SEMESTER Suriname study

The Suriname Meningo-encephalitis Study (SMS)

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Introduction

Meningitis/encephalitis

Meningitis is an acute inflammation of the protective membranes covering the brain and spinal cord, and encephalitis is inflammation of the brain itself. These inflammations can be caused by viruses, bacteria, or other microorganisms. Meningitis and encephalitis can be life-threatening diseases and prompt initiating of treatment is warranted. Despite availability of antibiotics, antiviral medication and adjunctive dexamethasone mortality and morbidity rates are still substantial.

The symptoms and signs of meningitis are headache, fever, neck stiffness and altered consciousness, but not in all cases all symptoms are present. In case of encephalitis and meningo-encephalitis there can also be behavioral changes, seizures and focal neurological deficits like aphasia. Children more often present with non-specific symptoms like irritability, poor feeding, vomiting and lethargy. A lumbar puncture is important to diagnose or exclude meningitis and encephalitis. The first treatment in acute bacterial meningitis consists of promptly administered antibiotics together with adjunctive dexamethasone and – in case of possible viral meningitis or encephalitis - antiviral drugs like acyclovir. Meningitis and encephalitis can be lethal, but can also lead to serious long-term consequences such as deafness, epilepsy, hydrocephalus and cognitive deficits, especially in case of delay in treatment. Some forms of meningitis and encephalitis may be prevented by immunization.
Guillain-Barré syndrome

The Guillain-Barré syndrome (GBS) is a post infectious autoimmune disease resulting in polyradiculoneuropathy with a variable clinical presentation. GBS is most commonly antecedent by an infection with the Campylobacter Jejuni bacteria but can also occur after viral infections. Patients with GBS suffer from rapid-onset symmetrical muscle weakness of the extremities. GBS can be life threatening; about 25% of the patients with GBS develop respiratory insufficiency. The pathogenesis of GBS is not fully elucidated, part of the pathogenesis is molecular mimicry of pathogen antigens that leads to cross-reactive ganglioside antibodies.

The Guillain-Barre syndrome is not a single disorder but a heterogeneous syndrome with several variant forms. The most common form of Guillain-Barré syndrome is the acute inflammatory demyelining polyradiculoneuropathy (AIDP). Acute motor axonal neuropathy (AMAN) and acute sensorimotor axonal neuropathy (AMSAN) are primary axonal forms of GBS. The Miller Fisher syndrome, characterized by ophthalmoplegia, ataxia and areflexia, is another variant of the Guillain-Barré syndrome.

Current clinical situation Paramaribo

Since mid-March 2014, an abnormal increase (more than a doubling compared to normal rates) in the number of patients presenting with the clinical picture of meningitis/encephalitis was noticed within the Academic Hospital Paramaribo (AZP). Typical signs and symptoms of meningitis such as fever and headache were usually present, but also behavioral changes and lethargy, indicating that there is possibly also inflammation of the brain itself (encephalitis). Cerebral spinal fluid (CSF) examination showed elevated protein and low cell counts (predominantly monocytes). The infection parameters in CSF and peripheral blood tend to favor more for a viral pathogen than for a bacterial or a fungal infection. No pathogen was found in the microbiology department of AZP. Till thus far most cases have been treated empiric with intravenous acyclovir and in some instances combined with amoxicillin. Sequelae consisted of lethargy and headaches several weeks after the episode of meningomyelitis. After noticing this increase of meningitis/encephalitis cases, it was decided to set up a prospective observational cohort study to identify the cause of this increase.

Since the end of 2015/start 2016 an increase in the incidence of GBS like symptoms was noticed in the Academic Hospital Paramaribo (AZP). The increase of these symptoms occurred simultaneous with the outbreak of the Zika virus in Suriname and other countries in South-America. This raises the question if an infection with the Zika virus can lead to the GBS or Guillain-Barré like symptoms. Because of the serious health risks that are associated with GBS it is important to investigate the etiology and epidemiology of this sudden rise of incidence of the GBS/GBS like symptoms.

