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Previous hepatitis E virus infection is associated with increased liver stiffness in patients with autoimmune hepatitis

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What's new?

The hepatitis E virus (HEV) is recognized globally as a leading cause of acute hepatitis, which is typically self-limited. However, in immunocompromised individuals, it may progress to chronic hepatitis. In our study involving 374 patients with autoimmune hepatitis (AIH), the seroprevalence of HEV IgG was 15%. This prevalence was notably higher in older

patients, those with higher BMI, and those exhibiting more advanced liver fibrosis. We found a robust impact of HEV on the progression of AIH, with a 3.69-fold increase in the risk of advanced fibrosis according to multivariable analysis. Overall, the seroprevalence of HEV among the study participants was lower than reported previously in Polish blood donors.

Abstract

Introduction: Autoimmune hepatitis (AIH) is a chronic, progressive liver disease which, in most cases, may require lifelong immunosuppression. Hepatitis E virus (HEV) is a leading cause of acute, typically self-limited, hepatitis worldwide, although immunocompromised patients may develop chronic hepatitis.

Objectives: Here, we evaluated the impact of HEV seropositivity on the clinical course of AIH.

Patients and methods: A group of 374 adult patients with AIH (female 68%, age 34 (18–83) years, 38% with liver cirrhosis) was analyzed. Serum HEV IgG and IgM antibodies were measured by enzyme-linked immunosorbent assay, liver fibrosis was assessed by liver stiffness measurement (LSM), and liver cirrhosis was confirmed with liver histology or LSM.

Results: Fifty-five (15%) patients with AIH were HEV IgG-positive. These patients were older (P <0.001) and had higher BMI and higher LSM (both P <0.05). In a multivariable model including ALT and immunoglobulin G, the HEV seropositive status was associated with an increased risk of advanced liver fibrosis with OR 3.69 (95% CI 1.26–10.77, P = 0.02) as reflected by liver stiffness ≥10.5 kPa. HEV IgG seropositivity was, however, not linked with the type of treatment or worse AIH outcome. The seroprevalence of HEV in patients with AIH was lower compared to results available for Polish blood donors (43%).

Conclusions: Patients with AIH and HEV IgG-positive status seem to be at risk of more advanced liver fibrosis. However, the overall seroprevalence of HEV IgG is a lower in patients with AIH than in the blood donors in Poland.

Key words

autoimmune hepatitis, elastography, hepatitis E virus, liver fibrosis, seroprevalence

INTRODUCTION

Autoimmune hepatitis (AIH) is a chronic liver disease whose incidence and prevalence have been consistently increasing worldwide over time, with higher rates observed in women than men[1]. The incidence and prevalence of AIH among adults in Poland are not precisely known; however they are estimated to be 1.8 and 23.1 per 100 000 people, respectively[1]. The clinical course of AIH varies from asymptomatic to liver failure[2]. Characteristic presentation includes icteric acute hepatitis, presence of antibodies, hypergammaglobulinemia and typical histological findings[3]. The absence of viral hepatitis, mainly cytomegalovirus (CMV), hepatitis A virus (HAV), hepatitis B virus (HBV) and hepatitis C virus (HCV), represents one of the four elements included in the simplified diagnostic criteria for AIH[3,4]. Treatment is based on steroids and azathioprine, and most patients require lifelong immunosuppression[2], which may increase the risk of infectious complications.

The pathogenesis of AIH has not fully elucidated. However, it is believed that an unknown factor triggers the loss of self-tolerance to liver autoantigens in patients with AIH, leading to self-perpetuated liver inflammation[2]. Common viral infections, such as CMV, Epstein-Barr virus (EBV), herpes simplex virus (HSV), HBV, HCV, and hepatitis E virus (HEV), have been suggested as potential precipitating factors for AIH[2,5,6]. This association might be attributed to molecular mimicry between immunogenic antigens (i.e., nuclear,

smooth muscle and liver kidney microsome type 1 antigen) and HSV, HBV or HCV. Still, none of these viruses has been definitively identified as a cause of autoimmune hepatitis[6,7].

