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SHORT COMMUNICATION

Hepatitis E virus markers in hematological patients in a highly endemic country

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Introduction

The hepatitis E virus (HEV) infects humans almost everywhere in the world. In developed countries, genotypes 3 (HEV-3) and sporadically 4 (HEV-4) are transmitted to humans, mainly through infected pork or game meat consumption. In Europe, in healthy individuals, HEV usually causes a self-limiting asymptomatic or subclinical infection that does not require treatment. It is estimated that less than 5% of infections may be accompanied by acute hepatitis symptoms such as elevated liver enzymes, jaundice, and other non-specific symptoms [1]. However, in immunocompromised patients such as oncological patients, solid organ or hematopoietic stem cell transplant (HSCT) recipients, due to basic illness or immunosuppressive therapy, HEV infections can lead to chronic hepatitis [2, 3].

HEV can also be transmitted by blood and its components, as well as by solid organ and tissue transplantation [4–6]. Due to the semi-enveloped structure of HEV virions, there are doubts about what is the effectiveness of currently available methods of pathogen reduction technology (PRT) [7–9]. Therefore, HSCT recipients receiving frequent transfusions are at risk of HEV infection transmitted by blood and its components [3].

The interest in HEV infection in hematological patients results from the increasingly frequent observations of serious complications. In hematological and oncological patients with reduced immunity, acute or chronic hepatitis, and even deaths, have been reported [2, 6, 10]. HEV infection may also cause non-hepatic manifestations, including neurological, hematological, nephrological and cardiovascular [6].

The epidemiological situation around the world is diverse. Poland is one of the countries with the highest frequency of specific antibodies indicating past infection (IgG — 43.52% in blood donors) and a high frequency of recent infection markers (IgM — 1.27%, HEV RNA — 1/2.109 blood donors) [11].

The aim of this study was to assess the presence of HEV infection markers in hematological patients in Poland, including allo-HSCT recipients.

Patients and methods

A retrospective study of acute HEV infection markers, including viral RNA or Ag and specific IgM, was performed in recipients of allo-HSCT treated in the Hematopoietic Stem Cell Transplantation Ward (HSCTW) at the Institute of Hematology and Transfusion Medicine (IHTM) in Warsaw, Poland (these patients were named Group I). HEV markers testing was performed also in patients' samples from other hematological wards referred to the Department of Virology for the diagnosis of viral hepatitis (these patients were named Group II). Additionally in Group II, anti-HEV IgG, indicating past infection, was tested.

428 plasma samples from 214 recipients aged 18–71 years (median: 49, mean: 49; 102 women, 112 men) were collected from Group I between January 2019 and January 2023 at two time points, i.e. c.30 and c.60 days

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after allo-HSCT. 138 patients underwent allo-HSCT from unrelated donors, 59 from related donors, and 17 from haploidentical donors. Group I consisted of patients with different types of malignancies (leukemia, lymphomas, myelomas) or transplanted due to other serious illnesses (myelodysplastic syndrome, leukoencephalopathy, myelofibrosis, amyloidosis). Acute infection status was investigated in this group: RNA HEV in 166 patients and HEV Ag in another 48.

Group II consisted of 213 patients aged 8–89 years (median: 62, mean: 59; 92 women, 121 men) primarily referred for other hepatitis viruses (HBV and/or HCV) markers testing. In this group, IgM anti-HEV was tested in all 213 patients (with an ELISA or rapid test) and specific IgG and HEV RNA were tested in 148. Plasma or serum samples were collected between January 2021 and April 2023. In this group, there were 20 HCV RNA and six HBV DNA positive patients.

HEV RNA testing was performed using the reverse transcription polymerase chain reaction method (RT-PCR: RealStar® HEV RT-PCR Kit 2.0, Altona Diagnostics GmbH, Germany) on the Rotor-Gene Q MDx 5plex HRM platform (Qiagen GmbH, Germany) or Light Cycler 480 II (Roche Diagnostics, Germany). The analytical sensitivity of the assay was estimated for 50 IU/mL [95% confidence interval (CI): 30–112.5 IU/mL] and the linear range for 2.5×10^3–2.5×10^9 IU/mL. RNA was isolated from 200 µL of plasma or serum by the automated NucliSENS® easyMag® (bioMérieux, France) method using specific protocol Generic 2.0.1 and nucleic acid was eluted with 50 µL of extraction buffer. For amplification, 25 µL of eluate was used.

