Prospective multicentre observational cohort study on perinatal bacterial infections

Part 1 of the Netherlands observational study on group B streptococcal disease, bacterial virulence and protective serology

(NO GBS)

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PROTOCOL TITLE 'Prospective multicentre observational cohort study on perinatal bacterial infections. Part 1 of the Netherlands observational study on group B streptococcal disease, bacterial virulence and protective serology (NO GBS)'

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Pharmacy	Not applicable

PROTOCOL SIGNATURE SHEET

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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)**
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] GBS meningitis is associated with neurological sequelae. A recent meta-analysis showed that one in threes survivors had neurodevelopmental impairment at 18 months of follow-up.[4] Long-term follow up outcome data is lacking.[4]

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 sensitivity of Dutch risk-based prevention guidelines to identify cases of invasive disease caused by GBS or *E. coli* in 0-3 months old patients. Furthermore, we will collect invasive bacterial isolates and blood from patients and their mothers to perform whole genome sequencing of invasive GBS isolates and determine empirical reverse cumulative distributions of specific IgG concentrations against vaccine targets in GBS patients and their mothers. 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 objective of the *NO GBS* study part 1 is to determine clinical characteristics, the prevalence of risk factors recommended for screening by national guidelines, and outcome of GBS and *E. coli* meningitis in patients aged 0-3 months in the Netherlands.
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-centre cohort study on GBS and *E. coli* meningitis and sepsis in patients 0-3 months old. We will collect detailed clinical data, invasive bacterial isolates, and blood and non-invasive bacterial cultures from patients and their mothers.

Study population: All patients 0-3 months of age with blood or cerebrospinal fluid culture confirmed invasive GBS or *E. coli* disease in the Netherlands and their mothers are eligible for this study.

Main study parameters/endpoints:
- Presence of risk factors recommended for screening by current and updated Dutch GBS prevention guidelines in cases of neonatal GBS disease
- Genetic profiles of invasive GBS isolates
- Specific IgG concentrations against vaccine targets in GBS patients and their mothers

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Patients will be treated according to national and local guidelines. We will ask for a maximum of 2 ml extra blood to be collected if the patient has blood tests ordered by the treating physician in routine medical care. If cerebrospinal fluid/blood was obtained at diagnosis surplus material will be collected from the laboratory. Based on previous experience, we expect to be able to collect material in the majority of patients in this manner. This amount will not lead to hemodynamic changes or anaemia. 10 ml of blood will be drawn from the mothers of included patients. Non-invasive bacterial cultures will be obtained from the patient and mother. Participants will have no direct benefit by participating in this study but will contribute to the prevention of future cases of GBS disease in neonates.

It is not possible to answer these research questions in other patient groups because perinatal invasive GBS disease is specific for this age group.

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] GBS meningitis is associated with neurological sequelae, data from a recent meta-analysis showed that 32% (95% CI, 25%–38%) had neurodevelopmental impairment at 18 months of follow-up, including 18% (95% CI, 13%–22%) with moderate to severe neurodevelopmental impairment.[4] Long-term follow up data is lacking. 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.[14,15] 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.[16] Protective IgG antibodies are actively transported over the placenta and can circulate in the newborn's bloodstream, reducing susceptibility to invasive disease.[17]

Vaccination against GBS during pregnancy may reduce invasive disease in neonates.[18] 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.[18]

In this observational cohort study we will determine clinical characteristics and outcome of invasive GBS disease, the sensitivity of Dutch risk-based guidelines to detect perinatal bacterial infection in patients 0-3 months old with culture proven GBS or *E. coli* invasive disease. Furthermore, we will collect invasive bacterial isolates and blood from the patients and their mothers. We will perform whole genome sequencing of invasive GBS isolates and determine empirical reverse cumulative distributions of specific IgG concentrations against vaccine targets in GBS patients and their mothers. 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 *Prospective multicentre observational cohort study on perinatal bacterial infections* 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 1 are to:

- determine the clinical characteristics and outcome, and the prevalence of risk factors used by Dutch guidelines for risk assessment in GBS and *E. coli* meningitis and sepsis cases aged 0-3 months in the Netherlands.
- determine the genetic profile of invasive GBS isolates by whole genome sequencing

The secondary objectives are to:

- develop a methodology to measure antibody concentrations against bacterial antigens in dried blood spots;
- determine antibody concentrations 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 and *E. coli* meningitis and sepsis in patients 0-3 months old. Cases will be identified through the Netherlands Reference Laboratory for Bacterial Meningitis or reported by treating physicians. We will collect detailed clinical data, invasive bacterial isolates, and blood from patients, and from patients, and blood non-invasive bacterial cultures, breastmilk and urine samples from mothers. We will ask permission to collect left-over material from routine pregnancy screening (prenatal screening infectious diseases and erythrocyte immunisation) and routine neonatal screening (heel prick test). We will ask permission to contact parents for follow-up studies (e.g. long-term outcome).

