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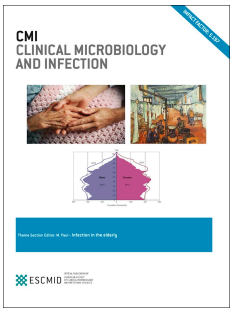

The added value of hepatitis E diagnostics in determining causes of hepatitis in routine diagnostic settings in the Netherlands

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Abstract:

Objectives: Hepatitis E virus (HEV) genotype 3 is endemic in Europe and an underdiagnosed and emerging (public) health issue. In recent years commercial enzyme immunoassays (EIAs) that detect antibodies to HEV more adequately, became available. We investigated the added value of this HEV serology in the diagnostic work flow to detect viral causes of recent hepatitis.

Methods: During a two year period (May 2013 - May 2015), HEV serology was added to the hepatitis work flow, consisting of serological detection of hepatitis A-B-C virus (HAV, HBV, HCV), Epstein-Barr Virus (EBV) and Cytomegalovirus (CMV). Samples positive for HEV IgM were also analysed using PCR to detect HEV RNA. If positive, HEV sequencing was performed for genotyping purposes.

Results: In 235 out of 2521 patients (9.3%), a viral cause for hepatitis was found. Recent HAV, HBV, HCV, EBV or CMV infections were serologically diagnosed in three, 34, 10, 69 and 42 patients respectively. Seventy-eight patients (3.1%) had a recent HEV infection. In 49 of them, sufficient HEV RNA was present for genotyping. All patients were infected with HEV genotype 3.

Conclusions: In our region, a HEV infection is the most frequently diagnosed viral cause for a recent hepatitis. These results indicate that, in a country where HEV is endemic, serological HEV diagnostics should be added to the standard work-up for viral hepatitis.

Introduction

For decades, hepatitis E virus (HEV) is known to be a causative agent of human viral hepatitis. Hepatitis E viruses are recently described as members of the genus orthohepevirus in the family Hepeviridae. This genus comprises in total four major human genotypes. Genotypes 1 and 2 are strictly human viruses and are identified as a cause of epidemic hepatitis in developing countries, associated with waterborne and fecal-oral transmission. Genotypes 3 and 4 are swine viruses (common in domestic and wild pigs, boar and deer) and infect humans as an accidental host.[1] In industrialised countries, non-travel-related HEV infections are caused mainly by genotype 3.[2-3]

Usually HEV infections cause a mild self-limiting hepatitis. Incidentally, a fulminant hepatitis may develop. In immunocompromised patients however, HEV genotype 3 viral infections may develop into a chronic infection with rapidly progressing cirrhosis.[4-7]

Global immunisation programs have changed the epidemiology of viral hepatitis. In the Netherlands, viral hepatitis caused by hepatitis A virus (HAV) and hepatitis B virus (HBV) has decreased considerably. Recently, HEV has been recognised as an increasingly important cause of viral hepatitis.[8-9] Often, a recent HEV infection may be mistaken for drug-induced hepatitis, especially in the elderly where poly-pharmacy is more common. In the Netherlands, the majority of patients with a HEV infection are immunocompetent with no travel history.[10]

HEV testing in healthy blood donors showed an overall immunoglobulin G (IgG) seroprevalence of 27% in 2011.[11] The prevalence appears to increase in time and with age.[12]

In recent years, commercially available HEV-immunoglobulin M (IgM) enzyme immunoassay (EIA) kits detect HEV infections more accurately, with a described sensitivity of 96.7% in immunocompetent and 83.3% in immunocompromised patients, and a specificity

of above 99%.[13-14] We therefore investigated the value of adding HEV serology in our routine workflow of viral causes of hepatitis.

Methods

Between May 2013 and May 2015, we prospectively added HEV serology (IgM and IgG) in all samples submitted to our laboratory for detecting viral causes of hepatitis. The samples were obtained from patients seen by general practitioners (GPs) and medical specialists working in the province of Friesland. Indication for testing was at the discretion of the attending physician. This could be anything from mildly elevated liver enzymes only (ALAT > 45 IU/ml) to severe hepatitis or during regular monitoring for e.g. potential hepatotoxic drug use.

