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Evaluation of the VIDAS hepatitis E IgM test in a nonendemic region

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ABSTRACT

Hepatitis E virus (HEV) causes worldwide more than 20 million new hepatitis cases yearly. These infections can take on fulminant forms or cause severe extrahepatic manifestations, and integration into hepatitis differential diagnosis is recommended. In developed countries, genotypes 3 and 4 are most common, mainly through zoonotic transmission. HEV diagnosis is usually first approached with serological methods, sometimes completed with PCR. In this study, we tested the VIDAS anti-HEV immunoglobulin M assay for its specificity on 181 serum samples from patients infected with selected pathogens possibly causing comparable symptoms or false-positive hepatitis E IgM, such as cytomegalovirus, enterovirus, or other hepatitis viruses. Additionally, serum samples were included from 29 patients who tested positive for autoantibodies in immune-mediated liver disease. We report 8 false-positives (specificity 96%), predominantly for hepatitis A, Epstein–Barr virus, and cytomegalovirus, with numbers that are generally lower than reported for comparable assays. A small sensitivity study showed that its use is limited in immunocompromised patients, for whom molecular detection is recommended as first-line test.

1. Introduction

Worldwide, hepatitis E virus (HEV) is one of the most common causes of viral hepatitis. Most references indicate 20 million new cases per year (Rein et al., 2012). This is an underestimate since it is based solely on genotype 1 or 2 infections in developing countries (Kamar et al., 2017). Even though infections with HEV remain mostly asymptomatic in immune competent patients, it can take on fulminant forms. Therefore, it is strongly recommended to include HEV in the differential diagnosis of acute hepatitis (European Association for the Study of the Liver, 2018; Kamar et al., 2017). However, since HEV manifestations can extend beyond hepatitis, HEV testing is also recommended—irrespective of the liver function—in patients with neuralgic amyotrophy, Guillain–Barré syndrome, renal disease, aplastic anemia, and encephalitis/myelitis, among others (European Association for the Study of the Liver, 2018).

In developed countries, the clinical features of acute hepatitis E are mostly caused by genotype 3 and occasionally genotype 4 (European Association for the Study of the Liver, 2018; Kamar et al., 2017). These subtypes are typically transmitted via the zoonotic route, often due to poorly cooked pork or game. In addition, transmission via the water borne route is still possible—for example, shellfish or contaminated river water (Kamar et al., 2017). The relative contribution of this route, however, cannot be well quantified. Above all, transmission via blood donation or tissue transplantation has been gaining more attention recently (Kraef et al., 2018; Vollmer et al., 2016), and therefore, the screening of blood donors has become a discussable topic. Notably, HEV genotype 3 infection may become chronic in immunocompromised patients, while to this date, no cases of chronic infection have been reported for genotypes 1, 2, and 4 (Kamar et al., 2014).

HEV is considered an infrequent cause of acute hepatitis in Belgium, although the seroprevalence of HEV immunoglobulin G (IgG) in this country is approximately 12–14% (De Keukeleire & Reynders, 2015; Van Hoecke et al., 2012). Currently, the first approach towards the diagnosis of acute hepatitis E in immunocompetent adults in daily clinical practice is usually based on the detection of serum/plasma IgM and IgG antibodies directed against hepatitis E virus, sometimes completed by PCR. Various commercially available immunoassays for HEV have been developed that achieve sensitivities >97% in immunocompetent patients and a specificity of >99.5% (Kamar et al., 2017). In 2017, the new Vidas immunoassay for HEV was launched by bioMérieux (France), with similar performance characteristics (Abravanel et al., 2019).

In this low-incidence context, with sometimes few clinical clues towards a causing agent of acute hepatitis, the diagnostic performance of an HEV immunoglobulin M (IgM) test will rely mainly upon the specificity of the assay. Therefore, this study focuses on the evaluation of the specificity of the VIDAS anti-HEV IgM test on serum samples from 181 patients with alternative proven acute and chronic active infectious diseases. In addition, serum samples at disease onset were evaluated from 29 patients who were suspected of autoimmune liver disease to exclude cross-reactivity since the biochemical and histopathological

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picture of autoimmune liver disease can be very similar to HEV (van Gerven et al., 2016). To our knowledge, only 1 research group so far investigated the performance of the Vidas anti-HEV IgG and IgM kit due to its recent launch (Abravanel et al., 2017; Abravanel et al., 2019).

