission to the hospital for delivery. We will collect clinical data on the obstetric history and outcome of the current pregnancy. A blood sample from the mother will be obtained at delivery in a participating hospital. Blood from the newborn will be collected from the umbilical cord from the placenta after the cord is cut at delivery, we also collect left-over material from blood from the umbilical cord collected for routine care (e.g. material from syringe for blood gas analysis). We will ask permission to collect left-over material (blood spots) from routine neonatal screening.

4. STUDY POPULATION

4.1 Population (base)

All pregnant women that are planning to deliver in a participating hospital in the Netherlands are eligible for this study.

4.2 Inclusion criteria

- Pregnant women who have obstetric care and are expected to deliver in a participating hospital
    - In case the newborn of the included pregnant women develops culture positive invasive GBS disease in the first 90 days of life, the serological data and bacterial sequencing data will also be included as a case in the NO GBS study part one and part three.

4.3 Exclusion criteria

- Oral or intravenous antibiotic treatment in the month prior to the first GBS colonization culture

4.4 Sample size calculation

Primary outcomes

Number of inclusions

We aim to include 300 GBS carriers and 300 non-carriers and their newborns. With an estimated GBS carriage rate of 20%, an estimated 1500 women will have to be included.[19]

Number of participating centres

Based on data from a single medical centre with1400-1500 clinical deliveries per year, approximately 280-300 inclusions will have positive rectovaginal cultures for GBS. With an inclusion rate of 20% we would need around 5 medical centres to include 300 GBS carriers in one year.

Prevalence of GBS carriage

With a sample size of 1500 women, the carriage prevalence can be determined with a 95% confidence interval of 4% using the Wilson procedure for determining the confidence interval of a proportion with a correction for continuity.

Secondary outcomes

Bacterial virulence genes

To increase power for the GWAS study, 1000 colonizing GBS isolates and 1300 invasive GBS isolates obtained in part 3 of the NO GBS study (see *NO GBS* study part 3) will be added to the 300 colonizing isolates obtained in this part of the NO GBS study.
Based on simulation experiments, and an estimated minor allele frequency of 0.2, odds ratios of 1.6 or higher for genetic variants predisposing for invasion could be identified with 80% power using a conservative correction for multiple testing ($p=1x10^{-8}$).

Antibody concentrations correlated to invasive disease

We will construct empirical reverse cumulative distributions of specific IgG concentrations against vaccine targets in serum and blood spots of 300 pregnant GBS carriers and their newborns. Results will be compared to IgG concentrations in patients aged 0-3 months of age and their mothers, included in part 1 of the NO GBS study. Based on previous findings[20], a geometric mean IgG concentration difference of 0.460 µg/ml can be identified with 50 GBS cases and 150 controls (with 80% power and a two-sided significance value of 0.05).

5. METHODS

5.1 Study parameters/endpoints

5.1.1 Main study parameter/endpoint

- Prevalence of GBS carriage in pregnant women in the Netherlands
- Whole genome sequencing of colonizing GBS isolates with Illumina HiSeq at the Wellcome Trust Sanger Institute
- Specific IgG concentrations by enzyme-linked immunosorbent assay (ELISA) and functionality against GBS vaccine targets in maternal serum from pregnant women colonized with GBS and non-carriers, and cord blood from their newborns.

5.1.2 Secondary study parameters/endpoints

- Comparison of specific IgG distributions and functionality against vaccine targets at delivery in pregnant women colonized with GBS and mothers of patients with invasive GBS disease
- Comparison of specific IgG distributions and functionality against vaccine targets in newborns from pregnant women colonized with GBS (blood spots and cord blood) and patients with invasive GBS disease (blood spots and serum)
- Genome wide association study comparing invasive to colonizing GBS isolates

5.2 Study procedures

5.2.1 Inclusion procedures

Pregnant women will be approached by the treating physician or midwife when they have a routine visit to the outpatient clinic before admission for delivery. The women will receive written information and will be asked to give written informed consent for participation before or on admission for delivery.

