Prospective single-centre observational cohort study on viral encephalitis in adults

Autoimmune inflammation as cause of RelapsIng Symptoms in patients with viral Encephalitis

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- Version 1.2

PROTOCOL TITLE

Autoimmune inflammation as cause of relapsing symptoms in patients with viral encephalitis

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Subsidising party	Stichting de Merel
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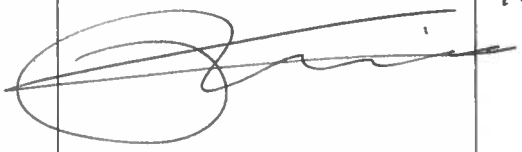
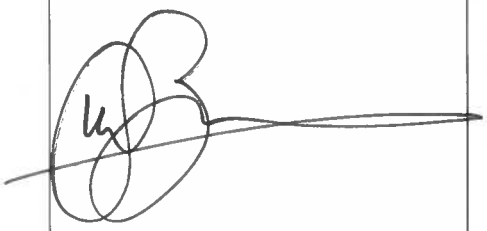
Pharmacy	Not applicable

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Deputy Head of Department of Neurology: *Prof. Dr. Y.B.W.E.M. Roos, MD, PhD* *(Neurologist, AMC)*		10-9-'19
Coordinating Investigator *Dr. M.C. Brouwer, MD, PhD* (Neurologist, AMC)		10-9-'19

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, the application form that is required fo submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
Amsterdam UMC	**Amsterdam University Medical Centers**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
IC	**Informed Consent**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
MRI	**Magnetic Resonance Imaging**
PCR	**Polymerase Chain Reaction**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, fo**

	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 a subsidising party.
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Background:

Encephalitis is a severe inflammation of the brain (1). Patients with viral encephalitis frequently fail to improve after the initial phase of the disease has passed and some even deteriorate weeks after the infection started (2, 3). It has been shown that activation of the immune system can lead to a secondary inflammation of the brain, which clinically manifests itself with memory deficits and behavioural changes, but also movement disorders or epileptic seizures can occur (2-4). However, there are no systematic studies evaluating this immune response after the acute phase of viral encephalitis and investigating whether this is correlated to neuropsychological deficits or increased brain inflammation on cranial MRI.

Objective:

The objective of this project is to identify causes of secondary deterioration or failure to improve after viral encephalitis by studying activation of the immune system and production of autoantibodies.

Study design:

We will conduct a prospective, observational, single-centre cohort study on viral encephalitis in adult patients. We will collect detailed clinical data and leftover cerebrospinal fluid. We will perform three blood withdrawals at 1, 4, 12 weeks and we will perform a study MRI after 12 weeks. Neuropsychological investigations will be performed after 1, 4 and 12 weeks, in which the clinical condition of the patient will determine the extent of the testing. After one year we will contact the patients by telephone to answer the final questionnaires.

Study population:

All adult patients with viral encephalitis proven by PCR and/or serology admitted to the Amsterdam UMC

(location AMC), and patients with proven viral encephalitis since <4 weeks, admitted to hospitals other than the Amsterdam UMC, who were referred to the ARISE study by their treating physician , are eligible for this study.

Main study parameters/endpoints:

- Proportion of patients with viral encephalitis that fail to improve or have secondary deterioration
- Clinical characteristics during admission
- Outcome as scored on the Glasgow Outcome Scale
- Neuropsychological deficits scored using: Cognitive Basic Assessment Test set (COGBAT) of the Vienna Test System (VTS), Cognitive and emotional consequences of stroke (CLCE-24), Profiles of mood states (POMS), Research and development (RAND-36), Beck depression inventory, the Montreal Cognitive Assessment (MOCA) and the mini-mental state examination (MMSE)
- Blood or cerebrospinal fluid tests on brain degradation products S100β, glial fibrillary acidic protein (GFAP), Tau and neuron-specific enolase (NSE), and the presence of anti-neuronal antibodies (LGI-1, anti-CASPR2, anti-NMDA, AMPAR, anti-GABA, anti-Gly, DPPX, anti-GAD) and cytokine levels indicative of ongoing inflammation.
- Cranial MRI abnormalities consistent with inflammation of the brain (MRI performed according to Imaging manual (appendix A).
    - **T1**
        - may show general edema in the affected region
        - if complicated by subacute hemorrhage there may be areas of hyperintense signal
    - **T1 C+ (Gd)**
        - enhancement is usually absent early in the disease

