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Performance of anti-HEV assays for diagnosing acute hepatitis E in immunocompromised patients

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ABSTRACT

Hepatitis E virus is an emerging concern in immunocompromised patients, who may become chronically infected. This prompted us to assess the performance of two anti-HEV IgG and IgM assays for diagnosing acute HEV infections. The specificities of the assays were estimated by testing samples from 2 to 3 year-old French children and blood donors and their sensitivities by testing 40 immunocompromised patients acutely infected. Both anti-HEV IgM assays were highly specific (99.6% and 100%). The sensitivity of the Adaltis was 87.5%, and that of Wantai was 85%. The specificities of anti-HEV IgG Wantai (97.8%) and Adaltis tests (89.5%, $p = 0.1$) were similar but the Wantai test was more sensitive (45%) than the Adaltis test (15%, $p < 0.001$). None of the samples was anti-HEV IgM negative and IgG positive. We conclude that these anti-HEV IgM assays performed well in immunosuppressed subjects with acute hepatitis E and can be used as first line virological tools. Testing for anti-HEV IgG and IgM simultaneously at the acute phase did not improve the diagnostic performance. In contrast, molecular detection of HEV RNA appears essential to exclude an HEV infection in patients who are negative for anti-HEV IgM and to assess the evolution of hepatitis E 3 months thereafter.

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1. Background

The hepatitis E virus (HEV) has been recognized for decades as a major cause of outbreaks associated with faecal contamination of drinking water in developing countries. This pathogen is now also recognized as a major etiologic agent of acute hepatitis in industrialized countries that is transmitted zoonotically [1,2].

HEV is an RNA virus with a single-stranded, positive sense approximately 7.2 kb genome containing partially overlapping open reading frames (ORFs). ORF1 encodes a nonstructural protein, ORF2 encodes the capsid protein and ORF3 encodes a phosphoprotein required for virion assembly [3]. Several immunoreactive domains have been identified in the ORF2 and ORF3 proteins [4].

There are 4 major genotypes of HEV, but only a single known serotype [5]. HEV1 and HEV2 are endemic in developing countries, where they cause outbreaks and sporadic cases of acute hepatitis, and HEV3 is prevalent in many industrialized countries. HEV4 is mainly found in Asia [5].

Within the last few years, it has been shown that an HEV genotype 3 infection can lead to chronic infection, defined as HEV RNA persisting in the serum and/or stool, in immunocompromised patients. Chronic hepatitis occurs in immunocompromised patients, such as solid-organ transplant recipients [6–9], patients with haematological disorders who have had chemotherapy [10–12], and patients infected with the human immunodeficiency virus [13–15]. Chronic infection may lead to cirrhosis in these patients [6,16]. Halac et al. recently reported a first case of HEV-related cirrhosis in a child who had been given a bone marrow transplant [10]. The incidence of HEV among 700 patients who had undergone solid-organ transplantation in southwest France was 3.2 cases/100 person-years [17]. In the Netherlands, the incidence of HEV infection among 331 liver transplant patients was 2% [18]. In Germany, 2.9% of liver transplant patients, with increased alanine aminotransferase activity of unknown origin, had detectable HEV

Abbreviations: HEV, hepatitis E virus; IQR, interquartile range; IU, international unit; ORF, open reading frames.

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RNA in their serum [19]. Thus, HEV is a major public health concern in immunocompromised patients [20].

Cases of acute hepatitis E are primarily diagnosed by detecting anti-HEV antibodies, mainly anti-HEV IgM, or by detecting viral RNA in the serum and/or faeces during the acute phase of the disease [21]. The appearance of anti-HEV IgM is rapidly followed by anti-HEV IgG in immunocompetent patients, so that they seem to appear almost simultaneously in the acute phase of infection. The performance of anti-HEV assays in immunocompetent patients has been studied. One study found that the sensitivity of anti-HEV IgG assays in HEV RNA positive sera may vary from 17% to 100% [22]. Another found that the sensitivity and specificity of anti-HEV IgM assays varied appreciably [23]. However, no data are yet available for immunocompromised patients, who might not mount a good antibody response to HEV, and might thus test negative for anti-HEV antibodies.

