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Diagnostic Challenges and Clinical Characteristics of Hepatitis E Virus–Associated Guillain-Barré Syndrome

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Supplemental content

IMPORTANCE Hepatitis E virus (HEV) recently has been shown to be an antecedent infection in Guillain-Barré syndrome (GBS), but the clinical spectrum of HEV-associated GBS is not yet documented, and diagnosing acute HEV infection can be a challenge.

OBJECTIVES To determine the prevalence of HEV-associated GBS in a Belgian cohort, study the clinical spectrum of HEV-associated GBS, and discuss difficulties in diagnosing acute HEV infection.

DESIGN, SETTING, AND PARTICIPANTS This single-center, retrospective cohort study was conducted between January 1, 2007, and November 1, 2015. All patients with GBS or a GBS variant who presented to the adult neurology department of the University Hospital Leuven were identified via a search of the electronic medical records. Hepatitis E virus IgM and IgG reactivity was determined. In a subgroup, polymerase chain reaction for HEV was performed.

MAIN OUTCOMES AND MEASURES Hepatitis E virus IgM and IgG reactivity.

RESULTS Of 73 eligible patients (mean [SD] age, 52 [18] years; 29 females and 44 males), 6 (8%) showed positive reactivity on IgM assays for HEV, indicating a possible acute HEV infection. Four of the 6 patients (67%) had increased alanine aminotransferase levels of more than 1.5 times the upper limit of normal, while 4 of 22 patients (18%) with increased alanine aminotransferase levels showed positive reactivity on HEV IgM assays. Serum samples of 2 of 6 patients with positive reactivity on HEV IgM assays also revealed positive test results for cytomegalovirus or Epstein-Barr virus, indicating possible cross-reactivity. Thus, 4 patients (6%) in our cohort had probable acute HEV infection. Two of these patients presented with an infrequent GBS variant.

CONCLUSIONS AND RELEVANCE Acute HEV infection was frequently associated with GBS in our cohort. Abnormal alanine aminotransferase levels at admission can indicate the presence of an associated HEV infection. When HEV testing is considered, it is important to test for other infectious agents in parallel, as cross-reactivity can occur. Further studies are required to guide neurologists in their workup of underlying triggers of GBS.

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Guillain-Barré syndrome (GBS) is characterized in its classic form by a rapidly progressive bilateral and relatively symmetric weakness of the limbs with or without involvement of respiratory muscles or muscles innervated by the cranial nerves, with a maximum level of weakness reached within 4 weeks.[1] Many clinical variants of GBS have been described according to the regional distribution of weakness (eg, limbs, ocular, or bulbar) or the relative involvement of motor vs sensory nerves. A postinfectious, immune-mediated disorder of the peripheral nerves and nerve roots is the most likely disease mechanism for GBS.[1] Studies in humans and animals have provided convincing evidence that at least some cases of GBS are caused by an infection-induced aberrant immune response that damages the peripheral nerves.[2-9] About two-thirds of patients have symptoms of an infection in the 3 weeks before the onset of weakness. The most common infectious causes of GBS are *Campylobacter jejuni*, cytomegalovirus (CMV), Epstein-Barr virus (EBV), *Mycoplasma pneumoniae*, and *Haemophilus influenzae*.[10,11] An association between infection with hepatitis E virus (HEV) and GBS has been suspected in several case reports and retrospective cohort studies of patients with HEV infection.[12-29] In 2013, a prospective case-control study reported positive IgM antibodies against HEV in 11% of patients with GBS in Bangladesh.[30] A second case-control study identified an associated acute HEV infection in 5% of patients with GBS in the Netherlands.[31]

Hepatitis E virus is a nonenveloped RNA virus belonging to the *Hepeviridae* family that has an icosahedral capsid and 3 open reading frames. Four HEV genotypes have been described. It is a common virus that is the most frequent cause of viral hepatitis worldwide and is endemic in one-third of the world population. In the Western world, HEV genotypes 3 and 4 are principally transmitted zoonotically to humans from an animal reservoir (eg, domesticated pig, wild boar, deer, and rabbits) by eating undercooked meat or via contaminated water.[32-34] Infection with HEV has been associated with many neurological diseases apart from GBS, such as neuralgic amyotrophy,[35-39] meningoencephalitis,[25,40,41] myelitis,[42,43] myositis,[22,44] cranial neuropathies,[29,45-47] myasthenia gravis,[48] and pseudotumor cerebri.[21,49-51]

In this study, we retrospectively assessed the prevalence of acute HEV infection in a cohort of Belgian patients with GBS or a variant of GBS and compared this prevalence with other infections known to be associated with GBS. Furthermore, we explored GBS disease parameters that are specific to HEV.

