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PREVALENCE AND CLINICAL CHARACTERISTICS OF HEPATITIS E VIRUS INFECTION AMONG INDIVIDUALS FROM RISK GROUPS, 2018–2022

AUTHOR'S ABSTRACT

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The dissertation is written on 138 standard pages and includes 39 figures, 28 tables and 31 appendices.

The reference list includes 403 sources: 37 in Cyrillic and 366 in Latin script; 55 by Bulgarian authors and 348 by international authors.

*The numbering of tables and figures in this author's abstract differs from that used in the full dissertation.

ABBREVIATIONS

anti-*CMV* IgM – class M antibodies against cytomegalovirus
anti-*EBV* IgM – class M antibodies against Epstein–Barr virus
anti-*HEV* IgA – class A antibodies against hepatitis E virus
anti-*HEV* IgM – class M antibodies against hepatitis E virus
anti-*HEV* IgG – class G antibodies against hepatitis E virus
ALP – alkaline phosphatase
ALT – alanine aminotransferase
AP – acute pancreatitis
AST – aspartate aminotransferase
CHE – chronic hepatitis E
CKD – chronic kidney disease
CMV – cytomegalovirus
CLD – concomitant liver disease
CVD – cardiovascular disease
EBV – Epstein–Barr virus
ELISA – enzyme-linked immunosorbent assay
EMA – European Medicines Agency
ESS – euthyroid sick syndrome
FH – fulminant hepatitis
FHE – fulminant hepatitis E
fT3 – free triiodothyronine
fT4 – free thyroxine
GBS – Guillain–Barré syndrome
GGT – gamma-glutamyl transferase
GN – glomerulonephritis
HAV – hepatitis A virus
HBV – hepatitis B virus
HBV DNA – hepatitis B virus deoxyribonucleic acid
HCV – hepatitis C virus
HD – maintenance haemodialysis
HEV – hepatitis E virus
HIV – human immunodeficiency virus
HRS – hepatorenal syndrome
IgA – immunoglobulin A
IgG – immunoglobulin G
IgM – immunoglobulin M
IS – immunosuppression
LF – liver failure
Med – median
NAAT – nucleic acid amplification test
PI – prothrombin time expressed as a prothrombin index, %
RF – risk factors
RNA – ribonucleic acid
RT-PCR – reverse-transcription polymerase chain reaction
VH – viral hepatitis
WHO – World Health Organization

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INTRODUCTION

Hepatitis E virus (*HEV*) is the fifth recognised hepatotropic virus. It causes acute and, in some cases, chronic hepatitis and is associated with a range of extrahepatic manifestations (Anderson, 2010; Donnelly et al., 2017; Mast & Krawczynski, 1996; Pérez-Gracia et al., 2015). According to some authors, *HEV* is the most common cause of enterically transmitted hepatitis and is associated with considerable morbidity and mortality (Dalton et al., 2018; Kumar et al., 2013).

An analysis of 287 studies, including 1 099 717 individuals from the general population and published up to July 2019, estimated the global *anti-HEV IgG* seroprevalence at 12.47%. Approximately 939 million people were estimated to have been exposed to *HEV*, while 15–110 million were considered to have a recent or ongoing infection (Li et al., 2020). According to the World Health Organization (WHO), approximately 20 million new *HEV* infections occur annually, of which only 3.3 million (16.5%) are clinically apparent (World Health Organization, 2022). In Europe, more than 20,000 cases were reported between 2005 and 2015, most of them autochthonous, with a marked increase after 2011 (European Centre for Disease Prevention and Control, n.d.). These data originate from countries with established systems for disease surveillance and case registration; Bulgaria was not included in the cited report. It is estimated that *HEV* infection accounts for 5–15% of cases of acute hepatitis of unspecified aetiology in Europe (Pishmisheva-Peleva, 2022; Aspinall et al., 2017).

Hepatitis E has not been comprehensively studied in Bulgaria. From the first reported case in 1995 (Teoharov et al., 1995) until 2008, only isolated cases were described. Thereafter, the number of confirmed cases and publications by Bulgarian authors increased, reflecting both growing interest in the disease and improved access to diagnostic testing. In 2019, M. Pishmisheva presented the first comprehensive study of acute HEV infection in Bulgaria in the dissertation entitled Clinical and Epidemiological Aspects of Acute Hepatitis E Virus Infection in the Pazardzhik Region (Pishmisheva-Peleva, 2018).

Since 15 January 2019, viral hepatitis E has been included in Ordinance No. 21 governing the registration, notification and reporting of communicable diseases in Bulgaria (Ministry of Health of the Republic of Bulgaria, 2019). This is expected to contribute to a more accurate assessment of the prevalence of HEV infection and to improve knowledge of its clinical manifestations and complications.

Several aspects of *HEV* infection remain insufficiently understood, including its epidemiology, extrahepatic manifestations, complications of acute and chronic infection, transmission among close contacts and individuals from risk groups, and preventive measures for vulnerable populations.

The present study investigates the prevalence and clinical characteristics of *HEV* infection among individuals from risk groups.

ORIGINAL RESEARCH

Aim and Objectives. Materials and Methods.

1. AIM:

To investigate the prevalence and clinical characteristics of hepatitis E virus infection among individuals from risk groups (those with concomitant liver disease, immunocompromised individuals, and individuals who misuse alcohol) and among individuals without identified risk factors, including individuals of the general population and blood donors, during the period 2018–2022.

2. OBJECTIVES:

2.1. To study the prevalence of HEV infection among individuals in risk groups from the Pazardzhik Region.

2.2. To study the prevalence of HEV infection among individuals without identified risk factors.

2.3. To study the clinical characteristics of HEV infection among individuals in risk groups.

2.4. To perform a comparative analysis of the prevalence and clinical clinical course of HEV infection in individuals with and without identified risk factors.

2.5. To propose an algorithm for testing donated blood for HEV and recommendations for testing individuals in risk groups.

3. MATERIALS:

A total of 616 adults from the Pazardzhik Region were studied and allocated to the following groups:

3.1. Individuals with risk factors for infection and severe HEV infection – 371, distributed as follows:

3.1.1. *Individuals with pre-existing liver disease* – 98 (28 with liver cirrhosis of different aetiology; 27 with chronic viral hepatitis; 28 with hepatic steatosis; 15 with more than one liver disease).

3.1.2. *Individuals with immunosuppression* – 78 (42 with diabetes mellitus; 23 with autoimmune or oncological disease; 1 HIV-positive individual; 12 with more than one disease).

3.1.3. *Individuals with end-stage renal disease on maintenance haemodialysis* – 102.

3.1.4. *Individuals with more than one risk factor* (pre-existing liver disease and immunosuppression) – 73.

3.1.5. *Individuals who misuse alcohol* – 20.

3.2. Individuals without identified risk factors – 245, of whom:

3.2.1. *Individuals from the general population without identified risk factors* – 151 clinically healthy individuals, recruited at random from persons attending the outpatient consulting rooms of the Infectious Diseases Department at Pazardzhik Hospital for various reasons: tick removal, bites from mammals, administration of rabies vaccination, or preparation of medical documentation for various purposes.

3.2.2. *Blood donors* – 94 donors at the Department of Transfusion Haematology of Pazardzhik Hospital during 2021.

3.3. Clinical specimens examined:

3.3.1. Serum for serological and molecular testing from all participants.

3.3.2. Blood and urine for clinical laboratory investigations.

4. METHODS

4.1. Epidemiological investigation – collection and analysis of demographic data (sex, age, ethnic affiliation, place of residence, occupation and activity); information on travel to regions endemic for hepatitis E; dietary habits – consumption of raw meat products from domestic animals and game; contact with persons affected by hepatitis; and vaccination against hepatitis A and B.

4.2. Clinical methods – medical history (current complaints, previous and comorbidities, family history, harmful habits – alcohol consumption), physical examination, clinical observation and follow-up.

4.3. Questionnaire – prepared as a clinical study record; in blood donors, the standard questionnaire used in transfusion-haematology centres was also applied.

4.4. Clinical laboratory investigations – haematological parameters: complete blood count and differential blood count; biochemical parameters: serum glucose; total and conjugated bilirubin; hepatocellular injury enzymes – AST and ALT; cholestatic enzymes – GGT and ALP; residual nitrogen compounds – urea and creatinine; serum electrolytes; total protein and albumin; total cholesterol and triglycerides; serum cholinesterase; haemostatic parameters (fibrinogen, prothrombin index, INR and D-dimer); urine – clinical chemistry examination and sediment.

4.5. Serological testing for detection of anti-HEV IgM and anti-HEV IgG. Testing was performed using the following assays in accordance with the manufacturers' instructions:

Dia.Pro anti-HEV IgM and IgG ELISA kit, manufactured in Italy, with reported diagnostic sensitivity and specificity of 100%.

Euroimmun anti-HEV IgG ELISA kit, manufactured in Germany, with reported diagnostic sensitivity and specificity of 100%.

Euroimmun combined anti-HEV IgA+IgM+IgG ELISA – used in patients with end-stage renal disease.

Markers of viral hepatitis B and C were also tested in all individuals in risk groups and in blood donors.

4.6. Molecular investigations for detection of HEV RNA in serum from individuals with high anti-HEV IgM and/or IgG levels.

Testing was performed by RT-PCR for HEV RNA using the RealStar HEV RT-PCR 2.0 assay, ***Altona Diagnostics, Germany***. The analytical sensitivity was 0.20 IU/μL, determined against the First WHO International Standard for HEV NAT-based techniques. No cross-reactivity was identified with EBV, HAV, HBV, HCV, parvovirus B19, CMV, HSV-1, HSV-2, VZV or HIV-1.

Serological and molecular investigations were performed at the National Reference Laboratory "Hepatitis Viruses", Department of Virology, National Centre of Infectious and Parasitic Diseases, Sofia.

Serological investigations in individuals with end-stage renal disease were performed at the Virology Laboratory, Acibadem City Clinic University Hospital Tokuda.

4.7. Imaging diagnostics – abdominal ultrasonography in individuals in risk groups; in some of them, endoscopic examinations (gastroscopy and colonoscopy), computed tomography and magnetic resonance imaging of the abdominal organs.

4.8. Statistical methods:

4.8.1. Descriptive statistical methods – quantitative variables were presented as arithmetic mean and standard deviation, median and interquartile range, and minimum and maximum values; categorical variables were presented as absolute and relative frequencies.

