Paediatric and Adult Causes of Encephalitis and Meningitis: The PACEM study.

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Aim of the study

Meningitis and encephalitis are feared for their high morbidity and mortality rate. Since the introduction of vaccines against the most common causative bacteria, the incidence of bacterial meningitis has dropped significantly. Enterovirus and herpes simplex virus are thought to be the most common causes of viral meningitis and encephalitis respectively, varying in severity from mild disease to destructive encephalitis with permanent neurological sequelae and death.

However, in the majority of patients with a clinical suspicion of meningitis and/or encephalitis no causative agents can be found. Many other viruses, bacteria and other micro-organisms as well as immunologic processes are known to cause occasionally meningitis and/or encephalitis. Routine testing of these pathogens is not done, therefore incidence numbers and more important, clinical relevance including morbidity and mortality, are missing.

In recent years molecular diagnostic tests have been improved rapidly, making it possible to detect many more pathogens in CSF with a high sensitivity and specificity. Interpretation of these results for clinical relevance and implementation in routine laboratory testing is lagging behind, since systematic registration is missing.

This project focuses on the initiation of a regional surveillance study to systematically monitor the causes of meningitis and encephalitis in children and adults, and register both clinical and laboratory findings in these patients to gain more insight into these potential devastating diseases.

Background

Meningitis (inflammation of the meninges) and encephalitis (inflammation of the brain in association with clinical evidence of neurologic dysfunction) are potential life-threatening conditions that can rapidly progress to permanent brain damage, neurologic problems, and even death. Since vaccination against the most common causative bacteria (*H. influenza* type b, *S. pneumonia*, *N. meningitidis* serogroup C), the incidence of bacterial meningitis has dropped significantly [1-3]. Therefore, non-bacterial pathogens, such as viruses and tick-born pathogens are increasingly recognized as important causes of central nervous system (CNS) infections. In addition, immune-mediated processes (like ADEM and antibody associated encephalitis) seem to play an important role. For example, in a study in the UK, immune mediated processes were found in 20 percent of patients with encephalitis [4].

In contrast with bacterial meningitis, for which an extensive surveillance system has been set up in many countries, viral or non-bacterial CNS infection is not monitored systematically and proper incidence numbers of the different causative agents and mortality and morbidity rates are missing. Recently it has been propagated that it is important to optimize the surveillance for viral encephalitis/meningitis in Europe to gain more insight in the aetiological pattern as well as to optimize therapy to improve survival and reduce brain injury [5].

The most common causes for aseptic meningitis are human enterovirus (HEV) [6-10] and human parechovirus (HPeV) [11, 12]. These viruses give usually mild and self-limiting symptoms, but in neonates and patients with a deficient humoral immune response severe disease with permanent neurologic sequelae has been described [13-15]. Moreover, in recent years Enterovirus 71 caused epidemics of severe meningo-encephalitis in healthy children and adults in Asia with an unprecedented high mortality rate [16, 17].

Herpes simplex virus (HSV) is thought to be the main cause of encephalitis, but in the majority (60-70%) of patients no cause is found [18-20]. With a systematic approach the detection rate can be much higher as has been shown in a recent prospective study in the UK where a cause was found in 63% of patients with encephalitis [4].

A variety of other micro-organisms can occasionally cause meningitis and/or encephalitis like Epstein-Barr virus (EBV), cytomegalovirus (CMV), adenovirus, varicella zoster virus (VZV), human herpes virus (HHV) 6 and 7, measles virus, mumps virus and the bacteria *Borrelia burgdorferi* [7, 21-24]. Arbo-viruses such as Tick-borne encephalitis virus (TBEV), Japanese encephalitis virus (JEV) and West-Nile Virus (WNV) are more prevalent in the third world countries, but the introduction of WNV in the first world has lead to extreme epidemic outbreaks in the USA [22, 25]. In Europe human cases of WNV have been reported in South and East Europe in the last decade [26]. Routine screening of CSF for these viruses is not done in most diagnostic laboratories, therefore proper incidence numbers are missing.

The majority of these meningitis/encephalitis causing agents are detected today by highly sensitive molecular diagnostic tests, but the clinical interpretation of the results is difficult because comparisons of clinical signs and symptoms and laboratory findings are missing. In contrast with bacterial meningitis, the CSF of patients with viral meningitis does not display extreme abnormalities of leukocyte count, protein and glucose levels [9, 27, 28]. In young children even normal leukocyte counts in CSF are seen in the presence of viral agents [29, 30]. In addition, in patients with viral meningitis/encephalitis, viral presence can often be detected in other specimen than CSF, such as faeces, blood and nasopharyngeal aspirates [28].

The contribution of immune mediated processes like ADEM and antibody-associated encephalitis is not clear, especially in children. ADEM usually affects children and young adults with an incidence varying from 0.07 per 100.000 per year in children <16 years in Germany to 1.1 per 100.000 per year in children < 10 years in Italy [31, 32]. Anti-N-Methyl-D-Aspartate (Anti-NMDA) receptor encephalitis seemed to be associated with teratoma in young women, but it is also seen in patients without malignancy as well as men and children [33, 34].

Prospective monitoring and systematic registration of CNS infections is of clinical importance to increase insight in the incidence and pathogenesis of meningitis and encephalitis in children and adults. Several countries like Germany, United Kingdom and France have recently started to monitor the epidemiology of viral meningitis/encephalitis in nationwide studies [5]. In the Netherlands there is only laboratory-based enterovirus surveillance conducted by the RIVM (National institute for public health and the environment) for detection of any case of polio, but this surveillance does not focus on other causes of meningitis/ encephalitis in the Netherlands.