2. Objectives

We have assessed the performance of two commercially available anti-HEV IgM and IgG tests, the Adaltis and the Wantai tests, the main serological assays employed in Europe, for diagnosing acute HEV infections in immunosuppressed patients.

3. Study design

3.1. Serum samples

All samples were collected in France. The specificities of the anti-HEV IgM assays were assessed by testing samples from 233 HEV-negative blood donors (HEV RNA negative, anti-HEV IgG negative with the HEV IgG ELISA kit; Wantai). Those of the anti-HEV IgG assays were assessed by testing samples from 180 2–3 year-old children hospitalized for surgery or trauma. The sensitivities of the HEV serological assays were determined using sera collected from 40 HEV immunocompromised patients whose sera were positive for HEV RNA (Panel 1). These samples included 35 solid-organ transplant recipients and 5 haematological patients. A second panel (Panel 2), consisting of sera from 44 acutely-infected immunocompetent patients, was also used for comparison. All of them had a resolving infection and none had a fulminant hepatitis. We used the first HEV RNA positive blood sample collected when an elevation of the ALT activity was observed. This was a non-interventional study with no addition to the usual procedures. Biological material and clinical data were obtained only for standard viral diagnosis following physicians' orders (no specific sampling, no modification of the sampling protocol, no questions in addition to the national standardized questionnaire). Data were analyzed using an anonymized database. According to the Public Health French law (CSP Art L 1121-1.1), such a protocol does not require written informed consent.

HEV RNA was detected in plasma samples by real time RT-PCR as previously described [24]. The detection limit of the test was 100 copies/ml. Sequence analysis indicated that all HEV samples were HEV3, but HEV1 was identified in samples from two immunocompetent patients who had recently travelled to India [25].

3.2. Anti-HEV antibodies assays

We used the EIAgen HEV IgG and IgM kits (Adaltis, Eurobio, France), based on synthetic antigens encoded by the ORF2 and ORF3 genes of HEV1 and HEV2. Samples were also tested with the HEV IgM and IgG ELISA 3.0, Wantai kits (Wantai Biological Pharmacy Enterprise Co., China), based on a recombinant HEV1 antigen, pE2. This is a structural peptide encoded by an ORF-2 sequence derived from a Chinese strain of HEV1. All tests were performed as recommended by the manufacturers. The WHO international standard 94/854 (supplied by the National Institute for Biological Standards and Control, South Mimms, UK), was used to determine the limit of detection of IgG assays. The limit of detection is 0.25 U/ml for the Wantai assay and 2 U/ml for the Adaltis assay. The Wantai assay is linear from 0.25 to 5 U/ml. The blood samples were diluted if anti-HEV IgG were >5 U/ml. The linearity of the Adaltis test was not determined.

Table 1
Performance of the anti-HEV IgM assays.

	HEV IgM ELISA, Wantai	EIAgen HEV IgM, Adaltis
% Specificity (95%CI)	99.6 (98.7–100)	100 (99.8–100)
% Sensitivity (95%CI)		
Panel 1	85 (70.2–94.3)	87.5 (73.2–95.8)
Panel 2	97.7 (87.9–99.9)	97.7 (87.9–99.9)
% PPV (95%CI)		
Panel 1	97.1 (91.6–100)	100 (99.7–100)
Panel 2	97.7 (93.3–100)	100 (99.7–100)
% NPV (95%CI)		
Panel 1	97.5 (95.5–99.5)	97.9 (96.1–99.7)
Panel 2	99.6 (98.7–100)	99.6 (98.7–100)

Note: 95%CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value.

3.3. Statistical analysis

We used the McNemar test to determine the significance of differences in sensitivity and specificity for the anti-HEV IgM and IgG assays. We used the Mann–Whitney test to compare anti-HEV IgG concentrations and HEV RNA concentrations. We used the Spearman test to determine the correlation between anti-HEV antibodies concentrations and HEV RNA concentrations.

4. Results

4.1. Performance of the anti-HEV IgM tests

Specificity was determined by testing 233 anti-HEV IgG and HEV RNA negative samples. The Wantai ELISA for HEV IgM found that 232 of them were negative (specificity: 99.6%, 95% confidence interval (95%CI): 98.7–100%), while the Adaltis EIAgen HEV IgM kit found that all 233 samples were negative (specificity: 100%, 95%CI: 99.8–100, $p = 0.49$) (Table 1).

