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Neurological Sequelae in Adults After *E coli* O104:H4 Infection-Induced Hemolytic–Uremic Syndrome

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Abstract: In an outbreak of shiga toxin-producing Escherichia coli infections and associated hemolytic-uremic syndrome (STEC O104:H4) in Germany in the year 2011 neurological complications in adult patients occurred with an unexpected high frequency (up to 100%). Little is known about the long-term effects of these complications. Therefore, we performed follow-up examinations on 44 patients treated for STEC-HUS at Hannover Medical School in this observational study.

We performed standardized follow-up examinations including neurological and neuropsychological assessments, laboratory testing, magnetic resonance imaging, and electroencephalographies. Subgroups were examined 2 (n = 34), 7 (n = 22), and 19 (n = 23) months after disease onset. Additionally, at the 19-month follow-up, the quality of life, sleep quality, and possible fatigue were assessed.

Nineteen months after the disease onset 31 patients were reassessed, 22 of whom still suffered from symptoms including fatigue, headache, and attention deficits. Only 39% had normal neuropsychological assessments. Sixty-one percent of the patients were in the borderline or pathological range. At follow-up, there was a secondary decline of cognitive function in about one-quarter of the patients. The outcome was not related to acute phase treatment or laboratory data or the length of hospitalization.

Prognosis of STEC-HUS associated brain dysfunction in adults with regard to severity of symptoms is mostly good. However, some Patients' caretakers have to be aware of possible secondary decline of brain function as was observed in this study.

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INTRODUCTION

In May 2011, an unusual serotype of *Escherichia coli* (O104:H4) caused an outbreak of diarrhea-associated hemolytic–uremic syndrome (HUS) in Germany. This strain combined the virulence potentials of 2 different pathotypes: enterohemorrhagic *E coli* with Shiga-Toxin 2 (Stx) production (also called Shiga toxin-producing *E coli* or STEC) and enteroaggregating *E coli* with aggregative adherence to endothelial cells.[1] The combination of the prophage encoding Stx and multiple resistance factors probably caused the spread of the outbreak and its severity.[2] The Robert Koch Institute, the federal public health institute in Germany, registered 855 cases of confirmed STEC O104:H4 infection with HUS in addition to 2987 pure gastrointestinal infections. Twenty-two percent of the adult patients affected developed HUS.[3] In this group, more than half had involvement of the central nervous system.[4,5] The majority (58%) met the ICD-10: F06.8 criteria for a mental disorder.[6] At Hannover Medical School 48 patients with STEC-HUS were treated between May and July 2011, 47 of whom displayed neurological symptoms varying from slight headaches or trouble finding words, to severe alterations of consciousness, epileptic seizures, and need for mechanical ventilation.[5] Although the majority of patients made a rapid recovery, some patients still report impairment of their daily activities and cognitive skills.

In the current study, we performed standardized long-term follow-up examinations including clinical, neuropsychological, and neuroradiological assessments in adult STEC-HUS patients after infection with *E coli* O104:H4 serotype to more completely describe and classify the neurological sequelae.

SUBJECTS AND METHODS

Of the 48 adult STEC O104:H4-HUS patients, treated at the Hannover Medical School between May and July 2011, 44 were examined by a neurologist during the acute phase of the disease (T1) and/or after and thus were considered eligible for the study (Figure 1). Patients gave written informed consent for subsequent examinations and testing. About 2 months after the onset of symptom (T2, median 51 days, range 33–124 days), 34 patients underwent an initial follow-up examination, during which 28 underwent electroencephalography (EEG). After 7 months (T3, median 203 days, range 168–241), 22 patients underwent a second follow-up examination. Magnetic resonance imaging (MRI) was obtained on 20. Between 17 and 27 months after symptom onset (T4, median 576 days, range 524–820 days), 31 patients answered standardized self-report questionnaires, of whom 23 underwent the third clinical and neuropsychological follow-up examination. EEG was performed in 20 and MRI in 13 of these 23 patients (Figure 1, Table 1).