- enhancement occurs later and is variable in pattern
    - gyral enhancement
    - leptomeningeal enhancement
    - ring enhancement
    - diffuse enhancement

- **T2**
    - hyperintensity of affected white matter, or grey matter or both compatible with demyelination or inflammation.
    - hyperintensity in one or both medial temporal lobes
    - more established hemorrhagic components may be hypointense

- **DWI/ADC**
    - more sensitive than T2 weighted images
    - restricted diffusion is common due to cytotoxic edema
    - restricted diffusion is less intense compared to infarction
    - beware of T2 shine through due to vasogenic edema

- **GRE/SWI:** may demonstrate blooming if hemorrhagic

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

We will ask for three blood withdrawals in week 1, 4 and 12. Risks of a blood withdrawal are negligible. Leftover cerebrospinal fluid from the diagnostic puncture from which the virus was isolated and all subsequent lumbar punctures during clinical course will be used to study parameters of inflammation (e.g. protein, white cell count, cytokines and chemokines) and the presence of anti-neuronal antibodies. No

additional lumbar punctures will be performed for study purposes.

Cranial MRI is commonly performed in the first week of the diagnosis and within a week of deterioration as standard clinical care. Additionally, we will perform a study MRI after 12 weeks.

Neuropsychological investigations will be performed after 1, 4 and 12 weeks, in which the clinical condition of the patient will determine the extent of the testing. Filling in the questionnaires will take less than 30 minutes. The cognitive functioning will be assessed with an online assessment taking one hour. After one year, patients will be contacted by telephone to answer the final questionnaires.

1. INTRODUCTION AND RATIONALE

Encephalitis is a rapidly progressive neurological disorder caused by inflammation of the brain. The most common etiology is infection but also autoimmune encephalitis have been frequently identified (5). Acute encephalitis is characterized by headache, altered mental status, fever and seizures (1). Approximately 800 patients per year in the Netherlands develop encephalitis of which two thirds have an infectious cause and one third is caused by autoimmune or paraneoplastic disease (6). Encephalitis is a severe disease with a mortality rate of 10-20% and half of the survivors have cognitive deficits or behavioural problems (7-9). Patients with encephalitis frequently fail to improve after the initial phase of the disease has passed, and some even deteriorate weeks after the infection started (2, 3). It has been suggested that activation of the immune system leads to a secondary inflammation of the brain, which clinically presents with an increase in memory deficits and behavioural changes, but also movement disorders or epileptic seizures can occur (2-4). The theory is that due to neuronal damage and disruption of the blood-brain barrier during encephalitis neuron specific proteins enter the blood stream. Some of these proteins are thought to invoke an immune response in the blood stream that subsequently targets the recovering brain tissue resulting in new symptoms or failure to recover. Several case reports supports this theory and have described a viral induced autoimmune encephalitis, such as anti-N-methyl-D-aspartate (NMDA) receptor antibodies in patients with

herpes simplex encephalitis (HSE) and Japanese encephalitis virus (2-4). Anti-NMDAR encephalitis is characterized by a multi stage progression and manifests within weeks or, rarely months and is recognisable on these clinical grounds (4, 5). However, existing criteria for autoimmune encephalitis are still too reliant on antibody testing and response to immunotherapy, which might delay the diagnosis (5). Moreover, there are no systematic studies evaluating this immune response after the acute phase of viral encephalitis and investigating whether this is correlated to neuropsychological deficits or increased brain inflammation on cranial MRI.