In this study, we chose to challenge the anti-HEV IgM kit with a broad panel of infected serum samples. The selection of these agents was based on the following conditions: the selected pathogens have been described to cause false-positive anti-HEV IgM results or potentially show comparable symptoms. Using this selection, we arrived at cytomegalovirus (CMV), Epstein–Barr virus (EBV), *Toxoplasma gondii* (TG), hepatitis A (HAV), hepatitis B (HBV), hepatitis C (HCV), enterovirus, and HIV.

Possibly because of a polyclonal stimulation of the immune system, it has been described that an EBV infection may cause false reactivity for anti-HEV IgM (Fogeda et al., 2009; Ghinoiu et al., 2009; Hyams et al., 2014). Since mononucleosis-like syndromes are not only described in acute CMV or EBV (Bottieau et al., 2006) but among others also in TG, we considered it valuable to also evaluate the VIDAS anti-HEV IgM in patients with acute toxoplasmosis. False-positive anti-HEV IgM in infections with other hepatitis viruses (A, B, C) was demonstrated previously with other assays (Herremans et al., 2007). The results for hepatitis A were confirmed by Jang et al. (2011). Abravanel et al. demonstrated positive anti-HEV IgG results in hepatitis C in the VIDAS anti-HEV IgG kit. Because of the genetic similarities between HAV and enteroviruses, acute infections with these agents were also included in this study. Various studies around the world have tried to evaluate the seroprevalence of HEV in the HIV-infected population. When the HIV-positive population was compared with the HIV-negative population, most studies found a pronounced HEV seroprevalence in HIV-infected patients (Debes et al., 2016). In all these studies, however, little attention was paid to possible false-positive anti-HEV results, although it was suggested by 1 group (Furukawa et al., 2016). In addition to the specificity study, the sensitivity of this kit was investigated to a limited extent in patients with HEV-RNA detection in blood.

2. Materials and method

The VIDAS HEV IgM test (Biomérieux, France) was performed according to the manufacturer's specifications on serum samples (Sarstedt Monovette® serum-geltubes, serum isolation by centrifugation 10 min × 2000 rpm) from 181 patients with alternative proven acute or chronic active infectious diseases and 29 patients with anti–smooth muscle antibody (ASMA) or anti–mitochondrial antibody (AMA) (Table 1). All tests were retrospectively tested on defrosted archive samples (stored at −80 °C), taken between March 2009 and July 2018 in AZ Sint-Jan Hospital (Bruges, Belgium) and Algemeen Medisch Laboratorium (Antwerp, Belgium).

Unless specified otherwise, all antibodies or antigens were determined on Architect® i2000 (Abbott, IL) in both centers.

The VIDAS IgM Assay is an automated qualitative test based on the ELFA technology (Enzyme Linked Fluorescent Assay) that uses 100-μL serum volume. This method is based on a 2-step enzyme immunoassay sandwich that is combined with a final fluorescent detection (results within 40 min). Every kit contains 30 Solid Phase Receptacles (SPR®) that each serves as the pipetting device and the solid phase, and 30 sealed reagent strips that each consists of 10 wells that contain the reagents necessary for the assay. The last well has a cuvette functionality that allows for the fluorescent detection.

The diagnosis of the acute HAV infections was based on a positive HAV IgM result accompanied by a significant (>2 times) IgG increase over time in a patient. For HBV, this was based on the following serological profile for every patient: positive HBV surface antigen, positive HBV e antigen, negative HBV surface antibody, and positive HBV core IgM (VIDAS 3, Biomérieux, France). Diagnosis of active HCV infection was performed by PCR (Abbott HCV test m2000rt, IL). For the acute CMV, TG, and EBV infections, the diagnosis was based on a positive CMV IgM, *Toxoplasma* IgM, and EBV viral capsid IgM result accompanied by a significant (>2 times) IgG increase over time. Moreover, a low IgG avidity (VIDAS) was ascertained for CMV and TG, and the absence of IgG towards EBNA-1 for EBV. Enteroviral and HEV RNA was detected by using in-house PCR methods targeting the 5′ noncoding region for enteroviruses and the ORF3 region for HEV (AZ Sint-Jan Hospital, Bruges) to prove active viremia for both viruses (detection limit: 90 copies/mL and 400 IU/mL, respectively). The HIV viral load was analyzed by the Aids Reference Laboratory Ghent by the COBAS HIV-1 test (COBAS 4800, Roche, Switzerland).