HEV infection is the most common cause of acute viral hepatitis worldwide, which affects approximately 20 million people each year, most of them in developing countries through contaminated water[8,9]. In developed countries, including those in Europe and North America, HEV affects immunocompromised patients through zoonotic transmission, primarily by the consumption of undercooked, contaminated meat, and this might lead to chronic hepatitis[8]. Immunoglobulin G (IgG) seroprevalence analysis is the standard method to assess past and present infections in a community[9]. The prevalence of HEV IgG in Europe varies from 0.6% to 52.5%[10]; a relatively high prevalence of 43% was reported in Polish blood donors[11].

The impact of HEV infection on patients with AIH was analyzed in several studies, yielding inconsistent results. The seroprevalence of HEV IgG antibodies being reported from 7.7% to 40% varied across different European regions [12-15]. These rates were typically higher than those observed in general population. The relationship between HEV seropositivity and liver fibrosis has also been explored, though with varying methodologies and inconclusive outcomes. Notably, Eder et al[13] observed a trend towards a higher prevalence of cirrhosis (42% vs 21%, $P = 0.053$) in HEV IgG-positive patients with AIH[13]. Furthermore, a recent study in non-alcoholic fatty liver disease (NAFLD) demonstrated an association between HEV seropositivity and increased liver stiffness measurements (LSM), as well as liver cirrhosis[16].

The HEV might play a role as a potential trigger of AIH (acute hepatitis), as well as a hypothetical modulator of clinical course (chronic hepatitis). The aim of the study was (a) to evaluate the impact of previous HEV infection on AIH course, (b) to study a potential link

between HEV-IgG status and liver fibrosis, and (c) to quantify the seroprevalence of HEV antibodies in adult Polish patients with AIH.

METHODS

Study population

A group of 398 Polish adult patients with AIH was recruited in the Department of Hepatology, Transplantology and Internal Medicine of the Medical University of Warsaw between 2015 and 2019. Diagnosis of AIH was established according to current European guidelines, and liver biopsy was obligatory at the time of diagnosis[17]. Patients with active or past HBV (tested as HBs antigen and HBc antibodies) or HCV (tested as anti-HCV antibodies) infections and those who tested positive for the HEV IgM antibodies and liver transplant recipients were excluded from analyses. The local ethics committee approved the study protocol (KB/128/2015) according to the ethical guidelines of the 1975 Declaration of Helsinki (latest revision, 2013), and written, informed consent was obtained from all participants.

Clinical variables and liver fibrosis assessment

Fasting venous blood samples were collected for tests – including blood count, biochemical tests, and serological markers – as part of the regular standard of care of the Department of Hepatology, Transplantology, and Internal Medicine of the Medical University of Warsaw, Poland. Complete biochemical response (CBR) was defined as serum ALT and IgG within the normal limits[18]. Liver fibrosis was assessed on the day of enrolment to the study by liver stiffness measurement (LSM) and fibrosis-4 (FIB-4) serum fibrosis index. Liver and spleen stiffness was measured using 2D-Shear Wave Elastography (Aixplorer, SuperSonic), as presented earlier[19]. We used the cutoffs of 10.5 kPa and 16.0 kPa for advanced liver

fibrosis and liver cirrhosis, respectively[19]. FIB-4 was calculated according to Sterling et al[20] and the cutoff of 2.67 was used to define patients at risk of advanced liver fibrosis[21].

Testing for hepatitis E infection markers

The detection of HEV IgG and IgM-specific antibodies was performed in the Department of Medical Biology (Pomeranian Medical University, Szczecin, Poland) by enzyme-linked immunosorbent assay (ELISA) manufactured by EUROIMMUN Medizinische Labordiagnostika AG (Luebeck, Germany). The antigen for this test were recombinant structural proteins of the HEV (genotype 1 and 3), and the test was calibrated using the World Health Organization reference serum (reference reagents HEV antiserum, human, 1st IS NIBSC Code 95/584). The assays were used according to the producer's instructions. Out of 379 patients tested using ELISA; five (1%) were positive for HEV IgM. To avoid bias due to the possible acute HEV infection, these HEV IgM positive patients were excluded, as illustrated in Figure 1. The aim of this study was to evaluate the impact of previous HEV infection (indicated by HEV IgG positivity) on the clinical course of AIH. Thus, the analysis proceeded with the remaining 374 patients, all of whom HEV were IgM negative, as shown in Figure 1.