Relevance for science, technology or society

Although viral encephalitis is a relatively rare disease, it strikes persons in the prime of their lives and results in long-term morbidity due to cognitive defects. Acute encephalitis constitutes a high economic burden of disease to society, costing $2.0 billion per year in hospitalization costs alone in the United States (10). For individual patients encephalitis is an important cause of functional, social and economic impairment (11).

This project will study activation of the immune system and production of autoantibodies in order to identify causes of secondary deterioration or failure to improve after viral encephalitis. We will study blood samples and cerebrospinal fluid for brain degradation products S100β, glial fibrillary acidic protein (GFAP), Tau and neuron-specific enolase (NSE). Furthermore, we will check for the presence of anti-neuronal antibodies and cytokine levels indicative of ongoing inflammation. Neuropsychological tests and questionnaires will be performed to investigate the correlation to neuropsychological deficits, and cranial MR will be performed after 3 months to evaluate brain inflammation.

This project we will perform a standardized follow-up of patients with viral encephalitis to identify the cause of secondary deterioration and failure to improve. This may lead to early identification of these patients and improved treatment strategies with immunosuppressive treatments. Furthermore, the mechanisms identified

by this project may be applicable for other infectious disease of the central nervous system. For instance in bacterial meningitis, up to 25% of patients were found to have neuropsychological sequelae that may in part be caused by prolonged activation of the immune response.

2. OBJECTIVES

Primary Objective is to:

- Identify causes of secondary deterioration or failure to improve after viral encephalitis by studying activation of the immune system and production of autoantibodies.

Secondary Objectives are to:

- Determine if the delayed inflammatory response of the brain can be predicted by clinical characteristics upon presentation, blood or cerebrospinal fluid tests and cranial imaging.

3. STUDY DESIGN

We will conduct a prospective, observational, cohort study on viral encephalitis in adult patients.
With a standardized follow up of 1, 4, 12 weeks and 1 year. This follow up time is based on previous data that described a median of 40.5 days between viral encephalitis and relapsing symptoms (4).
We will collect detailed clinical data and leftover cerebrospinal fluid. To evaluate the autoimmune mechanism, we will perform laboratory tests, neuropsychological investigation and brain imaging with MRI scan. Blood or cerebrospinal fluid tests on brain degradation products S100β, glial fibrillary acidic protein (GFAP), Tau and neuron-specific enolase (NSE), and the presence of anti-neuronal antibodies and cytokine levels indicative of ongoing inflammation.

Patients admitted to the Amsterdam UMC will have a first blood withdrawal after 1 week and when the clinical condition will allow, a neuropsychological investigation will be performed.
All patients will be asked to visit the outpatient clinic of the Amsterdam UMC in week 4 and 12 during which

blood samples will be withdrawn. Furthermore, the patient will fill in the questionnaires and performs the neuropsychological assessment, in which the clinical condition of the patient will determine the extent of the testing. In week 12 additionally a cranial MRI will be performed. After one year, patients will be contacted by telephone to answer the final questionnaires.

4. STUDY POPULATION

4.1. Population (base)

All adult patients with PCR and/or serology proven viral encephalitis admitted in the Amsterdam UMC and patients included in the I-PACE biobank that have given informed consent to participate in future research projects are eligible for this study. The patient or their legal representative will be asked for informed consent to participate in this study and provide access to clinical data.