Samples were tested for HAV, HBV, hepatitis C virus (HCV), Cytomegalovirus (CMV), Epstein-Barr Virus (EBV) and HEV. Work-up is illustrated in Figure 1. In accordance with the manufacturer's instructions, HAV, HBV and HCV serological testing was performed using an i2000 Architect analyzer (Abbott, Wiesbaden, Germany). For EBV (VCA-IgM, VCA-IgG and EBNA-IgG) testing an indirect chemiluminescence-assay (CLIA) Liaison (DiaSorin, Saluggia, Italia) was used. For CMV (IgM and IgG, and if necessary IgG avidity) a VIDAS (bioMérieux, Marcy l'Etoile, France) was used during the first year and the a CLIA Liaison was used during the second year.

A recent HAV was diagnosed by a positive anti-HAV IgM. A recent HBV was diagnosed by HBsAg, HBeAg and anti-HBc IgM positivity and first detection of chronic HBV by positive HBsAg, negative anti-HBcore IgM and positive anti-HBc IgG, and six months later unchanged serological profile. First detection of HCV was diagnosed by positive anti-HCV IgG confirmed by HCV-RNA positivity. A recent EBV was diagnosed by high positive VCA-IgM and low VCA-IgG titer and negative EBNA-IgG. A recent CMV was diagnosed by positive IgM titers and low IgG titers with a low avidity index of the IgG.

HEV IgM and IgG testing was performed using a Wantai ELISA kit (Biological Pharmacy Enterprise, Beijing, China). The Wantai HEV IgM kit is a capture EIA using a HEV ORF 2 recombinant antigen conjugate. The Wantai HEV IgG ELISA kit is a binding EIA using microwell strips pre-coated with HEV ORF2 recombinant antigen.

Hepatitis E serology was interpreted as follows: if both IgM and IgG were positive the patient was considered as having a recent HEV infection. If IgM was borderline (OD/CO > 0.9 and < 1.1) and IgG was positive (OD/CO ≥ 1), a HEV RNA PCR was performed.[15] If PCR was positive, these patients were regarded as having had a recent HEV. If HEV IgM was found positive or borderline and IgG was negative, a HEV RNA PCR was performed and a follow-up sample was requested for serology. If HEV RNA PCR was found positive and/or subsequent HEV-IgG seroconversion was detected in the follow-up sample, the patient was considered as suffering from a recent HEV infection.

Sera from all other HEV IgM positive patients were analysed for HEV RNA as well. If HEV RNA was detected and the viral load was high enough for sequencing, a phylogenetic analysis was performed as described before.[11,16]

This study was reviewed by the ethical commission of the University Medical Center Groningen, who concluded that it did not fall within the scope of the rules on medical research involving human subjects. Therefore ethical approval was not applicable to our study.

Results

From May 2013 until May 2015, we received 2567 serum samples from 2521 patients for the diagnosis of viral hepatitis. The age of the patients varied from six months to 93 years (median age 42). In 235 patients (9.3%), a viral cause of their hepatitis was found. A recent

HAV was diagnosed in three patients. A recent HBV or first detection of chronic HBV was found in 34 patients. First detection of HCV occurred in 10 patients. A recent EBV was found in 69 patients. A recent CMV was found in 42 patients (see Figure 1).

Recent HEV infections

In 94 patients, a positive or borderline HEV-IgM was detected. In 74 patients, both HEV IgM and IgG was found. In 45 of these 74 patients, HEV-RNA was also detected. In eight patients, HEV-IgM, but no HEV-IgG was found. In four of these eight patients, HEV RNA was found while in the remaining four patients, no RNA was found and no HEV-IgG seroconversion was observed in the follow-up sample. In 12 patients, a borderline IgM and positive IgG was detected but none of them were positive for HEV-RNA. In summary, 78 patients (3.1% of all patients tested) were regarded as having a recent HEV infection. Among the 78 patients with a recent HEV infection, men were more often affected than women (ratio 2:1), whereas in our total study population men did not outnumber women. The median age of those recently affected by HEV was 56 years for women and 59 years for men (range 11-85 years) (see Figure 2). Of the patients with a recent HEV, 68% were seen by medical specialists and 32% by GPs.