Key Points

Question What is the importance of, and what are the challenges in, diagnosing an acute hepatitis E virus (HEV) infection as a preceding infection in patients with Guillain-Barré syndrome or a variant of Guillain-Barré syndrome?

Findings This cohort study finds that acute hepatitis E virus infection is frequently associated with Guillain-Barré syndrome, presenting with a heterogeneous phenotype. Cross-reactivity with other pathogens must be taken into account to make a certain diagnosis of infection with hepatitis E virus.

Meaning In a classic or atypical presentation of Guillain-Barré syndrome, one should consider testing the patient for an associated hepatitis E virus infection but always in parallel with testing for other pathogens, since cross-reactivity is possible.

Methods

Patients

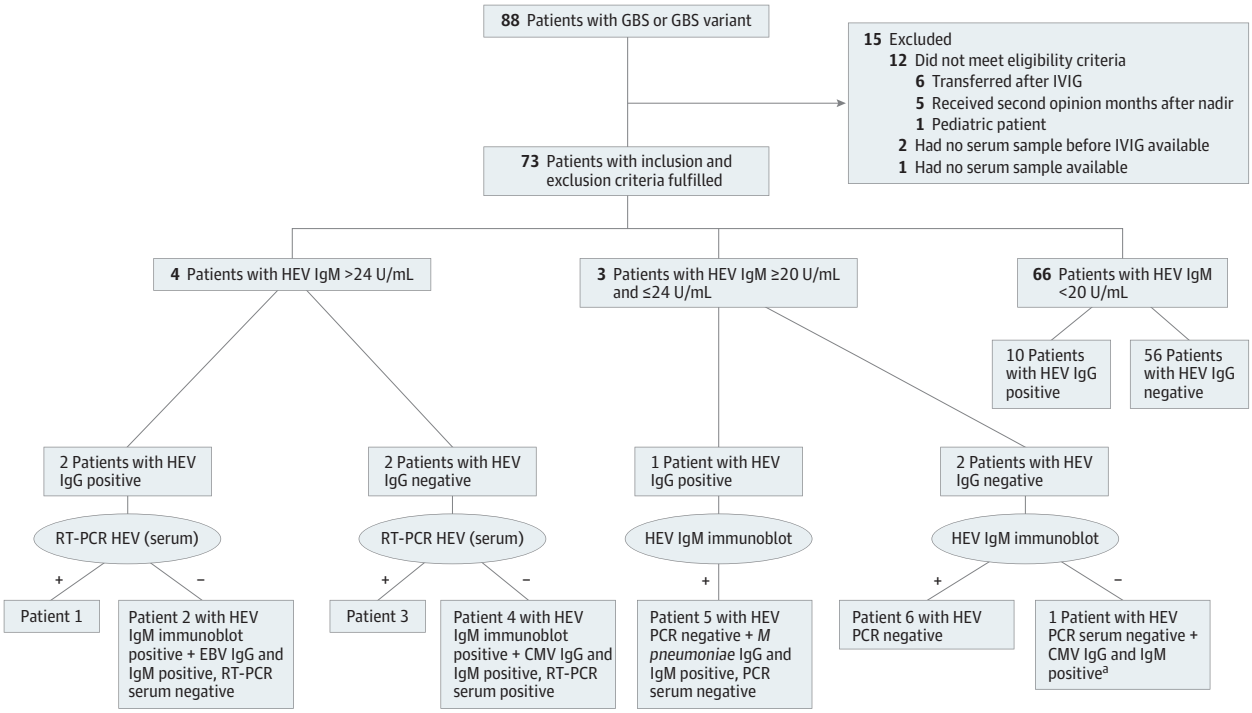
We searched the electronic medical records to identify all patients with GBS or a variant of GBS who presented to the adult neurology department of the University Hospital Leuven between January 1, 2007, and November 1, 2015. All patients with a diagnosis of GBS or a variant who presented within the first 4 weeks after the onset of neurological symptoms were included. Using criteria described in the literature, all patients were divided into the following clinical categories: pure motor GBS, Miller-Fisher syndrome, Bickerstaff brainstem encephalitis, acute ataxic neuropathy, bifacial weakness with distal paresthesias, acute multiple cranial neuropathic conditions, pharyngeal-cervical-brachial variant, and overlap syndromes (eAppendix 1 in the Supplement).[52-61] We excluded patients who were transferred to our hospital after administration of intravenous immunoglobulins as well as patients without a usable serum sample in the laboratory serum bank. This study was approved by the ethical committee and the biobank of the University Hospital Leuven. As this was a retrospective study, the University Hospital Leuven Institutional Review Board did not require informed consent.