In all these patients, if multiple consecutive samples over time were available, only the first sample at diagnosis was used for the VIDAS anti-HEV IgM evaluation. If a positive anti-HEV IgM result was obtained in this first sample, a hepatitis E viral load was determined. If follow-up samples were available, a VIDAS anti-HEV IgG test was performed to show possible IgG seroconversions or significant IgG level changes in the latter sera compared to the initial sera. For enteroviruses, serum samples came from patients with a PCR-proven virosis (PCR-positive nasopharyngeal aspirates, bronchoalveolar fluids, or CSF) in the course of the last 10 days.

The detection of autoimmune antibodies in immune mediated liver disease is based on an indirect immunofluorescence assay (NOVA Lite ANA KSL Kit, Inova Diagnostics, CA) that utilizes optimally fixed mouse kidney, stomach, and liver sections. The intended use of this assay is the screening and semiquantitative determination of antinuclear, antimitochondrial, anti–smooth muscle, and gastric parietal antibodies in human serum.

To observe the clinical sensitivity of the assay in a limited way, 9 patient samples who showed positivity for HEV RNA were tested with the VIDAS anti-HEV IgM test.

Table 1
Frequency of positive VIDAS anti-HEV IgM results in the specificity panel.

Initial diagnosis	*n*	Positive	Negative	Cross-reactivity (%)
Hepatitis A	47	4	43	8.5
Hepatitis B	20	0	20	0.0
Hepatitis C	20	0	20	0.0
Toxoplasma gondii	11	0	11	0.0
CMV	23	1	22	4.3
EBV	25	2	23	8.0
Enterovirus	15	0	15	0.0
HIV	20	1	19	5.0
Autoimmune liver disease	29	0	29	0
Total	210	8	202	3.8

3. Results

The frequency of positive anti-HEV IgM results in this specificity panel is shown in Table 1. A total of 8 patients were found to be positive for anti-HEV IgM. These patients were further investigated to confirm if these were indeed false-positives (Table 2). As can be observed, in none of the 8 patients could hepatitis E RNA be detected. Moreover, in 7 of these patients, follow-up samples were available, and no anti-HEV IgG seroconversion or significant IgG level changes could be shown. Therefore, these 8 samples were considered to be false-positives, leading to a specificity of the VIDAS anti-HEV IgM for the diagnosis of an acute hepatitis E infection of 96.2% (202/210) in this study.

When we regard the false-positive results more closely, we find that among the hepatitis viruses, only false-positive VIDAS anti-HEV IgM results were observed in hepatitis A in 8.5% (4 of the 47) of the patients. In acute CMV and EBV infections, some false-positives were observed (3 in 48 patients). Furthermore, 1 false-positive was detected in an HIV-infected patient. No false-positives were found in patients with TG and enterovirus infections, or in patients with positive ASMA and AMA autoimmune antibodies.

Table 2
Detailed results from the 8 patients with initial positive anti-HEV IgM results.