Statistical analyses

Statistical analyses were performed using SPSS (SPSS Statistics, version 27.0. IBM Corp., USA) and GraphPad Prism (GraphPad Prism, version 10.1.1, GraphPad Software, USA). Continuous variables are shown as median with a range (not normally distributed), and categorical variables are expressed as absolute and relative (in per cent) frequencies. The Kolmogorov-Smirnov test was applied to determine whether continuous variables were normally distributed. The Wilcoxon Mann-Whitney U test was used to analyze continuous

variables. The chi^2 and Fisher´s exact tests were used to compare categorical variables. Logistic regression analyses tested the associations between HEV IgG status and clinical data. Statistical procedures were performed two-sided, and *P* values < 0.05 reflected to be statistically significant.

RESULTS

Clinical characteristics of the study cohort

The baseline characteristics of the AIH study cohort are presented in Table 1. Of 374 patients, 68% were females, and the median age was 34 (range 18–83) years. The diagnosis of AIH was established at the median age of 27 (range 3–83) years, and liver cirrhosis was present in 23% of patients at the time of the diagnosis. More than half of the cohort (53%) was diagnosed before the age of 30 years. The median liver stiffness was 11 (range 4–74) kPa. In 183 (49%) patients, the LSM was >10.5 kPa, suggesting advanced liver fibrosis. The median MELD score was 8 (range 6–37) points, and 39 (10%) patients had MELD score >15 points. Most of the patients in the study group (73%) were on azathioprine with or without steroids, 21% were without any immunosuppressive therapy at inclusion, and 6% were on mycophenolate mofetil with or without calcineurin inhibitors and steroids.

The HEV serological status was assessed in median of 4 (range 0–32) years after the diagnosis. Fifty-five (15%) patients in the entire cohort were HEV IgG-positive. Among patients recruited at the moment of AIH diagnosis (n = 105), 20% were positive for HEV IgG antibodies.

As shown in Table 1, patients with HEV IgG-positive were older at the diagnosis of AIH and at enrolment to the study (both *P* <0.001) and had higher BMI as compared to patients with AIH and HEV IgG-negative (*P* = 0.047). Among clinical variables, patients who were seropositive for the HEV IgG had higher serum concentrations of immunoglobulin A

(IgA) (P = 0.047) and showed a trend for higher serum concentrations of γ-globulins (P = 0.058). In terms of markers of liver scarring, patients positive for the HEV IgG antibodies had significantly increased liver fibrosis markers, such as LSM (P = 0.03, Figure 2A), spleen stiffness (P = 0.04, Figure 2B) and FIB-4 results (P = 0.02, Figure 2C). On the other hand, these antibodies were not associated with other disease activity markers such as ALT, IgG, or MELD score (Table 1).

HEV serological status during the AIH course

From the entire study cohort, 269 patients were treated ≥1 year for AIH. Overall, 39 (14%) patients were positive for HEV IgG. They had lower albumin concentrations (4.1 (2.7–5.0) vs. 4.3 (2.2–5.7) g/dl, reference range, 3.5–5.5 g/dl; P = 0.009) than the HEV IgG-negative cases. The HEV IgG positivity was associated with higher LSM (12.6 (5.4–48.0) vs. 10.2 (3.9–74.0) kPa, P = 0.03) in this sub-cohort. On the other hand, the presence of the IgG antibodies was not associated with reaching a complete biochemical response after 12 months of therapy (P = 0.63). Moreover, we did not detect any significant associations between the HEV IgG status and treatment regimen, the odds of requiring liver transplantation or liver-related death.