4.8.2. Analytical methods – Pearson's χ^2 test, Fisher's exact test, Student's t-test, Mann–Whitney test, ANOVA, Kruskal–Wallis test, and linear and binary logistic regression analysis. Data were processed using Microsoft Office Excel 2025 and the AI-based online statistical platform DATAtab. A result with $p<0.05$ was considered statistically significant.

4.9. Study design:

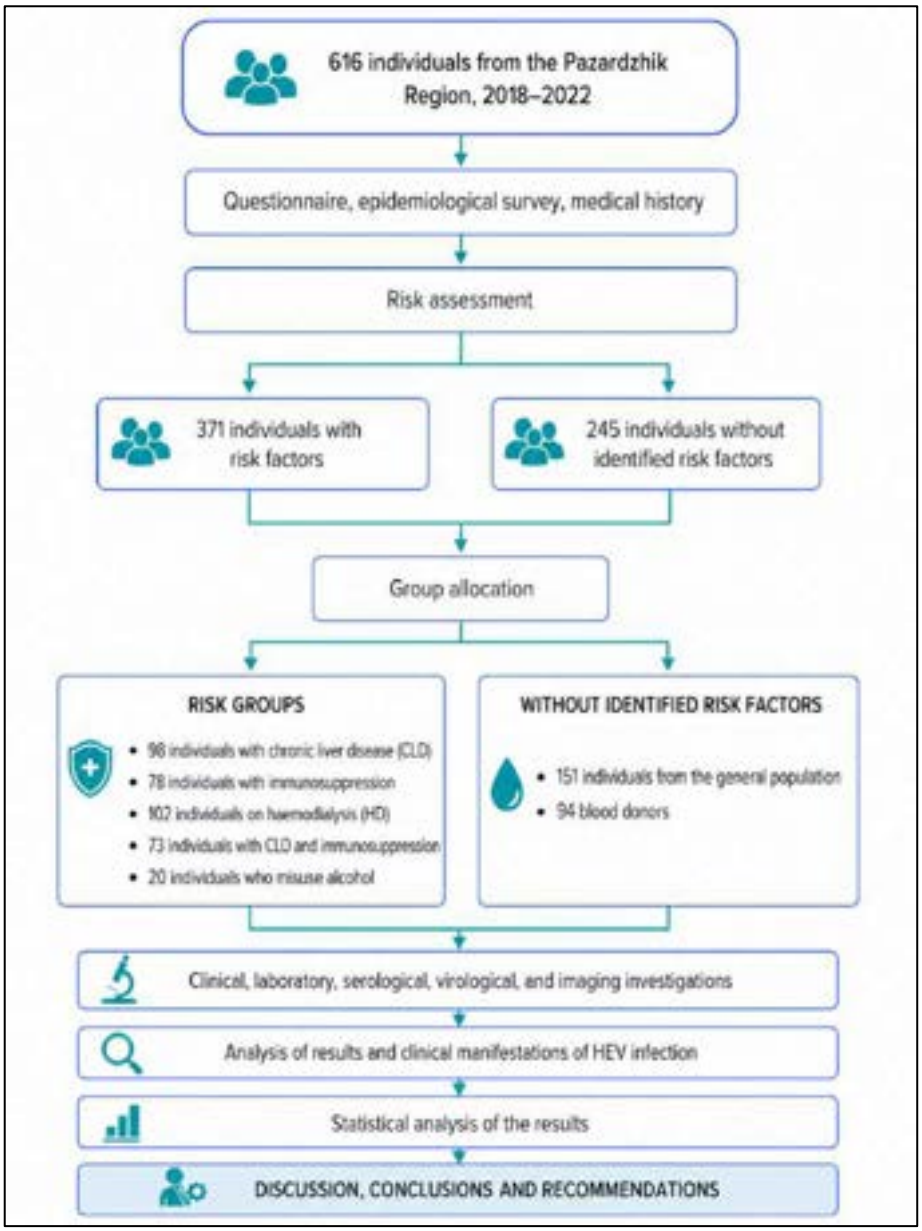


Figure 1. Study design.

4.10. Definitions of the severity of the clinical course in individuals with manifestations of hepatitis:

Mild hepatitis – mildly expressed asthenia, dyspeptic complaints are present, without general toxic manifestations. Jaundice is mild to moderate or absent. Serum bilirubin concentrations are <100 μmol/L, transaminases are within 500–2000 IU/L, and haemostatic parameters are normal.

Moderately severe hepatitis – a well-expressed asthenic-adynamic and dyspeptic syndrome and mild manifestations of intoxication are present. Jaundice is pronounced, serum bilirubin concentrations are between 100 and 200 μmol/L, transaminases reach 3000 IU/L, and coagulation parameters are mildly decreased.

Severe hepatitis – pronounced manifestations of intoxication and intense jaundice are present. Serum bilirubin concentrations are >200 μmol/L, transaminases are >3000 IU/L, and haemostatic parameters are moderately decreased.

The study was conducted with the patient's written consent after the patient had been informed about the risks and expected benefits of the investigation.

The study was conducted in accordance with established standards of scientific integrity and research ethics, including:

- the ethical principles set out in the Declaration of Helsinki;
- the Bulgarian legislation and regulations governing clinical and scientific research involving human participants;
- the principles of Good Clinical Practice.

A favourable opinion was issued by the Research Ethics Committee at the Medical University – Plovdiv, registration No. ***P-KHE29/21.10.2025***.

RESULTS

1. Prevalence of Hepatitis E Virus Infection in the Pazardzhik Region, 2018–2022

A total of 616 individuals were examined during the study period, and specific *anti-HEV* antibodies (*IgM* and/or *IgG*) were detected in 34% (210/616). Seropositive individuals were more frequently male and of Bulgarian ethnic background, and the differences were statistically significant (Table 1). *Anti-HEV(+)* individuals were older than *anti-HEV(–)* individuals (55.57 ± 15.3 vs 53.76 ± 16.7 years), although the difference was not statistically significant.

Table 1. Frequency of *anti-HEV* (+) individuals according to sex and ethnic background

Demographic characteristic	Total examined, N	anti-HEV(+)		p-value
		n	%	
SEX				**p=0,006**
Male	379	145	38%	
Female	237	65	27%	
ETHNIC BACKGROUND				**p<0,001**
Bulgarian	488	184	38%	
Other	128	26	20%	

Antibodies of different classes were detected, with *anti-HEV IgG* positivity accounting for the largest proportion (28.9%) (Figure 2).

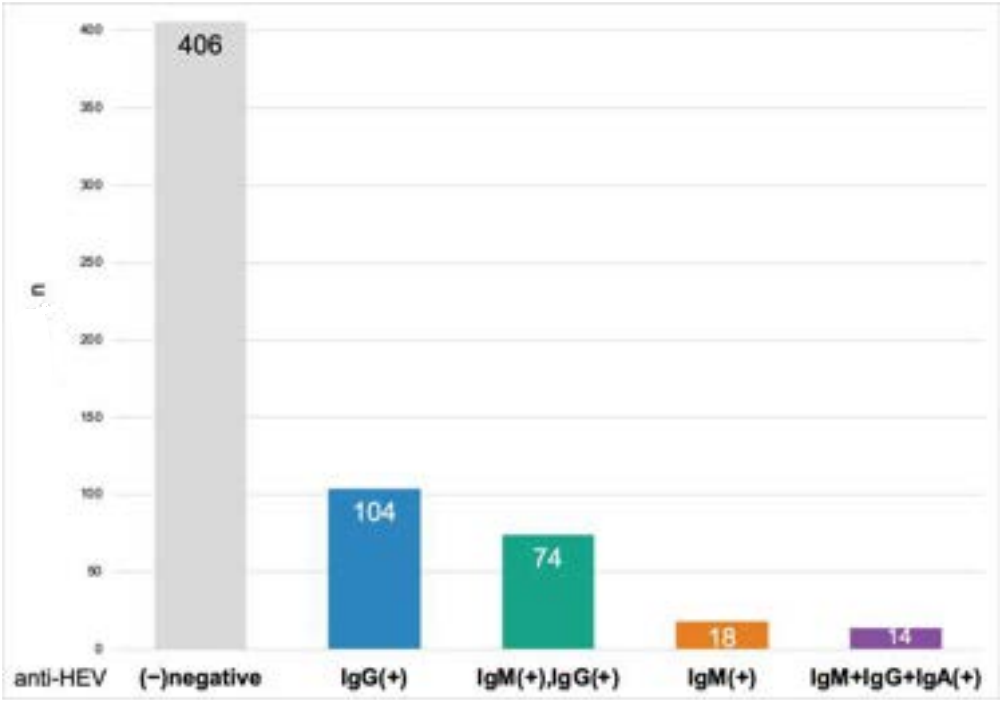


Figure 2. Identified *anti-HEV* of different classes among the examined individuals, N=616

Fourteen of the participants reported an occupation or activity associated with an increased risk of *HEV* exposure; 6 of the 14 were anti-HEV positive.

Anti-HEV IgM predominated among younger individuals (mean age 45.19 ± 14.83 years), whereas *anti-HEV IgG* predominated among older individuals (mean age 59.01 ± 15.88 years). The differences in age were statistically significant (Figure 3).