No seasonal pattern in HEV infections was observed and no multiple infections were diagnosed.

Seven patients with a recent HEV infection turned out to be immunocompromised. In four of them, HEV RNA was found. HEV infection resolved in all four of them, in two by discontinuing the immune-suppression, while in the other two patients this had to be combined with an oral ribavirin treatment.

HEV IgG seroprevalence

For the HEV seroprevalence rate calculation, patients with recent HEV were excluded. HEV-IgG was found in 475 out of 2443 patients (18.8%). IgG was detected in 20.4% of male patients, and in 17.6% of female patients. The seroprevalence steadily increased from 4.9% in those younger than 10 years, to 40.2% in those over 70 years of age.

Phylogenetic analysis of HEV

Forty-nine out of 78 patients with a recent HEV infection tested positive for HEV RNA. In 30 patients HEV could also be genotyped (Figure 1). All 30 appeared to be genotype 3 and 25 patients had subtype c (Figure 3). No epidemiological link was observed.

Discussion

In our region, HEV infection appeared to be the most frequently diagnosed viral cause of hepatitis in patients seen by medical specialists and GPs. Physicians sent in serum samples for viral hepatitis testing if abnormal liver function (ALAT > 45 IU/ml) was found, either in patients with complaints of hepatitis or during regular monitoring for e.g. potential hepatotoxic drug use. This open approach, reflecting 'routine diagnostics', probably contributed to our finding that a viral cause of hepatitis was only discovered in a relatively low percentage of patients (9.3%). To our surprise HEV was most prevalent. It outnumbered EBV and CMV, which are regularly observed causes of abnormal liver function and hepatitis in our population. In particular the GPs were quite often surprised by the outcome, as they frequently suspected alcohol consumption as cause of the diseased liver.

In several other European studies looking for recent HEV infections, percentages are reported in the same range as in our study[17-20] or higher (up to 11%)[21-23]. Differences in regional epidemiology may cause this variability, although differences in test selection and study population may also play an important role.

Despite the described high specificity and sensitivity of the Wantai ELISA, positive cases during early infection could have been missed. Borderline IgM with positive IgG was found in 12 patients. No HEV RNA was detected in these 12 patients, suggesting just resolved infection with cleared RNA and waning IgM or aspecific reaction in the IgM response.
Of eight patients with a positive IgM and negative IgG, in four patients the recent HEV infection could be confirmed by detection of HEV-RNA and a seroconversion to IgG positivity in the follow-up samples.

A limitation in our study is that detailed clinical information was not available for every patient. While HEV-serology alone is considered to be sufficient to diagnose a recent HEV infection in immunocompetent patients, it lacks sensitivity in the patient group most at risk of a complicated

course of disease, the immunocompromised.[24-26] The results could have been more complete if PCR would have been performed in all immunocompromised patients, allowing us to report in more detail

on sensitivity levels in this group of patients. This is a risk in our test algorithm. However, in our open population only a minority is expected to be immunocompromised. These findings underline a need to keep educating physicians in our region to adequately inform the laboratory about the immune status of patients for whom tests are requested. For the 'high risk' immunocompromised patient group testing primarily with PCR is advised, similar to HCV diagnostics.

In accordance with other findings in the Netherlands, HEV IgG seroprevalence in our total study population was 18.8%, suggesting that asymptomatic HEV-infections do occur frequently.[25, 27] HEV seroprevalence varies both between and within countries and is higher in individuals exposed to swine and wild animals. The predominance of HEV that we found might be related to the importance of the pig industry in the Netherlands compared to

surrounding countries such as Belgium and Northern France.[28] We also observed that older people were significantly more likely to test positive for HEV IgG, suggesting a lifelong cumulative exposure of HEV.