HEV serological status and liver fibrosis markers

To further explore the impact of HEV seropositive status on liver fibrosis, we analyzed a sub-cohort of patients treated for AIH ≥1 year with normalized ALT activity (n = 160, ALT 28 (8–55) U/l; reference range, <55 U/l; IgG 1387 (677–1600) mg/dl; reference range, 800–1600 mg/dl), to avoid the inflammation bias on the fibrosis markers, in particular on LSM. multivariable regression analysis demonstrated that seropositive HEV status (OR 3.69, 95% CI 1.26–10.77, P = 0.02), ALT activity and IgG serum concentration are independently

associated with increased risk of advanced liver fibrosis (LSM ≥10.5 kPa), as presented in Table 2.

Moreover, in univariable regression analyses the seropositivity of HEV was linked to a higher risk of liver cirrhosis, defined as increased LSM (≥16.0 kPa) with OR 2.78 (95% CI 1.03–7.45, *P* = 0.04), as well to a higher risk of advanced liver fibrosis, defined as FIB-4 >2.67 with OR 2.82 (95% CI 1.09–7.28, *P* = 0.03); however, these two associations were not more significant in the multivariable model.

DISCUSSION

In this study, we evaluated the potential role of previous HEV infection on the clinical course of 374 patients with AIH and assessed the HEV seroprevalence among these patients. One of our major findings is that patients with AIH and HEV IgG-positive status had more pronounced liver fibrosis markers: liver and spleen stiffness and FIB-4 and were at higher risk of advanced liver fibrosis. To the best of our knowledge, this observation was not previously reported in patients with AIH. In 2019, Eder et al[13] evaluated the effects of the previous HEV infections in 92 patients with AIH and noted a trend, but not statistical significance, of higher prevalence of cirrhosis (42% vs 21%, *P* = 0.053) in HEV IgG-positive patients with AIH at the time of the first diagnosis[13]. Authors of other reports[15,22] stated no differences in AIH staging related to HEV IgG positivity. However, these studies primarily based their findings on histological fibrosis assessment at the time of diagnosis (cirrhosis or Ishak Score)[13,15,22,23]. One study reported using elastography but did not provide results specific to HEV IgG status[23]. Additionally, none of these studies assessed liver fibrosis at the time of sampling or reported on LSM.

Our observation regarding increased LSM in HEV IgG seropositive patients with AIH aligns with the results presented by Paternostro et al[16] in 177 patients with NAFLD[16].

The authors of that study[16] found that besides diabetes mellitus and older age, HEV IgG positivity was independently linked with increased liver stiffness (>10 kPa) and liver cirrhosis. Being aware of LSM limitation in AIH, we evaluated this aspect in a well-characterized group of treated at least one-year patients[24] with normalized ALT activity to limit the bias of hepatic inflammation on LSM[19,24]. In our cohort, HEV IgG seropositive status was associated with an increased liver fibrosis risk but not with the development of cirrhosis, which might be caused, however, by low statistical power due to a small cohort of patients with liver cirrhosis.

The seroprevalence of HEV IgG in patients with AIH has been assessed in several studies. In 2014, Pischke et al[12] analyzed anti-HEV antibodies in 208 patients with AIH, finding a 7.7% positivity rate, which was higher than in healthy controls[11]. A Dutch study investigated HEV-IgG in 354 patients with AIH, with 30% testing positive. This study also observed a higher frequency of anti-smooth muscle antibody (ASMA) in patients who were HEV-IgG positive [14]. However, these authors noted no evidence of a differing clinical course of AIH in these patients[13]. An analysis of German patients with AIH showed HEV IgG positivity in 40% of 109 patients at diagnosis, highlighting a higher prevalence in older individuals[14]. A 2019 study of 92 Austrian AIH patients found a 21% HEV IgG positivity rate, exceeding that of in the general population (12–14%)[15] and confirmed the relationship with patients age[12]. Contrastingly, we found lower seroprevalence of HEV IgG in patients with AIH (15%) compared to healthy blood donors (43%)[11], liver transplant recipients (41%)[25], as well patients with advanced chronic liver diseases (48%)[25] in Poland. These findings are in contrast to reports from Germany[12,15] and Austria[13], where HEV seroprevalence was found to be higher in patients with AIH compared to the general population. However, the reports from the Netherlands[14] and Spain (Catalonia)[23] showed

HEV seroprevalence rates similar to those of the general population; therefor, the published results appear inconsistent.