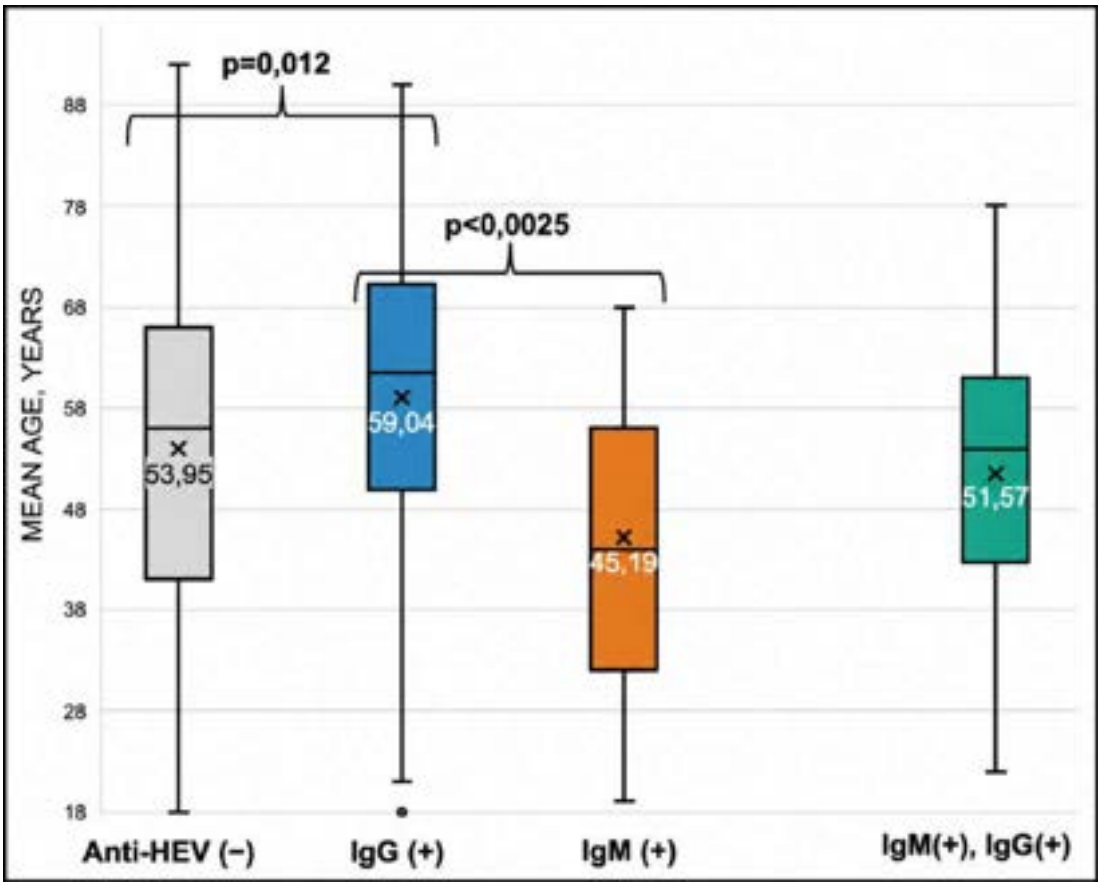


Figure 3. Mean age of the individuals with different serological result – comparison.

2. Studies among Individuals from Risk Groups

Of the 616 individuals examined, 371 had concomitant risk factors for *HEV* infection and severe disease. Specific *anti-HEV* antibodies (*IgM* and/or *IgG*) were detected in 35% (129/371), with men of Bulgarian ethnic background predominating among the seropositive individuals (Figure 4).

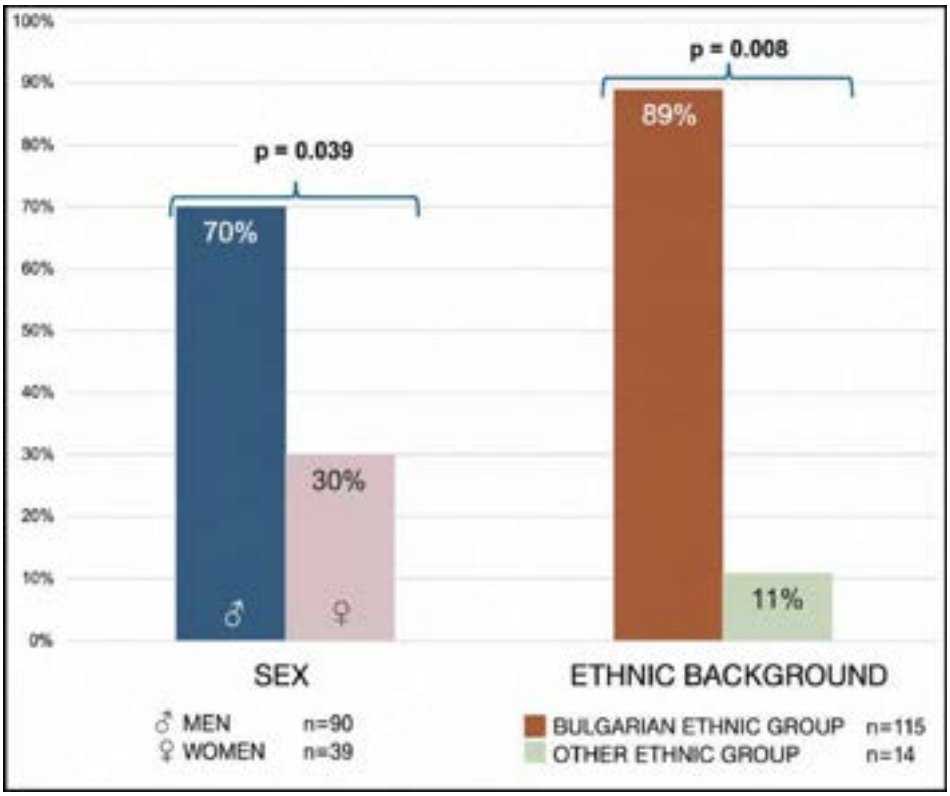


Figure 4. Distribution of *anti-HEV(+)* individuals from risk groups by sex and ethnic background.

Figure 5. presents the percentage distribution of *anti-HEV(+)* individuals according to their serological profile. *Anti-HEV IgG* were most frequently detected, whereas individuals with isolated *anti-HEV IgM* positivity accounted for the smallest proportion.

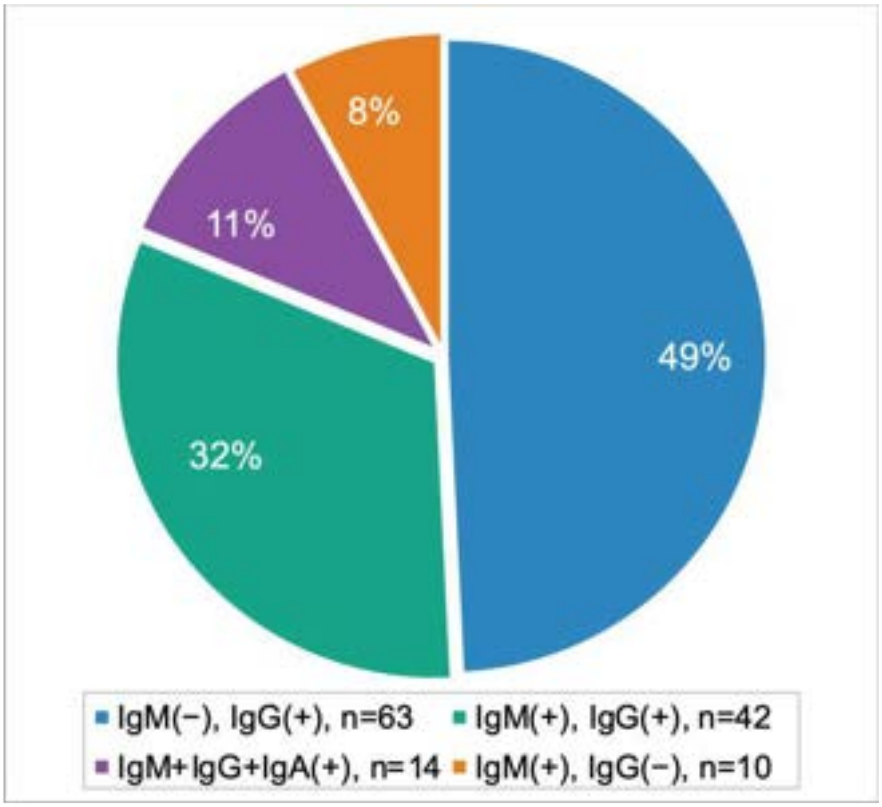


Figure 5. *Anti-HEV(+)* individuals from risk groups – frequency of the identified antibodies of different classes, n=129.

The examined individuals with risk factor were stratified in 5 risk groups – table 2.

Table 2. Distribution of the individuals with risk factors by risk groups and sex.

Risk group	sex	n	Total
Individuals with concomitant liver diseases	**M**	77	**98**
	F	21	
Individuals with immunosuppression	**M**	34	**78**
	F	44	
Individuals with chronic kidney diseases n haemodialysis	**M**	60	**102**
	F	42	
Individuals with concomitant liver diseases and immunosuppression	**M**	42	**73**
	F	31	
Individuals who misuse alcohol	**M**	18	**20**
	F	2	

2.1 Study among Individuals with Concomitant Liver Diseases (CLD), n = 98

Specific *anti-HEV* antibodies were detected in 51% (50/98) of the individuals with concomitant liver diseases. Seropositive individuals most frequently had liver cirrhosis or hepatic steatosis; however, there were no statistically significant differences in the frequency of *anti-HEV* positivity between the subgroups (Table 3). *HEV RNA* was detected in one *anti-HEV IgM(+), IgG(-)* man with liver cirrhosis.

Table 3. Frequency of *anti-HEV(+)* individuals according to the concomitant liver disease.

CONCOMITANT LIVER DISEASE	Total, N	Anti-HEV (+)		p-value
		n	%	
SUBGROUPS				>0,05
Liver cirrhosis	28	15	54%	
Chronic viral hepatitis	27	13	48%	
Hepatic steatosis	28	15	54%	
>1 CLD	15	7	47%	

Anti-HEV IgG antibodies were detected in 47.96% of cases. The distribution of *anti-HEV(+)* individuals with CLD according to the serological profile is presented in Figure 6.