4. STUDY POPULATION

4.1 Population (base)

All new-borns 0-3 months of age in the Netherlands with blood or cerebrospinal fluid culture confirmed invasive GBS or *E. coli* disease and their mothers are eligible for this study. Hospitals throughout the Netherlands will be approached for participation following approval of the AMC Medical Ethical Committee.

4.2 Inclusion criteria

- Patient aged 0-90 days
- GBS or *E. coli* cultured from blood or cerebrospinal fluid
- Admitted to participating hospital
- Legal representative(s) able and willing to give informed consent

4.3 Exclusion criteria

- Neurosurgical device such as cerebrospinal fluid drain in situ prior to development of meningitis

4.4 Sample size calculation

Primary outcomes

Number of inclusions

Based on a conservative estimate of 0.2 culture positive early-onset, 0.1 late-onset GBS, and 0.1 *E. coli* cases per 1000 live births[1], 170 500 live births per year (CBS) and an estimated notification rate of 85% to the NRLBM [19] we expect to include at least 30 early-onset GBS cases, 15 late-onset GBS cases and 15 *E. coli* and cases per year.

Risk factors for perinatal bacterial invasive disease

Obstetric risk factors for perinatal GBS disease are present in an estimated 50% of early-onset GBS cases.[3,20] With 150 cases of early-onset GBS, 75 cases of late-onset GBS and 75 cases of *E. coli* disease, an absolute difference of approximately 20% in the proportion of risk factors between early-onset and late-onset GBS disease, and a difference of approximately 18% in the proportion of risk factors between between *E. coli* and all GBS cases with a two-tailed Fisher Exact test at a significance level of $p = 0.05$.

Secondary outcomes

Antibody concentrations correlated to invasive disease

We will construct empirical reverse cumulative distributions of specific IgG concentrations against vaccine targets in blood and blood spots of included GBS patients and blood of their mothers. Results will be compared to IgG concentrations in a control group of pregnant GBS colonized woman and their newborns included in part 2 of the NO GBS study. Based on previous findings[21], a geometric mean IgG concentration difference of 0.460 times the standard deviation on the logarithmic scale 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

- Clinical characteristics and outcome parameters
- Proportion of cases with risk factors recommended for screening by current and updated Dutch GBS prevention guidelines
- Whole genome sequencing of invasive GBS isolates with Illumina HiSeq at the Wellcome Trust Sanger Institute

5.1.2 Secondary study parameters/endpoints

- Comparison of reverse cumulative distributions of specific IgG concentrations determined by enzyme-linked immunosorbent assay (ELISA) against vaccine targets in pregnant women colonized with GBS and mothers of patients with invasive GBS disease
- Comparison of reverse cumulative distributions of specific IgG concentrations determined by ELISA 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 blood)

5.2 Study procedures

5.2.1 Inclusion procedures

Patients can be included by two procedures:

1. The Netherlands Reference Laboratory for Bacterial Meningitis will provide daily updates of names of hospitals where patients with bacterial meningitis have been admitted in the preceding two to four days. The Reference laboratory receives

cerebrospinal fluid (CSF) and blood isolates from approximately 85% of all patients with bacterial meningitis in the Netherlands. Physicians from the department where the culture was obtained are contacted by the researchers by telephone and are informed about the study. Subsequently, patients or their legal representatives and controls receive written information and are asked to give written informed consent for participation.

2. A 24-hour telephone service for participating physicians is provided. Physicians can consult one of our meningitis researchers 24/7 to include a patient. Subsequently, patients or their legal representatives and controls receive written information and are asked for written informed consent to participate.

5.2.2 Data collection and outcome score

Online case-record forms will be used to collect data on patients' history, symptoms and signs on admission, laboratory findings on admission, treatment (including adjunctive treatment), clinical course, outcome and findings at discharge. Severity and clinical deterioration will be evaluated. Data on complications will be collected according to predefined criteria. Results of neuroimaging will be collected.

5.2.3 Collection and storage of patient specimens

Collection of blood

Mothers will be asked to give 10ml of serum blood for immunological studies. Additional blood (maximum 2 ml) will be collected from the child, only if blood is drawn for routine clinical care. The child will not be subjected to additional dermal or vena punctures for this study. In general, most children that are admitted to hospital for treatment of an invasive perinatal infection will have blood drawn for monitoring of the success of therapy. Leftover patient blood from patient care will be collected if available. Blood will be processed and stored by local laboratory protocols of the participating hospitals. Researchers from the AMC will regularly visit the laboratories in which CSF is stored and transport the samples to the AMC.

We will also ask permission to collect left-over material from routine pregnancy screening (prenatal screening infectious diseases and erythrocyte immunisation) and routine neonatal screening (heel prick test).

