

Appendix 1 to MeninGene study
Serial Meningitis Sampling (SMS) study; A sub-study of the MeninGene study



MeninGene SMS

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TABLE OF CONTENTS

	SUMMARY	4
1.	INTRODUCTION AND RATIONALE	5
2.	OBJECTIVE	6
3.	STUDYDESIGN	6
4.	STUDYPOPULATION	6
	4.1 Population (base)	6
	4.2 Inclusion criteria	6
	4.3 Exclusion criteria	6
	4.4 Sample size calculation	6
5.	METHODS	7
	5.1 Study procedures	7
	5.1.1 Inclusion	7
	5.2 Collections and storage of patients specimen	7
	5.2.1 Serial blood sampling during admission	7
	5.2.3 Control sample after 3 months	8
	5.2.2 Throat swab	8
	5.3 Storage of blood and bacteria in MeninGene biobank	8
	5.4 Withdrawal of individual subjects	8
	5.5 Participation centres	8
6.	ETHICAL CONSIDERATIONS	8
	6.1 Regulation statement	8
	6.2 Recruitment and consent	8
	6.3 Benefits and risks assessment, group relatedness	9
	6.4 Compensation for injury	9
	6.5 Incentives	9
7.	ADMINISTRATIVE ASPECTS AND PUBLICATION	10
	7.1 Handling and storage of data and documents	10
	7.1.1 Storage of specimens in MeninGene biobank	10
	7.1.2 Coding of and access to data	10
	7.2 Amendments	10
	7.3 Annual progress report	11
	7.4 End of study report	11
	7.5 Public disclosure and publication policy	11
8.	REFERENCES	11
9.	APPENDIX 1.1	13

SUMMARY

Background

Genetic polymorphisms are major determinants of susceptibility, complications and outcome in bacterial meningitis patients. In 2006 we started a nationwide prospective genetic association study, which was continued in 2013 as MeninGene. Further characterisation of the functionality of genetic polymorphisms is needed to determine the nature of the influence on susceptibility and disease severity.

Objective

1. To characterize the functionality of genetic polymorphisms influencing susceptibility and disease severity during the acute phase of bacterial meningitis.
2. To evaluate the expression of identified risk genes and the effect on inflammation and coagulation in bacterial meningitis patients.
3. To determine genetic differences in bacterial strains from the nasopharynx, blood and cerebrospinal fluid in bacterial meningitis patients.

Study design

Sub-study in a nationwide prospective genetic association study (NL43784.018.13 Nationwide prospective study on community-acquired bacterial meningitis: from genetics to therapy; MeninGene).

Study population

We will prospectively enrol 150 patients admitted with bacterial meningitis included in MeninGene. Inclusion criteria will be: age >16 yr and suspected bacterial meningitis defined by CSF characteristics (a glucose level of less than 1.9 mmol/L, a ratio of CSF glucose to blood glucose of less than 0.23, a protein level of more than 0.220 g/L, or a white-cell count of more than 1,000 per 3mm³) or a positive CSF Gram stain.

European meningitis database

Data will be entered in a pseudonymized fashion in a European meningitis database (MeninGene) and will be made available to researchers upon request if scientific sound and free of direct commercial interest.

Nature and extent of the burden

From all patients participation will mean that 25 ml of blood will be obtained at 5 time points: admission (day 0), day 1, day 2, day 7 and 3 months after admission. Blood samples will be drawn from an intravenous-catheter, which is placed in all bacterial meningitis patients as part of standard care for antibiotic treatment. Furthermore, in some of the participating centres a throat swab will be obtained from meningitis patients at the day of admission for bacterial culture.

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 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.

Understanding host genetic factors in bacterial meningitis

In recent years it has been recognized that host genetic variations influence susceptibility, severity and outcome of bacterial meningitis, but also influence response to treatment.⁶⁻¹⁰ These genetic variations were identified in genes coding for the immune system (especially the complement system) but also in genes coding for coagulation and fibrinolysis factors. Often the mechanism by which the genetic variation influences disease is unclear, as genetic variations may influence protein concentrations, binding affinity but also degradation constants. Furthermore, the role of the protein itself during bacterial meningitis is poorly understood. To determine the pathophysiology of disease, study of the functionality of the specific genes and genetic variations is needed. The functionality of these genes and genetic variations can be studied during the acute phase of bacterial meningitis by measuring mRNA expression levels and protein levels at multiple time points. By correlating these to the patient's DNA profile, clinical characteristics and outcome, further insight in the pathophysiology of the disease will be acquired. Furthermore, this study will facilitate studying the interaction of proteins in the immune system, coagulation and fibrinolysis.