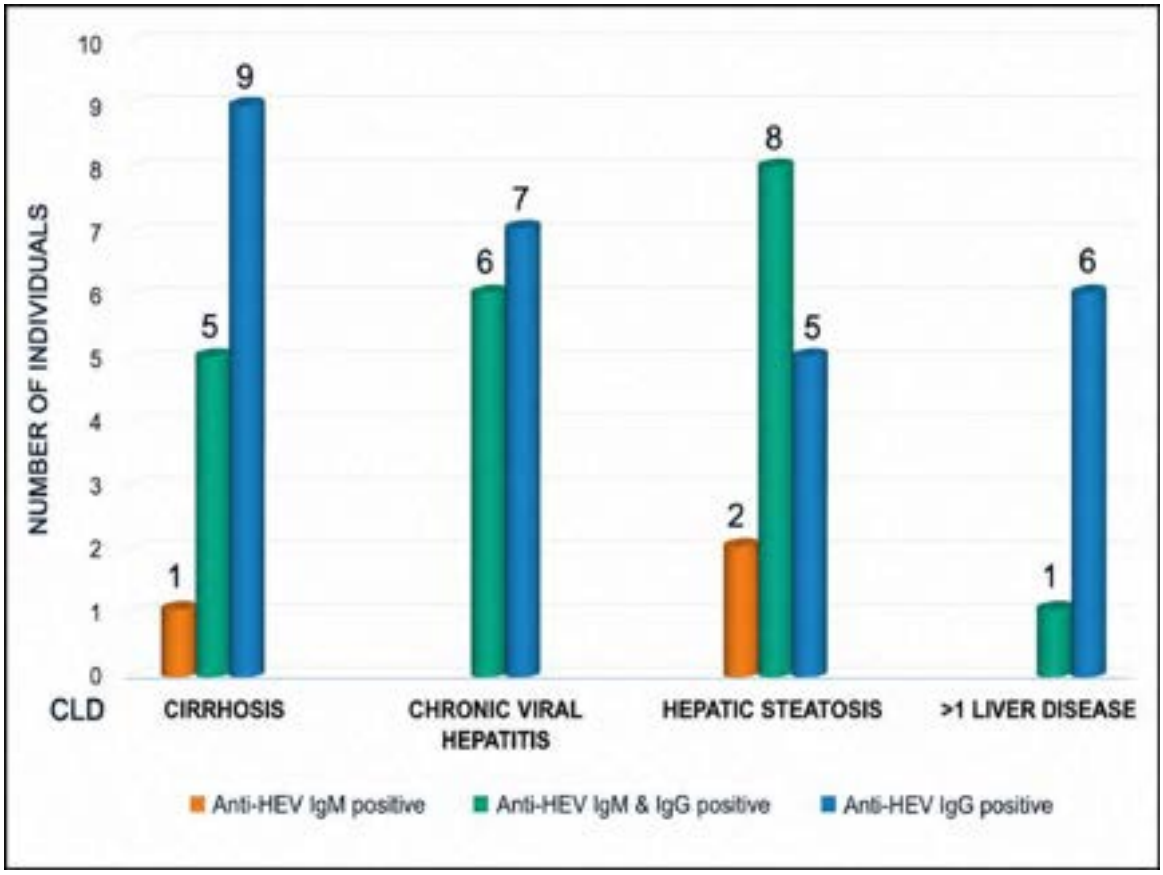


Figure 6. *Anti-HEV*(+) individuals with CLD: antibody classes detected across the subgroups, n = 50.

2.2 Study among Immunocompromised Individuals, n = 78

Specific *anti-HEV* antibodies were detected in 35% (27/78) of the immunocompromised individuals. Seropositive individuals most frequently had diabetes mellitus; however, there were no statistically significant differences in the frequency of *anti-HEV* positivity between the subgroups (Table 4).

Table 4. Frequency of anti-HEV positivity according to the cause of immunosuppression.
(*This group included one HIV-positive man)

IMMUNOSUPPRESSIVE CONDITION	Total, N	anti-HEV (+) n	anti-HEV (+) %	p-value
SUBGROUPS				>0,05
Diabetes mellitus	42	15	36%	
Autoimmune / oncological condition*	24	8	33%	
>1 Immunosuppressive condition	12	4	33%	

Anti-HEV IgG(+) was detected in 30.77% of cases. The distribution of *anti-HEV(+)* with immunosuppression according to serological profile is presented in Figure 7.

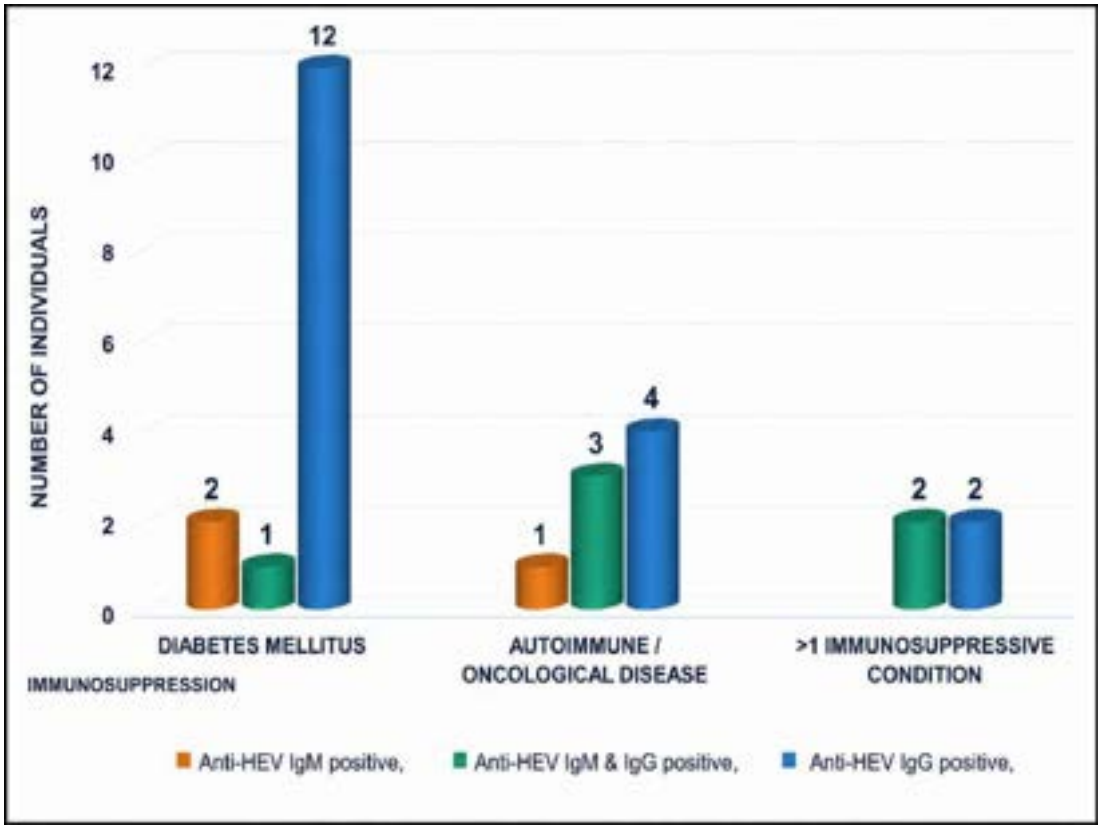


Figure 7. *Anti-HEV*(+) immunocompromised individuals: antibody classes detected across the subgroups, n = 27

2.3 Study among Individuals with Chronic Kidney Disease on Haemodialysis (CKD on HD), n = 102

A total of 102 individuals with chronic kidney disease on maintenance haemodialysis were examined. The mean duration of haemodialysis was 8.4 years (range, 1.6–21 years). All participants had multiple comorbidities (Table 5) and had received blood and blood products. The four individuals who had undergone kidney transplantation subsequently experienced graft rejection despite immunosuppressive treatment.

Table 5. Frequency of *anti-HEV* positivity among individuals with CKD on maintenance haemodialysis according to concomitant diseases

INDIVIDUALS ON HAEMODIALYSIS	Total, N	anti-HEV (+)	
		n	%
COMORBIDITIES			
Chronic viral hepatitis	10	1	7%
Post-transplant immunosuppression	4	1	7%
Diabetes mellitus	69	5	36%
Oncological disease	7	2	14%
>1 comorbidities	63	5	36%

Testing was performed using a combined *anti-HEV IgM/IgG/IgA* assay. A positive result was obtained in 14% (14/102) of the individuals, most frequently among those who also had diabetes mellitus.

2.4 Study among Individuals with Concomitant Liver Disease and Immunosuppression, n = 73.

Specific *anti-HEV* antibodies were detected in 38% (28/73) of individuals with concomitant liver disease and immunosuppression. Seropositive individuals most frequently had concomitant liver disease and diabetes mellitus. No statistically significant differences in the frequency of *anti-HEV(+)* were observed between the subgroups (Table 6).

Table 6. Frequency of *anti-HEV* positivity among individuals with concomitant liver disease and immunosuppression. (*This group included one HIV-positive man)

CLD AND IMMUNOSUPPRESSIVE CONDITION	Total, N	Anti-HEV (+)		p-value
		n	%	
SUBGROUPS				
CLD and diabetes mellitus	46	20	43%	>0,05
CLD and autoimmune / oncological disease	17	5	29%	
CLD and >1 immunosuppressive condition*	10	3	30%	

Anti-HEV IgG antibodies detected in 34.25% of cases. The distribution of *anti-HEV(+)* individuals with concomitant liver disease and immunosuppression according to serological profile is presented in figure 8.

