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Recovery of thalamic microstructural damage after Shiga toxin 2-associated hemolytic–uremic syndrome

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ABSTRACT

Introduction: The underlying pathophysiology of neurological complications in patients with hemolytic–uremic syndrome (HUS) remains unclear. It was recently attributed to a direct cytotoxic effect of Shiga toxin 2 (Stx2) in the thalamus. Conventional MRI of patients with Stx2-caused HUS revealed – despite severe neurological symptoms – only mild alterations if any, mostly in the thalamus. Against this background, we questioned: Does diffusion tensor imaging (DTI) capture the thalamic damage better than conventional MRI? Are neurological symptoms and disease course better reflected by thalamic alterations as detected by DTI? Are other brain regions also affected?
Methods: Three women with serious neurological deficits due to Stx2-associated HUS were admitted to MRI/DTI at disease onset. Two of them were longitudinally examined. Fractional anisotropy (FA) and mean diffusivity were computed to assess Stx2-caused microstructural damage.
Results: Compared to 90 healthy women, all three patients had significantly reduced thalamic FA. Thalamic mean diffusivity was only reduced in two patients. DTI of the longitudinally examined women demonstrated slow normalization of thalamic FA, which was paralleled by clinical improvement.
Conclusion: Whereas conventional MRI only shows slight alterations based on *subjective* evaluation, DTI permits *quantitative*, *objective*, and *longitudinal* assessment of cytotoxic cerebral damage in *individual* patients.

1. Introduction

Hemolytic–uremic syndrome (HUS), first described in 1955 by Gasser et al. [1], is a well-known complication of Shiga toxin (Stx)-producing *Escherichia coli* (STEC) infection. It comprises acute kidney injury, thrombocytopenia (platelet count < 150.000 cells/mm^3), and microangiopathic hemolytic anemia [2]. HUS is the most common cause of acute renal failure in childhood [3–7] and occurs predominantly in children younger than five years [2,6-11]. Between May and July 2011, the world's largest reported HUS outbreak affected northern Germany [8,12,13]. While previous outbreaks could all be traced back to the serotype O157:H7 [2,14,15], this one was caused by O104:H4 [2,12,14]. O104:H4 was characterized by the virulence properties of the typical enteroaggregative *E. coli* and the ability to produce Stx [2,14].

It was remarkable that unusually more adults (88%), primarily women (68–69%), were affected [2,8,12-14]. More people developed HUS after infection with O104:H4 as opposed to those infected with the common serotype O157:H7 (20–25% versus 10–15%) [2,4,8,15]. Neurological complications were severe and frequent (48–56%). The outcome with neurological sequelae in only 3% [13,14] was clearly better than that reported for children [3,5]. Most frequently, patients had alterations of consciousness, cognitive dysfunction (attention, orientation, working or short-term memory deficits), apraxia, dys- or aphasia, hyperreflexia, central oculomotor disturbances, visuoconstructive and psychiatric disorders, and epileptic seizures. Other symptoms were headaches and focal neurological deficits such as (extra-) pyramidal, cerebellar or brainstem symptoms [8,14].

Whereas the cause for acute kidney injury in patients with HUS due to occlusion of renal vessels by platelet–fibrin thrombi and fluid loss is well known, the underlying pathomechanism of central nervous system damage still remains enigmatic [11,16]. Multiple factors including thrombotic microangiopathy, hypertension [17],

Abbreviations: ADC, apparent diffusion coefficient; convMRI, conventional MRI; DTI, diffusion tensor imaging; DW, diffusion-weighted; DWI, diffusion-weighted imaging; EEG, electroencephalogram; EXM, examination; FA, fractional anisotropy; FLAIR, fluid attenuated inversion recovery; GM, gray matter; HC, healthy controls; HUS, hemolytic–uremic syndrome; MD, mean diffusivity; MNI, Montréal Neurological Institute; MRI, magnetic resonance imaging; N, number; NPA, neuropsychological assessment; p, probability of type I error; PLEX, plasmapheresis; POAS, position–orientation adaptive smoothing; ROIs, regions of interest; SCL-90-R, Symptom Checklist-90-Revised; SD, standard deviation; STEC, Shiga toxin-producing *Escherichia coli*; Stx, Shiga toxin; TE, echo time; TR, repetition time; T1w, T1 weighted; T2w, T2 weighted; WM, white matter; y, years.

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and secondary changes following epileptic seizures [16] have been suggested. Additionally, the following pathomechanisms have also been proposed: metabolic (azotaemia, hyponatremia with consequent osmotic derangements, and demyelination) [16], inflammatory [10,18], mixed toxic-inflammatory [11,14], mixed toxic-metabolic [8,19,20], and direct toxic effects [16]. However, the possibility that Stx may have a direct influence on neurons has not been systematically analyzed [9]. Recently, based on combinations of different experiments in Long–Evans rats, Meuth et al. found an enhanced expression of Gb3 receptors on thalamic neurons of female rats compared to other brain regions and to male animals. Using acutely prepared brain slices in this study, it was suggested that binding of Stx2 to the Gb3 receptor could directly induce neuronal and astrocytic apoptosis. Together with MRI findings and electroencephalogram (EEG) results of seven female patients suffering from neurological complications due to Stx2-associated HUS, the authors proposed that a direct cytotoxic effect of Stx2 in the thalamus contributes to the pathophysiology of neuronal complication following HUS [9].