We have assessed the sensitivity of the anti-HEV IgM tests by testing 2 panels of patients. The mean HEV RNA concentrations was 5.6 ± 1.1 log copies/ml and 4.7 ± 1.2 log copies/ml in immunocompromised patients and in immunocompetent patients, respectively ($p < 0.01$) (Fig. 1).

The Wantai test found that 34 of the samples from immunocompromised patients (Panel 1) were anti-HEV IgM positive (sensitivity: 85%, 95%CI: 70.2–94.3%); the Adaltis test detected 35 positive samples (sensitivity: 87.5%, 95%CI: 73.2–95.8%; $p = 0.3$) (Table 1). There was only one discrepant result (IgM positive with the Adaltis test and negative with the Wantai test).

Both tests indicated that 43 of the samples from acutely-infected immunocompetent patients (Panel 2) were anti-HEV IgM positive (sensitivity: 97.7%, 95%CI: 87.9–99.9%, $p = 0.97$). Two samples were discordant for IgM: one sample was anti-HEV IgM and IgG negative with the Adaltis tests and positive for both anti-HEV IgM and IgG with the Wantai test. The second sample was anti-HEV IgM negative with Wantai test and positive with the Adaltis test.

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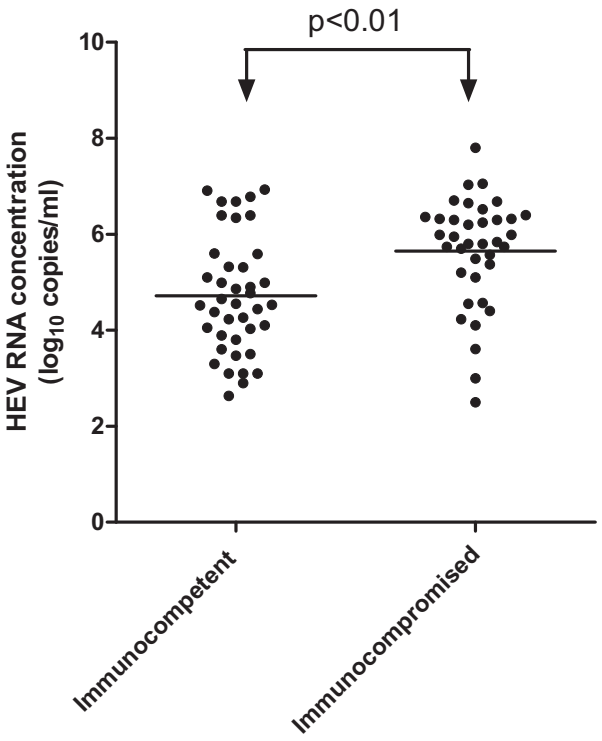

Fig. 1. HEV RNA concentration during the acute phase in immunocompetent and immunocompromised patients.

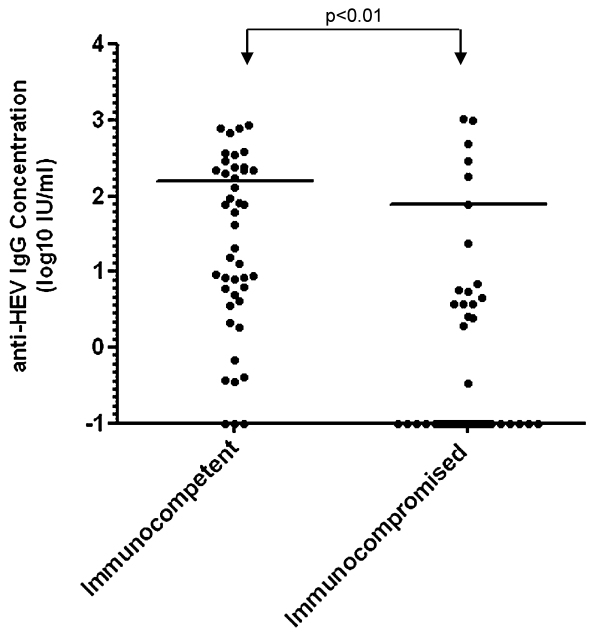

Fig. 2. Anti-HEV IgG concentration during the acute phase in immunocompetent and immunocompromised patients.