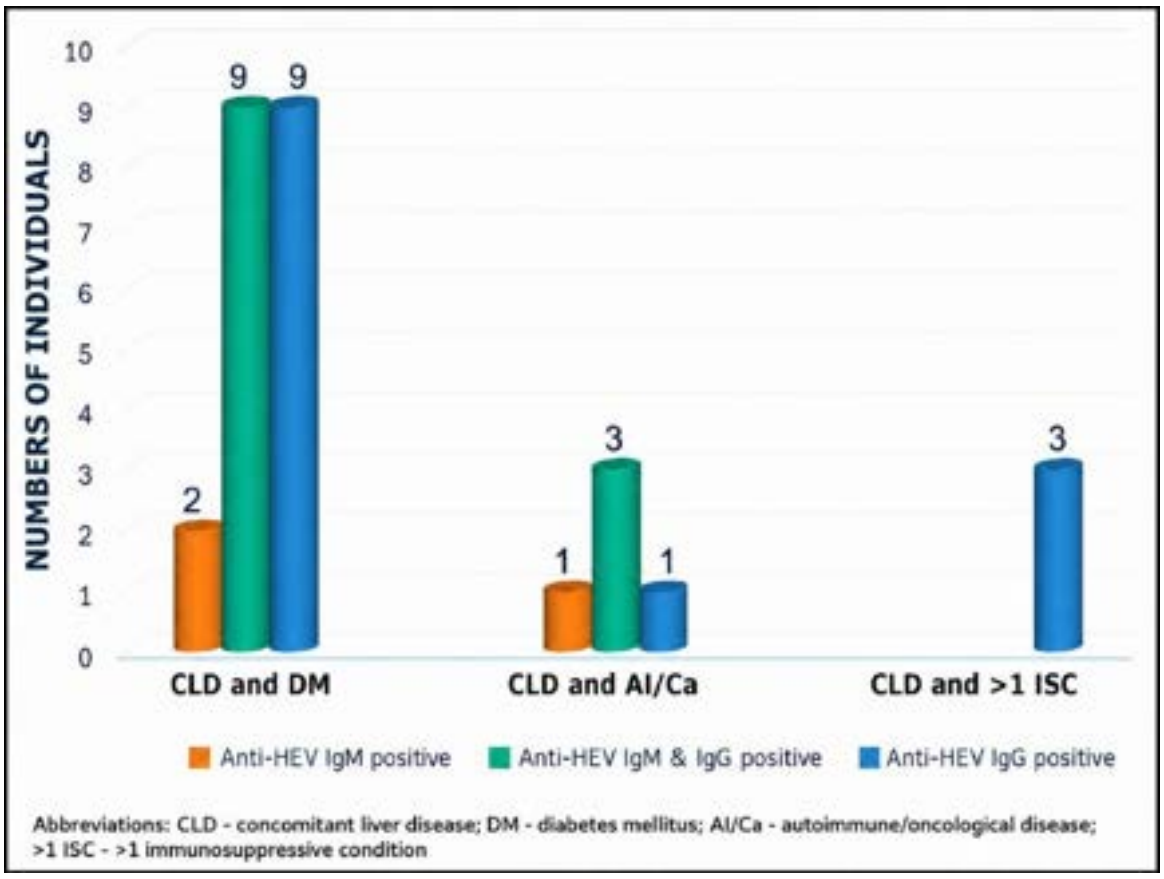


Figure 8. *Anti-HEV(+)* individuals with CLD and Immunosuppression, n=28

2.5 Study among Individuals who misuse alcohol, n=20

Specific *anti-HEV* antibodies were detected in 50% (10/20) of the individuals who misused alcohol. *Anti-HEV IgG* positivity was found in 45% (Figure 9).

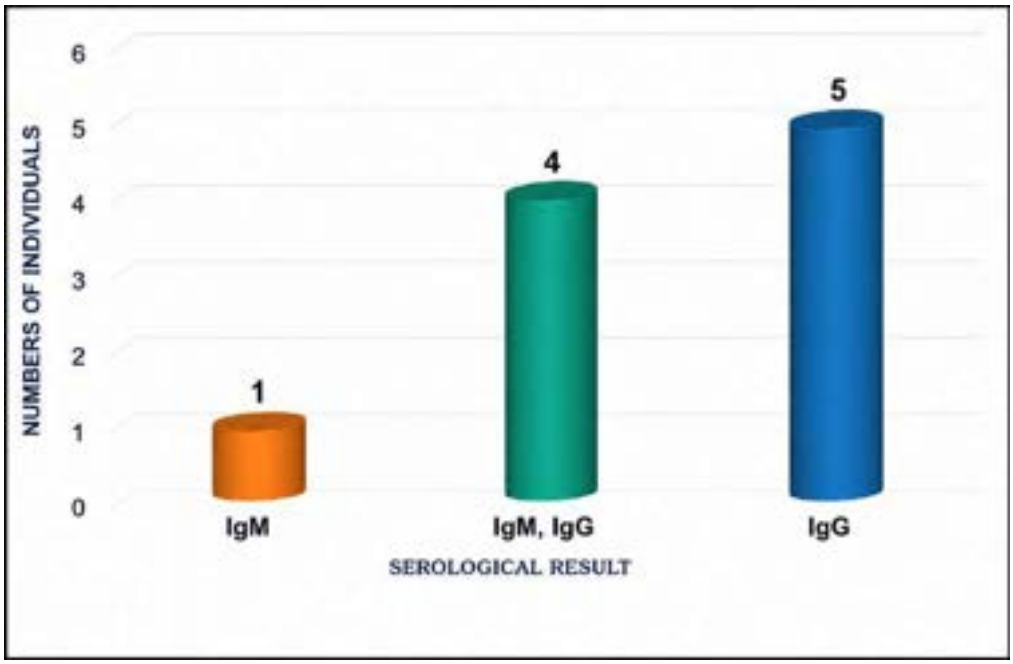


Figure 9. *Anti-HEV(+)* individuals who misuse alcohol – identified antibodies from different classes, n=10.

1. Comparative Analysis of Seropositive Individuals across the Risk Groups

According to the comparative analysis, *anti-HEV(+)* individuals were most frequently found among those with concomitant liver disease. Statistically significant differences in seropositivity were observed between individuals with concomitant liver disease and two other risk groups: immunocompromised individuals and individuals with chronic kidney disease on maintenance haemodialysis (Table 7).

Table 7. Frequency of *anti-HEV* positivity: comparative analysis across the risk groups.

INDIVIDUALS WITH RISK FACTORS	Total, N	Anti-HEV (+)	
		n	%
RISK GROUPS			
CLD	**98**	**50**	**51%**
Immunosuppressive condition*	78	27	35%
CLD and immunosuppressive condition	73	28	38%
CKD ON HD**	102	14	14%
Alcohol misuse	20	10	50%

*significant difference between those risk groups CLD and immunosuppressive condition ($\chi^2(1)=4,75$; $p=0,029$)
** significant difference between those risk groups CLD and CKD on HD ($\chi^2(1)=31,95$; $p<0,001$)

The sex distribution of *anti-HEV(+)* individuals across the risk groups is presented in Figure 10. A statistically significant difference was observed among individuals with CKD on HD. Immunocompromised individuals were the only group in which women predominated among the seropositive ones.

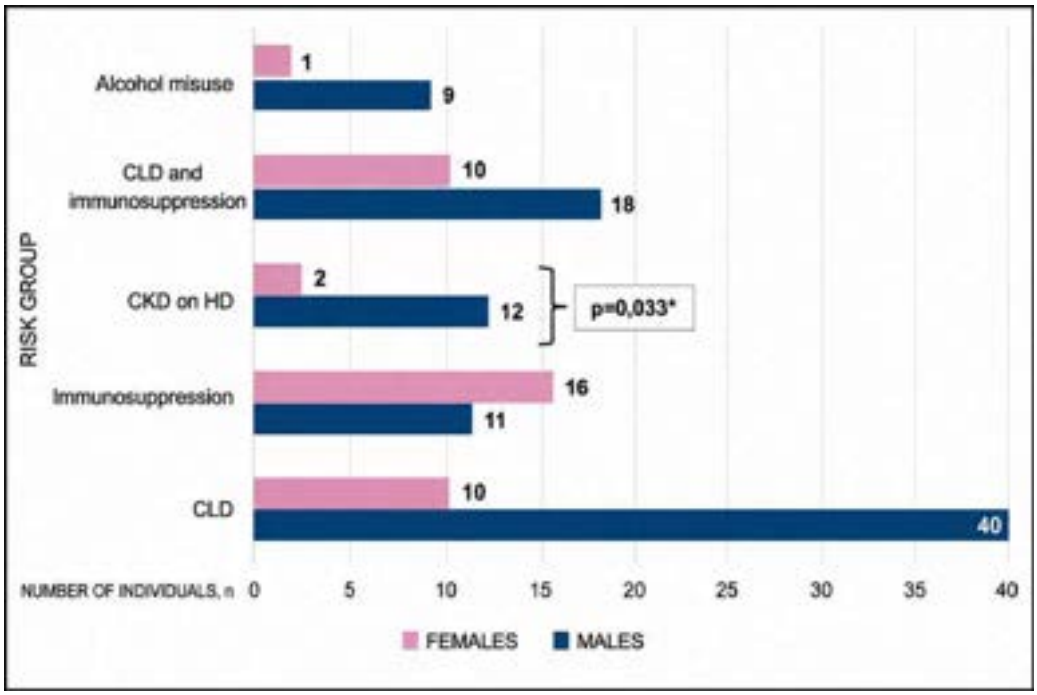


Figure 10. *Anti-HEV(+)* individuals across the risk groups – sex distribution, n=129.
*Fisher exact test

Individuals across the risk groups differed in age, with those with concomitant liver disease being the youngest (Table 8).

Table 8. Comparison of mean age across the different risk groups. (F = 10,09, p = <0,001)

RISK GROUP	Total, N	AGE	
		$\overline{X}$	SD
CLD	98	**55,52**	**± 13,16**
Immunosuppressive condition*	78	65,13	± 8,98
CLD and immunosuppressive condition	73	64,18	± 10,22
CKD ON HD**	102	58,74	± 12,97
Alcohol misuse	20	58,85	± 9,88

*significant difference between those risk groups CLD and immunosuppressive condition (p<0,001)
** significant difference between those risk groups CLD and CLD immunosuppressive condition (p<0,001)

The mean ages of individuals with different serological profiles are presented in Figure 11. *Anti-HEV IgM* positivity was observed in younger individuals, whereas those with *anti-HEV IgG* positivity were the oldest. A statistically significant difference in age remained between *anti-HEV IgG(+)* and seronegative individuals.

