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BRIEF REPORT

Pathological Cerebrospinal Fluid Findings in Patients With Neuralgic Amyotrophy and Acute Hepatitis E Virus Infection

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There is growing evidence that hepatitis E virus (HEV) infection can present with extrahepatic manifestations including neurological disorders. Among these, neuralgic amyotrophy (NA) has been reported to occur in some industrialized countries. We investigated 35 patients with NA and a control group for markers of HEV infection. Acute HEV infection was found in NA patients only and was associated with an inflammatory response in the central nervous system. Shedding of HEV RNA into the cerebrospinal fluid and intrathecal production of anti-HEV immunoglobulin M occurred in 1 patient, suggesting that HEV is neurotropic.

Keywords. hepatitis E virus; neuralgic amyotrophy; cerebrospinal fluid; control group.

Neuralgic amyotrophy (NA) is an acute, painful disorder, which typically affects the brachial plexus. The pathogenesis is largely unclear but assumed to be immune-mediated. Of note, preceding events suggestive of infections have been reported by 43% of patients [1]. There is growing evidence that hepatitis E virus (HEV) infection is associated with NA and other neurological disorders (eg, Guillain-Barré-syndrome, myelitis, and encephalitis) [2]. In particular, a recent study from the United Kingdom and the Netherlands found acute HEV infection in 10% of NA patients, possibly indicating a causal association [3]. This is further supported by experimental data using animal models indicating that HEV is neurotropic [4, 5]. However, data on HEV and NA from other geographic regions and a systematic analysis of cerebrospinal fluid (CSF) are still lacking.

HEV genotype 3 is endemic in Europe and the United States and associated with zoonotic and foodborne transmission. Clinically, most infections remain asymptomatic or present as generally mild and anicteric disease. Unlike previously thought, HEV infection is rather common but seroprevalence rates vary considerably and range between 12% and 49% among adults in Europe [6].

In this study, we aimed to describe the prevalence of HEV infection in a cohort of well-defined NA patients and a control group from southwestern Germany and to systematically analyze the CSF. Our results support the notion that HEV is neurotropic and can cause NA.

METHODS

Patients

Paired CSF and serum samples of 29 NA patients collected between 2000 and 2010 were retrospectively analyzed [7]. In 2016, another 6 NA cases (2014–2016) were identified after a systematic electronic database search but, between 2010 and 2014, no cases with sufficient leftover volume were available. Samples were stored at –80°C until use. All patients fulfilled the modified diagnostic criteria for hereditary NA (apart from the hereditary criterion) of van Alfen [8]. Paired CSF and serum samples of 37 patients with laboratory confirmed VZV-associated radiculitis between 2005 and 2013 served as controls [7]. Clinical data were obtained from the participant's medical records. The study was approved by the local ethics committee. All participants provided their written consent.

HEV Testing

All serum samples were tested for anti-HEV immunoglobulin M (IgM) and immunoglobulin G (IgG) using the *recom*Well HEV IgG and IgM test (Mikrogen, Neuried, Germany) according to the manufacturer´s instructions. Samples with borderline results were retested using the *recom*Line HEV IgG/IgM test (Mikrogen). Nucleic acid extraction of all serum and CSF samples was done using the QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany). All serum and CSF samples were analyzed for HEV RNA using the RealStar HEV reverse-transcription polymerase chain reaction (PCR) kit (Altona Diagnostics, Hamburg, Germany). HEV RNA–positive samples were genotyped by amplifying a 242-bp fragment of the HEV open reading frame 1 region as described previously [9].

HEV Antibody Index Determination

Paired CSF and serum samples of patients tested positive for anti-HEV IgG and/or IgM in serum were (re-)tested using the *recom*Well HEV IgG and IgM test. Optical density values of paired samples between 1.3 and 0.5 and which differed no more than 0.3 were included in the evaluation. Antibody indices (AIs) were calculated according to Reiber and Lange [10]. Additional details can be found in the Supplementary Data.

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Case Definitions

A case of acute HEV infection was defined by the presence of anti-HEV IgM and/or a positive HEV RNA in serum. Resolved HEV infection was defined by the detection of anti-HEV IgG only and a negative HEV PCR.