The neuropsychological assessments at each follow-up examination included the tests ''alertness,'' ''divided attention,'' ''orienting,'' ''attention shift,'' and ''working memory'' of the test battery for the assessment of attention,[7] the word-figure-memory test,[8] Luria list of words,[9] the Recurring Figures Test,[10] and the Rey–Osterrieth Complex Figure

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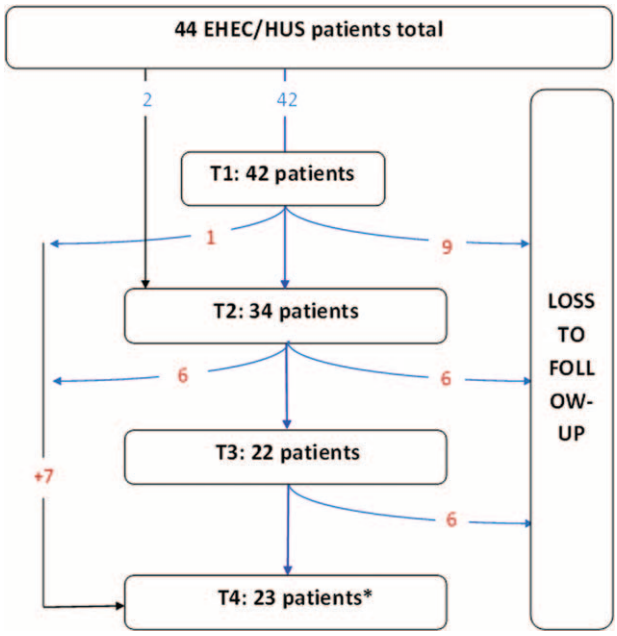

FIGURE 1. Diagram of loss to follow-up; T1 acute phase of infection, T2–T4 follow-up visits; left side: patients who missed 1 of the examinations inbetween (T1–T3), but long-term examination is (T4) available; right side: patients who left the study at this level due to different reasons, no long-term examination is available; *only patients with neuropsychological assessment considered.

Test.[11] The Mini Mental Status Examination (MMS) was also performed. Individual test results were evaluated according to normative data and converted to percentile rank (PR). The 10th percentile was used as a cutoff between normal and abnormal results (accordingly z scores ≤ -1.3), and scores lower than that were considered clinically relevant.

The EEG was recorded according to the International 10-20-System using the EPAS Harmony System (Schwarzer, Germany). MRI of the brain was performed using a 1.5-Tesla Avanto or a 3-Tesla Verio (Siemens Medical Systems, Erlangen, Germany) and identical imaging parameters as in.[5]

Additionally at T4, patients answered the Epworth Sleepiness Scale (ESS),[12,13] the Pittsburgh Sleep Quality Index (PSQI),[14,15] the Fatigue Impact Scale (FIS),[16,17] the Hospital Anxiety and Depression Scale (HADS),[18,19] and the Short Form-36 Questionnaire (SF-36).[20] All psychometric tests were completed in the German version and assessed using German norms. Furthermore, the patients answered a questionnaire addressing current symptoms, job status, and current medication.

The ESS is a very short test to measure daytime sleepiness. Each item is rated on a 4-point scale; 10 points being the cutoff score for pathological daytime sleepiness (min 0, max 24). The PSQI is a subjective measure of sleep quality in the 4 weeks before completing the form. The patients answer 17 questions assessing sleep quality, latency, duration, efficiency, disturbance, use of sleeping medication, and daytime dysfunction. A score ≥ 5 is suggestive of a significant sleep disturbance (min 0, max 21).

The FIS is a self-report scale to measure the impact of fatigue upon patients' daily activities. It contains 40 items, each scored on a scale from 0 to 4. Sixty is the cutoff for pathological fatigue (min 0, max 160).[16] The HADS serves for the detection of depression and anxiety in patients with internal and/or psychosomatic diseases. It is a 14 items scale, each scored from 0 to 3. A score ≥ 11 indicates anxiety or depression (min 0; max 21).

The Short-Form Questionnaire is a survey with 36 questions to measure health-related quality of life. It provides scores for 8 health domains, which can be summarized as a physical, and a mental score. As a cutoff, a value outside the 2-fold standard deviation was chosen.

