MeninGene Recall study; Follow-up study of the MeninGene study

Principal investigator
Prof. Dr. D. van de Beek, MD, PhD, Department of Neurology, Center of Infection and Immunity Amsterdam (CINIMA), AMC

Study group
Drs. A.T. Kloek, MD, Department of Neurology, Neuroinfections Amsterdam, AMC
Dr. M.C. Brouwer, MD, PhD, Department of Neurology, Neuroinfections Amsterdam, AMC
Prof. Dr. D. van de Beek, MD, PhD, Department of Neurology, Neuroinfections Amsterdam, AMC

Correspondence to:
Prof. Dr. D. van de Beek, MD, PhD, Department of Neurology, H2-217, Academic Medical Center University of Amsterdam, PO Box 22700, 1100 DE Amsterdam, The Netherlands

General Study Characteristics	
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Multicenter research per site	D. van de Beek, MD, PhD (AMC)
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Sponsor	AMC
Independent physician	R.M.A. Bie, MD, PhD R.M.debie@amc.uva.nl, 020-6921912 AMC, Department of Neurology

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SUMMARY

Background

In the MeninGene study, the genetic profile of the bacteria and patients are investigated to assess the influence of genetic variation on disease susceptibility and outcome. The effect of the genetic variation on activation of the immune system during bacterial infection, is however still unknown.

Objectives

- Study the effect of genetic variations associated with susceptibility and outcome of bacterial meningitis patients on the activation of the immune system.
- Determine the long term effects of bacterial meningitis on quality of life, quality of sleep and cognitive functioning
- Study antibody titers and unique expression patterns in B cells of survivors of bacterial meningitis

Study design and population

In this recall study we will invite 150 survivors of bacterial meningitis to come to the AMC for a blood withdrawal and a cognitive assessment. Partners or proxies of the patients will be requested to participate as controls (n=150). All patients have given informed consent for participation in the MeninGene study and granted permission to be approached for new research projects on bacterial meningitis. In the MeninGene study, patient DNA was analyzed for genetic variations that influence susceptibility and severity of disease. In this study effects of those genetic variations on the immune response are studied by stimulation experiments. Furthermore, blood samples will be used to analyze baseline activity of the immune system (cytokine, chemokine, complement and antibody levels) and to isolate B cells to assess the B-cell repertoire. After the blood withdrawal patients will have a neurological examination, fill in questionnaires and perform a neuropsychological assessment.

Nature and extent of the burden

A single blood withdrawal of maximum 100 cc will be performed in each participant. Risks of a blood withdrawal are negligible. Filling in the questionnaires will take less than 30 minutes. The cognitive functioning will be assessed with an online assessment taking one hour.

1. INTRODUCTION AND RATIONALE

Bacterial meningitis is a life-threatening disease.[1] The estimated incidence is 2–5 per 100.000 people per year in developed countries and is up to ten-times higher in resource poor countries.[1-4] The most common causative bacteria is the *Streptococcus pneumonia* in 70% of the cases. [1,2] Despite treatment with antibiotics and dexamethasone the mortality and morbidity is still high. Especially in pneumococcal meningitis the fatality rate is substantial (26%).[1,2]

Understanding host genetic factors by PBMC and whole blood stimulation

Bacterial meningitis is a complex disorder in which injury is caused, in part, by the causative organism and, in part, by the host's own inflammatory response.[5] Both host and bacterial genetic factors influence this process.[6-8] Further characterisation of the interplay between host and bacterial factors is needed to increase our understanding in the pathophysiology of bacterial meningitis.

Genetic variations influencing susceptibility and severity of bacterial meningitis are identified in genes coding for the immune system.[8,10-12] To enhance understanding of the functionality of these genetic variations stimulated peripheral blood mononuclear cells (PBMCs) and whole blood can be used to study the immune response.[9] After stimulation of PBMCs and whole blood with bacteria or pathogen-associated molecular patterns activation of the immune response can be analysed by measuring cytokine production and mRNA expression.[9]

Our hypothesis is that differences in the immune response activation will be found when stimulating PBMCs and whole blood of survivors of bacterial meningitis with different genetic profiles. The activation of the immune system will be compared between individuals with risk genotypes and those with protective genotypes. Also differences in the immune response activation between survivors of bacterial meningitis and healthy controls will be compared. Determination of differences in immune activation will give us more insight in the pathophysiology of bacterial meningitis and will help us to find new targets for prevention or treatment.