The notion of autochthonous zoonotic transmission is supported by phylogenetic studies that have shown that HEV strains circulating in human beings and pigs are closely related.[10,29] To clarify farm-to-table risk assessments, more studies on HEV circulation are necessary. Although most zoonotic HEV cases are sporadic, point source foodborne HEV outbreak has been documented.[30] In our study, virus RNA sequences were not identical, and were found over a longer time period of two years, making a common-source unlikely. If typable, all HEV infections found in our patients were genotype 3. HEV genotype 3 is the endemic genotype causing viral hepatitis in our population. The majority of HEV-RNA positive samples were genotype 3c. This is in accordance with the study of Rutjes et al.[29] who found that cluster 3c comprises 35% of animal and environmental HEV sequences and 75% of human HEV sequences.

In conclusion, HEV genotype 3 is more frequently diagnosed as a cause of viral hepatitis than HAV, HBV, HCV, EBV and CMV in our patients. Our results warrant the addition of HEV diagnostics in the standard diagnostic work-up for recent viral hepatitis, both in a hospital, as well as in a GP setting.

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Authors' contributions: MHED, AB and AR-B drafted the manuscript, AB contributed to the design of the study, JW and HGMN provided a detailed critical review of the manuscript. All authors approved the final manuscript.

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Figure 1. Flow diagram for the diagnosis of recent viral hepatitis