Follow-up visits T2 and T3 included an analysis of serum creatinine, urea, glomerular filtration rate (GFR), C-reactive protein, white blood cell count, and platelet count. At T1 S100-

TABLE 1. Distribution of Age and Sex in Study Subjects and Performed Examinations at Different Follow-Up Visits

Visit	T1	T2	T3	T4
Time since symptom onset, d	11 (5–28)	51 (33–124)	203 (168–241)	576 (524–820)
Number of patients	42	34	22	23
Age (median; range)	43 (17–82)	41.5 (18–75)	38.5 (18–68)	44 (19–75)
Sex (%)				
Female	73	76	86	74
Male	27	24	14	26
MRI (number of patients)	26	0	20	13
EEG (number of patients)	35	28	0	20
Neuropsychological assessment (no. of patients)	42	34	22	23
Percentage of patients who belonged to groups				
1	43	47	41	48
2	21	24	27	26
3	36	29	32	26
Considering grouping at baseline (5)				

EEG = electroencephalography, MRI = magnetic resonance imaging, MRS = modified ranking scale; for T4 only patients who underwent a clinical examination are considered in this table. MRI was performed at T4 only in those patients who had shown structural alterations before or who complained about new symptoms, T1 = acute phase between May and July 2011, T2 = 3 months after T1, T3 = 7 months after T1, T4 = 18 months after T1.

B and neuron specific enolase (NSE), both indicators of brain damage, were measured. Peak levels were selected and used for subsequent correlation analyses. All biochemical markers were determined using commercially available CE (Communautés Européennes)-certified reagents.

To identify prognostic variables, patients were subdivided into groups with good or poor outcome according to their SF-36 results and their answers to the questionnaire regarding current symptoms. Patients with remaining neuropsychiatric handicaps, reduced quality of life and/or >2 abnormal test results (PR $\leq$ 10) and/or abnormal results in the assessment after completely normal performance in earlier follow-up visits were assigned to the poor outcome group (n = 15). Those with no or only slight subjective symptoms without relevance to daily activities and less than 3 abnormal results were sorted into the good outcome group (n = 16).

Statistical Analysis

For comparison of outcome groups, the Mann–Whitney–Wilcoxon test was used for continuous data, the χ^2, and the Fisher exact test for categorical data, as appropriate. The Friedman test was used for comparison of the different points in time. Analyses were performed using the SPSS software package 21 and 22. $P < 0.05$ was considered statistically significant. The local ethics committee approved data collection and follow-up examinations.

RESULTS

Group Characteristics and Baseline Data

Data at the time of the initial examination, T1, have been reported.[5] Of the 47 STEC-HUS patients with neurological symptoms seen at the onset of their disease at Hannover Medical School, 42 underwent a standardized neurological assessment. Nine of these did not attend any of the neuropsychological follow-up visits (T2–T4): 1 patient died, 1 was transferred to a rehabilitation clinic with a posterior reversible encephalopathy syndrome, and 4 refused participation in the neuropsychological assessment. At T4 3 patients, who lived quite far from our clinic, did not come in for the examinations, but sent us the questionnaires via mail (see Figure 1).

At baseline (T1), patients were subdivided into 3 groups according to their worst neurological status during the hospital stay[5]: group 1 consisted of 18 patients (43%) who had presented with a normal MMS and normal Glasgow Coma Scale score but suffered from slight clinical signs such as hyperreflexia, dysmetria, dysphasia, or slight headache. Nine patients (21%) who had been alert, but had scored <28 points in the MMS were assigned to group 2, group 3 comprised 15 patients (36%) who had presented with alterations of consciousness during the acute phase. Two patients wanted to take part in the follow-up examinations who were not examined by a neurologist at T1 because they had been treated in another hospital in the acute phase. These 2 patients were retrospectively assigned to group 1 based on their medical history. At the follow-up examinations the distribution of these 3 groups remained the same as at T1 (Table 1).