If additional permission has been granted to share patient data and material with other research groups for new scientific research into bacterial meningitis, we will collect a saliva sample for genetic analysis if no blood was collected. Obtaining a saliva sample is a minimal burden and has negligible risk. DNA analysis will be performed anonymized.

Collection of invasive bacterial isolates

The Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) is a collaboration of the Academic Medical Centre 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. For children under the age of 1 year in addition to liquor also all positive blood cultures are sent to the NRLBM. Bacterial isolates will be stored according to existing NRLBM protocols. We will attempt to recover the maternal colonizing recto-vaginal GBS isolate if obtained in routine obstetric care.

Additional cultures for study purposes

Non-invasive culture swabs to determine GBS carrier status in mothers will be obtained. We will collect a breast milk sample and urine sample, and a swab of the lower vagina and rectum from mothers. Recto-vaginal swabs will be self-collected.[22,23] Local laboratory protocols with this purpose have been implemented in each hospital. Researchers from the AMC will visit the laboratories regularly and transport the samples to the AMC in dry ice, after which it will be stored until the analysis.

Collection of cerebrospinal fluid (CSF)

Leftover CSF from the diagnostic puncture will be collected if available and will be locally stored for study purposes. Local laboratory protocols with this purpose have been implemented in each hospital. Researchers from the AMC will regularly visit these local and transport the samples to the AMC on dry ice, after which it will be stored until the analysis.

Storage of data and samples in MeninGene biobank

All patient samples and data will be stored in the MeninGene biobank, which is located in the AMC, Amsterdam, the Netherlands. All patient samples and data will be stored in this 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 Biobank versie 1.1 d.d. 24-08-2017”.

Permission to use material for further research

We will ask additional permission to share the collected data and patient material included in the MenenGene Biobank with other research groups for new scientific studies into bacterial meningitis with a scientifically sound research proposal that has been approved by a medical ethics committee. If DNA sequencing is performed in one of these additional studies, participants will not be informed on their own genetic code. Participating in these new studies will have no impact on the patient or family members.

Permission for long-term follow up

We will ask additional permission to contact the patients or their parents to ask new permission for follow up studies (e.g. long-term outcome).

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 prospective cohort if: Funding is terminated or no new funding is found.

6. SAFETY REPORTING

Not applicable

7. STATISTICAL ANALYSIS

7.1 Primary study parameter(s)

Data on clinical presentation, laboratory results, treatment and outcome will be presented with descriptive statistics. Differences between early-onset GBS, late-onset GBS and set GBS and *E. coli* disease in the proportion of cases with guideline risk factors or family members that carry the invasive bacterial strain will be tested with the Fisher Exact test at a significance level of 0.05.

7.2 Secondary study parameters

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.[24] We will estimate risk of neonatal GBS disease by concentration of maternal IgG as previously described.[21,25-27]

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 patients fulfil the required inclusion criteria the treating doctor will inform parents about the study. They will ask for permission from parents to be contacted by the study group. The treating physician or the study group will inform parents in detail and will ask parents, or if applicable other patients' 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

Patients do not directly benefit from this study. The risks of the study are limited to those of a venous blood withdrawal and non-invasive bacterial culture swabs in mothers which are minor, and risks of an extra blood volume collection of 2 ml , which are negligible. This study aims to look at infections that only occur in new-borns specifically. Risk factors and disease course and outcome are specific to this age-group. Therefore this study is only possible in this particular population. Improved prevention measures as a result of this study may be beneficial to prevent future episode of sepsis and meningitis in newborns.

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 data analysis file will not be traceable to a patient. This will be assured by the following steps:
When patients are included culture samples will be taken and a Case Report Form will be filled out. 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, Dr. V. Bekker and Drs. M.N. 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 days/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