The aim of this project is to set up a regional pilot survey study that will systematically register both clinical and laboratory findings of adults and children with meningitis and/or encephalitis in the Netherlands to gain more insight in the causes and pathogenesis of these diseases. After one year, the results of the pilot survey will be evaluated to assess if the study will be set up nationwide.

This surveillance system will be set up in collaboration with the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) (a collaboration of the Academic Medical Center, Amsterdam and the National Institute of Public Health and the Environment in the Netherlands). The NRLBM has been set up to perform national surveillance of bacterial meningitis in the Netherlands. They have an excellent logistic network and receive CSF and blood isolates from approximately 85% of all adult patients with bacterial meningitis in the Netherlands, giving this study the opportunity to use this already established logistic network [35-37].

Objectives

To evaluate the incidence and characteristics of meningitis and/or encephalitis in patients of all ages:

1. To determine the causes of meningitis and/or encephalitis in children and adults
2. To evaluate the clinical signs and symptoms and CSF laboratory characteristics of children and adults with a diagnosis of meningitis and/or encephalitis
3. To determine what materials (CSF, blood, faeces/rectal swab, nasopharyngeal aspirate/swab or throat swab) are most suitable for the detection of meningitis/encephalitis in children and adults.
4. To determine the impact of meningitis and/or encephalitis on the health care systems in terms of costs, antibiotic use, morbidity and mortality.

Methods

A pilot-study (duration 1 year) will be initiated in 3 regional hospitals in Amsterdam and Almere, the Netherlands (Academic Medical Center, Onze Lieve Vrouwe Gasthuis, and Flevohospital).

Patients of all ages (including premature born infants) with a clinical suspicion of meningitis or encephalitis of whom CSF is obtained will be eligible for inclusion in this study if they do not met one of the following (exclusion) criteria:

- neurosurgical operation in recent past (last 3 months)
- drain with connection to cerebrospinal fluid

Informed consent is asked by the local attending pediatrician or neurologist. By questionnaire, information about clinical findings at presentation, demographic information, medical history including immune status, use of antibiotics and/or antivirals, results of laboratory, EEG and neuroimaging testing and outcome (Glasgow outcome scale) will be collected. In addition the discharge letters of the hospital and CD-ROMs of neuroimaging testing will be collected.

Simultaneously, the following additional materials will be collected for screening:

- faeces or rectal swab
- throat-swab and/or nasopharyngeal aspirate or swab
- blood: Adults and children >12 years: 10 ml blood

  Children <12 years: 5-10 ml blood

In children and persons who are incapable to give informed consent, blood samples will only be collected in combination with regular blood collections for diagnostic purposes.

Patients and/or their parents with a clinical suspicion of meningitis or encephalitis who are not included during the period they were hospitalized will be approached by mail to ask if they give informed consent to use the CSF and other materials that were collected during their hospitalization. This is possible due to the fact that in the clinical setting CSF and other materials are stored for several months before they are thrown away. Information about clinical findings at presentation, demographic information, medical history including immune status, use of antibiotics and/or antivirals, results of laboratory, EEG and neuroimaging testing and outcome (Glasgow outcome scale) will be collected from medical records. In addition the discharge letters of the hospital and CD-ROMs of neuroimaging testing will be collected.

CSF samples (and additional materials) of patients with a clinical suspicion of meningitis/encephalitis will be sent to the Meningitis Reference Laboratory (AMC) and onwards to the virology laboratory at the AMC.

At the Meningitis Reference Laboratory the CSF will be screened for bacterial pathogens by means of *N. meningitidis*, *S. pneumoniae* specific PCRs and of PCR targeting the gene encoding16SRNA. If the 16S PCR is positive, the bacterial pathogen will be further determined by sequencing of the 16S amplicon.

At the virology laboratory the following tests on CSF will be routineously done:

Panel 1: PCR on HEV, HPeV, HSV

Panel 2: PCR on Epstein-Barr virus, CMV, adenovirus, varicella zoster virus, human herpesvirus type 6 and 7

The following tests will only be done if results of the earlier test panels are negative:

Panel 3: PCR on measles virus, mumps virus

Panel 4: PCR on influenza virus, parainfluenza virus

Panel 5: PCR on West-Nile virus, tick borne encephalitis virus (and Japanese encephalitis virus)

Panel 6: Detection of anti-neural antibodies

Panel 7: Other viruses (if clinical suspicion) and virus discovery

Detection of viruses in CSF will be done by means of real time PCR as previously described by Benschop *et al* [38].

By using virus discovery method implemented at our laboratory, we will screen negative CSFs to identify whether we are dealing with a new virus type or strain. Documentation of these cases will aid in the evaluation of CSF screens to certain pathogens in cases of meningitis and encephalitis.

Ethical considerations

The study will be performed in accordance with the principles of the Declaration of Helsinki (version 22 October 2008) and the Medical Research Involving Human Subjects Act (WMO).

Patients (and their parents) will get information about the study from the local attending pediatrician or neurologist. Written information about the study will be given and explained orally. At a later moment patients (and their parents) will be asked by one of the doctors if they want to participate. If necessary, more time to consider their participation in the study will be given. As has been mentioned in the patient information, participation in the study has no influence on treatment and the relation with the attending pediatrician or neurologist.

The discomfort for the participant is minimal. For children and persons who are incapable to give informed consent, the collection of blood will only be done in combination with a regular vena puncture for diagnostic purposes. Collection of CSF is part of the regular diagnostic work-up. Collection of a nasopharyngeal aspirate or throat-swab and rectal swab can give some temporary discomfort. There is no additional health risk known concerning collection of the above mentioned materials.

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