Results of Neuropsychological Assessment, Laboratory Data, MRI and EEG at T2–T4

Details about the number of patients who participated into the follow-up examinations are given in Table 1. Two months after disease onset (T2) 29% of the patients (10/34) had normal results (PR > 10) in all subtests of the neuropsychological assessment, 62% scored at a borderline or lower level (PR $\leq$ 10) in 1 or 2 and 9% in >2 subtests. Sixty-five percent of the patients (22/34) reported slight neurological symptoms—mainly dysphasia, chronic fatigue, and headache. However, the neurological examination revealed no deficits in all patients. EEG was abnormal in 2 patients (7%), with general slowing in 1 and focal slowing in the other. Both had an abnormal EEG at T1 as well. Renal function was impaired in 35% (GFR level < 60 mL/min).

At T3, 59% (13/22) of the patients scored in the normal range on the neuropsychological assessment (PR > 10), 27% scored at a borderline or lower level (PR $\leq$ 10) on 1 or 2 subtests and 14% on >2. A majority of the patients (64%) reported neurological symptoms, mainly fatigue, and concentration deficits. Three still had impaired renal function (GFR < 60). White matter lesions, diagnosed in the acute phase on diffusion weighted MRI, had resolved completely. However, 11 of 20 studies showed at least 1 new microangiopathic lesion compared with baseline. Two patients had 8 or more new microangiopathic lesions.

At T4, 39% (9/23) of the patients scored in the normal range on the neuropsychological assessment, 44% scored at a borderline or lower level on 1 or 2 subtests and 17% on >2. 71% of the patients complained about slight neurological symptoms. Cognitive impairment and attention deficits were reported with the highest frequency (37% and 43%). The EEG was still abnormal in 1 patient with general slowing (theta waves). Her neuropsychological assessment was worse at T4 than at T3. The MRI showed an increase in the number of microangiopathic lesions in 2 cases, and no change in 9. In 2 patients who received a MRI at T4 for the first time the MRI was normal.

Table 2 summarizes the results of the neuropsychological assessment of 16 patients, who attended all follow-up visits. These 16 patients ''attention ability'' improved significantly over time, although they had a decrease in their verbal learning ability and their visuoconstructional ability at T4. Memory retrieval improved over time in the Rey Complex Figure Test, whereas there was no change in the recognition of words or figures in the word-figure-memory test, and no change in the recollection of words in the Luria List of Words test.

Change of Cognitive Function Between T2 and T4

In the follow-up examinations, some of the patients reported new symptoms at T3 and T4, with memory decline and loss of visual function being the most frequently mentioned. At T4, the performance on the neuropsychological assessment declined in 7 of the 23 patients tested compared with earlier examinations. Figure 2 shows the percentage of normal, borderline, and abnormal test results in 16 patients who underwent all follow-up examinations. The mean number of abnormal test results per patient declined between T2 and T3 (1.44–0.88) and increased between T3 and T4 (1.13) in these 16 patients. The total number of abnormal test results increased on memory function tests and decreased on attention tests between T2 and T4 (Figure 3). Significant improvement was seen between T2 and T3 in the Rey–Osterrieth Memory Test ($P = 0.017$) and in the attention shift test ($P = 0.001$). Between T2 and T4, significant improvement was seen in the simple reaction time in the alertness test ($P = 0.026$), the number of errors in the working memory test ($P = 0.035$), and 3 subtests of the orienting test (left-left $P = 0.024$, left-right $P = 0.001$, right-right $P = 0.008$). In Luria List of Words (sum score), however, patients scored

TABLE 2. Results of the Neuropsychological Assessment of the 16 Patients Who Attended All 3 Follow-up Visits, for the MMS, Luria List of Words, WFMT, RFT and Rey–Osterrieth a Higher Score Is Improvement, for the Subtests of the TAP-battery a Lower Score Is Improvement *P*-value, Using the Friedmann Test for Comparison of the 3 Different Points in Time