Statistical Analysis

Categorical data were analyzed using Fisher exact test and odds ratio; ordinal and interval data were tested using the Mann–Whitney *U* test. A *P* value of < .05 was considered statistically significant. All statistical analysis was performed using IBM SPSS Statistics version 22 (IBM).

RESULTS

Demographic Findings

A total of 72 patients were included in this study and 35 fulfilled the case definition for NA. NA patients were slightly younger (median, 54 years [range, 22–80 years]) than control cases (median, 59 years [range, 19–76 years]). There was no difference between the 2 groups in sex (26 males among cases and 20 males among controls).

HEV Seroprevalence

Of the NA patients, 11% (4/35) had laboratory evidence of acute HEV infection and 37% (13/35) were anti-HEV IgG positive only, a pattern typically seen in resolved HEV infection (Table 1). Overall, anti-HEV IgG was detected in 49% (17/35) of the NA patients. The mean anti-HEV IgM concentration in acute cases was 139.7 AU/mL (95% confidence interval [CI], 56.4–222.9 AU/mL) and the mean anti-HEV IgG concentration in resolved HEV infection was 78.1 AU/mL (95% CI, 50.5–105.7 AU/mL; Supplementary Figure 1). HEV-PCR in serum was positive in 3 of 4 anti-HEV IgM–positive patients. HEV RNA concentrations were 11 IU/mL (case 1), 420 IU/mL (case 2), and 43 000 IU/mL (case 3). In addition, 870 IU/mL was detected in the CSF of case 2. Genotyping of HEV was successful in 2 patients and showed genotype 3c (case 2) and 3f (case 3). In 1 sample (case 1), genotyping failed most likely due to the low viral RNA concentrations.

In the control group, 1 patient showed evidence of modestly elevated anti-HEV IgM reactivity with a negative anti-HEV IgG and negative HEV RNA (Supplementary Figure 1). This result was considered false positive. Thus, none of the control patients showed evidence of acute HEV infection (0% vs 11% of NA patients; odds ratio [OR], 10.71; Table 1). An association with overall anti-HEV IgG seropositivity was observed for the NA group compared with the control group (49% vs 24%; OR, 2.94), but not for resolved HEV infection (37% vs 24%; OR, 1.84). Of note, in 2 patients of the control group the anti-HEV IgG reactivity was in the gray zone of the *recom*Well assay with 21.6 AU/mL and 20.1 AU/mL, respectively (Supplementary Figure 1). Retesting with the *recom*Line assay confirmed anti-HEV IgG reactivity in 1 sample (21.6 AU/mL) but was negative in the other sample, indicating unspecific reactivity. None of the serum and CSF samples of the control group tested positive for HEV RNA.

CSF Findings

We calculated the AI for anti-HEV IgG and IgM to detect intrathecal synthesis of HEV-specific antibodies. One patient (case 2) of the NA group with acute HEV infection showed a highly pathological AI of 34.4 for anti-HEV IgM, demonstrating intrathecal synthesis of antibodies (Table 2). All other patients for whom AI calculation was feasible showed AI ≤1.5, consistent with no evidence of specific intrathecal antibody production.

Next, 75% of the acute HEV-associated NA cases showed a slightly elevated white blood cell (WBC) count in the CSF (5–9 WBC/µL). The elevated WBC in CSF was more common in acute HEV-associated NA cases compared with HEV-negative NA cases (75% vs 6%; *P* = .01) (Supplementary Table 1). Of the HEV-associated NA patients, 50% showed a slightly elevated protein level without increased albumin quotient (Table 2). In HEV-negative cases, CSF showed pathological findings (elevated WBC and/or protein) in 39% (7/18) and only 1 patient had a pleocytosis. In none of the HEV-associated NA patients were positive oligoclonal bands

Table 1. Hepatitis E Virus Status and Patient Characteristics in Patients With Neuralgic Amyotrophy Compared to a Control Group With Acute Varicella Zoster Virus Infection

Characteristic	NA Group (n = 35)	VZV Group (n = 37)	OR (95% CI)
Acute HEV infection			
HEV-IgM positive	4 (11)	0 (0)	10.71 (.56–206.75)
Positive HEV PCR in serum	3 (9)	0 (0)	8.08 (.4–162.27)
Positive HEV PCR in CSF	1 (3)	0 (0)	3.26 (.13–82.76)
Resolved HEV infection			
HEV-IgG only	13 (37)	9 (24)	1.84 (.66–5.08)

Data are shown as No. (%) unless otherwise specified.