Patient	Diagnosis	Date sample	Architect HAV/CMV/EBV IgM result	Anti-HEV IgM <1.00 negative ≥1.00 positive	Anti-HEV IgG <0.56 negative ≥0.56 positive	HEV RNA (PCR)
1	Hepatitis A	21/03/2017	10.16	7.41 (pos)	<0.10 (neg)	Negative
		31/03/2017		3.56 (pos)	<0.10 (neg)	
2	Hepatitis A	18/11/2013	7.73	1.52 (pos)	0.19 (neg)	Negative
		25/11/2013		1.45 (pos)	0.15 (neg)	
3	Hepatitis A	16/02/2015	11.54	1.02 (pos)	<0.10 (neg)	Negative
		25/02/2015		0.52 (neg)	<0.10 (neg)	
		11/10/2016		0.05 (neg)	<0.10 (neg)	
4	Hepatitis A	30/11/2015	10.07	1.74 (pos)	0.11 (neg)	Negative
		7/12/2015		0.77 (neg)	<0.10 (neg)	
		4/01/2016		0;35 (neg)	<0.10 (neg)	
5	Cytomegalovirus	4/06/2011	7.84	2.20 (pos)	<0.10 (neg)	Negative
		18/06/2011	6.66	1.08 (pos)	0.12 (neg)	
		5/07/2011		0.30 (neg)	<0.10 (neg)	
6	Epstein–Barr virus	15/01/2016	5.4	1.36 (pos)	6.65 (pos)	Negative
		29/01/2016	5.3	1.22 (pos)	7.95 (pos)	
		7/03/2016	0.87	1.01 (pos)	6.59 (pos)	
7	Epstein–Barr virus	21/08/2014	4.2	2.36 (pos)	<0.10 (neg)	Negative
		23/09/2014	0.5	0.85 (neg)	<0.10 (neg)	
8	HIV			1.38 (pos)	<0.10 (neg)	Negative

We also evaluated concisely the sensitivity of the VIDAS anti-HEV IgM assay on hepatitis E RNA positive serum samples from 9 patients. Two of these patients were immunocompetent, and both tested seropositive. Seven patients were considered immunodeficient, of whom only 3 tested serologically positive. These 3 were a kidney transplant patient and 2 patients with alcohol use disorder. The 4 that were missed also contained 1 kidney transplant patient, 1 hematological patient, 1 patient with trisomy 21, and another with ulcerohemorrhagic rectocolitis, under chronical therapy with monoclonal antibodies and corticosteroids.

4. Discussion

In this study, we investigate the specificity of the VIDAS anti-HEV IgM assay. It is a practical advantage that this assay allows automated individual testing (with random access) instead of testing in batch, which enables a simpler integration into laboratory routine. We find a specificity of 96.2%. Compared to other commercialized IgM assays, this is comparable with the percentages (86–100%) provided in literature for low-incidence regions (Legrand-Abravanel et al., 2009; Pas et al., 2013; Wu et al., 2014). It should be stressed, however, that the specificity in this study is expected to be an underestimate for the entire population since patients are explicitly selected for infections that could be prone to false-positive results with this assay. On the other hand, pregnant women as well as patients with rheumatoid factor are not investigated even though in the latter group positive results with the VIDAS anti-HEV IgG assay have been described (Abravanel et al., 2017), while the former group is generally considered to show false-positive assay results with immunoassays in general.

In this study, more false-positives were found compared to a similar investigation by the French National Reference Center for Hepatitis E and Biomérieux (Abravanel et al., 2019), who investigated cross-reactivity for mostly the same set of pathogens but on a smaller cohort. In that study, cross-reactivity was detected only for HIV. The most plausible explanation for the absence of cross-reactivity for the other viruses is most probably linked to the smaller sample size, which limits the detection of false-positivity with rates that we showed to lie mostly below 10%.

The 8.5% false-positivity rate that is found for HAV-infected patients compares well with other immunoassays, which report values from 6% to 9% (Herremans et al., 2007; Jang et al., 2011). This confirms that the coexistence of anti-HAV and anti-HEV IgM must be interpreted with caution, especially since these 2 infections present with similar symptoms. Similar false-positivity rates are reported in literature for HBV and HCV (Herremans et al., 2007), for which in contrast none are observed in this study using the Vidas IgM kit.

False-positivity rates in the EBV- and CMV-infected patients are 8% and 4.3%, respectively. This is significantly lower than the values found in literature for EBV (33–56%) and for CMV (11–24%) (Fogeda et al., 2009; Hyams et al., 2014). The very high specificity of the VIDAS anti-HEV IgM in the TG- and enterovirus-infected patients is in line with literature, for which, to the knowledge of the authors, no positive cases have been reported.

In this study, we detected a false-positivity rate of 5% for HIV. The single HIV patient who tested positive for anti-HEV IgM tested negative for hepatitis E with PCR. A follow-up sample was however not available. Since this patient was most likely not infected with HEV, we assumed a false-positive result. The insert of this method mentioned no false-positive results, except for 1 HIV-infected patient who showed a positive VIDAS anti-HEV IgM. Since, to our knowledge, no independent studies investigated false-positivity rates of HEV IgM tests in HIV infected patients, we suggest that such a study should be performed. This is especially relevant since multiple studies investigated the prevalence of HEV infections within the HIV infected population.