HEV Ag and anti-HEV IgG were tested using the Wantai HEV-Ag ELISA^{Plus} and Wantai HEV-IgG ELISA, respectively, whereas anti-HEV IgM alternatively with Wantai HEV IgM ELISA or rapid test for IgM Antibody to Hepatitis E Virus (Colloidal Gold Device) (all tests by Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., China).

All tests were performed according to the manufacturer's instructions.

Frequencies were expressed as percentages with 95%CI and differences between proportions using the relative risk (RR) factor. The significance of differences (p <0.05) was verified by the chi-squared test using the Tibco Statistica 13.3 program (Tibco Software, Palo Alto, CA, USA).

Results and discussion

The results of HEV infection markers testing in allo-HSCT recipients (Group I) and hematological patients (Group II) are set out in Table I.

In Group I, neither HEV RNA nor HEV antigen was detected. Similarly, in Group II, no HEV RNA or specific IgM in the ELISA test was detected, but a positive anti-HEV IgM result was obtained in a rapid test in one (0.47%) patient, aged 65 with factor VIII deficiency and liver cirrhosis. However, ELISA did not confirm this result and neither HEV RNA nor anti-HEV IgG was detected. The patient had a history of hepatitis C (anti-HCV reactive, HCV RNA negative).

44.59% of patients in Group II had a history of HEV infection (specific IgG detected), a proportion very similar (43.5%) to that observed in a nationwide study of donors conducted in 2015. In the current study including patients, no significant differences were found between men and women (RR 1.11; 95% CI: 0.77–1.59; p = 0.73), while the frequency of anti-HEV IgG in donors was higher in men (46.48%) than in women (38.15%) and increased with age (up to 56.00% in the group ≥58 years of age) [11]. In patients from IHTM hematology wards, the frequency of anti-IgG antibodies was also higher in older patients (>60 years) compared to younger individuals (50.00% vs. 37.88%), although these differences, probably due to the limited number of subjects, were not significant (RR 1.32; 95% CI: 0.91–1.92; p = 0.36).

It is worth noting that in both groups the percentage of seropositive individuals was lower (although again the differences were not significant, again most likely due to the relatively small number) compared to the groups of donors <58 years and ≥58 [11] — 37.88% vs. 42.65% (RR 0.88; 95% CI: 0.65–1.21; p = 0.62) and 50.00% vs. 56.00% (RR 0.89; 95% CI: 0.69–1.14; p = 0.61), respectively. It cannot be ruled out that the detection of specific antibodies indicating a past infection (anti-HEV IgG) was lower in patients than in donors because the level of antibodies could be influenced by immunodeficiencies and/or treatment among patients. The frequency of anti-HEV IgG we observed in Group II is higher compared to data reported by other centers from Europe and China in patients after HSCT (data for hematological patients are unavailable), where the average proportion of IgG antibodies was 11.4% [3], or from the United States — in a group of immunocompromised patients (including 35% with hematological malignancies), the frequency of anti-IgG was 16% [12]. However, a lower frequency of the acute phase of infection marker — IgM was observed compared to the general population obtained in previous years in blood donors in Poland, for whom it was 1.27% [11] and in HSCT recipients from European centers and China, where average frequency of anti-HEV IgM was 2.00% [3]. The lack of detectable HEV RNA among hematological IHTM patients, including those after HSCT, indicates a lower incidence of HEV compared to the results from the above-mentioned centers in Europe and China, where the incidence of HEV RNA was found to be 0–3.85% (average frequency: 1.5%) [3]. Other available studies on HEV infection in stem cell transplant recipients have shown the incidence of infection in the group of HSCT recipients to range from <1 to 4% [2]. However, HEV studies in HSCT recipients in Sweden showed the occurrence of HEV RNA in up to 3.4% of patients [13].