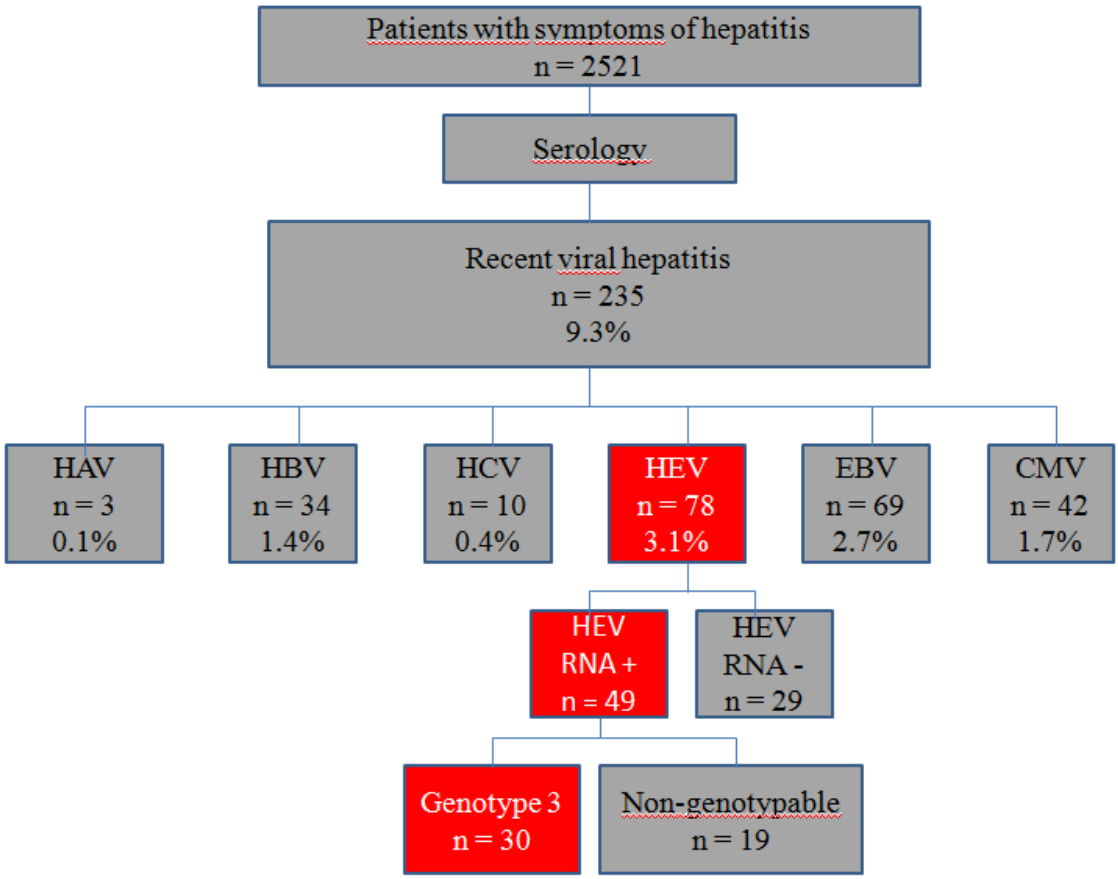

Figure 2. Age and sex distribution in 78 Dutch patients with recent HEV-infection

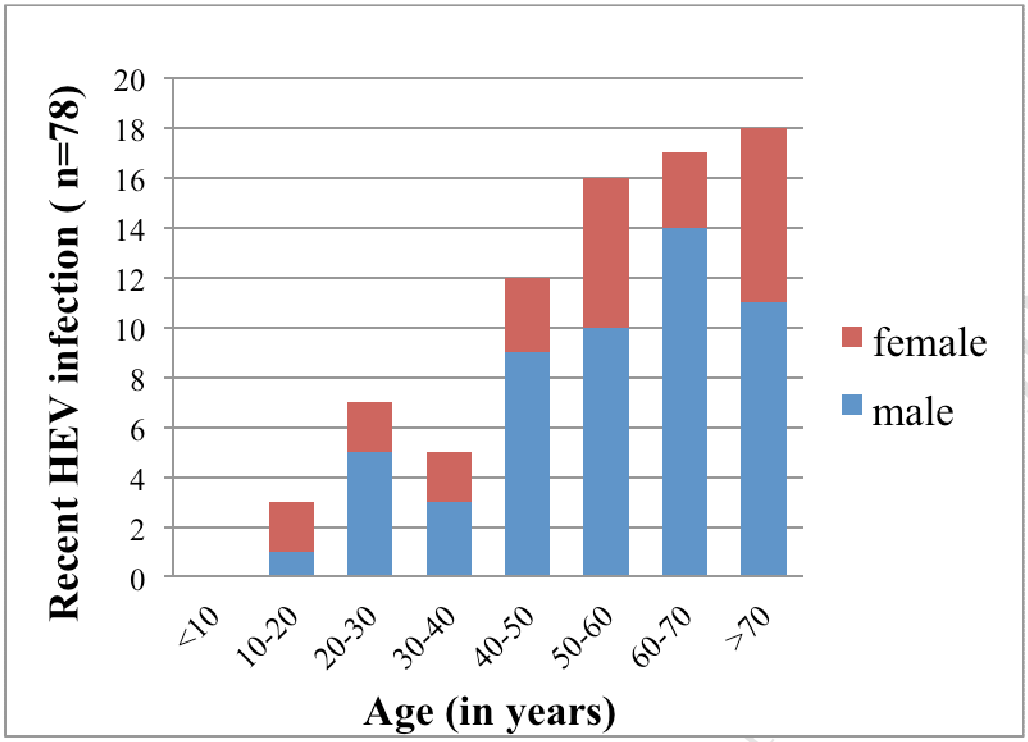

Figure 3. Maximum parsimony tree - phylogenetic analysis of hepatitis E virus in 30 Dutch hepatitis E patients (2013-2015). Patient sequences are in green, reference sequences in red. The following reference sequences of the Genbank were used: Genotype 3a I = AB074920, Genotype 3a II = HQ389543, Genotype 3c I = HF912156, Genotype 3c II = FJ705359, Genotype 3f I = AB291961 and Genotype 3f II = FJ956757. The following GenBank accession numbers were provided for the patient nucleotide sequences: KY086071-KY086100.