5.2.2 Data collection

We will ask consent to collect the medical correspondence about the obstetric history, outcome of the current pregnancy, and any hospital stay of the newborn in the first three months of life. We will collect blood samples and GBS isolates. Placental cord blood will be obtained from the newborn directly after birth. All other diagnostic procedures and treatment of study participants will be based on current obstetric guidelines and are not affected by participation in this study.

5.2.3 Collection and storage of patient specimens

Blood

Mothers will be asked to give 10ml of blood for immunological studies after inclusion on the day of delivery. Blood (1-5 ml in vacutainer and 200 ul on blood spot) from the newborn will be collected from the umbilical cord from the placenta after the cord is cut. We also collect left-over material from blood from the umbilical cord collected for routine care (e.g. material from syringe for blood gas analysis) if available. The child will not be subjected to dermal or vena punctures for this study. Blood will be processed and stored by local laboratory protocols of the participating hospitals. Researchers from the AMC will regularly visit the laboratories and transport the samples to the AMC.

We will also ask permission to collect left-over material from routine neonatal screening (heel prick test).

Bacterial isolates

GBS colonization cultures of the participating pregnant woman will be collected on admission for delivery . Swabs from the lower vagina and rectum will be obtained by the treating physician, midwife or the woman herself. Self-collection of swabs has been shown to be feasible and reliable. [21] Swabs obtained in the hospital will be collected, processed and stored according to local protocols.

The Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) is a collaboration of the Academic Medical Center and the National Institute of Public Health and the Environment. All clinical microbiology laboratories in the Netherlands collaborate by sending bacterial isolates from patients with meningitis and blood isolates from specific bacterial strains. We will ask participating hospitals to also send colonizing GBS isolates of included patients for this study.

Bacterial isolates will be cultured and stored according to existing NRLBM protocols.

Storage of data and samples

All patient samples and data will be stored in the MeninGene biobank, which is located in the AMC, Amsterdam. Bacterial isolates will be stored in the Netherlands Reference Laboratory for Bacterial Meningitis. All patient samples and data will be stored in the biobank and will only be used for research on bacterial meningitis and

sepsis. As research on epidemiology, pathophysiology and changes in management requires large number of patients and samples, all samples will be stored for a period of 50 years. Further information is available in the attachment "K6 Biobankreglement MeninGene versie 1.1 d.d. 24-08-2017".

5.3 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

5.4 Premature termination of the study

We will terminate the carriage cohort if funding is terminated or no new funding is found.

6. SAFETY REPORTING

Not applicable

7. STATISTICAL ANALYSIS

7.1 Primary study parameters

Overall prevalence of carriage will be presented as proportion with 95% confidence interval. The Fisher exact test will be used for comparison of cases and controls by demographic characteristics and obstetrical factors.

Specific IgG concentrations against vaccine targets in GBS patients and their mothers will be presented as median and interquartile range and geometric mean concentration and as empirical reverse cumulative distributions.

We will compare serotype specific (log-transformed) IgG concentrations and functionality in women colonized by a particular serotype to women colonized by a different serotype and to non-colonized women. The Mann–Whitney U test or 2-sample Student's t test will be used to compare the geometric means (GMs) of antibody levels depending on the assumption of normal distribution after log-transformation.

7.2 Secondary study parameters

New virulence factors for invasive disease will be identified by hypothesis-free association analyses, comparing single nucleotide variations or gene presence/absence in GBS isolates cultured from asymptomatic carriers with isolates that caused neonatal invasive disease. We will correct the significance level of $\alpha = 0.05$ for multiple testing with a Bonferroni based on the number of genes and SNP's included in the analysis.