Abbreviations: CI, confidence interval; CSF, cerebrospinal fluid; HEV, hepatitis E virus; IgG, immunoglobulin G; IgM, immunoglobulin M; NA, neuralgic amyotrophy; OR, odds ratio; PCR, polymerase chain reaction; VZV, varicella zoster virus.

Table 2. Hepatitis E Virus Antibody Index in Patients With Neuralgic Amyotrophy Compared to a Control Group With Acute Varicella Zoster Virus Infection

		Patient No.	HEV PCR (Serum/CSF)	Q_{Alb} ×10^{-3}	$Q_{IgG/M}$ ×10^{-3}	$Q_{aHEV\text{-}G/M}$ ×10^{-3}	Q_{Lim} ×10^{-3}	AI
IgG class	NA group	1	– / –	9.80	4.90	5.10	7.70	1.0
		2	– / –	7.72	4.40	2.54	5.83	<0.7
		7	– / –	4.54	1.81	2.00	3.10	1.1
		8	– / –	9.00	3.81	5.48	6.98	1.4
		9	– / –	4.13	1.86	0.30	2.77	<0.7
		15	– / –	7.09	3.08	3.67	5.28	1.2
		17	– / –	4.59	2.26	0.72	3.14	<0.7
		19	– / –	11.99	6.30	7.48	9.68	1.2
		20	– / –	6.38	3.46	1.68	4.65	<0.7
		22	– / –	3.93	2.25	0.82	2.60	<0.7
		24	– / –	9.63	4.68	5.64	7.54	1.2
		26	– / –	9.11	5.01	3.84	7.07	0.8
		27	– / –	5.78	2.33	2.94	4.14	1.3
		32	**+ / +**			aHEV-G <0.05 OD		
	VZV group	2	– / –	9.44	4.48	4.61	7.37	1.0
		3	– / –	7.52	4.25	4.77	5.65	1.1
		5	– / –	9.72	5.50	7.63	3.90	0.7
		6	– / –	9.55	4.74	7.47	2.77	<0.7
		7	– / –	6.71	3.48	4.94	4.04	1.2
		8	– / –			aHEV-G <0.05 OD		
		9	– / –	9.44	5.12	7.37	3.60	0.7
		10	– / –			aHEV-G <0.05 OD		
IgM class	NA group	32	**+ / +**	3.93	0.19	6.41	0.70	**34.4**
	VZV group	10	– / –	43.19	9.09	12.49	22.75	1.4

Positive test results are marked in bold.

Abbreviations: aHEV-G, anti–hepatitis E virus immunoglobulin G; AI, antibody index; CSF, cerebrospinal fluid; HEV, hepatitis E virus; IgG, immunoglobulin G; IgM, immunoglobulin M; NA, neuralgic amyotrophy; OD, optical density; PCR, polymerase chain reaction; $Q_{aHEV\text{-}G/M}$, specific HEV IgG/M CSF/serum quotient; Q_{Alb}, albumin CSF/serum quotient; Q_{Lim}, IgG fraction in CSF originating only from blood, calculation according to [10]; $Q_{IgG/M}$, total IgG/IgM CSF/serum quotient; VZV, varicella zoster virus.

detected, compared with 1 HEV negative NA case with positive oligoclonal bands.

CSF parameters of NA patients with a resolved HEV infection were pathological in 77%: namely, elevated WBC in 23%, elevated protein levels in 77%, and an elevated albumin quotient indicative of blood–brain barrier damage in 50% (Supplementary Table 1).