Numerous studies using MRI have also tried to identify characteristic findings with regard to pathophysiology, diagnosis, and clinical outcome in patients with neurological impairment due to Stx-associated HUS [16,19]. However, by examination of inhomogeneous and small patient cohorts with non-standardized MRI protocols, partially retrospectively, and almost never longitudinally, no uniform or pathognomonic MRI pattern could be detected [19]. Additionally, conventional MRI (convMRI) often depicted little to no cerebral alterations despite severe neurological symptoms [5,7]. Therefore, diffusion-weighted imaging (DWI) in combination with apparent diffusion coefficient (ADC) was increasingly applied for the detection of pathological changes in those patients. However, inconsistent results were achieved by this method [7,10,11,16,19]. A few studies succeeded to identify the thalamus as "main target structure" in patients with neurological complications due to Stx2-associated HUS [8,9,13,14,16,19]. Thus convMRI including DWI of seven patients of the study of Meuth et al. [9] revealed – despite severe neurological deficits – only slight thalamic hyperintensities in T2 weighted (T2w)/FLAIR images and mildly reduced ADC values in the thalamus, whereas T1 weighted (T1w) images were inconspicuous.

Here we use diffusion tensor imaging (DTI) in three of seven patients of the study of Meuth et al. [9] to address the following objectives: (i) Can DTI better monitor thalamic alterations in *individual* patients suffering from severe neurological deficits due to Stx2-caused HUS than convMRI? (ii) Do thalamic changes captured by DTI better correlate with neurological symptoms and more precisely reflect the disease course than those detected by convMRI? (iii) Are other brain regions also affected by the disease?

2. Methods

2.1. Patients and their main demographic, diagnostic, clinical, and therapeutic features

2.1.1. Recruitment

Patients 2, 5, and 6 (mean age 34.3 years (y)) from the study of Meuth et al. [9] agreed to participate in our study. Hereafter they were referred to as patients 1, 2, and 3. These three patients were treated in the university hospital in Münster between May and July 2011. Patients 1 and 2 had no medical history. Patient 3 was frequently treated with antibiotics in 2010 and 2011 because of recurrent angina tonsillaris. Ninety neurologically and psychiatrically healthy women (age: mean 34.6 y, median 32.0 y, range 26 y–49 y, SD 7.57 y) were included as control subjects (HC = healthy controls). HC were recruited by announcements in local newspapers. All subjects gave their written informed consent to participate in this study. Data collection and follow-up examinations have been approved by the local ethics committee.

2.1.2. Diagnosis

STEC presence was confirmed by enzyme-linked immunosorbent assay in all three patients. For diagnosis of HUS at least two of the following findings had to be present: 1) platelet count ≤ 150.000 cells/mm^3, 2) microangiopathic hemolytic anemia (LDH > 240 U/l and hemoglobin < 12 g/dl) and 3) acute kidney injury. Values of laboratory-chemical blood examinations of patients 1, 2, and 3 are listed in the paper of Meuth et al. [9].

2.1.3. Clinical symptoms and their development during the course of the disease

All patients developed severe and progressive neurological deficits that are listed in detail for each patient below and in Table 1.

2.1.3.1. Patient 1. Patient 1 was admitted to our hospital with generalized convulsive status epilepticus with rightward eye deviation, right-beating spontaneous nystagmus, and bilateral Babinski sign. Despite intravenous therapy with initially lorazepam followed by levetiracetam, the seizures continued. For this reason, she was intubated and deeply anesthetized with propofol. Cerebrospinal fluid examination revealed a leakage of blood–brain barrier on initial examination, which normalized within days. EEG under deep anesthesia showed burst suppression without electrographic seizure activity. Therefore, anesthesia was stopped. Despite effective termination of status epilepticus, she was comatose and showed decerebrate posturing. EEG demonstrated generalized severe delta slowing (~1 Hz) at that moment. Due to prolonged coma and thus prolonged mechanical ventilation, a percutaneous dilational tracheostomy had to be performed. One month after onset of neurological symptoms, she was discharged from the hospital and her therapy with levetiracetam was stopped. At that moment, she was irritable and had long-term memory deficits, trouble finding words, and concentration difficulties. Additionally, psychomotor slowing, an ataxia of the left arm, and brisk deep tendon reflexes were found in a neurological examination. Two months after onset of neurological symptoms, she was weak and exhausted, and had intermittent vertigo. Two months later she was fully recovered and had no physical or cognitive deficits. EEG at that time demonstrated only an unspecific, slightly dysfunctional temporal slowing over the right temporal lobe. Five months after onset of neurological symptoms, she was able to return to her professional life as teacher.

2.1.3.2. Patient 2. Five days after onset of enteritis (bloody diarrhea, nausea, fever, exhaustion, and weakness), patient 2 had intermittent double vision, exaggerated startle response, and brachial and foot myoclonus of the left side. Additionally, she was increased irritable and showed concentration difficulties and trouble finding words. Four days after admission to our hospital, she was initially somnolent and then later stuporous. She had increased muscle tone, brisk deep tendon reflexes, hyperactive patellar reflex, and exaggerated startle response. Two days later she suffered repeatedly from generalized epileptic seizures. The Babinski sign was positive on the right side. A therapy with levetiracetam was initiated and complemented by valproic acid. She was intubated because of respiratory insufficiency and aspiration, and was reanimated because of bradycardia and hypotonia. 3 weeks after admission to our hospital, the therapy with levetiracetam and valproic acid was finished. At that moment, patient 2 was agitated and easily startled. Furthermore, she complained of visual hallucinations, vertigo, and whole body paresthesias due to touch. She demonstrated latent paresis of the left arm and a truncal ataxia with a tendency to fall to the left side. After 44 days, she was discharged from the hospital. Two months after disease onset, she was physically exhausted and had significant cognitive deficits in a foreign neuropsychological assessment (NPA). Six months later she was completely recovered and could return to her work as teacher. She only suffered from a retrograde amnesia for the time on intensive care unit. EEG recordings, which earlier demonstrated moderate generalized slowing during the onset of neurological symptoms, also normalized at that time.

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