4.2. Performance of the anti-HEV IgG tests

The Wantai test for anti-HEV IgG antibodies was negative for 176 of the 180 children tested (specificity: 97.8%, 95%CI: 95.6–99.9%) and the Adaltis test was negative for 161 (specificity: 89.5%, 95%CI: 85–94%; $p = 0.10$) (Table 2). Three samples were anti-HEV IgG positive with both the Wantai and Adaltis tests.

Anti-HEV IgG was detected in 18 of the samples collected from immunocompromised patients at the acute phase (Panel 1) with the Wantai test (sensitivity: 45%, 95%CI: 29.3–61.5%), but in only 6 samples with the Adaltis test (sensitivity: 15%, 95%CI: 5.7–29.8%; $p < 0.001$) (Table 2).

Anti-HEV IgG was detected in 41 of the samples collected from 44 acutely-infected immunocompetent patients (Panel 2) with the Wantai test (sensitivity: 93.2%, 95%CI: 81.3–98.6%) and in 36 samples with the Adaltis test (sensitivity: 81.8%, 95%CI: 67.3–91.8%; $p = 0.10$). None of the anti-HEV IgM negative samples in either panel was anti-HEV IgG positive.

Table 2
Performance of the anti-HEV IgG assays.

	HEV IgG ELISA, Wantai	EIAgen HEV IgG, Adaltis
% Specificity (95%CI)	97.8 (95.6–99.9)	89.5 (85.0–94.0)
% Sensitivity (95%CI)		
Panel 1	45 (29.3–61.5)	15 (5.7–29.8)
Panel 2	93.2 (81.3–98.6)	81.8 (67.3–91.8)
% PPV (95%CI)		
Panel 1	81.8 (87.2–76.4)	24 (7.58–40.52)
Panel 2	91.1 (82.8–99.4)	65.4 (52.9–78.02)
% NPV (95%CI)		
Panel 1	88.9 (84.5–93.3)	82.6 (77.2–87.89)
Panel 2	98.3 (96.4–100)	95.2 (92.1–98.5)

Note: 95%CI, 95% confidence interval, PPV, positive predictive value; NPV, negative predictive value.

The HEV RNA concentrations were similar in concordant and discordant samples collected in immunocompromised patients (mean HEV RNA concentration: 5.6 *versus* 5.7 log copies/ml, $p = 0.71$) and in immunocompetent patients (4.5 *versus* 5.6 log copies/ml, $p = 0.09$) suggesting no relationship between HEV RNA concentration and the sensitivity of the anti-HEV IgG assays.

The mean anti-HEV IgG concentration measured with the Wantai test was 78.3 ± 236.6 IU/ml in immunocompromised patients and 153.7 ± 233.7 IU/ml in immunocompetent patients ($p < 0.01$) (Fig. 2).

No correlation was found between anti-HEV IgG concentration and HEV RNA concentration in any panels. By using the Wantai assay, HEV RNA concentrations were similar in anti-HEV negative and anti-HEV positive immunocompromised patients (5.8 *versus* 5.5 log copies/ml, $p = 0.20$). HEV RNA concentrations were higher in anti-HEV IgG negative immunocompetent patients compared with anti-HEV positive immunocompetent patients (mean concentration: 6.6 log copies/ml *versus* 4.5 log copies/ml, $p < 0.01$). By using the Adaltis assay, HEV RNA concentrations tended to be higher in anti-HEV IgG negative in immunocompromised patients (5.7 *versus* 4.9 log copies/ml, $p = 0.06$) and in immunocompetent patients (5.3 *versus* 4.7 log copies/ml, $p = 0.06$).

5. Discussion

We have evaluated the performance of two commercial anti-HEV IgG and IgM assays in immunocompromised patients during the acute phase. Both anti-HEV IgM assays were highly specific and sensitive (87.5% and 85%). The specificities of the anti-HEV IgG assays were similar but they were rather insensitive and did not contribute to the diagnosis of acute hepatitis E.