Table I. Detection of HEV infection markers in hematological patients

Tested marker	Group	Number of tested person (samples)	Positive patients	
			number	frequency in % (95% CI)
Ia. RNA HEV				
	Group I	166 (332)	0	0.00 (0.00–2.26)
	Group II	148 (148)	0	0.00 (0.00–2.53)
Ib. Ag HEV ELISA				
	Group I	48 (96)	0	0.00 (0.00–7.41)
IIa. Anti-IgG HEV ELISA				
	Group II	148 (148)	66	44.59 (36.82–52.64)
females		71 (71)	30	42.25 (31.45–53.85)
males		77 (77)	36	46.75 (36.03–57.78)
	>60	82 (82)	41	50.00 (39.42–60.58)
	≤60	66 (66)	25	37.88 (27.15–49.94)
IIb. Anti-IgM HEV ELISA				
	Group II	148 (148)	0	0.00 (0.00–2.53)
females		71 (71)	0	0.00 (0.00–5.13)
males		77 (77)	0	0.00 (0.00–4.75)
	>60	82 (82)	0	0.00 (0.00–4.48)
	≤60	66 (66)	0	0.00 (0.00–5.50)
IIc. Anti-IgM HEV Rapid Test				
	Group II	65 (65)	1	1.54 (0.27–8.21)
females		21 (21)	0	0.00 (0.00–15.46)
males		44 (44)	1	2.27 (0.40–11.81)
	>60	30 (30)	1	3.33 (0.59–16.67)
	≤60	35 (35)	0	0.00 (0.00–9.89)
Anti-IgM HEV (ELISA+Rapid — IIb+IIc in total)				
	Group II	213 (213)	1	0.47 (0.08–2.61)
females		92 (92)	0	0.00 (0.00–4.01)
males		121 (121)	1	0.83 (0.15–4.53)
	>60	112 (112)	1	0.89 (0.16–4.88)
	≤60	101 (101)	0	0.00 (0.00–3.66)

CI — confidence interval

Several potential explanations exist for the lack of markers of acute infection in patients intensively treated with blood components. In most similar studies, such cases were recorded, although there are also analyses that did not allow the frequency of infection to be determined. This may happen when the study group is too small, considering the relatively low incidence of TTI-HEV. In the case of our study, this is surprising, because previous studies showed a rather high incidence of HEV in Poland compared to other countries (frequency in blood donors: IgM 1.27%, HEV RNA 1:2.109) [11]. We cannot rule out that the epidemiological situation has changed, as in the Netherlands and Denmark, where significant fluctuations in the detection of viral RNA among donors have been observed over several years [14]. It should be noted that our study concerns a recent period, for which we yet do not have data available on the frequency of acute phase markers that would enable the assessment of incidence in the general population. It should also be noted that the samples we tested came from wards supplied with blood and blood components by RCKiK Warszawa, which uses PRT for most FFPs and KKPs. It is true that these preventive methods seem to have limited effectiveness against HEV, but the virus reduction factor (VRF) at the level of 2–3.5 log observed in previous studies [15–17] allows us to predict a certain degree of TTI-HEV risk reduction. Of note, in 2015 we documented the occurrence of anti-IgG and IgM HEV in 35.63% and 1.21% of donors from Warsaw, respectively [11].

Although there is a shortage of publications with case descriptions, and our study did not demonstrate active HEV infection in Polish patients, our preliminary observations present the risk of HEV infection and the occurrence of significant clinical complications also in our country — this is indicated by the recent diagnosed case of chronic HEV infection in the IHTM ward — HEV infection lasting at least seven months, accompanied by hepatitis, initially attributed to the treatment received by the patient [data not published].

In conclusion, reported high HEV seroprevalence in hematological patients indicates the significant past prevalence of HEV infections among immunocompromised patients, although no ongoing infections were revealed.

Available data on the risk of HEV in HSCT recipients and clinical complications resulting from infection indicate the importance of HEV testing, especially using molecular biology methods, which are essential for correctly diagnosing liver diseases in patients with immune deficiencies. Because in Poland, the testing of HEV RNA has not been introduced as an obligatory screening in blood donors, the European recommendation encouraging the performance of diagnostics for HEV in all patients after transfusion who develop elevated levels of liver enzyme activity is especially important [1, 18].

It should be underscored that this kind of diagnostics is important in risk groups including in immunocompromised patients, in which the most effective is RNA HEV testing [2, 6]. Some authors have postulated screening for HEV RNA in transplant patients, including all HSCT recipients, 100 days after transplantation [19].

Article information and declarations

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Authors' contributions

AK — literature analysis, data analysis, original draft preparation; PG — conceptualization, literature analysis, original draft preparation, review and editing; JŚ — testing of samples; KH, BN-A, BC — data concerning patient groups; HD, JJ — conceptualization, literature analysis, original draft preparation, review and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

None.

Ethics statement

The authors declare that informed consent for publication was not obtained, as published data does not allow for patient identification.

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Supplementary material

None.

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