Identifying bacterial genetic factors influencing bacterial meningitis

Genetic variation in bacteria has been recognized to determine the invasiveness of the bacteria and disease course in the patient.¹¹⁻¹³ The bacteria causing meningitis reside in the nose and throat of many healthy people. Subtle spontaneous mutations in the bacterial DNA may cause the bacteria to change from a colonizing to disease causing bacterium. By studying the genetic characteristics of bacterial strains from nasopharynx, blood and cerebrospinal fluid, variations in bacterial DNA that determine virulence may be detected. To this end throat swabs will be performed in patients and proxies living in the same dwelling, and bacteria cultured from these swabs will be compared with blood and CSF strains that are already present in the Netherlands Reference Laboratory for Bacterial Meningitis.

2. OBJECTIVE

1. To characterize the functionality of genetic polymorphisms influencing susceptibility and disease severity during the acute phase of bacterial meningitis.
2. To evaluate the expression of identified risk genes and the effect on inflammation and coagulation in bacterial meningitis patients.
3. To determine genetic differences in bacterial strains from the nasopharynx, blood and cerebrospinal fluid in bacterial meningitis patients.

3. STUDYDESIGN

Sub-study in a nationwide prospective genetic association study (MeninGene).

4. STUDYPOPULATION

4.1 Population (base)

All adult patients with suspected bacterial meningitis acquired in the community are eligible for the study.

4.2 Inclusion criteria

1. Age > 16 yr
2. Suspected bacterial meningitis defined by CSF characteristics (a glucose level of less than 1.9 mmol/L, a ratio of CSF glucose to blood glucose of less than 0.23, a protein level of more than 0.220 g/L, or a white-cell count of more than 1,000 per 3mm³) or a positive CSF Gram stain.

4.3 Exclusion criteria

1. Neurosurgical operation in the month previous to the meningitis episode
2. Head trauma in the month previous to the meningitis episode
3. Presence of neurosurgical devices in the central nervous system such as cerebrospinal fluid catheters or deep brain stimulator.

4.4 Sample-size calculation

We aim to include 150 patients in this substudy during a period of 5 years. This number of patients is based on the estimated number of patients available in the Netherlands in centres including >10 patients per year. With this number of patients we will be able 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 Study procedures

5.1.1. Inclusion

Prior to initiation of the study, the investigators will give presentations in the participating hospitals to explain study procedures and distribute pocket cards as reminder. The treating physician will contact the investigators to assess eligibility of the patient for the SMS MeninGene. A 24-hour telephone service for participating physicians is provided to include a patient. Subsequently, patients or their legal representatives and controls receive written information and are asked for written informed consent for participation. Patients can be included in the study until 48 hours after start with treatment for bacterial meningitis (at that time point at least two serial measurements still can be performed).

Three months after admission, patients will be asked for a fifth blood withdrawal. These blood samples will be used as baseline control samples. For this withdrawal the patients will be invited to visit the AMC or the centre that participates in this study. If possible this blood withdrawal will be combined with an appointment with the neurologist on the outpatient clinic. .

5.2 Collections and storage of patients' specimen

5.2.1. Serial blood sampling during admission

Serial blood samples will be drawn from existing intravenous or intra-arterial infusion systems on the day of admission, and day 1, 2 and 7 of admission to evaluate the expression of identified risk genes and their effect on inflammation and coagulation. We chose for these time points since they can reveal both the early immune response and subsequent normalisation after 1 week following recovery of the patient. Each day, a maximum of 25 millilitres will be drawn from an iv-inserted catheter and blood samples will be collected in EDTA, citrated, heparin-gel, serum and Pax-gene tubes to determine gene en protein expression (see APPENDIX 1).