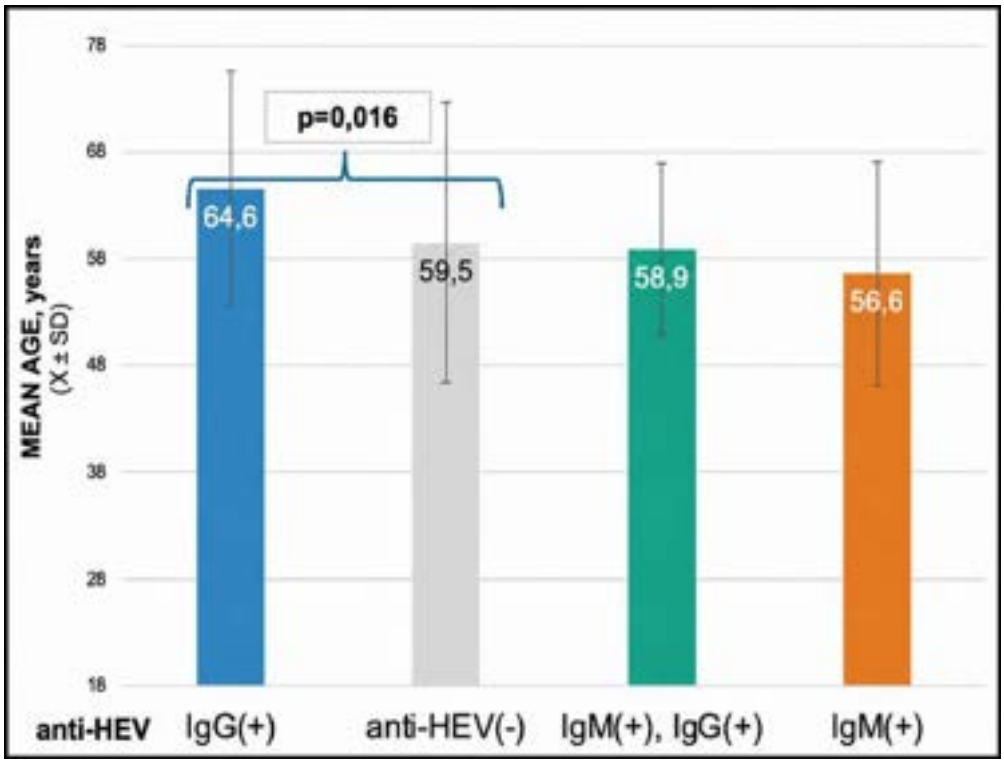


Figure 11. Comparison of mean age among individuals with risk factors and different serological profiles, n = 371.

The frequency of *anti-HEV IgG(+)* individuals across the risk groups is presented in Figure 12.

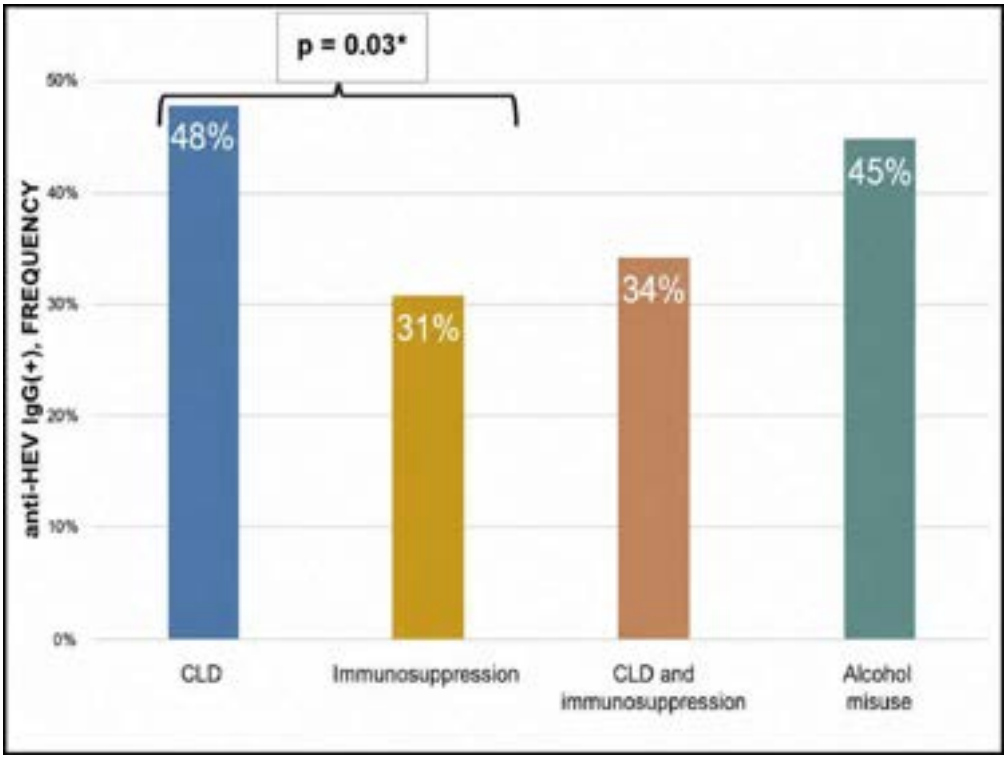


Figure 12. Frequency of *anti-HEV IgG* positivity across the risk groups: comparison
*Fisher's exact test.

The proportion of *anti-HEV IgG(+)* individuals was highest among those with concomitant liver disease and lowest among immunocompromised individuals. The difference in the frequency of anti-*HEV IgG* positivity between the two groups was statistically significant.

Anti-HEV antibodies were detected more frequently among individuals with only one risk factor—concomitant liver disease, immunosuppression, or alcohol misuse, and the difference was statistically significant (Table 9).

Table 9. Frequency of anti-HEV positivity according to the number of concomitant risk factor.

CATEGORY	Total, N	Anti-HEV (+)		p-value
		n	%	
NUMBER OF RISK FACTORS				**p<0,001**
1 risk factor	196	87	44%	
>1 risk factor	175	42	24%	

($\chi^2(1) = 16,94$, $p<0,001$).

4. Studies among Individuals without Identified Risk Factors

Of the 616 individuals examined, 245 had no concomitant risk factors. Specific *anti-HEV* antibodies were detected in 33% (81/245) of these individuals. Seropositive individuals were predominantly male and of Bulgarian ethnic background (Figure 13). *Anti-HEV*-positive and *anti-HEV*-negative individuals were of similar mean age: 43.53 and 43.33 years, respectively.

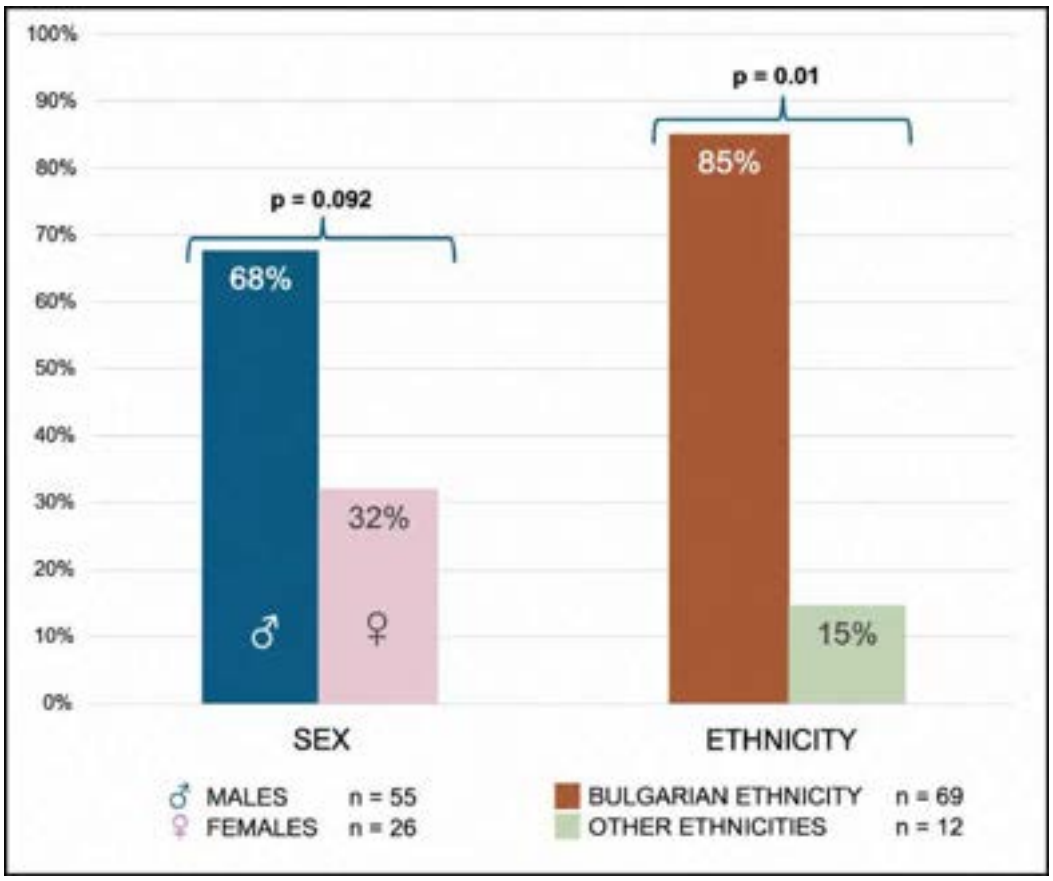


Figure 13. Distribution of *anti-HEV (+)* individuals without risk factors by sex and ethnicity background.

Figure 14. presents the percentage distribution of *anti-HEV(+)* individuals according to the antibody class detected. *Anti-HEV IgG* was detected most frequently, whereas individuals with isolated *anti-HEV IgM* positivity accounted for the smallest proportion.

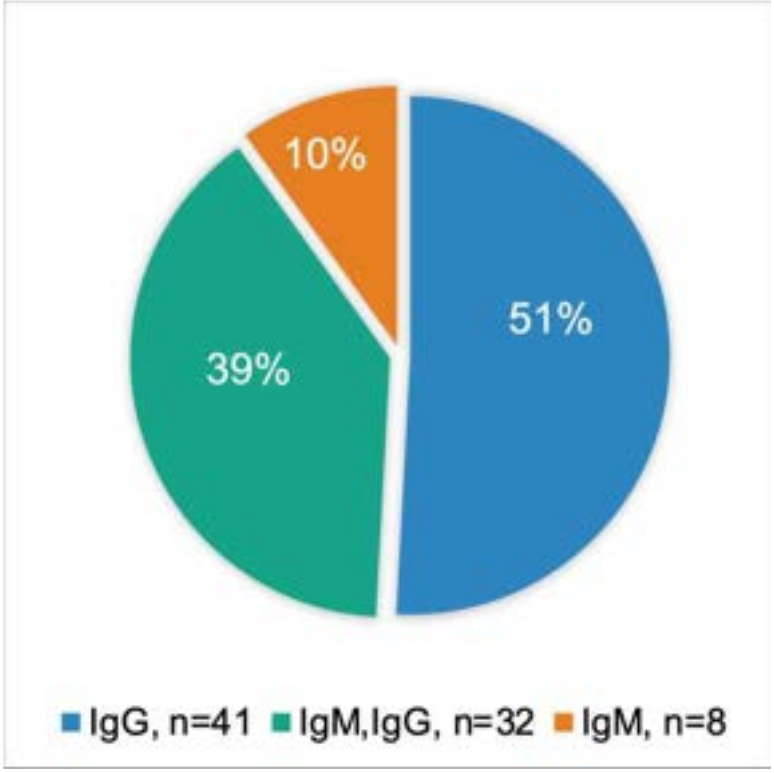


Figure 14. *Anti-HEV(+)* individuals without risk factors – frequency of the detected antibodies of different classes, n=81.