The varied prevalence of the anti-HEV IgG in European cohorts of patients with AIH seems to depend on the geographical location and assay selection. The differences between patients with AIH and a healthy population in Poland might be explained by (i) the assay used in the studies, (ii) the age of the study cohorts, and (iii) the patients' awareness of infection factors. First, we used the Euroimmune assay with previously reported 61.5% sensitivity and 98.8% specificity. In contrast, in other studies[11,12,23,25], the Wantai assay was used, which has the highest sensitivity across all available assays[10,26] and a bit lower specificity (96.5%) than Euroimmune[27]. Nevertheless, available enzyme immunoassays for HEV have yet to be standardized, leading to uncertainty in comparing published studies [9]. Second, our cohort seems to be younger (mean age 38 ± 16 years) than the reported Polish transplant recipients (mean age 52 ± 13 years)[25], as well as Polish patients with liver cirrhosis (mean age 58 ± 11 years)[25]. Finally, we speculate that patients with autoimmune liver diseases might be more aware of potential viral infections and prevent them with a diet excluding raw or undercooked meat (preventing HEV-3 and HEV-4) compared to the general population, however, no data support our assumption.

Finally, regarding autoimmune diseases, we should consider the possible cross-reactivity with antibodies commonly occurring in AIH, such as polyclonal γ-globulins, antinuclear antibodies (ANA) and ASMA. This aspect was evaluated from the HEV perspective by Terziroli et al[28], who found that among 48 patients with acute HEV infection, 50% of them had at least one autoantibody (ANA 33%, ASMA 21% and anti-neutrophil cytoplasmic antibody (ANCA) in 15%)[28]. Previously, it was also reported that patients with AIH and anti-HEV IgG positivity had higher serum IgG levels, gamma-globulins, as well as higher ANA and ASMA titres, which might suggest the occurrence of

cross-reactivity between HEV and liver antigens[14,23]. In our cohort, we found only elevated serum IgA and a trend with higher γ-globulins concentrations in patients with AIH and HEV IgG positivity.

Our study has some limitations which need to be addressed. First, by using different assays as compared to the previous studies, we were not able to directly compare the seroprevalence with data regarding Polish blood donors as well as other Polish patients with chronic liver diseases. Moreover, the HEV RNA PCR was not tested in HEV IgG-positive and HEV IgM-negative patients, as it should be considered in patients treated with immunosuppressants, particularly in highly suspected cases. We have no data on antibodies at the time of sampling, and we cannot correlate the titres of antibodies and HEV IgG status. Finally, the study's cross-sectional nature cannot demonstrate a cause-and-effect relationship, which might require a prospective, multicenter study.

CONCLUSION

HEV IgG seropositive status seems to correlate with more advanced liver fibrosis in patients with AIH, highlighting the influence of HEV infection on disease progression in this population. The relatively low frequency of HEV IgG seropositivity in our study cohort might be attributed to different assays used in the studies or hygienic awareness in those patients. The exact role of the HEV infection as a potential trigger of AIH needs to be elucidated in additional studies.

Article information

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Contribution statement PM conceived the concept of the study. MKJ, PM and MM contributed to the research design. MKJ coordinated funding for the project. AKP and MM performed serological testing. MKJ, JRW and PM were involved in data collection. MKJ and MK analyzed the data and wrote the manuscript. All authors edited and approved the final version of the manuscript.

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References

1 Hahn JW, Yang HR, Moon JS, et al. Global incidence and prevalence of autoimmune hepatitis: a systematic review and meta-analysis. eClinicalMedicine. 2023; 65.

2 Muratori L, Lohse AW, Lenzi M. Diagnosis and management of autoimmune hepatitis. Bmj. 2023; 380.

3 Dalekos GN, Samakidou A, Lyberopoulou A, et al. Recent advances in the diagnosis and management of autoimmune hepatitis. Polish Arch Intern Med. 2022; 132.