We recorded the following clinical features from the medical records: date of onset of neurological symptoms, date of onset and type of antecedent infection, age at onset, sex, immune status before symptom onset, clinical variant of GBS, cranial nerve involvement, immunomodulatory treatment, need for intubation, and GBS disability score at nadir and after 3 to 6 months.[62] If present, the following laboratory results were recorded: alanine aminotransferase (ALT) and total bilirubin levels at admission or highest level before presentation and before the start of any medical therapy, date and results of lumbar puncture, presence of anti-ganglioside or anti-sulfatide antibodies (IgM and IgG against GM1, GM2, GM3, GM4, GD1a, GD1b, GD2, GD3, GT1a, GT1b, GQ1b, and sulfatides), and infectious serologic tests in serum and stool cultures. The date and results of nerve conduction studies (NCS) and needle electromyography were also recorded.[63] Patients were classified into different electrophysiologic variants of GBS (acute inflammatory demyelinating polyneuropathy, demyelinating, acute motor axonal neuropathy, acute motor and sensory axonal neuropathy, or inexcitable) according to Hadden et al[63] (eAppendix 2 in the Supplement).

Microbiological Testing on Stored Sera

Serum samples—aliquoted, stored at -20°C, and collected before the administration of intravenous immunoglobulins—were retrieved from the laboratory serum bank and used for HEV serologic testing. All samples were previously tested for *C jejuni* antibodies, *M pneumoniae*, IgG and IgM for EBV, and

Figure. Flowchart of Patient Inclusion and Hepatitis E Virus (HEV) Testing



Patients were divided according to anti-HEV IgM levels on enzyme immunoassay. They were then subdivided according to anti-HEV IgG levels on enzyme immunoassay and subdivided further according to real-time reverse transcriptase polymerase chain reaction (RT-PCR) for HEV RNA, anti-HEV IgM levels on a recombinant immunoblot test, and serologic markers for other pathogens. CMV indicates cytomegalovirus; EBV, Epstein-Barr virus; GBS, Guillain-Barré syndrome; IVIG, intravenous immunoglobulins; *M pneumoniae*, *Mycoplasma pneumoniae*.
[a]Patient with acute CMV infection.

IgG and IgM for CMV as part of the routine diagnostic workup. Serologic testing for *H influenzae* was not routinely performed.

Testing for HEV was performed as follows. First, an enzyme immunoassay (EIA) was performed (*recom*Well HEV IgM/IgG enzyme immunoassay, MIKROGEN Diagnostik) following the manufacturer's recommendations: positive when the results are above 24 U/mL, equivocal when they are between 20 and 24 U/mL, and negative when they are less than 20 U/mL. In cases of equivocal reactivity for HEV IgM or in the case of concurrent presence of positive serologic findings for other infectious agents (ie, CMV or EBV) and an equivocal or positive EIA result for HEV IgM, a confirmatory recombinant immunoblot test (RIBT) for HEV IgM (*recom*Line HEV IgM, MIKROGEN Diagnostik) and EIA for HEV IgM and IgG on later serum samples was performed, with remaining serum samples (if available) sent to the Belgian Scientific Institute of Public Health for HEV reverse transcriptase polymerase chain reaction (RT-PCR) (RealStar HEV RT-PCR kit 1.0, Altona Diagnostics). An acute HEV infection was considered possible in the case of positive results on anti-HEV IgM EIA and/or positive results on anti-HEV IgM RIBT. In cases with positive serologic markers for anti-HEV IgM and other pathogens, RT-PCR for those other pathogens was performed on the serum samples used for HEV assays to exclude false anti-HEV reactivity by these agents. Probable acute HEV infection was defined as possible infection in the absence of significant cross-reactivity to other pathogens. The additional presence of anti-HEV IgG antibodies or positive results on RT-PCR for HEV on serum samples was considered as suggestive of a definite HEV infection.