The mean age of the individuals with different serological profiles is presented in Figure 15. Isolated *anti-HEV IgM* positivity was observed at a younger age, whereas *anti-HEV IgG(+)* individuals were the oldest; the difference approached statistical significance.

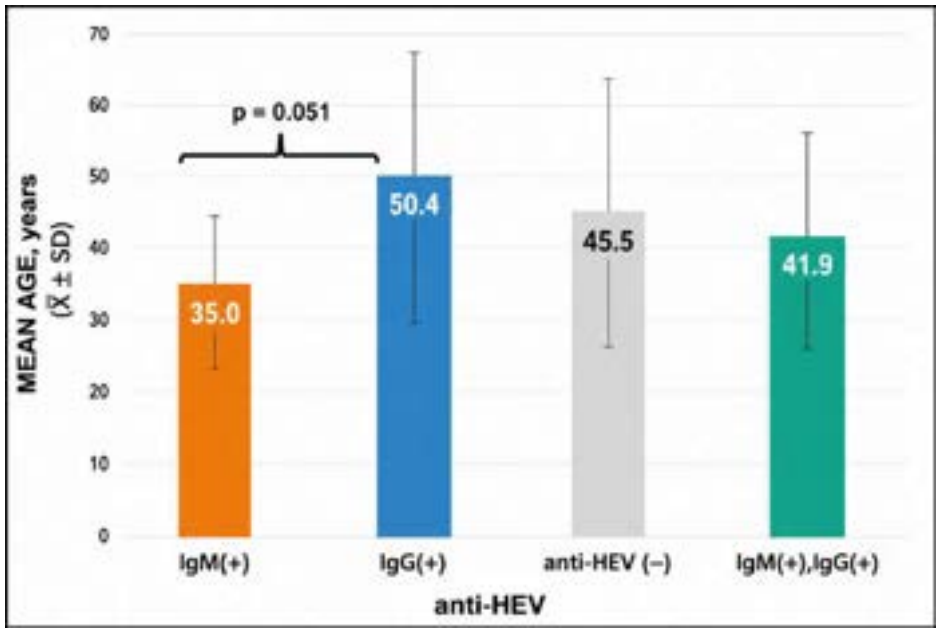


Figure 15. Comparison of mean age among individuals without risk factors and different serological profiles,n=245.

4.1 Prevalence of Hepatitis E Virus Infection among Blood Donors in the Pazardzhik Region, n = 94

A total of 94 blood donors were examined, comprising 77 men and 17 women. Their distribution by age group and ethnicity is presented in Table 10.

Table 10. Distribution of blood donors by age group and ethnicity.

AGE GROUPS, years	Total, N	Ethnicity background	
		Bulgarian, n	Roma, n
18 – 30	27	21	6
31 – 40	34	22	12
41 – 50	17	10	7
>50	16	11	5

Blood donors aged up to 40 years predominated (61/94), as did repeat donors (78/94).
Specific anti-HEV antibodies were detected in 40% (38/94) of the blood donors. The seropositive donors were most frequently men (33/38), of Bulgarian ethnic background (29/38), and repeat donors (32/38).
The proportion of anti-HEV-positive blood donors increased with age and was highest among donors aged >50 years (Table 11).

Table 11. Frequency of *anti-HEV* positivity among blood donors by age group.

	Total, N	Anti-HEV (+)		p-value
		n	%	
AGE GROUPS, years				p>0,05
18 – 30	27	10	37%	
31 – 40	34	13	38%	
41 – 50	17	7	41%	
>50	16	8	50%	

Anti-HEV IgG positivity was the highest (27.65%), whereas the percent of the *anti-HEV IgM(+), IgG(–)* blood donors was 12.77%.

Blood donors with isolated *anti-HEV IgM* positivity were predominantly <40 years of age (9/12), whereas *IgM(−), IgG(+)* donors were predominantly aged >40 years (7/13) (Figure 16).

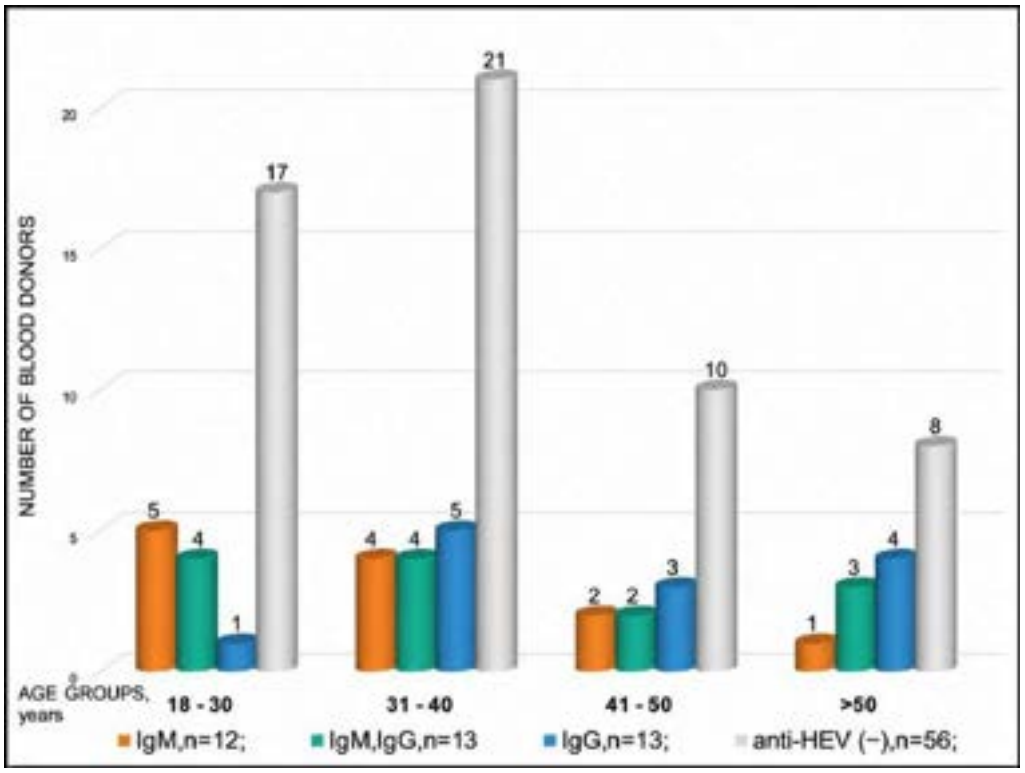


Figure 16. Anti-HEV antibody classes detected among blood donors by age group, n = 94.

Anti-HEV(+) blood donors were also tested for *HEV RNA*, and all results were negative.

5. Comparative Analysis of Seropositive Individuals with and without Risk Factors

The frequency of *anti-HEV(+)* individuals was higher among those with risk factors (Table 12).

Table 12. Frequency of anti-HEV positivity among individuals with and without risk factors.

CATEGORY	Total, N	Anti-HEV (+)		p-value
		n	%	
CONCOMITANT RISK				p>0,05
Individuals with concomitant risk factors	371	129	35%	
Individuals without concomitant risk factors	245	81	33%	
				$\chi^2(1) = 0,19, p=0,661$

Anti-HEV(+) individuals with risk factors were older than seropositive individuals without concomitant risk factors, and the difference was statistically significant (Figure 17).

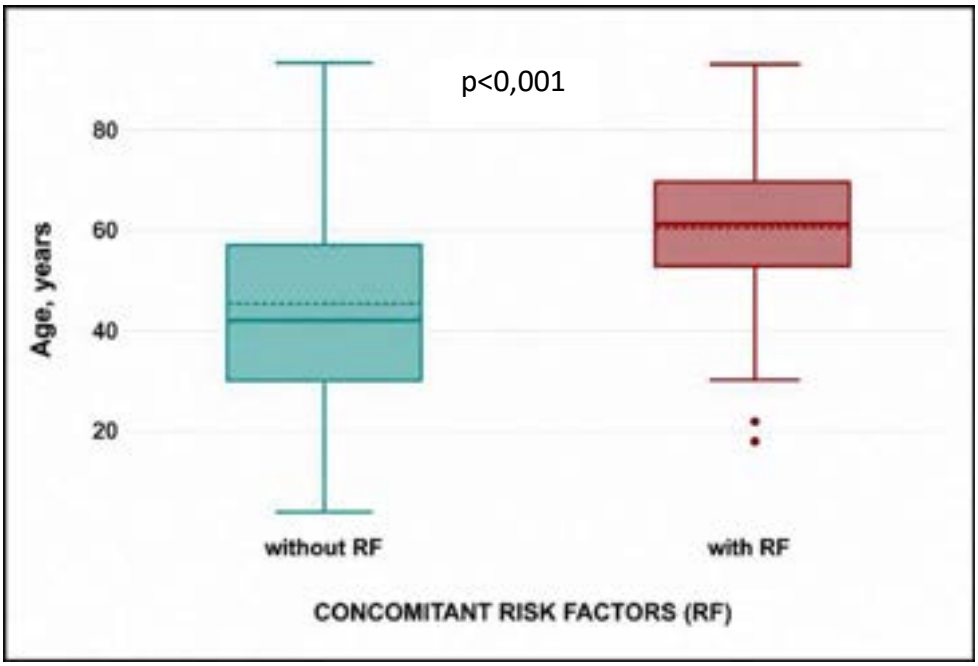


Figure 17. Mean age of anti-HEV-positive individuals with and without concomitant risk factors.
Mann–Whitney U test: U = 22,010.5, p < 0.001.

The frequency of *anti-HEV* positivity was higher among individuals from the risk groups than among those without risk factors. The exception was individuals receiving maintenance haemodialysis, in whom specific antibodies were detected less frequently (Table 13).

Table 13. Frequency of *anti-HEV* positivity among individuals from the risk groups and those without concomitant risk factors.