Aim

To describe the epidemiology, etiology and clinical picture of meningitis/encephalitis and the Guillain-Barré syndrome in the AZP and based on the results develop and introduce the diagnostic laboratory tools to identify the causative organisms of meningitis/encephalitis.
Objectives

Primary objectives

- To identify the causative microorganisms in patients presenting with meningitis and encephalitis in the AZP.
- To identify the incidence and type of GBS/GBS like syndromes and to identify the antecedent infections resulting in GBS

Secondary objectives

- To describe the clinical findings in patients presenting with meningitis/encephalitis and GBS in the AZP
- To examine risk factors associated with meningitis/encephalitis and GBS including demographic characteristics (i.e. age, sex), area of likely acquisition, mosquito bites, animal contact, and other characteristics.
- To describe demographic, clinical, biological, bacteriological, viral factors associated with severe complications of viral meningitis/encephalitis and GBS (e.g. death, disability).
- Capacity building: to strengthen diagnostic tools for the (early) identification of viral meningitis and encephalitis in the AZP and to strengthen the diagnostic tools for the Guillain-Barré syndrome.

Tertiary objectives

- To develop clinical guidelines for the diagnosis and treatment of (viral) meningitis/encephalitis and GBS in the AZP.
- To explore preventive (public health) interventions.

Methods

Design: prospective cohort study

Inclusion criteria:

- All patients (≥18 years) presenting with one or more of the following (neurological) symptoms:
  - Group 1, symptoms of meningitis/encephalitis (e.g. fever (>38 °C), headache, seizures, altered mental status, behavioural changes, neck stiffness or focal neurological deficits (e.g. aphasia, hemiparesis)).
  - Group 2, patients with symptoms of GBS (e.g. progressive symmetrical weakness, decreased tendon reflexes).

Study set-up:

After signing informed consent, participant will be divided in one of the two study groups of this study according to their symptoms. These study groups are: 1. Clinically suspected meningitis or encephalitis, and 2. Clinically suspected Guillain-Barré syndrome. It is expected that a lumbar puncture will be performed on patients in both groups for routine diagnostics. For this study we ask
the participants for permission to store some of the CSF collected/to be collected with the first lumbar puncture to perform more comprehensive viral pathogen detection tests in the CSF.

Only if there was no causative pathogen found in the first (routine) lumbar puncture in the meningitis/encephalitis group, an extra lumbar puncture will be performed for this study at day 10 or at discharge.

To establish the diagnosis GBS or Miller Fisher syndrome (a syndrome related GBS) in the participants in group 2, the criteria in supplement 1 and 2 will be used. If necessary to establish the diagnosis, an electromyography (EMG) will be performed.

Data collection:
Different case record forms (CRF) will be filled out for the included patients in group 1 and group 2 on the day of inclusion and during follow up. Date will be entered in OpenClinica®. All participants will receive a unique and anonymized study code.

Sampling:
Study material will be obtained and will be stored at - 80C (CSF) and -20C (serum)
- CSF: on day of admission (T=0) and when no causative organism is established: on day 10 (T=10) or on discharge (T=D). CSF will be stored in aliquots.
- Blood: serum [10mL] for serology on day of admission (T=0) and on day 10 (T=10) or on discharge (T=D).
- Blood: plasma (10mL) for potential biomarker studies on day of admission (T=0) and on day 1 (T=1) and day 5 (T=5) and on day 10 (T=10) or on discharge (T=D).
- Of both CSF and serum 100µl will be stored in 400 µl of RNA lysis buffer to serve as a backup for molecular diagnostics.
- Peripheral blood mononuclear cells will be isolated from 1 tube of EDTA plasma within 24 hours after blood withdrawal on day of admission.
- A nasal swab and throat swab will be performed on day of inclusion (T=0) and will be stored in Universal Transport Medium (UTM™)
- Urine and blood will be tested on Zikv RNA via PCR on day of inclusion

Routine diagnostics will be done in the AZP and include:
- Haematology (including leucocyte differentiation)
- Chemistry (including CRP, Creatinine, ALT)
- Serology: HIV, syphilis
- CRF: opening pressure, cell count and leucocyte differentiation, glucose and glucose ratio, protein. Gram staining and culture (aerobe, anaerobe,) and cryptococcal staining (India ink), culture and Ag test. PCR: Herpes simplex 1 and 2 and mycobacteria.
- If necessary according to the treating physician: CT-scan of the brain or MRI-scan of the brain.