Long term effects of bacterial meningitis

In survivors of bacterial meningitis neurological and neuropsychological sequelae occur frequently. Even in patients with a good recovery, cognitive impairments are common and affect one third of patients with pneumococcal and meningococcal meningitis.[13] The affected cognitive domains differ between studies, but a study with pooled data showed most of cognitive impairments were associated with a decline in cognitive speed.[13-16] Despite concerns about possible harmful effects of adjunctive dexamethasone therapy on cognition, earlier studies did not pointed out an association

between dexamethasone and cognitive impairments in bacterial meningitis patients.[16] Nowadays dexamethasone is a routine therapy in adults with bacterial meningitis, therefore evaluation of the long term affect on cognitive functioning is still important.

Another long term finding in bacterial meningitis patients is a decreased quality of sleep. Sleep disturbance is common in survivors of meningitis (58%), but the pathophysiological understanding is still unknown.[17] Possibly, structural brain lesions due to the inflammatory disease underlie sleep disturbances in meningitis patients. Still, large patient groups in order to assess the extent and frequency of sleep disorders in bacterial (and in particular pneumococcal) meningitis are lacking. In this study we will assess quality of sleep and screen for possible related depressive mood disorders in patients and controls.

Adaptive immune system after bacterial meningitis

The innate immune system plays an important role in bacterial meningitis, but the role of the adaptive immune system is still unclear. In this study, we want to sequence gene expression of B cells in search for long term memory signals that are unique to survivors of bacterial meningitis. Furthermore we will measure pneumococcal and anti-neuronal antibodies to determine if an adequate immune response did take place and patients did acquired long term protection for new pneumococcal infections.

2. OBJECTIVES

1. Characterize the interplay between host factors and bacterial factors and the specific activation of inflammatory pathways.
2. Evaluate the expression of identified risk genes after activation of inflammatory pathways in host immune mediating cells.
3. Determine differences in expression of immune genes in survivors of bacterial meningitis and healthy controls.
4. Determine the long term effects of bacterial meningitis on quality of life, quality of sleep and cognitive functioning
5. Evaluate pneumococcal and anti-neuronal antibody titers and expression patterns in B cells of survivors of bacterial meningitis

3. STUDYDESIGN

Follow up study of a nationwide prospective genetic association study (MeninGene).

4. STUDYPOPULATION

4.1 Population (base)

Adult survivors of community acquired bacterial meningitis included in the Dutch Bacterial Meningitis Study II and MeninGene study and corresponding control persons who participated in those studies are eligible for this recall study.

4.2 Inclusion criteria

Adult survivors of community acquired bacterial meningitis who:

1. Participated in the Dutch Bacterial Meningitis Study II or MeninGene study
2. Gave informed consent to be approached for new research projects

Control persons who:

1. Participated in the Dutch Bacterial Meningitis Study II or MeninGene study
2. Gave informed consent to be approached for new research projects

4.4 Exclusion criteria

Patients and control persons who:

1. Had a meningitis episode in the 6 months previous to participation

4.4 Sample-size calculation

We aim to include 150 patients and 150 controls in this study during a period of 1,5 year. With this number of patients we are able to find significant differences ($p<0.05$) in cognitive functioning (estimated needed number of patients is > 90)[13] and in quality of sleep (estimated needed number of patients is > 25)[17].

We expect to need 150 patients in order to determine functional differences in genetic variations with a minor allele frequency of over 10% ($\alpha=0.05$, $\beta=0.80$).

5. METHODS

5.1 Inclusion procedure

We will first check in the Dutch Municipal Population Register (Basis Registratie Personen) whether the participating patients in the MeninGene study are still alive. Investigators of the MeninGene study will contact by phone the survivors of bacterial meningitis that have given informed consent to participate in future research projects and provided contact information. After permission by phone we will send the patients the patient information letter. Informed consent form will be signed during the visit in the AMC.

5.2 Study procedures

On the outpatient clinic of the AMC blood samples will be withdrawn from the former patient and healthy control. After this blood withdrawal, the patient has a neurological examination, will fill in the questionnaires and performs the neuropsychological assessment. The neuropsychological assessment is a digital assessment and will be supervised by a medical (PhD) student or physician. As compensation for patients visiting the AMC travel costs reimbursement and lunch will be provided.