	T2	T3 7 mo	T4 18 mo	*P*
MMS	29.2 ± 1.3	29.4 ± .7	28.7 ± 1.5	0.47
Luria				
Run 1–5 (sum)	43.8 ± 3	46.6 ± 2.4	44 ± 2.8	<0.001*
Run 6/5 (quotient)	.89 ± .1	.91 ± .1	.87 ± .1	0.25
WFMT				
Words	13.13 ± 5.0	12.44 ± 4.5	14.13 ± 4.8	0.16
Figures	16.38 ± 4.9	17.50 ± 3.7	17.19 ± 4.9	0.16
Concrete items	16.00 ± 4.4	16.61 ± 3.6	17.31 ± 3.6	0.19
Abstract items	13.50 ± 4.4	13.31 ± 4.3	14.00 ± 4.9	0.27
RFT				
Geometrical	17.81 ± 2.1	17.94 ± 2.1	18.19 ± 2.3	0.51
Nonsense	5.56 ± 5.2	6.19 ± 6.4	7.5 ± 6.1	0.41
Rey–Osterrieth				
Copy	35.75 ± .4	35.56 ± 1.1	35.06 ± 1.3	0.03*
Memory	23.60 ± 5.5	27.16 ± 5.3	26.66 ± 5.8	0.005*
TAP				
Attention shift RT, ms	925.8 ± 838	584.0 ± 114	604.3 ± 131	0.001*
Attention shift errors	2.06 ± 2.8	1.19 ± 1.8	1.94 ± 2.3	0.36
Div.attention visual RT median, ms	790.8 ± 119	758.4 ± 91	745.0 ± 81	0.14
Div.attention auditory RT median, ms	603.1 ± 125	608.1 ± 72	581.5 ± 96	0.87
Div.attention errors	1.0 ± 1.5	.6 ± 1.3	.7 ± .9	0.63
Div.attention misses	1.8 ± 3.1	1.1 ± .9	.9 ± 1.3	0.39
Alertness simple RT, ms	315.2 ± 167	256.6 ± 36	215.1 ± 39	0.047*
Alertness warned RT, ms	302.1 ± 142	261.8 ± 48	247.1 ± 38	0.06
Phasic alertness	.031 ± .073	−045 ± .156	.016 ± .083	0.11
Working memory RT, ms	652.7 ± 188	581.7 ± 121	591.3 ± 122	0.17
Working memory errors	2.4 ± 4.5	.9 ± 1.9	.7 ± 1.0	0.04*
Working memory misses	2.2 ± 3.2	1.6 ± 2.0	1.9 ± 2.1	0.91
Orienting left-left, ms	376.2 ± 246	308.2 ± 57	291.7 ± 66	0.02*
Orienting left-right, ms	456.8 ± 397	339.3 ± 60	321.4 ± 73	0.001*
Orienting right-left, ms	471.9 ± 419	362.1 ± 73	354.2 ± 71	0.47
Orienting right-right, ms	392.2 ± 275	304.3 ± 51	298.9 ± 71	0.01*

Div. = divided, FU = follow-up, RFT = Recurring Figures Test, RT = reaction time, s = signal, T1 = acute phase between May and July 2011, T2 = 3 months after T1, T3 = 7 months after T1, T4 = 18 months after T1, TAP = test battery for the assessment of attention; WFMT = word-figure-memory test.

* Significant differences (*P*-value <0.05).

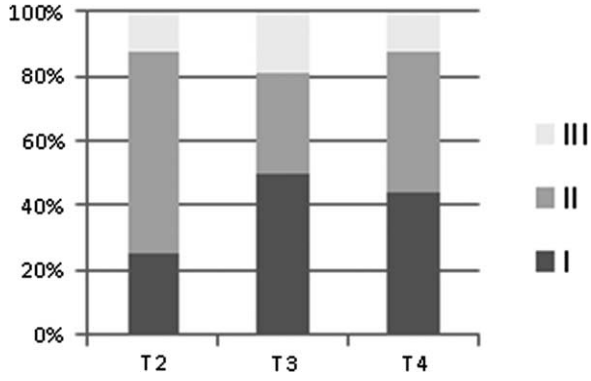

FIGURE 2. Results of the neuropsychological assessment for the 16 patients who attended all 3 follow-up visits, I normal result in all subtests (PR > 10), II 1-2 subtests with abnormal results (PR ≤ 10), III > 2 subtests with abnormal results (PR ≤ 10).

significantly worse at T4 compared with T3 ($P = 0.002$), whereas they had significantly improved from T2 to T3 ($P = 0.001$) (for subscores see Figure 4). In the MMS, which was the only test we could perform in the acute phase (MMS (T1) mean: 21.0 ± 11.2), the score significantly improved between T1 and T3 ($P = 0.013$).