The following additional tests will be performed at the Erasmus MC, department of Virology:
- PCR CSF: Enterovirus, West Nile virus (WNV), chikungunya (CHIKV), Lymphocytic Choriomeningitis Virus (LCMV), Zika virus (ZIKV).
- Serology: IgG and IgM WNV, LCMV, Dengue, CHIKV, ZIKV.
• Microarray: Dengue 1-4, WNV, St. Louis encephalitis virus (SLEV), Yellow fever virus (YFV), CHIKV, ZIKV.
• IFA: equine encephalitis viruses.
• Antiganglioside antibodies (GM1, GM2, GD1a, GD1b en GQ1b and GalC) in the participants in group 2 (GBS).
• When indicated tests for other viruses based on epidemiologic findings or specific clinical findings.

Study site:
Suriname, with a population of approximately 566,000, is a country on the northeastern Atlantic coast of South America. It is bordered by French Guiana to the east, Guyana to the west and Brazil to the south. Suriname has a tropical climate with a small and large rainy season. The study will be performed in the Academic Hospital Paramaribo (AZP), in the city of Paramaribo, which is the capital city of Suriname located at the north coast. The AZP is the only academic hospital in the country and has 465 beds. Paramaribo has three other, smaller general hospitals. Although the patients may be recruited mainly from the AZP, medical doctors in the other hospitals will be informed about the study and encouraged to submit patients under their care that meet the inclusion criteria.

Table 1: blood sampling/testing

<table>
<thead>
<tr>
<th>Assay</th>
<th>Collection container</th>
<th>Moment of collection</th>
<th>Centralized/ Decentralized testing</th>
<th>Routine/ research purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard hematology and chemistry testing</td>
<td>EDTA vacutainer</td>
<td>At T=0 and at T=10/T=D</td>
<td>Centralized</td>
<td>Routine</td>
</tr>
<tr>
<td>HIV and Lues serology</td>
<td>SST vacutainer</td>
<td>At T=0</td>
<td>Centralized</td>
<td>Routine</td>
</tr>
<tr>
<td>Serology: IgM and IgG for WNV, LCMV, dengue, CHIKV, ZIKV</td>
<td>SST vacutainer</td>
<td>At T=0 and T=10/T=D</td>
<td>Decentralized</td>
<td>Research</td>
</tr>
<tr>
<td>Microarray: dengue 1-4, WNV, SLEV, YFV, CHIKV, ZIKV</td>
<td>SST vacutainer</td>
<td>T=0</td>
<td>Decentralized</td>
<td>Research</td>
</tr>
<tr>
<td>PCR: ZIKV detection</td>
<td>SST vacutainer</td>
<td>T=0</td>
<td>Centralized</td>
<td>Routine</td>
</tr>
<tr>
<td>IFA: equine encephalitis viruses</td>
<td>SST vacutainer</td>
<td>T=0</td>
<td>Decentralized</td>
<td>Research</td>
</tr>
<tr>
<td>PBMC isolation</td>
<td>EDTA vacutainer/BD vacutainer PCT</td>
<td>At T=0</td>
<td>Decentralized</td>
<td>Research</td>
</tr>
<tr>
<td>Plasma for potential biomarkers</td>
<td>EDTA vacutainer</td>
<td>T=0, T=1, T=5, T=10/T=D</td>
<td>Decentralized</td>
<td>Research</td>
</tr>
</tbody>
</table>

WNV; West Nile virus, LCMV; Lymphocytic Choriomeningitis Virus, CHIKV; Chikungunya virus, ZIKV; Zika virus, SLEV; St. Louis encephalitis virus, YFV; Yellow Fever virus
Table 2: sampling/testing of other materials

<table>
<thead>
<tr>
<th>Assay</th>
<th>Collection container</th>
<th>Moment of collection</th>
<th>Centralized/Decentralized testing</th>
<th>Routine/research purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF: 1: opening pressure, cell count, L-diff, glucose (ratio), protein, gram staining, cryptococcal staining, PCR: HSV 1 and 2 2: PCR enterovirus, WNV, CHIKV, LCMV, ZIKV</td>
<td>Aliquots</td>
<td>At T=0, when negative also at T=1 and T=10/T=D</td>
<td>Centralized/Decentralized</td>
<td>1: Routine 2: Research</td>
</tr>
<tr>
<td>Nasal swab</td>
<td>UTM viral transport media</td>
<td>At T=0</td>
<td>Decentralized</td>
<td>Research</td>
</tr>
<tr>
<td>Throat swab</td>
<td>UTM viral transport media</td>
<td>At T=0</td>
<td>Decentralized</td>
<td>Research</td>
</tr>
<tr>
<td>Urine: PCR for ZIKV detection</td>
<td>Universal sterile</td>
<td>At T=0</td>
<td>Centralized/Decentralized</td>
<td>Routine</td>
</tr>
</tbody>
</table>