1. Bekker V, Bijlsma MW, van de Beek D, Kuijpers TW, van der Ende A. Incidence of invasive group B streptococcal disease and pathogen genotype distribution in newborn babies in the Netherlands over 25 years: a nationwide surveillance study. The Lancet Infectious diseases 2014;14:1083-9.
2. Kwatra G, Cunnington MC, Merrall E, et al. Prevalence of maternal colonisation with group B streptococcus: a systematic review and meta-analysis. The Lancet Infectious diseases 2016;16:1076-84.
3. Trijbels-Smeulders M, de Jonge GA, Pasker-de Jong PC, et al. Epidemiology of neonatal group B streptococcal disease in the Netherlands before and after introduction of guidelines for prevention. Archives of disease in childhood Fetal and neonatal edition 2007;92:F271-6.
4. Kohli-Lynch M, Russell NJ, Seale AC, et al. Neurodevelopmental Impairment in Children After Group B Streptococcal Disease Worldwide: Systematic Review and Meta-analyses. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2017;65:S190-S9.
5. Stoll BJ, Hansen NI, Sanchez PJ, et al. Early onset neonatal sepsis: the burden of group B Streptococcal and E. coli disease continues. Pediatrics 2011;127:817-26.
6. Landwehr-Kenzel S, Henneke P. Interaction of Streptococcus agalactiae and Cellular Innate Immunity in Colonization and Disease. Front Immunol 2014;5:519.
7. Maisey HC, Doran KS, Nizet V. Recent advances in understanding the molecular basis of group B Streptococcus virulence. Expert reviews in molecular medicine 2008;10:e27.
8. Tazi A, Bellais S, Tardieux I, Dramsi S, Trieu-Cuot P, Poyart C. Group B Streptococcus surface proteins as major determinants for meningeal tropism. Curr Opin Microbiol 2012;15:44-9.
9. Ohlsson A, Shah VS. Intrapartum antibiotics for known maternal Group B streptococcal colonization. The Cochrane database of systematic reviews 2014;6:CD007467.
10. Schrag SJ, Zywicki S, Farley MM, et al. Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. N Engl J Med 2000;342:15-20.
11. Thigpen MC, Whitney CG, Messonnier NE, et al. Bacterial meningitis in the United States, 1998-2007. N Engl J Med 2011;364:2016-25.
12. Glasgow TS, Young PC, Wallin J, et al. Association of intrapartum antibiotic exposure and late-onset serious bacterial infections in infants. Pediatrics 2005;116:696-702.
13. Van Dyke MK, Phares CR, Lynfield R, et al. Evaluation of universal antenatal screening for group B streptococcus. N Engl J Med 2009;360:2626-36.
14. gynaecologie. Nvvoe. Prevention of neonatal group-b streptococcal disease in the Netherlands [Preventie van neonatale groep-B-streptokokkenziekte]. version 20 NVOG-richtlijn 2012 2012.
15. NVK N. Preventie en behandeling van early-onset neonatale infecties (Adaptatie van de NICE-richtlijn). 2017.
16. Borghesi A, Stronati M, Fellay J. Neonatal Group B Streptococcal Disease in Otherwise Healthy Infants: Failure of Specific Neonatal Immune Responses. Frontiers in immunology 2017;8:215.
17. Dangor Z, Kwatra G, Izu A, Lala SG, Madhi SA. Review on the association of Group B Streptococcus capsular antibody and protection against invasive disease in infants. Expert Rev Vaccines 2015;14:135-49.

18. Dangor Z, Lala SG, Kwatra G, Madhi SA. Group B Streptococcus: developing a correlate of protection for a vaccine against neonatal infections. Curr Opin Infect Dis 2016;29:262-7.
19. Bijlsma MW, Bekker V, Brouwer MC, Spanjaard L, van de Beek D, van der Ende A. Epidemiology of invasive meningococcal disease in the Netherlands, 1960-2012: an analysis of national surveillance data. The Lancet Infectious diseases 2014;14:805-12.
20. Kuhn P, Dheu C, Bolender C, et al. Incidence and distribution of pathogens in early-onset neonatal sepsis in the era of antenatal antibiotics. Paediatr Perinat Epidemiol 2010;24:479-87.
21. Lin FY, Weisman LE, Azimi PH, et al. Level of maternal IgG anti-group B streptococcus type III antibody correlated with protection of neonates against early-onset disease caused by this pathogen. The Journal of infectious diseases 2004;190:928-34.
22. Price D, Shaw E, Howard M, Zazulak J, Waters H, Kaczorowski J. Self-sampling for group B streptococcus in women 35 to 37 weeks pregnant is accurate and acceptable: a randomized cross-over trial. J Obstet Gynaecol Can 2006;28:1083-8.
23. Arya A, Cryan B, O'Sullivan K, Greene RA, Higgins JR. Self-collected versus health professional-collected genital swabs to identify the prevalence of group B streptococcus: a comparison of patient preference and efficacy. Eur J Obstet Gynecol Reprod Biol 2008;139:43-5.
24. Heath PT, Jardine LA. Neonatal infections: group B streptococcus. BMJ Clin Evid 2014;2014.
25. Baker CJ, Carey VJ, Rench MA, et al. Maternal antibody at delivery protects neonates from early onset group B streptococcal disease. The Journal of infectious diseases 2014;209:781-8.
26. Fabbrini M, Rigat F, Rinaudo CD, et al. The Protective Value of Maternal Group B Streptococcus Antibodies: Quantitative and Functional Analysis of Naturally Acquired Responses to Capsular Polysaccharides and Pilus Proteins in European Maternal Sera. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2016;63:746-53.
27. Dangor Z, Kwatra G, Izu A, et al. Correlates of protection of serotype-specific capsular antibody and invasive Group B Streptococcus disease in South African infants. Vaccine 2015;33:6793-9.