Many investigations were published on the interaction between HEV and autoimmune hepatitis (AIH) since it is known that, in AIH patients, antibodies against HEV (IgG) may be present and vice versa (Terziroli Beretta-Piccoli et al., 2018; van Gerven et al., 2016). Although the pathogenesis and the role of HEV in the development of autoimmune liver disease are not the scope of this publication, cross-reactivity with anti-HEV IgM was investigated in patients with positive ASMA and AMA autoimmune antibodies in this study, with negative results.

To obtain an impression of the sensitivity of this test, a random cohort was selected from patients who tested positive for HEV by PCR. This cohort contained mostly immunocompromised patients. The only 2 immunocompetent patients were detected successfully by the VIDAS HEV IgM assay, but no definitive conclusions could be drawn due to the low numbers. Only few immunocompromised patients tested positive for HEV IgM. These results lie lower than the study by Abravanel et al., who report a sensitivity of 78% in a larger cohort of 57 patients (Abravanel et al., 2019). Nevertheless, these results confirm that the diagnosis of HEV within this population is highly complicated since the IgM and IgG response is delayed or even remains absent. This underlines the necessity of molecular detection of HEV in these patients, in addition to serology (Kamar et al., 2017).

Despite the positive confirmation for the IgM response of both immunocompetent patients, it should not be forgotten that the immune response for HEV is relatively slow, with an initial incubation period of 2–6 weeks (Kamar et al., 2017). Moreover, in a study of antibody-naïve blood donors, even longer incubation periods are reported. Two out of 27 HEV RNA-positive donors had become IgM positive after more than 100 days (Kraef et al., 2018). The latest hepatitis guidelines indeed recommend a combination of serology and NAT testing to diagnose HEV infection (European Association for the Study of the Liver, 2018).

5. Conclusion

This study shows that the VIDAS anti-HEV IgM assay specificity compares well with other commercial IgM assays when applied in a low-incidence region on samples infected with a selection of pathogens such as other hepatitis viruses and samples from patients who tested positive for autoantibodies in immune-mediated liver disease. For HAV, the false-positivity rate is the most pronounced, while for EBV and CMV, less false-positive results are observed. Our data on HIV infected patients illustrate the need for further independent studies on the performance of the HEV IgM tests in HIV-infected patients. Based on a small number of HEV-RNA–positive sera, HEV IgM testing appears to be too insensitive in immunocompromised patients. For this group, molecular detection is absolutely indispensable.

References

Abravanel F, Goutagny N, Perret C, Lhomme S, Vischi F, Aversenq A, et al. Evaluation of two VIDAS ®prototypes for detecting anti-HEV IgG. J Clin Virol 2017 Apr;89:46–50.

Abravanel F, Goutagny N, Joffray R, Eichenlaub E, Baron S, Aversenq A, et al. Performance characteristics of the VIDAS® ANTI-HEV IgM and IgG assays. J Clin Virol 2019 Mar;112:10–4.

Bottieau E, Clerinx J, Van den Enden E, Van Esbroeck M, Colebunders R, Van Gompel A, et al. Infectious mononucleosis-like syndromes in febrile travelers returning from the tropics. J Travel Med 2006 Jul-Aug;13(4):191–7.

De Keukeleire S, Reynders M. Hepatitis E: an underdiagnosed, emerging infection in nonendemic regions. J Clin Transl Hepatol 2015 Dec 28;3(4):288–91.

Debes JD, Pisano MB, Lotto M, Re V. Hepatitis E virus infection in the HIV-positive patient. J Clin Virol 2016 Jul;80:102–6.

European Association for the Study of the Liver. EASL clinical practice guidelines on hepatitis E virus infection. J Hepatol 2018 Jun;68(6):1256–71.

Fogeda M, de Ory F, Avellón A, Echevarría JM. Differential diagnosis of hepatitis E virus, cytomegalovirus and Epstein–Barr virus infection in patients with suspected hepatitis E. J Clin Virol 2009 Jul;45(3):259–61.