4.2. Inclusion criteria

Patient aged 16 years or older

Viral encephalitis proven by PCR and/or serology

4.3. Exclusion criteria

1. Neurosurgical operation in the 3 months previous to the encephalitis episode
2. Neurotrauma in the 3 months previous to the encephalitis episode
3. Presence of neurosurgical devices in the central nervous system such as cerebrospinal fluid catheters or deep brain neurostimulator
4. Insufficient mastery of the Dutch language
5. Severe cognitive impairment prior to the encephalitis episode

4.4. Sample size calculation

This is an exploratory study on immune activation in encephalitis patients in which we aim to include 25 patients to get an estimate of how often this complication occurs. Based on previous data the estimated incidence of encephalitis in high-income countries is about 5-10 per 100.000 inhabitants annually (5). In a previous study of 363 patients, 29% of the patients with suspected CNS infections fulfilled the diagnostic criteria of an encephalitis, and the most common cause of encephalitis was viral infections (16%) (6). The estimated 25 patients are thought to be included in a period of 2 to 3 years.

5. METHODS

5.1. Study parameters/endpoints

- Proportion of patients with viral encephalitis that fail to improve or have secondary deterioration
- Clinical characteristics and outcome parameters
- Neuropsychological deficits scored using: Cognitive Basic Assessment Test set (COGBAT) of the Vienna Test System (VTS), Cognitive and emotional consequences of stroke (CLCE-24), Profiles of mood states (POMS), Research and development (RAND-36), Beck depression inventory, the Montreal Cognitive Assessment (MOCA) and the mini-mental state examination (MMSE).
- Blood or cerebrospinal fluid tests on brain degradation products S100β, glial fibrillary acidic protein (GFAP), Tau and neuron-specific enolase (NSE), and the presence of anti-neuronal antibodies (LGI-1, anti-CASPR2, anti-NMDA, AMPAR, anti-GABA, anti-Gly, DPPX, anti-GAD) and cytokine levels indicative of ongoing inflammation.
- Cranial MRI abnormalities consistent with inflammation of the brain

- **T1**
    - may show general edema in the affected region
    - if complicated by subacute hemorrhage there may be areas of hyperintense signal
- **T1 C+ (Gd)**
    - enhancement is usually absent early in the disease
    - enhancement occurs later and is variable in pattern
        - gyral enhancement
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- **T2**
    - hyperintensity of affected white matter, or grey matter or both compatible with demyelination or inflammation.
    - hyperintensity in one or both medial temporal lobes
    - more established hemorrhagic components may be hypointens
- **DWI/ADC**
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    - restricted diffusion is common due to cytotoxic edema
    - restricted diffusion is less intense compared to infarction
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- **GRE/SWI:** may demonstrate blooming if hemorrhagic

5.2. Study procedures

5.2.1. Inclusion procedures

Patients can be included by two procedures:

1. We will daily check if there are patients admitted to the Amsterdam UMC with proven viral encephalitis. Subsequently, patients or their legal representatives receive written information and are asked to participate and give written informed consent for participation.
2. Patients, admitted to hospitals other than the Amsterdam UMC and included in the I-PACE biobank with a proven viral encephalitis, are eligible for the study. The researchers will be informed about these patients via either the I-PACE database or the treating physician. The treating physicians will be requested by phone to ask the patients for permission to be contacted for the follow up study. Subsequently, the patients will be phoned by the researcher and receive written information by post. Written informed consent will be signed during the visit in the Amsterdam UMC.

This study will consist of laboratory investigations, questionnaires, neuropsychological evaluation and cranial MRI in patients with confirmed viral encephalitis. All patients are asked to visit the outpatient clinic of the Amsterdam UMC after discharge in week 4 and week 12. Patient admitted in the Amsterdam UMC will have a first blood withdrawal after 1 week and when the clinical condition will allow a neuropsychological investigation will be performed. Patients initially admitted to another hospital will be included from week 4. In the outpatient clinic of the Amsterdam UMC, blood samples will be withdrawn from the former patient in week 4 and 12. After this blood withdrawal, the patient will fill in questionnaires and performs the neuropsychological assessment. The neuropsychological assessment is a digital assessment and will be supervised by a medical (PhD) student or physician. In week 12 a cranial MRI will be performed.