Results of Standardized Questionnaires (T4)

At T4 patients, complained of headache (23%), loss in physical fitness (30%), chronic fatigue (30%), sleeping disorders (23%), dysphasia (23%), gait disorder (30%), attention-deficit (43%), visual disturbances (27%), and cognitive impairment (37%). However, only 1 patient reported having lost her job due to the aftereffects of the disease. One of the 31 patients did not answer this questionnaire.

The ESS and PSQI scores indicated that 55% of the patients had significant sleep disturbances (ESS score ≥10 and/or PSQI score ≥5) at T4 and 19% were affected by fatigue in their daily life (FIS > 60). One of the patients had an

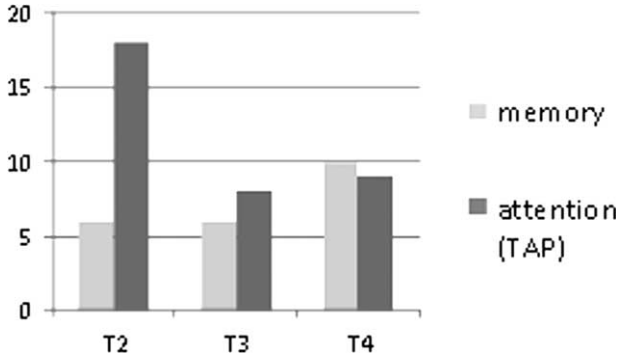

FIGURE 3. Number of abnormal test results, attributed to the main functions tested: ''memory and attention'' at the 3 follow-up examinations for the 16 patients.

abnormal depression score in the HADS, and another 1 showed an abnormal anxiety score (>10); 2 of the 31 patients did not complete the HADS questionnaire. In the SF-36 1 patient scored abnormal results in the physical sum score and 4 patients in the mental sum score (<2 SD).

Outcome Parameters

The poor (n = 15) and the good outcome group (n = 16), as defined in subjects and methods, did not differ regarding age or sex nor with regard to questionnaire results other than the SF-36 (Table 3). Furthermore there were no significant differences in laboratory results such as the minimum platelet count, maximum white blood cell count, S100max, neuronspecific enolase at T1, minimum serum sodium at T1, GFR levels at T1, T2 or T3, and length of hospital stay and MMS at T1. Moreover, there were no differences regarding treatment at T1 (tested for immunoadsorption, plasma exchange, Eculizumab). By defining the outcome groups only according to the patients' subjective impairment (at least 2 symptoms at T4 which affect daily activities), the poor outcome group (n = 12) was significantly older ($P = 0.010$) and more severely affected in the acute phase ($P = 0.010$).

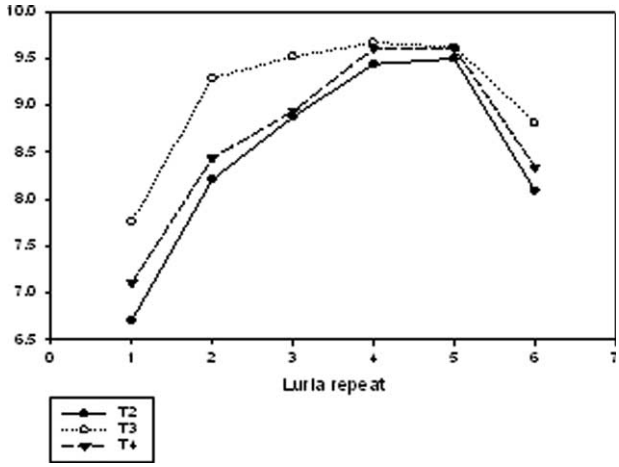

FIGURE 4. Recalled items of Luria List of words for run 1–6 at the 3 follow-up visits. Significant differences between the 3 points-in-time could be observed for Luria 1 ($P = 0.013$), Luria 2 ($P = 0.009$) and for the sum score ($P < 0.001$, not illustrated in the graph). Data are presented as mean.