Statistical Analysis

Data are presented as medians (ranges) or means (SDs). Normal distribution of data sets was determined with the Shapiro-Wilk normality test. A *t* test was used to compare the quantitative variables if they were normally distributed. A 2-tailed, unpaired Mann-Whitney test was used if variables were not normally distributed. $P < .05$ was considered significant.

Results

We identified 88 patients with GBS or a variant of GBS, of which 73 fulfilled our inclusion criteria (**Figure**). Two of the 88 patients were excluded because they had no serum sample before the start of intravenous immunoglobulins, and a third patient was excluded because no serum sample was available.

The demographic and clinical characteristics of our cohort, as well as results of laboratory tests, NCS, and electromyography, are summarized in **Table 1**.[63] Classic GBS and acute inflammatory demyelinating polyneuropathy were the most common clinical and electrophysiologic variants, respectively. Three patients had acute motor axonal neuropathy associated with *C jejuni*

Table 1. Demographic, Clinical, Biochemical, and Electrophysiologic Features of Study Population

Variable	Value[a]
Demographic features	
Age, mean (SD) [range], y	52 (18) [16-85]
Female	29 (40)
Clinical features	
Immunocompetent	68 (93)
Clinical variant	
Classic, sensory-motor	56 (77)
Pure motor	2 (3)
MFS, no limb weakness	4 (6)
MFS-GBS overlap	2 (3)
Bickerstaff brainstem encephalitis	2 (3)
Acute ataxic neuropathy	3 (4)
Bifacial weakness with distal paresthesia	2 (3)
Acute multiple cranial neuropathies	1 (1)
Pharyngeal-cervical-brachial	1 (1)
Severity	
Intubation	12 (16)
GBS at nadir, median (range)	3 (1-6)
GBS at 3-6 mo, median (range)	1 (0-6)
No information	7 (10)
Preceding illness	51 (70)
No information	1 (1)
Flulike	9 (12)
Gastroinstestinal	24 (33)
Respiratory	15 (21)
Zona	1 (1)
Other[b]	2 (3)
Immunotherapy	66 (90)
IVIG	64 (88)
Plasmapheresis	2 (3)
No therapy[c]	7 (10)
Laboratory features	
Preceding infection identified[d]	29 (40)
No information[e]	3 (4)
Cytomegalovirus[f]	11 (15)
Campylobacter jejuni[g]	9 (12)
Epstein-Barr virus[h]	2 (3)
Mycoplasma pneumoniae[i]	6 (8)
Varicella zoster virus[j]	1 (1)
Anti-ganglioside or anti-sulfatide antibodies[k]	16 (22)
Antecedent infection	
No information[e]	1/16 (6)
Campylobacter jejuni[g]	7/16 (44)
Cytomegalovirus[f]	2/16 (3)
Mycoplasma pneumoniae[i]	1/16 (6)
No preceding infection identified	7/16 (44)
GBS variant	
GQ1b, MFS, GBS-MFS	4/6 (67)
GQ1b, Bickerstaff brainstem encephalitis	1/2 (50)
GQ1b, acute ataxic neuropathy	1/3 (33)
GD1a, acute motor axonal neuropathy	1/3 (33)
GM1, acute motor axonal neuropathy	2/3 (67)
Albuminocytologic dissociation, CSF	36 (49.3)
Days after onset of neurological symptoms, median (range)	7 (1-31)
Electrophysiologic variant	
Acute inflammatory demyelinating polyneuropathy	39 (53)
Demyelinating[l]	22 (30)
Acute motor axonal neuropathy	3 (4)
Acute motor and sensory axonal neuropathy	2 (3)
Sensory neuro(no)pathy	1 (1)
Other[m]	1 (1)
Normal	4 (6)

Abbreviations: CSF, cerebrospinal fluid; GBS, Guillain-Barré syndrome; IVIG, intravenous immunoglobulins; MFS, Miller-Fisher syndrome.