4 Hennes EM, Zeniya M, Czaja AJ, et al. Simplified criteria for the diagnosis of autoimmune

hepatitis. Hepatology. 2008; 48: 169-176.

5 Ferri S, Muratori L, Lenzi M, et al. HCV and autoimmunity. Curr Pharm Des. 2008; 14: 1678-1685.

6 Christen U, Hintermann E. Pathogens and autoimmune hepatitis. Clin Exp Immunol. 2019; 195: 35-51.

7 Czaja AJ. Incorporating the Molecular Mimicry of Environmental Antigens into the Causality of Autoimmune Hepatitis. Dig Dis Sci. 2023; 68: 2824-2842.

8 Damiris K, Aghaie Meybodi M, Niazi M, Pyrsopoulos N. Hepatitis E in immunocompromised individuals. World J Hepatol. 2022; 14: 482-494.

9 Willauer AN, Sherman KE. Hepatitis E virus: has anything changed? Curr Opin Gastroenterol. 2023; 39: 169-174.

10 Hartl J, Otto B, Madden RG, et al. Hepatitis E Seroprevalence in Europe: A Meta-Analysis. Viruses. 2016; 8.

11 Grabarczyk P, Sulkowska E, Gdowska J, et al. Molecular and serological infection marker screening in blood donors indicates high endemicity of hepatitis E virus in Poland. Transfusion. 2018; 58: 1245-1253.

12 Pischke S, Gisa A, Suneetha PV, et al. Increased HEV seroprevalence in patients with autoimmune hepatitis. PLoS One. 2014; 9: e85330.

13 Eder M, Strassl R, Beinhardt S, et al. High seroprevalence of anti-Hepatitis E antibodies in Austrian patients with autoimmune hepatitis. Liver Int. 2019; 39: 640-645.

14 van Gerven NMF, van der Eijk AA, Pas SD, et al. Seroprevalence of Hepatitis E Virus in Autoimmune Hepatitis Patients in the Netherlands. J Gastrointestin Liver Dis. 2016; 25: 9-13.

15 Taubert R, Diestelhorst J, Junge N, et al. Increased seroprevalence of HAV and parvovirus B19 in children and of HEV in adults at diagnosis of autoimmune hepatitis. Sci Rep. 2018; 8.

16 Paternostro R, Traussnigg S, Staufer K, et al. Prevalence of anti-Hepatitis E antibodies and

impact on disease severity in non-alcoholic fatty liver disease. Hepatol Res. 2020; hepr.13581.

17 Lohse AW, Chazouillères O, Dalekos G, et al. EASL clinical practice guidelines: Autoimmune hepatitis. J Hepatol. 2015; 63: 971-1004.

18 Pape S, Snijders RJALM, Gevers TJG, et al. Systematic review of response criteria and endpoints in autoimmune hepatitis by the International Autoimmune Hepatitis Group. J Hepatol. 2022; 76: 841-849.

19 Janik MK, Kruk B, Szczepankiewicz B, et al. Measurement of liver and spleen stiffness as complementary methods for assessment of liver fibrosis in autoimmune hepatitis. Liver Int. 2021; 41: 348-356.

20 Sterling RK, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology. 2006; 43: 1317-1325.

21 McPherson S, Hardy T, Dufour J-F, et al. Age as a Confounding Factor for the Accurate Non-Invasive Diagnosis of Advanced NAFLD Fibrosis. Am J Gastroenterol. 2017; 112: 740-751.

22 Van Gerven NMF, Verwer BJ, Witte BI, et al. Epidemiology and clinical characteristics of autoimmune hepatitis in the Netherlands. Scand J Gastroenterol. 2014; 49: 1245-1254.

23 Llovet LP, Gratacós-Ginés J, Ortiz O, et al. Higher seroprevalence of hepatitis E virus in autoimmune hepatitis: Role of false-positive antibodies. Liver Int. 2020; 40: 558-564.