DISCUSSION

This study presents follow-up data from a cohort of adult patients from the German STEC O104:H4 induced HUS outbreak in 2011 2, 7, and 19 months after the disease onset. Overall, the psychometric test results showed an improvement over time. However, more than half of the patients scored at borderline or abnormal levels on a formal neuropsychological assessment 19 months after disease onset. The percentage of patients who complained about neuropsychiatric symptoms and the percentage of patients who scored at borderline or lower level in the neuropsychological tests increased at 19 months after an intermittent improvement at 7 months compared to 2 months after disease onset.

The main unexpected features of the 2011 outbreak were the high rate of adult patients (approximately 88%) and the frequent neurological impairment.[4,5] Until now little was known about the long-term course of STEC-HUS in adults. The higher rate of neurological involvement in the acute phase in adults compared with children (20%–30% versus 48%–100%)[4,5,21,22] suggests the importance of long-term monitoring of neurological sequelae in addition to renal function.

In children, neurological sequelae of STEC-HUS are reported in only 4% of all patients, but 50% of those with initial neurological complications have a neurological sequelae after 4 to 7 years.[23] Most frequent sequelae are hemiparesis, cortical blindness, and epilepsy.[24,25] In contrast none of our adult patients had seizures or clinical signs such as paresis or aphasia in the long-term follow-up. Our patients (27%) reported visual disturbances similar to those previously observed in preschool children with the disorder.[26,27] One of our patients developed a bilateral anterior ischemic optic neuropathy with persistent visual field defects and decrease of visual acuity to 70%.

The white matter lesions that had been observed especially in diffusion-weighted magnetic resonance images of the brain in about half of the patients had resolved after 7 months (T3). In another cohort from this outbreak, the MRI showed persistent lesions in 40% of the patients 37.1 ± 24.1 days after the infection.[28] This discrepancy suggests a recovery between 2 and 7 months after HUS onset. Detailed data are missing. Several of our patients complained of limitations in daily tasks including leading a professional discussion, handling things simultaneously (''multitasking'') or doing mental calculations. These complaints are reflected in the reductions we observed in verbal learning and visuoconstructional ability at T4.

So far only a few studies have addressed cognitive impairment after HUS. In 2 pediatric studies no significant cognitive impairment was detected 6 and 12 months after diarrhea-associated HUS although a comprehensive neuropsychological test battery was applied.[25,29] Recently, early treatment with eculizumab has been suggested to improve neurological outcome in children.[30]

A recent study that assessed 20 adult patients 3 months and 1 year after the acute disease, reported cognitive impairment in almost half of the patients 1 year after infection.[31] Like in our study fatigue, psychomotor slowing and concentration problems were reported frequently. Neuropsychological assessments were performed at 1 year only in those patients and in those tests where results below average had been observed in the first follow-up 10 to 30 weeks after disease onset. In our study, the complete neuropsychological assessment was repeated at every follow-up. Therefore, we were able to detect a secondary decline in performance after an initial improvement in about one-quarter of our patients. This biphasic course is a new aspect

TABLE 3. Results of the Questionnaires, n = 31, Except for the HADS (n = 29)