[a] Data are presented as number (percentage) of patients unless otherwise indicated.

[b] One patient with abdominal wound infection, 1 patient with aphthous ulcerations in the mouth; causal relation with GBS unclear.

[c] Mild symptoms.

[d] Hepatitis E virus not included.

[e] No or only limited serologic testing; insufficient information to exclude preceding infection.

[f] Positive cytomegalovirus IgM and IgG.

[g] Positive results on stool culture and/or serologic testing.

[h] Positive Epstein-Barr virus IgM and IgG, negative anti–Epstein-Barr virus nuclear antigen.

[i] Positive *Mycoplasma pneumoniae* IgM and IgG.

[j] Positive varicella zoster virus IgM and IgG.

[k] The following anti-ganglioside antibodies (IgG and IgM) were tested: ganglioside GM1, GM2, GM3, GM4, GD1a, GD1b, GD2, GD3, GT1a, GT1b, GQ1b, and sulfatides.

[l] Demyelinating features, but insufficient for criteria for acute inflammatory demyelinating polyneuropathy.[63]

[m] One patient with only signs of axonal loss in the arms (patient 3).

infection. Four patients had normal results of NCS; they were diagnosed with either Miller-Fisher syndrome, Bickerstaff brainstem encephalitis, or acute multiple cranial neuropathies.[63] The median delay between symptom onset and serum sampling was 7 days (range, 0-31 days). The results of HEV serologic testing in our cohort are presented in the Figure.

When assessing possible acute HEV infection preceding GBS, we found positive anti-HEV IgM on EIA and/or RIBT in 6 patients (8%). Of those, 4 (67%) had ALT levels more than 1.5 times the upper limit of normal. We found no significant age difference between patients with anti-HEV IgM antibodies vs those without such antibodies (mean age, 61 vs 52 years; $P = .12$). Thirteen patients (18%) had positive IgG serologic findings for HEV.

The characteristics of patients with a possible acute HEV infection are presented in **Table 2.**[63] There was a striking heterogeneity in phenotypes observed. Patient 1 presented with an acute ataxic neuropathy, which was previously described in more detail.[64] Patient 3 developed rapidly progressive, painful paresthesia and paresis in both arms, with development of scapular winging on the right side, only 1 day after reporting general discomfort and fever. Consecutive NCS and the electromyogram showed signs of progressive axonal loss in both sensory and motor nerves in both arms. The patient developed global areflexia, paralysis of the vocal cords on the left

Table 2. Patients With Possible Acute Hepatitis E Virus Infection

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age, y/sex	54/M	68/M	67/M	41/F	62/F	75/M
Time from onset of neurological symptoms to serum sample, d	6	16	2	8	4	19
Preceding symptoms	Gastrointestinal	Flulike	Flulike	Flulike	Respiratory and gastrointestinal	Respiratory and gastrointestinal
Cranial nerve deficit	+	+	+	+	–	+
Clinical variant	Acute ataxic neuropathy	Mild, predominantly sensory	Pharyngeal-brachial-cervical	Classic	Classic	Classic
Immunotherapy	Plasmapheresis	None	IVIG	IVIG	IVIG	IVIG
GBS disability score at nadir	4	2	2	4	3	6
GBS disability score after 3-6 mo	2	1	1	1	1	[a]
NCS and EMG	Sensory neuro(no)pathy	Equivocal[b]	Axonal loss in both arms (sensory-motor)	AIDP	Demyelinating[c]	Demyelinating[c]
CSF cell count, /μL	6.0	16.0	4.4	0.4	0.8	1.4
CSF protein level, mg/L[d,e]	1028 (7)	724 (16)	531 (2)	689 (8)	296 (4)	897 (21)
ALT, U/L[f]	**3023**	**57**	**636**	**792**	**50**	15
Total bilirubin, mg/dL[g]	9.61	1.24	1.11	0.41	0.35	1.06
Anti-ganglioside and anti-sulfatide antibodies	–	–	–	–	–	–
HEV IgM, U/mL (+ or –)	133 (+)	41 (+)	135 (+)	29 (+)	23 (+/–)[h]	20 (+/–)[i]
HEV IgG, U/mL (+ or –)	119 (+)	119 (+)	10 (–)	5 (–)	74 (+)[h]	19 (–)[i]
HEV IgM immunoblot	NA	+	NA	+	+	+
HEV RT-PCR (serum)	+	–	+	–	–	–
Other positive serology/PCR	–	EBV (IgG +, IgM +, RT-PCR serum –, EBNA –)	–	CMV (IgG +, IgM +, RT-PCR serum +)	*M pneumoniae* (IgG +, IgM limit value, RT-PCR serum –)	–