Furukawa NW, Teshale EH, Cosmas L, Ochieng M, Gikunju S, Fields BS, et al. Serologic evidence for hepatitis E virus infection among patients with undifferentiated acute febrile illness in Kibera, Kenya. J Clin Virol 2016 Apr;77:106–8.

van Gerven NM, van der Eijk AA, Pas SD, Zaaijer HL, de Boer YS, Witte BI, et al. Dutch Autoimmune Hepatitis Study Group. Seroprevalence of hepatitis E virus in autoimmune hepatitis patients in the Netherlands. J Gastrointestin Liver Dis 2016 Mar;25(1):9–13.

Ghinoiu M, Naveau S, Barri-Ova N, Thaury J, Grangeot-Keros L, Perlemuter G. Acute hepatitis E infection associated with a false-positive serology against Epstein–Barr virus. Eur J Gastroenterol Hepatol 2009 Dec;21(12):1433–5.

Herremans M, Bakker J, Duizer E, Vennema H, Koopmans MP. Use of serological assays for diagnosis of hepatitis E virus genotype 1 and 3 infections in a setting of low endemicity. Clin Vaccine Immunol 2007 May;14(5):562–8.

Hyams C, Mabayoje DA, Copping R, Maranao D, Patel M, Labbett W, et al. Serological cross reactivity to CMV and EBV causes problems in the diagnosis of acute hepatitis E virus infection. J Med Virol 2014 Mar;86(3):478–83.

Jang JH, Jung YM, Kim JS, Lee SH, Kim JW, Hwang SG, et al. Coexistence of IgM antihepatitis A virus and IgM antihepatitis E virus in acute viral hepatitis: a prospective, multicentre study in Korea. J Viral Hepat 2011 Oct;18(10):e408–14.

Kamar N, Dalton HR, Abravanel F, Izopet J. Hepatitis E virus infection. Clin Microbiol Rev 2014 Jan;27(1):116–38.

Kamar N, Izopet J, Pavio N, Aggarwal R, Labrique A, Wedemeyer H, et al. Hepatitis E virus infection. Nat Rev Dis Primers 2017 Nov 16;3, 17086.

Kraef C, Schlein C, Hiller J, Westhölter D, Denzer U, Horvatits T, et al. Course of HEV viremia and anti-HEV IgM/IgG response in asymptomatic blood donors. J Clin Virol 2018 Aug;105:26–30.

Legrand-Abravanel F, Thevenet I, Mansuy JM, Saune K, Vischi F, Peron JM, et al. Good performance of immunoglobulin M assays in diagnosing genotype 3 hepatitis E virus infections. Clin Vaccine Immunol 2009 May;16(5):772–4.

Pas SD, Streefkerk RH, Pronk M, de Man RA, Beersma MF, Osterhaus AD, et al. Diagnostic performance of selected commercial HEV IgM and IgG ELISAs for immunocompromised and immunocompetent patients. J Clin Virol 2013 Dec;58(4):629–34.

Rein DB, Stevens GA, Theaker J, Wittenborn JS, Wiersma ST. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. Hepatology 2012 Apr;55(4):988–97.

Terziroli Beretta-Piccoli B, Ripellino P, Gobbi C, Cerny A, Baserga A, Di Bartolomeo C, et al. Swiss Autoimmune Hepatitis Cohort Study Group. Autoimmune liver disease serology in acute hepatitis E virus infection. J Autoimmun 2018 Nov;94:1–6.

Van Hoecke F, Van Maerken T, De Boulle M, Geerts A, Vlierberghe V, Colle I, et al. Hepatitis E seroprevalence in east and west Flanders. Belgium Acta Gastroenterol Belg 2012 Sep;75(3):322–4.

Vollmer T, Diekmann J, Eberhardt M, Knabbe C, Dreier J. Monitoring of anti–hepatitis E virus antibody seroconversion in asymptomatically infected blood donors: systematic comparison of nine commercial anti-HEV IgM and IgG assays. Viruses 2016 Aug 22;8(8).

Wu WC, Su CW, Yang JY, Lin SF, Chen JY, Wu JC. Application of serologic assays for diagnosing acute hepatitis E in national surveillance of a nonendemic area. J Med Virol 2014 Apr;86(4):720–8.