Test	Patients (Poor Outcome)	Patients (Good Outcome)	Norm	*P*
ESS	7.0 ± 3.6	6.73 ± 3.4	5.7 ± 3	0.892
FIS	20.0 ± 42.11	38.47 ± 20.34	26 ± 22	0.338
PSQI	6.20 ± 4.6	4.79 ± 2.9	3.3 ± 1.8	0.682
HADS A	4.33 ± 0.6	3.79 ± 3.4	4.7 ± 3.5	0.780
HADS D	3.73 ± 3.6	2.36 ± 2.6	4.7 ± 3.9	0.354
SF-36 physical functioning	88.67 ± 11.3	97.14 ± 5.8	85.71 ± 22.10	0.037*
SF-36 role physical	68.33 ± 34.7	96.43 ± 9.1	83.70 ± 31.73	0.078
SF-36 bodily pain	77.47 ± 23.2	91.29 ± 13.1	79.08 ± 27.38	0.163
SF-36 global health	61.87 ± 22.2	83.00 ± 14.6	68.05 ± 20.15	0.004*
SF-36 vitality	57.67 ± 22.5	62.5 ± 18.9	63.27 ± 18.47	0.682
SF-36 social functioning	86.67 ± 21.4	87.5 ± 17.7	88.76 ± 18.40	0.861
SF-36 role emotional	75.56 ± 38.8	92.86 ± 14.2	90.35 ± 25.61	0.520
SF-36 mental health	74.93 ± 20.2	76.29 ± 13.1	73.88 ± 16.38	0.830
SF-36 physical component summary	48.74 ± 6.9	56.32 ± 4.1	50.21 ± 10.24	0.008*
SF-36 mental component summary	49.28 ± 7.4	50.12 ± 11.8	51.54 ± 8.14	0.487

ESS = Epworth Sleepiness Scale, FIS = Fatigue Impact Scale, for the FIS norm data from a big Austrian cohort were used,[23] HADS = Hospital Anxiety and Depression Scale, PSQI = Pittsburgh Sleep Quality Index, SF-36 = Short Form-36 questionnaire; Data are presented as median (interquartile range).

* $P < 0.05$ is considered statistically significant.

of STEC-HUS in adult patients and must be considered when making plans for follow-up care.

Secondary decline after initial improvement has been described several times for renal function in children with HUS,[22,32–34] but the mechanism behind is still unknown—as it is for secondary cognitive decline.

Neurological symptoms in the acute phase of the disease are thought to be due to Shiga-toxin induced neuronal damage or antibody-related neuroinflammation. The latter hypothesis is supported by the delayed onset of neurological complications and their excellent response to immunoadsorption.[35] A systemic inflammatory response to the infection may play a role as well. Serum IL-6, soluble tumor necrosis factor receptor 1 (sTNFR1) and tissue inhibitor of metalloproteinase-1 levels are elevated in HUS encephalopathy compared with HUS alone.[36] Proinflammatory cytokines and especially tumor-necrosis-factor (TNF)-alpha are known to induce neurodegeneration directly through signaling death pathway of TNF-α/p55 TNF receptor-1 in neurons.[37]

Stx consists of an enzymatic subunit A and 5 receptor-binding B subunits, which bind to the glycolipid receptor globotriaosylceramide (Gb3) on the surface of endothelial cells and neurons, whereupon Stx enters the cell by endocytosis. Subunit A inactivates protein synthesis and induces cell death. By damaging endothelial cells, Stx impairs the blood–brain barrier function, thereby getting access to brain cells as well.[38] Animal experiments showed microglial activation and neuronal lesions with focal dendritic thickening and swelling in response to Stx.[37,39,40]

The hippocampus and the basal ganglia appear to be particularly vulnerable to Stx. Intravenous administration of sublethal doses of Stx in mice showed a correlation between neurological symptoms assessed by motor behavioral tests and the damage observed in the striatum and the hippocampus via transmission electron microscopy.[40] Interestingly, we were able to show microstructural alterations in the basal ganglia during the acute phase of STEC-HUS using quantitative MRI.[41] In contrast, postmortem examination of the brain of five STEC-HUS patients from the 2011 outbreak did not reveal any endothelial or neuronal injury, but upregulation of the Stx receptor CD77/Gb3, a higher neuronal expression of interleukin 1β and slight microglia activation.[42] Thus, a possible mechanism of secondary brain damage after the acute phase of the disease remains elusive.

One limitation of our study was the small sample size, which precluded multivariate analyses. However, we were able to follow-up a very valuable subgroup of 16 patients by performing extensive examinations at all time points, to understand the specific time course of the disease. The lack of baseline data is a further limitation of our study, but cannot be avoided.

Future studies should address chronic microstructural alterations or ongoing microglial activation as possible causes of the 2-phasic course of cognitive dysfunction in adult STEC-HUS patients.

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