Abbreviations: AIDP, acute inflammatory demyelinating polyneuropathy; ALT, alanine aminotransferase; CMV, cytomegalovirus; CSF, cerebrospinal fluid; EBNA, Epstein-Barr virus nuclear antigen; EBV, Epstein-Barr virus; EMG, electromyography; GBS, Guillain-Barré syndrome; HEV, hepatitis E virus; IVIG, intravenous immunoglobulins; *M pneumoniae*, *Mycoplasma pneumoniae*; NA, not available; NCS, nerve conduction study; RT-PCR, reverse-transcriptase polymerase chain reaction; +, positive; –, negative.

SI conversion factors: To convert ALT to microkatals per liter, multiply by 0.0167; bilirubin to micromoles per liter, multiply by 17.104; IgG to grams per liter, multiply by 0.01; and IgM to grams per liter, multiply by 10.

[a] Not applicable since patient died at nadir.

[b] Electromyographic results normal on day 1 after symptom onset. Results after 1 month include development of fibrillation potentials and positive sharp waves and decrease of sensory nerve action potential amplitudes that remain above the upper limit of normal.

[c] Demyelinating features on NCS not fulfilling criteria for AIDP.[63]

[d] Reference value, 150 to 600 mg/L.

[e] Numbers in parentheses indicate days from onset of neurological symptoms to CSF sample.

[f] Upper limit of normal, ≤31 U/L for females; ≤41 U/L for males. In bold if >1.5 times the upper limit of normal.

[g] Reference value, ≤1.00 mg/dL.

[h] Serum sample 10 days later: HEV IgM, 21.5 U/mL; HEV IgG, 81.6 U/mL.

[i] Serum sample 2 days later: HEV IgM, 22.4 U/mL; HEV IgG, 18.3 U/mL (no serum sample from a later date available for further testing).

side, phrenic nerve involvement on the left side, and dysautonomic features with orthostatic hypotension, compatible with a pharyngeal-cervical-brachial variant of GBS. The rapid development of neurological symptoms after 1 day of flulike illness can explain the positive IgM and RNA results for HEV, with negative IgG results.

Patient 6 had positive anti-HEV IgM RIBT results with a borderline EIA anti-HEV IgM result 16 days after the onset of neurological symptoms. A serum sample 2 days later showed no significant increase of HEV IgG or IgM levels. No later time points were available for serologic study. The patient had a classic presentation of GBS, with demyelinating features seen on NCS, without elevation of ALT levels. The patient never received mechanical ventilation. He died after cardiac arrest 1 month after the onset of neurological symptoms. No autopsy was performed. The patient had a medical history of ischemic and valvular cardiomyopathy, complicated with episodes of ventricular tachycardia, for which a cardioverter defibrillator had been implanted.

Patient 5 had a classic presentation of GBS. She had a positive serologic result for anti-HEV IgG and borderline or equivocal anti-HEV IgM EIA results but a positive serologic result for anti-HEV IgM RIBT and increased ALT levels. She had a borderline value for IgM against *M pneumoniae*, which was deemed not relevant. Results of RT-PCR performed on serum samples for *M pneumoniae* were negative.