The presence of anti-HEV IgM is a marker of acute infection. A recent study evaluated the performance of six IgM anti-HEV enzyme immunoassays using sera from immunocompetent patients infected with one of each of the 4 main HEV genotypes [23]. The specificities of the 6 assays, 2 in-house and 4 commercial assays, ranged from 78.2% to 95.6% [23]. But neither the Adaltis nor the Wantai tests were evaluated in [23], though these tests are frequently used in Europe. We find that these two anti-HEV IgM

tests are more specific than those tested previously by Drobeniuc et al. [23]. This agrees well with our previous estimate of the specificity of the Adaltis anti-HEV IgM test (100%, 95%CI: 99.1–100.0%) [26]. Similarly, Khan et al. estimated the specificity of the Wantai anti-HEV IgM test to be 100% [27].

The sensitivity of the anti-HEV IgM assays evaluated by Drobeniuc et al. ranged from 72% to 98% in immunocompetent patients [23]. We assessed the performance of the Wantai and Adaltis anti-HEV IgM tests on samples from immunocompromised patients because data for these patients are scarce and their antibody response might be lower at the acute phase. The sensitivity of the anti-HEV IgM tests in this population was about 85%, which appears quite good. Their sensitivity in immunocompetent patients was excellent (97.5%). This high sensitivity in immunocompetent patients is in agreement with a previous study in England with the Wantai test (95%) [28] and with our previous estimate of the sensitivity of the Adaltis test (90%) [26].

It has been suggested that the IgM antibody index could be useful for identifying recent infections [28]. These authors suggest that an acute HEV infection should not be diagnosed from a low IgM antibody index (<5) [28]. However, 7 samples (17.5%) from our immunocompromised patients and 7 samples (16%) from our immunocompetent patients had IgM indices <5. Therefore, in the light of the good specificity of these IgM assays, a low IgM index must be taken into account.

While anti-HEV IgG was frequently detected in samples from immunocompetent patients at the acute phase, it seems to be more difficult to detect anti-HEV IgG in samples from immunocompromised patients due to the lower concentrations of anti-HEV antibodies. The Wantai test detected anti-HEV IgG in acute phase immunosuppressed patients more frequently than did the Adaltis test. However, this assay found that only 45% of the patients were anti-HEV positive. Also, no sample collected from acutely infected patients was anti-HEV IgM negative and IgG positive. Thus, testing acute phase samples for anti-HEV IgG and IgM simultaneously did not improve the diagnostic performance of HEV serology.

Several seroprevalence studies have highlighted the great sensitivity of the Wantai test in immunocompetent patients [29–33]. The Wantai assay may be more sensitive because the pE2 peptides associate to form dimers which react more strongly with HEV-reactive sera than do the linear monomeric antigens used in the Adaltis test [34]. Thus, the Wantai assay is particularly useful for detecting low concentrations of antibodies and identifying previous exposure to HEV.

The good performances of the anti-HEV IgM assays indicate that they can be used as first line tools for the routine diagnosis of acute hepatitis E, even in immunocompromised patients. However, the molecular detection of HEV RNA is essential for the diagnosis of acute hepatitis E in anti-HEV IgM negative patients. HEV RNA testing is crutial for assessing the evolution of an HEV infection after 3 months to determine whether the HEV has been cleared or persists [35].

Nevertheless, only validated HEV RNA assays should be used. A study has demonstrated the performances of homebrew RT-PCRs tests vary widely [36]. The genotype 3 diversity may also influence HEV RNA assays [24]. Commercial assays for detecting HEV RNA have only recently become available. A recent evaluation of their performance concluded that both the assays tested are suitable for detecting HEV infections in industrialized countries where genotype 3 is predominant [37].

In conclusion, the anti-HEV IgM assays performed well in immunosuppressed subjects with acute hepatitis E and can be used as first line virological tool. In contrast, it appears essential to use molecular detection of HEV RNA to exclude an HEV infection in patients with negative anti-HEV IgM and to assess the evolution of hepatitis E.

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Competing interests

The authors declared no conflict of interest with respect to this manuscript.

Ethical approval

Not required.

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