24 Hartl J, Denzer U, Ehlken H, et al. Transient elastography in autoimmune hepatitis: Timing determines the impact of inflammation and fibrosis. J Hepatol. 2016; 65: 769-775.

25 Parfieniuk-Kowerda A, Jaroszewicz J, Łapiński TW, et al. High prevalence of anti-HEV antibodies among patients with immunosuppression and hepatic disorders in eastern Poland. Arch Med Sci. 2021; 17: 675-681.

26 Rossi-Tamisier M, Moal V, Gerolami R, Colson P. Discrepancy between anti-hepatitis E virus immunoglobulin G prevalence assessed by two assays in kidney and liver transplant recipients. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2013; 56: 62-64.

27 Al-Absi ES, Al- Sadeq DW, Younis MH, et al. Performance evaluation of five commercial assays in assessing seroprevalence of HEV antibodies among blood donors. J Med Microbiol. 2018; 67: 1302-1309.

28 Terziroli Beretta-Piccoli B, Ripellino P, Gobbi C, et al. Autoimmune liver disease serology in acute hepatitis E virus infection. J Autoimmun. 2018; 94: 1-6.

Table 1 Characterization of the study cohort and its subset regarding HEV serological status

	Total cohort n = 374	HEV IgG (+) n = 55	HEV IgG (-) n = 319	*P* value
Age at the study, years	34 (18–83)	43 (18–73)	32 (18–83)	<0.001
Age at diagnosis, years	27 (3–83)	38 (10–72)	26 (3–83)	<0.001
Female, n (%)	256 (68%)	40 (72%)	216 (68%)	0.53
BMI, kg/m^2	23 (16–41)	25 (18–34)	23 (16–41)	0.047
ALT, U/L **reference range, <55 U/L**	55 (8–3400)	55 (15–930)	57 (8–3400)	0.70
Immunoglobulin A, mg/dL **reference range, 70–400 mg/dL**	233 (3–1480)	260 (70–1054)	230 (3–1480)	0.047
Immunoglobulin G, mg/dL **reference range, 800–1600 mg/dL**	1488 (318–6508)	1592 (567–3490)	1466 (318–6508)	0.62

Immunoglobulin M, mg/dL reference range, 50–280 mg/dL	159 (14–1035)	166 (27–904)	156 (14–1035)	0.58
Spleen length, cm	12 (8–25)	13 (8–22)	12 (8–25)	0.06
MELD, points	8 (6–37)	8 (6–34)	8 (6–37)	0.99

All quantitative data shown as median and range

The Chi^2 and U Mann-Whitney tests were used to compared between subgroups and *P* <0.05 was considered as significant

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; MELD, model of end stage liver disease

Table 2 Regression analysis for increased liver stiffness ≥10.5 kPa in patients treated at least one year and with normalized ALT (n = 161)

	Univariable		Multivariable	
	***P* value**	**OR (95% CI)**	***P* value**	**OR (95% CI)**
ALT, U/L	<0.001	1.08 (1.04–1.11)	<0.001	1.07 (1.03–1.11)
Immunoglobulin G, mg/dL	0.002	1.01 (1.00–1.01)	0.01	1.01 (1.00–1.01)
HEV IgG positive	0.04	2.73 (1.07–7.00)	0.02	3.69 (1.26–10.77)
Age at sampling, years	0.055	1.02 (1.00–1.05)	0.67	1.01 (0.98–1.03)

Duration of the disease, years	0.13	1.04 (0.99–1.09)	-	
Age at diagnosis, years	0.31	1.01 (0.99–1.03)	-	
Female sex	0.39	1.40 (0.65–2.99)	-	
First line therapy or nil	0.72	1.36 (0.26–6.96)	-	
Abbreviations: ALT, alanine aminotransferase; CI, confidence interval; HEV, hepatitis E virus, OR odds ratio				