The last 2 patients with positive HEV serologic findings also tested positive for other pathogens. Patient 2 developed mild GBS, with predominantly sensory symptoms (paresthesia and pain) and mild paresis (limbs and mild dysarthria) without

ataxia or areflexia; he received no immunomodulatory treatment. The patient had positive serologic results for anti-HEV IgG and IgM, as well as for anti-EBV IgG and IgM. Results of RT-PCR performed on serum samples for EBV were negative. Patient 4 had a classic presentation of GBS, with an acute inflammatory demyelinating polyneuropathy seen on NCS. She also had an elevated ALT level at admission and positive results for anti-CMV IgM and IgG. She had positive serologic results for anti-HEV IgM on EIA without anti-HEV IgG only 10 days after the onset of neurological symptoms or 18 days after the onset of flulike symptoms. Results of RT-PCR performed on serum samples for CMV were positive.

We found no significant correlations between clinical parameters and the presence of HEV antibodies. Results of antiganglioside and anti-sulfatide antibody testing were negative in all 6 patients with HEV IgM antibodies. Four of the 6 patients had ALT levels more than 1.5 times the upper limit of normal. Bilirubin levels were slightly elevated in 3 patients and were significantly elevated in 1 patient with icterus.

We studied the contribution of HEV infection to elevated ALT levels[65] in the entire cohort and found 22 patients with elevated (>1.5 times the upper limit of normal) ALT levels. In 8 of those cases (36%), positive anti-CMV IgM reactivity was found. The second most common finding was a possible acute HEV infection seen in 4 patients (18%). One patient demonstrated positive reactivity for EBV and 1 for *M pneumoniae*. For patients with positive serologic markers for multiple pathogens, every pathogen was counted. Of the 36 patients with normal ALT levels, 1 (3%) had HEV IgM antibodies (patient 6). Of the 51 patients with ALT levels less than 1.5 times the upper limit of normal, 2 (4%) had positive HEV IgM serologic findings (patients 2 and 6).

Discussion

The demographic and clinical characteristics, as well as results of laboratory tests, NCS, and electromyography, of our cohort of patients with GBS were comparable with those of earlier published cohorts. Guillain-Barré syndrome affected males 1.5 times more often than females.[1,66] The mean age in our cohort was slightly higher than anticipated, but this finding may be owing to the fact that we did not include children.[66] Similar to findings in previous reports, the sensory-motor (classic) variant of GBS was the most frequent, seen in 77% of patients, compared with rates of 75% to 80% in an earlier study.[54] We found antibodies to various gangliosides and sulfatides in only 16 patients (22%), while other studies have reported the presence of those antibodies in about half of the patients with GBS or variants.[1] In several case reports of GBS with associated HEV infection, anti-ganglioside antibodies to GM1 or GM2 were found.[14,15,17,24,25,28] In line with a larger case-control series with 10 patients,[31] none of the HEV-associated cases in our cohort had anti-ganglioside or anti-sulfatide antibodies. An acute demyelinating neuropathy was the most frequent electrophysiologic variant in our cohort. Only 7% of patients had an axonal variant (acute motor axonal neuropathy or acute motor and sensory axonal neuropathy), which is similar to findings in other reports.[66] In line with other studies, we also observed that most patients with Miller-Fisher syndrome or Bickerstaff brainstem encephalitis had no or only slight abnormalities on results of NCS. Overall, our cohort has the characteristics of a representative sample of patients with GBS and variants.

The seroprevalence of anti-HEV IgG in our cohort is lower (18%) than that found in a cohort with GBS from the Netherlands (46%).[31] Moreover, they found no significant difference in seroprevalence between controls and the patients with GBS. The exact seroprevalence of anti-HEV IgG in Belgium is not known, but our value in patients with GBS or a variant was similar to the seroprevalence of 14% seen in a small cohort of Belgian orthopedic and obstetric patients.[67] This percentage is lower than findings, mostly in blood donors, from the Netherlands (27%), Germany (29%), Bangladesh (50%), and southwest France (34%-52%),[31,68] and suggests that important locoregional differences in HEV prevalence occur.