Specific IgG concentrations against vaccine targets in GBS patients and their mothers will be presented as median and interquartile range and geometric mean concentration and as empirical reverse cumulative distributions. Cases will be compared to controls matched for maternal carriage status. The Mann–Whitney U test or 2-sample Student's t test will be used to compare the geometric means (GMs) of antibody levels depending on the assumption of normal distribution after log-transformation. We will use conditional logistic regression analysis to correct for established risk factors for invasive disease.[25] We will estimate risk of neonatal GBS disease by concentration of maternal IgG as previously described.[20,26-28]

8. ETHICAL CONSIDERATIONS

8.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (version of 2013, Fortaleza, Brazil) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts. Data management, monitoring and reporting of the study will be performed in accordance with the ICH GCP guidelines. Technicians and data managers of the AMC Clinic Research Unit (CRU) will perform central data management. Internet based remote data capture will be used for entering, managing and validating data from the investigative sites.

8.2 Recruitment and consent

When a pregnant woman fulfils the required inclusion criteria the treating doctor or midwife will inform her about the study in detail and will ask her, or if applicable her representatives, for written informed consent, in accordance with the guidelines of the local medical ethics committee.

8.3 Objection by minors or incapacitated subjects

The code of conduct in case of objection of minors who participate in non-therapeutic research is applicable to this study.

8.4 Benefits and risks assessment, group relatedness

Screening for GBS colonization is part of standard obstetric care in many developed countries. In the Netherlands only women with identified risk factors for disease transmission are screened. Study participants will be screened for GBS colonization. If GBS colonization is detected, we will inform the midwife or gynaecologist responsible for her obstetric care. Dutch guidelines recommend discussing the possibility of antibiotic prophylaxis during delivery for pregnant women with GBS colonization without other risk factors and GBS screening in a subsequent pregnancy.[4] A possible benefit is the option to receive antibiotic prophylaxis during delivery for women who are colonized with GBS and would not have been tested in routine obstetric care, thereby reducing the chance of early onset neonatal bacterial infection.

The risks of the study are limited to those of a venous blood withdrawal in mothers and increased chance of receiving antibiotic prophylaxis, which are minor, and risks of cord blood and colonizing culture sampling which are negligible. This study aims to study GBS colonization in pregnancy and the neonatal period. Therefore this study is only possible in this particular population.

8.5 Compensation for injury

This study is exempt from insurance obligations as there are no significant risks attributable to participation to this study.

8.6 Incentives (if applicable)

Included patients will not receive any special incentives, compensation or treatment through participation in this study.

9. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

9.1 Handling and storage of data and documents

The study will be conducted according to the principles of the Declaration of Helsinki (version of 2013, Fortaleza, Brazil) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts. Data management, monitoring and reporting of the study will be performed in accordance with the ICH GCP guidelines. Technicians and data managers of the AMC Clinic Research Unit (CRU) will perform central data management. Internet based remote data capture will be used for entering, managing and validating data from the investigative sites. When the study is finished, all essential documents (Case Record Forms, Informed Consent

forms, patient files) will be archived and stored for the next 50 years, in accordance to GCP guidelines.

Data will be coded and data in the analysis file will not be traceable to a patient. This will be assured by the following steps:
When patients are included each patient will be assigned a patient ID. The subject identification code list will connect patient ID and data which could lead to the patient, such as birth-date and patient number assigned by the hospital. Only Prof. Dr. D. van de Beek, Dr. M.C. Brouwer, Dr. M.W. Bijlsma and Dr. V. Bekker and PhD Drs. M van Kassel will have access to this list. The data analysis file will be a pseudonymized limited dataset. There will be no name, date of birth (age in years only), hospital, etc in the data analysis file or biobank.
Variables in the pseudonymised limited dataset will be coded.

9.2 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

9.3 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

9.4 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. We will end the study or amend the METC protocol five years after the first patient is included. The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

9.5 Public disclosure and publication policy

The coordinating investigators will have the responsibility for decisions regarding publication of data for scientific purposes.

There are no arrangements with the sponsor that jeopardize the publication of the data

10. REFERENCES

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