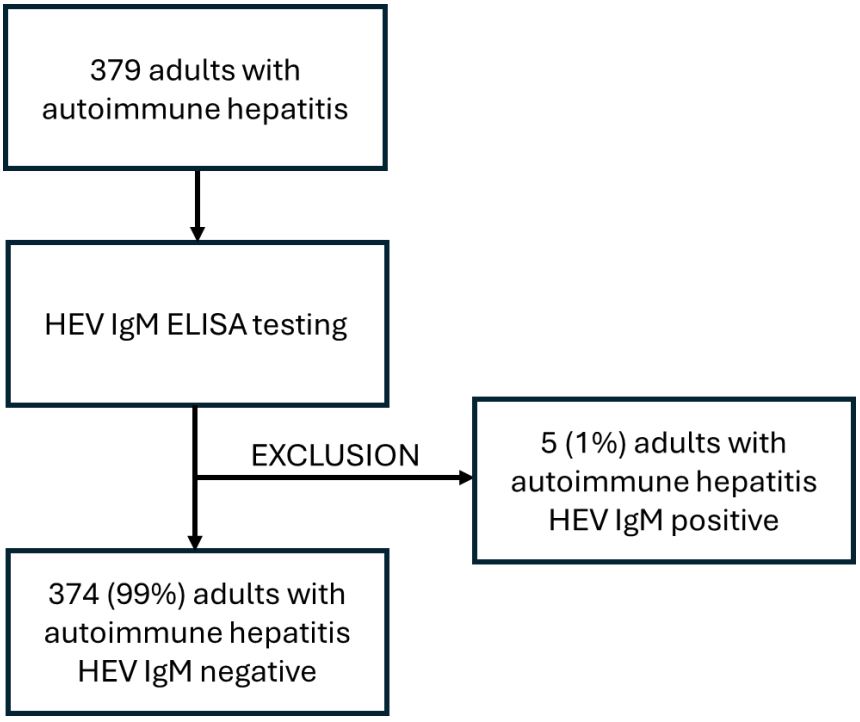

Figure 1 A workflow of the overall study

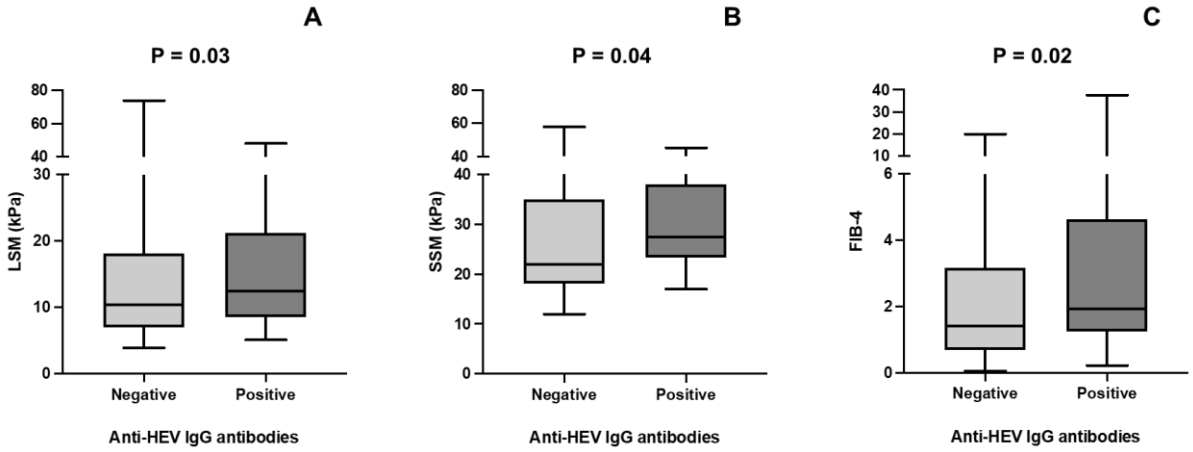

Figure 2 Liver stiffness (panel A), spleen stiffness (panel B) and Fibrosis-4 index (panel C) in patients with autoimmune hepatitis in relation to the HEV serological status. Groups of patients were compared using the Mann-Whitney U test, data presented as mean value and standard errors. Abbreviations: FIB-4, Fibrosis-4 index; LSM, liver stiffness measurements; SSM, spleen stiffness measurements

Short title: HEV antibodies in autoimmune hepatitis