We found that 8% of our patients had positive anti-HEV IgM on EIA and/or RIBT, indicating a possible acute HEV infection. This rate is lower than that in a cohort of patients with GBS from Bangladesh (11%), where HEV is endemic,[30] but higher than the rate found in a Dutch cohort (5%).[31] However, in the latter study, a more strict definition of acute HEV infection was used: positive serologic results for anti-HEV IgM and IgG or, if results for anti-HEV IgG were negative, positive serologic results for IgM and RT-PCR performed on serum samples.[31] Using this definition, 4 of our patients (6%) would have met the criteria for acute HEV infection (excluding patients 4 and 6). Patients 1 and 3 are clear cases of acute HEV infection, also following this more strict definition. Patients 2 and 5 also fulfill these serologic criteria. However, patient 6 is also a likely case of an acute HEV infection with positive serologic results for anti-HEV IgM. Unfortunately, a serum sample from this patient at a later time point to demonstrate seroconversion for HEV IgG was not available. Use of RT-PCR performed on serum samples for HEV has a limited added value, as negative results of RT-PCR do not exclude a recent infection because HEV RNA is rapidly cleared and typically becomes undetectable within 3 weeks after onset of the infectious episode.[32]

One of the most important diagnostic challenges we encountered, which was not fully addressed in previous studies, was the co-occurrence of positive results of infectious serologic tests for different pathogens at the same time. The limit value of *M pneumoniae* in patient 5 was deemed irrelevant, and cross-reactivity between HEV and *M pneumoniae* also has not been described. We thus retain patient 5 as a case of definite acute HEV infection. Patients 2 and 4, however, had positive serologic results for CMV or EBV IgM and IgG. Cross-reactivity on serologic tests between HEV, CMV, and EBV has been described, giving rise to false-positive results of HEV IgM owing to CMV or EBV infection or giving rise to false-positive results of CMV or EBV IgM owing to HEV infection.[69-71] This cross-reactivity is mentioned in the manufacturer's instructions for the *recom*Line test. Cross-reactivity with EBV, but not with CMV, is described in the manufacturer's instructions for the *recom*Well test. However, cross-reactivity with CMV when using the older version of the test has been described.[69] These

issues suggest that the assays are not perfect and that it is difficult to obtain a definite diagnosis in some patients. It is our hope that further refinement of the immunoassays will eliminate the issue of cross-reactivity in the future. This refinement might also help in resolving the possibility of a coinfection. Since patient 4 had a positive result for RT-PCR performed on serum samples for CMV, she probably had an acute CMV infection or reactivation of CMV. Taking these factors into account, we had 4 cases (6%) of definite or probable acute HEV infection in our cohort (excluding patients 2 and 4, not 4 and 6, when following the serologic criteria from the Dutch cohort[31]).

Similar to an older report in the literature,[65] we observed elevated liver enzymes in about one-third of the patients with GBS. Eight of these patients (36%) had positive reactivity for CMV IgM. Reactivity for HEV IgM appears to be the second most common finding in patients with GBS who have elevated ALT levels, seen in 18% of our cohort. When examining only cases of probable or definite acute HEV infection in our cohort, 3 of 22 patients (14%) had significant elevation of ALT levels. This finding suggests that HEV is the second most common cause of elevated ALT levels in patients with GBS. However, not all patients with HEV-associated GBS have elevated ALT levels. Therefore, testing for HEV should be considered in patients with normal liver enzyme levels.

Limitations

Our study has several limitations. As it is a retrospective study, not every patient received the same diagnostic workup. Another limitation was the absence in particular cases of follow-up serum samples at later time points, which could have helped to demonstrate seroconversion.

Conclusions

Acute HEV infection was frequently associated with GBS or variants of GBS in our cohort. Depending on the stringency of the definition of HEV positivity, 8% or 6% tested positive. The phenotypes associated with GBS ranged widely from classic GBS to a pharyngeal-cervical-brachial variant and a sensory ataxic variant. Abnormal ALT levels at admission can indicate an associated HEV infection. Testing for other infectious agents in parallel is important since cross-reactivity with EBV and CMV is possible with current assays. Performing RT-PCR to detect viral nucleic acids does not always allow us to make definitive conclusions, as the test results may be negative owing to the short viremia in an acute HEV infection. Further studies are required to guide neurologists in their workup of the underlying causes of GBS.

ARTICLE INFORMATION

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