If possible, we will draw the blood samples from an iv-catheter, which all meningitis patients receive in standard hospital care. Subsequent analysis will be performed in the AMC. Local laboratory protocols will be implemented in each collaborating hospital for adequate storage of the samples. Blood samples will be stored at -80 °C and will be transported every two-three months to the AMC.

5.2.2. Throat swab

In centres with sufficient microbiology laboratory facilities, a throat swab will be performed in patients and controls (for example partner or proxy living in the same dwelling) on the day of

admission, or the day after admission. These samples will be cultured and if the culture is positive, the bacterial strain will be isolated. The isolates will be stored in a -20 freezer and will be transported to the AMC at the same time with the blood samples.

5.2.3 Control sample 3 months after discharge

Three months after discharge patients will be contacted for another blood withdrawal. The blood samples will be identical to those withdrawn during admission in the hospital. All blood samples will be processed immediately and will be stored at -80°C in the AMC.

5.3 Storage of blood and bacteria in MeninGene biobank

All patient 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 number 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.

5.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. If patients will be discharged or die before day 7 after admission, blood sampling will be stopped prematurely. If the treating physician decides to withdraw active treatment in a patient with a very poor prognosis, blood sampling will also be stopped.

5.5 Participating centres

Hospitals in the Netherlands including over 10 patients per year will be approached for participation in the SMS MeninGene following approval of the AMC Medical Ethical Committee.

6. ETHICAL CONSIDERATIONS

6.1 Regulation statement

The 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. Technicians and data managers of the AMC Clinic Research Unit (CRU) will perform central data management.

6.2 Recruitment and consent

When the selection criteria are fulfilled, the patient or, if necessary, the patient's representative will be 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 sub-study. When the patient has diminished decision-making capacity as result of the meningitis e.g. due to aphasia or cognitive impairment informed consent will be obtained from the patient's representative. Exclusion of these non-communicative meningitis patients would lead to a selective patient sample. Information materials for patients and patients' relatives are attached separately. For patients of 16 and 17 years old, the parents or legal guardian will be asked to co-sign the informed consent form.

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%).

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 ml (4x25ml) which is less than 1 percent of the total circulating volume, and therefore will not attribute to any of the patient's burdens, nor result in an additional risk. The fifth blood withdrawal after 3 months (25 ml) will not result in additional risk. Taking the throat swab may cause an unpleasant sensation, but does not bring any risks. The swab can provoke the pharyngeal reflex (gag reflex) which is part of the normal neurological examination.

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

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

7. ADMINISTRATIVE ASPECTS AND PUBLICATION

7.1 Handling and storage of data and documents

The MeninGene study and this substudy 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 Storage of specimens in MeninGene biobank

The blood samples and bacterial strains of throat swabs will be stored in the MeninGene biobank, which is located in the AMC, Amsterdam, the Netherlands in the Reference Laboratory for Bacterial Meningitis. Data will only be used for research on bacterial meningitis. 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 “Biobank Protocol MeninGene” and the MeninGene protocol.

7.1.2 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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Appendix 1 MeninGene study
Study-protocol SMS substudy V 3
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APPENDIX 1.1

Table 1. Samples collected in this substudy

	Analysis of:	Volume	Day 0	Day 1	Day 2	Day 7	Month 3
Throat swab Patient and control	Microbial flora nasopharynx	NA	1x	-	-	-	
Blood Citratated sample	Coagulation and fibrinolysis factors	6,0 ml	1x	1x	1x	1x	1x
Blood Heparin-gel	Cytokines, antibodies, complement factors	4,5 ml	1x	1x	1x	1x	1x
Blood Serum sample	Cytokines and complement components	5,0 ml	1x	1x	1x	1x	1x
Blood EDTA sample	Cytokines, markers of inflammation	4,0 ml	1x	1x	1x	1x	1x
Blood Pax-gene tube	mRNA	2.5 ml	1x	1x	1x	1x	1x
Total per day		22 ml	22 ml	22 ml	22 ml	22 ml	22 ml

In total, for this substudy a maximum of 110 ml blood is obtained from each patient. For MeninGene, another 14 ml EDTA blood is obtained once only.