After one year, patients will be contacted by telephone and some questionnaires to score neuropsychological deficits will be repeated. As compensation for patients visiting the AMC travel costs reimbursement and lunch will be provided.

5.3. Data collection and outcome score

The neuropsychological assessment is a digital assessment. These data will be collected in a dataset and coded with a unique number. Data in the analysis file will be pseudonymized. The online case-record form of the I-PACE biobank will be used to collect data on patients' history, symptoms and signs on admission, laboratory findings on admission, cranial imaging findings, treatment (including adjunctive treatment), clinical course, outcome and findings at discharge. Data on complications will be collected according to predefined criteria.

5.4. Collection and storage of patient specimens

Collection of cerebrospinal fluid (CSF):

Leftover CSF from the diagnostic puncture from which the virus was isolated and all subsequent lumbar punctures during clinical course will be collected and used for study purposes. No additional lumbar punctures will be performed for study purposes. Laboratory protocols with this purpose have been implemented in the hospital for the I-PACE biobank study. Leftover CSF from patients included in the I-PACE, and initially admitted in another hospital, will be stored according to the I-PACE protocol that have been implemented in the laboratory. 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.

Collection of blood:

In week 1, 4 and 12, from each participant blood samples will be withdrawn by puncture of the cubital vein, a maximum of 25ml. Blood will be processed and stored by local laboratory protocols of the participating hospitals.

5.5. Withdrawal of individual subjects

Subjects can withdraw their consent at any time for any reason if they wish to do so without any consequences.

6. SAFETY REPORTING

6.1. Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed. There are no expected serious adverse events for this study.

7. ETHICAL CONSIDERATIONS

7.1. 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.

7.2. Recruitment and consent

When the inclusion criteria are fulfilled, the patient or, if necessary, the patients representative will be asked for written informed consent, in accordance with the guidelines of the local medical ethics committee (METC). The patients or their representative will be informed in detail about the participation in the study. Patients who are included in the IPACE study will be asked for permission by their treating physician to be contacted by the study group for future research.

7.3. Benefits and risks assessment, group relatedness

Patients do not directly benefit from this study. Therapies and diagnostic approaches identified as a result of this study may be beneficial to a future episode. The risks of the study are limited to those of a venous blood withdrawal and MRI, which are minor. The maximum amount of blood withdrawn from patients during admission will be 25ml, 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. Cranial MRI is without using any ionizing radiation, and therefore risks of MRI are negligible.

7.4. Compensation for injury

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

7.5. Incentives (if applicable)

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

8. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

8.1. Handling and storage of data and documents

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 and monitor the study. Internet based remote data capture will be used for entering, managing and validating data from the investigative sites. Human material and all documents (Case Record Forms, Informed Consent forms, patient files) will be archived and stored for the next 20 years in the I-PACE biobank, in accordance to GCP guidelines.

8.2. Coding of and access to data

Patients' data will be coded with a unique number and data in the analysis file will be pseudonymized, according to the General Data Protection Regulation (GDPR) of May 2018 The patient 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 the investigators of the study, Dr. M.C. Brouwer and Drs. L. ter Horst, will have access to this list. In case of unexpected findings during the study that could indicate a certain disease, or a higher risk for a certain disease, of which treatment is available we will inform the patients.

It is possible that in the future human material and (coded) data will be shared with foreign research institutes outside of the European Union. In those countries the level of protection of privacy can differ from within the European Union because different rules apply. On the consent form patients can indicate whether or not they give permission to send their data to countries outside of the European Union.

8.3. Monitoring

Monitoring of the conduct of the study will be performed by the Clinical Research Unit (CRU).

The CRU is informed about the study and a monitoring plan will be constructed.

8.4. 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.

8.5. Annual progress report

The 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 study, serious adverse events/ serious adverse reactions, other problems, and amendments.

8.6. Temporary halt and (prematurely) 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.

8.7. 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.

9. REFERENCES

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