Clinical Features

Apart from involvement of the brachial plexus, 3 of 4 HEV-associated NA cases had additional neurological symptoms, a finding which differed significantly from HEV-negative NA patients (75% vs 11%; P = .02). Two HEV-positive patients showed signs of phrenical nerve affection. Consistent with the detection of HEV RNA in the CSF, case 2 not only suffered from a right brachial plexus deficit (scapula alata and hypoesthesia of the whole arm), but showed signs of a cranial radiculitis (trigeminal hypoesthesia on the right side). There was no other difference in clinical presentation (Supplementary Table 2). Time from symptom onset to presentation was equal to sampling time. Electrophysiological exams were available for 34 NA patients and showed signs of axonal damage in 28 cases, without any difference between the HEV-associated NA patients and the HEV-negative NA patients (50% vs 82%; P = .228). Plexus magnetic resonance imaging scans were done in 14 NA patients and were without pathological findings in all cases. Liver aminotransferases were available in 25 NA cases only and were elevated in all 3 HEV RNA–positive cases, which was significantly different from NA cases without markers of HEV infection (75% vs 14%; P = .04).

Discussion

We found laboratory markers of acute HEV infection in 11% of patients presenting with NA in southwestern Germany. In one of these patients, HEV RNA was detected in the CSF. There was further evidence of neurotropic HEV infection in this patient by demonstrating intrathecal synthesis of anti-HEV IgM. In addition, an inflammatory response of the CSF was found more frequently in patients with HEV-associated NA compared with HEV-negative NA. These results extend the findings of van Eijk et al (who found acute HEV in 10% of patients with NA) and add weight to the notion that NA is associated with acute HEV infection in a subgroup of patients [3].

Importantly, we provide evidence for the intrathecal synthesis of anti-HEV IgM, consistent with HEV infection present in the CNS. An increased AI for anti-HEV IgM was reported in a single patient with HEV-associated NA recently but, to our

knowledge, CSF has never been systematically analyzed in HEV-positive NA patients [11]. Thus, AI analysis might prove a beneficial diagnostic tool in the acute phase, similar to other CNS-associated infections [12]. In a literature review and case report by Dartevel et al, 36% (4/11) of HEV-positive NA patients had elevated CSF protein levels and/or WBC count [13]. In a Dutch NA study, pathological CSF parameters were reported in 12.5% (4/32) [1]. We found inflammatory CSF changes, especially in acute infections with detection of HEV RNA in CSF and/or serum. We were able to detect HEV from the CSF, which further substantiates our findings and supports the hypothesis that HEV may directly infect cells of the peripheral nervous system, causing an acute damage of nerve roots. This assumption is further supported by the higher HEV RNA concentration in the CSF compared to serum. Of note, the CSF was clear without the presence of blood rendering spillover of HEV-contaminated blood into the CSF unlikely. Drave and colleagues showed that HEV can replicate in vitro in various human neuronal-derived cell lines [5]. The detection of HEV RNA in the CSF of one patient is in line with these findings. As a limitation, we were not able to retrieve HEV sequences from serum and CSF to address a possible quasispecies compartmentalization that warrants further investigations.

Recent epidemiological data obtained from Germany found an anti-HEV IgG–positive serology in 16.8% of the adult population, increasing with age up to 26% in the group 60–69 years of age [14]. This matches the prevalence of our control group (24%; median age, 59 years) indicating the validity of our control group. Testing archived samples without signs of acute infection showed a comparable HEV prevalence (data not shown). HEV infections were most likely acquired autochthonous as evidenced by the close homology to European HEV strains.

From a clinical perspective, only half of the HEV-associated patients had a bilateral involvement. This contrasts with recent findings of van Eijk at al and Dartevel et al, who reported a bilateral plexus involvement in the majority of HEV-associated NA cases [13, 15]. Whether this phenomenon is HEV subgenotype specific, driven by host factors, or related to the small number of patients remains unclear.

In summary, we were able to show that acute HEV infection in NA patients is associated with pathological CSF parameters, thus further challenging the current dogma of HEV as an exclusively hepatotropic virus. Based on our findings, we propose that patients presenting with NA should be tested for markers of HEV infection. A prospective multicenter study is necessary to further examine the association between HEV infection and neuralgic amyotrophy, including clinical outcome, and a possible benefit of antiviral treatment.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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