5.3 Collections and storage of patients' specimen

From each participant a maximum of 100 cc blood will be sampled by puncture of the cubital vein. The investigators will immediately process the samples to isolate the PBMCs. After PBMCs are harvested, part of them are used for PBMC stimulation and part for cell sorting and B cell isolation. B cell isolation will be done with a B cell isolation kit and after isolation the cells are stored at -80°C.

Cell sorting will be done with Magnetic Activated Cell Sorting (MACS) and after cell sorting PBMC subsets will be used for stimulation experiments. PBMC subsets, PBMCs and whole blood will be stimulated with: 1. Bacteria (causative agents of bacterial meningitis), 2. Bacterial extracts (positive controls), 3. Culture medium (negative controls). PBMC subsets, PBMCs and whole blood samples will be stimulated for 4 hours and 24 hours. After 4 hours mRNA will be collected to study mRNA expression. After 24 hours, supernatant will be collected to determine cytokine responses. mRNA and supernatant will be stored at -80°C. Further analysis of samples will be done after all samples are collected.

Blood samples to measure antibody titres will be processed and stored at -80°C. Further analysis of samples will be done after all samples are collected.

5.4 Storage of specimens in MeninGene biobank

All participants' samples will be stored in the MeninGene biobank, which is located in the AMC, Amsterdam, and will only be used for research on bacterial meningitis. As research on epidemiology, pathophysiology and changes in management requires large numbers of patients and

samples, all samples will be stored for a period of 50 years. Further information is available in the "Biobank Protocol MeninGene" and the MeninGene protocol.

5.5 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences.

5.6 Participating centres

Blood samples need to be processed immediately in identical conditions. Therefore the AMC is the only participating centre to obtain samples for the recall study.

6. ETHICAL CONSIDERATIONS

6.1 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.

6.2 Recruitment and consent

When the inclusion criteria for the MeninGene study have been fulfilled, patients are asked for written informed consent, in accordance with the guidelines of the medical ethics committee (METC) of the AMC. Additional written informed consent will be requested for this study.
Provided informed consent procedures and privacy measures and safeguards are in accordance with the Dutch Personal Data Protection Act and the Medical Treatment Contract Act and the EU-Directive 95/46/EC on the protection of individuals with regard to the processing of personal data and on the free movement of such data.

6.3 Benefits and risks assessment, group relatedness

Patients do not have direct benefit from this study. Therapies identified as a result of this study may be beneficial to a future episode of meningitis in patients with recurrent meningitis (approximately 5%). A possible benefit could be the opportunity to ask questions to a bacterial meningitis expert.

The risks of the study are limited to those of a venous blood withdrawal, which are minor. The maximum amount of blood withdrawn from patients during admission will be 100 cc, which is less than 2 percent of the total circulating volume, and therefore will not attribute to any of the patients burdens, nor result in an additional risk.

6.4 Compensation for injury

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

6.5 Incentives

Participants in this study will receive travel costs reimbursement and if desired a lunch will be provided in the AMC.

7. ADMINISTRATIVE ASPECTS AND PUBLICATION

7.1 Handling and storage of data and documents

The MeninGene study and this study will be conducted according to the principles of the Declaration of Helsinki (version of 2008, Seoul, South-Korea) 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.
When the study is finished, all essential documents (Informed Consent forms, test reports) will be archived and stored for the next 15 years, in accordance to GCP-guidelines.

7.1.1 Coding of and access to data

Patient's data are coded with a unique number (the same number as is used in the MeninGene). This consists of four numbers, which will be used sequentially for included patients. The study code does not include data that may be used for identification of the patient such as date of birth, initials or codes for hospital of admission. The key to this code is known in the AMC; the coordinating researchers and research team will have entry to this code. This code will be used to store blood and test results.
The health-inspection (inspectie voor gezondheidszorg – IGZ), the METC, and audits will have access to source documents.

7.2 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the study;
- the scientific value of the study;
- the conduct or management of the study.

All substantial amendments will be notified to the METC and to the competent authority.
Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

7.3 Annual progress report

The investigator will submit a summary of the progress of the study to the accredited METC yearly. 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 study, and amendments.

7.4 End of study report

The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's follow-up. In case the study is ended prematurely, the investigator will notify the accredited METC, 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.

7.5 Public disclosure and publication policy

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

8. REFERENCES

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