L-diff: leucocytes differentiation, HSV: Herpes Simplex virus, WNV: West Nile virus, CHIKV: Chikungunya virus, LCMV: Lymphocytic Choriomeningitis Virus, ZIKV: Zika virus

Figure 1: Algorithm of study procedures
Data analysis

Statistical analysis

All statistical analyses will be performed using GraphPad Prism 5.01 for Windows. When comparing two groups we will make use of a Student’s t test or Mann Whitney U, depending on the distribution of the data. For the comparison between multiple groups non-parametric Kruskal-Wallis test will be used with Dunn’s multiple comparison test or a one-way ANOVA with Tukey’s multiple comparisons test, again depending on the distribution of the data. P values ≤0.05 will be considered significant.

Study initiation and duration:

The study will be initiated as soon as funding and ethical approval has been obtained. Since the occurrence of meningitis and encephalitis seems to be seasonal, we aim to continue with inclusions for at least two years, in order to also catch the expected seasonal trend.

Ethical and legal aspects:

The protocol will be submitted for approval to the ethical board of the Ministry of Health in Suriname.

The AZP has a research centre that facilitates research that is being initiated by hospital staff (www.researchcentersuriname.org); this includes administrative support and guidance in preparing a study budget and obtain funding to carry out the study.

The results from the study will be reported in manuscripts submitted to peer reviewed journals with shared authorships between the investigators from the participating institutes.

Expected results

• Establishing the actual incidence of admitted meningitis and encephalitis and GBS cases in the AZP and possibly other hospitals in Paramaribo.
• Finding the cause of the recent increase of cases of meningitis and encephalitis and GBS, with taking demographic and climatologic factors into consideration.
• Describe the clinical findings of the cases of meningitis, encephalitis and GBS.
• Establishing risk factors for meningitis and encephalitis and GBS in Suriname.
• To evaluate the outcome (prognosis) in these cases of meningitis and encephalitis and GBS.
Supplement 1: Diagnostic criteria for Guillain-Barré syndrome (GBS)

Features required for diagnosis
Progressive weakness in both arms and legs (might start with weakness only in the legs)
Areflexia (or decreased tendon reflexes)

Features that strongly support diagnosis
Progression of symptoms over days to 4 weeks
Relative symmetry of symptoms
Mild sensory symptoms or signs
Cranial nerve involvement, especially bilateral weakness of facial muscles
Autonomic dysfunction
Pain (often present)
High concentration of protein in CSF
Typical electrodiagnostic features

Features that should raise doubt about the diagnosis
Severe pulmonary dysfunction with limited limb weakness at onset
Severe sensory signs with limited weakness at onset
Bladder or bowel dysfunction at onset
Fever at onset
Sharp sensory level
Slow progression with limited weakness without respiratory involvement (consider subacute inflammatory demyelinating polyneuropathy or CIDP)
Marked persistent asymmetry of weakness
Persistent bladder or bowel dysfunction
Increased number of mononuclear cells in CSF (>50×10E6/L)
Polymorphonuclear cells in CSF

Reference
Adapted from Asbury AK, Cornblath DR. Assessment of current diagnostic criteria for Guillain-Barré syndrome. Ann Neurol 1990;27 Suppl:S21-4
Supplement 2: Diagnostic criteria for Miller Fisher Syndrome (MFS)

Features required for diagnosis
Bilateral ophthalmoparesis or ophthalmoplegia
Ataxia
Areflexia (or decreased tendon reflexes)

Features that support diagnosis
Progression of symptoms over days to 4 weeks
Relative symmetry of symptoms
Mild limb weakness (in case of prominent limb weakness, consider GBS-MFS overlap syndrome)
Mild sensory symptoms or signs (in case of prominent sensory symptoms or signs, consider GBS-MFS overlap syndrome)
Facial palsy and/or bulbar palsy
Presence of serum IgG antibodies against ganglioside GQ1b
Nerve conduction studies: no changes in extremities
High concentration of protein in CSF, cytoalbuminologic dissociation

Features that should raise doubt about the diagnosis
Alterations in consciousness
Corticospinal tract signs
Fever at onset
Marked persistent asymmetry of weakness

Reference