РИСКОВА ГРУПА	Общо изследвани, N	Anti-HEV (+)	
		n	%
CLD	98	50	51%
Immunosuppressive condition	78	27	35%
CLD and immunosuppressive condition	73	28	38%
CKD on HD	102	14	14%
Alcohol misuse	20	10	50%
Individuals without risk factors	245	81	33%

Binary logistic regression analysis, using individuals without risk factors as the reference group, identified statistically significant differences. Concomitant liver disease was an independent risk factor associated with an increased likelihood of *anti-HEV* positivity, whereas maintenance haemodialysis was associated with a lower likelihood of a positive result (Table 14).

Table 14. Effect of risk factors on *anti-HEV* positivity: binary logistic regression analysis.

Risk groups	B	SE	OR	95% CI	p-value
CLD	**0,75**	**0,24**	**2,11**	**1,31 – 3,4**	**0,002**
Immunosuppressive condition	0,07	0,27	1,07	0,63 – 1,83	0,8
CLD and Immunosuppressive condition	0,23	0,28	1,26	0,73 – 2,17	0,403
CKD on HD	**-1,13**	**0,32**	**0,32**	**0,17 – 0,6**	**<0,001**
Alcohol misuse	0,71	0,47	2,02	0,81 – 5,06	0,131

B, regression coefficient; **SE**, standard error; **OR**, odds ratio; **95% CI**, 95% confidence interval; **p-value**, level of statistical significance. Nagelkerke $R^2 = 0.08$; $\chi^2(5) = 36.58$, $p < 0.001$; $n = 616$.
Reference group: individuals without risk factors.

Anti-HEV IgG positivity was more frequent among individuals with risk factors than among those without risk factors ($p < 0.05$; Figure 18).

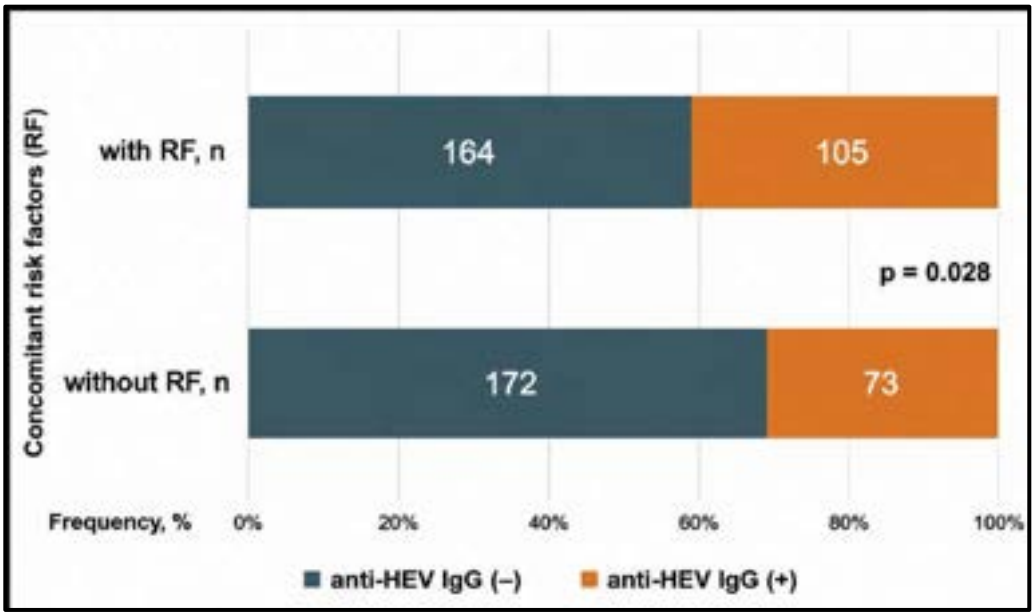


Figure 18. Frequency of *anti-HEV IgG (+)* among individuals with and without risk factors. $\chi^2(1) = 4,83$, $p = 0,028$.

6. Epidemiology, Clinical Manifestations, Laboratory Abnormalities, and Course of Hepatitis E Virus Infection among Individuals from Risk Groups

Clinically apparent hepatitis developed in 46 of the 210 *anti-HEV(+)* individuals and occurred more frequently among those with concomitant risk factors (44/46). The difference between the two groups was statistically significant (Table 15).

Table15. Distribution of individuals with acute hepatitis E according to the presence of concomitant risk factors.

CATEGORY	Anti-HEV (+), N	Individuals with clinically manifested hepatitis E		p-value
		n	%	
CONCOMITANT RISK				**p< 0,00001**
Individuals from risk groups	129	44	34	
Individuals without risk factor	81	2	2	

$\chi^2(1)=29,12$; $p<0,00001$

6.1 Analysis of Selected Epidemiological Characteristics

Individuals with clinical manifestations of hepatitis were predominantly men (39/46; $p < 0.05$), of Bulgarian ethnic background (43/46), and older than 55 years (mean age ± SD, 57.59 ± 10.45 years).

The highest number of cases was recorded in 2018 (Figure 19). The disease occurred throughout the year, with a higher number of cases during the autumn–winter season (Figure 20).

All individuals with clinically apparent hepatitis E reported consuming pork, and five had received blood products during the preceding year.

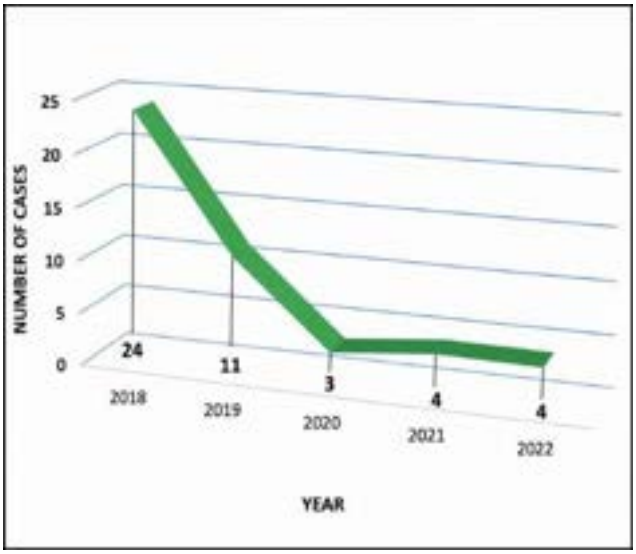


Figure 19. Hepatitis E cases by year, 2018–2022, n = 44.

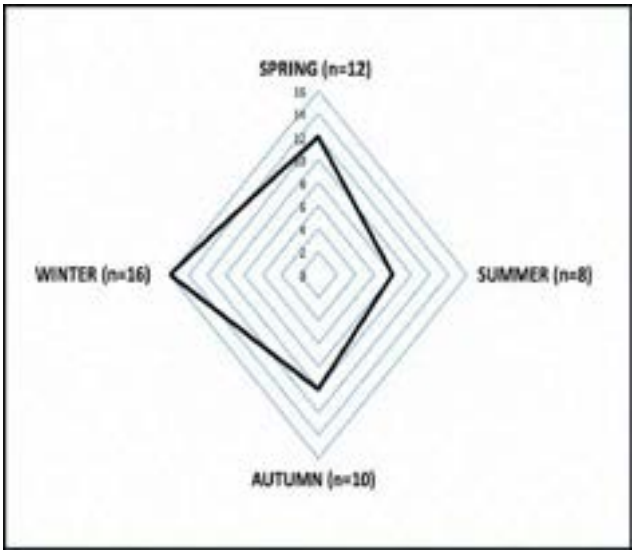


Figure 20. Seasonal distribution of hepatitis E cases, n = 44.

6.2 Clinical Manifestations of Hepatitis E Virus Infection among Individuals from Risk Groups

Clinically apparent hepatitis developed most frequently among individuals with concomitant liver disease and least frequently among immunocompromised individuals (Table 16).

Table 16. Distribution of individuals with acute hepatitis E across the risk groups, n = 44.

CATEGORY	Anti-HEV (+), N	Individuals with clinically manifested hepatitis E		p-value
		n	%	
РИСКОВИ ГРУПИ				**p<0,05**
CLD*	50	24	48	
CLD and immunosuppressive condition	28	14	50	
Immunosuppressive condition*	27	4	15	
Alcohol misuse	10	2	20	

* A statistically significant difference in the frequency of acute viral hepatitis was observed between the concomitant liver disease and immunosuppression groups: $\chi^2 = 8.34$; $p = 0.004$.

Fifty per cent reported regular alcohol consumption. Fifty-two per cent were coinfected with another hepatotropic virus (n = 23; $p < 0.05$): 18 had chronic hepatitis B and/or C, four had acute hepatitis A, and one had acute hepatitis B.

The distribution of patients with acute hepatitis E according to the type of concomitant liver disease is presented in Figure 21.

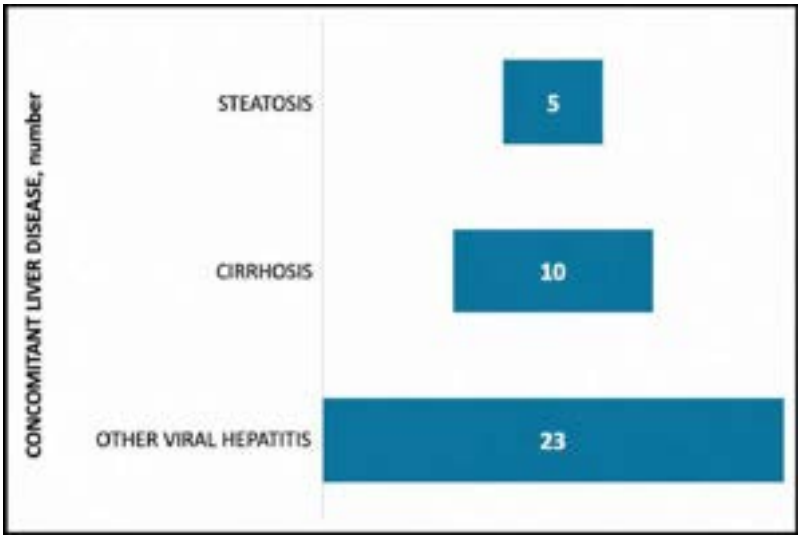


Figure 21. Concomitant liver disease among individuals with clinical manifestations of hepatitis E.

6.3 Analysis of Clinical Manifestations

- **Pre-icteric period**

The prodromal period ranged from 2 to 30 days, with a mean duration of 8.82 ± 6.14 days, and varied among individuals with different risk factors (Figure 22; Table 17).

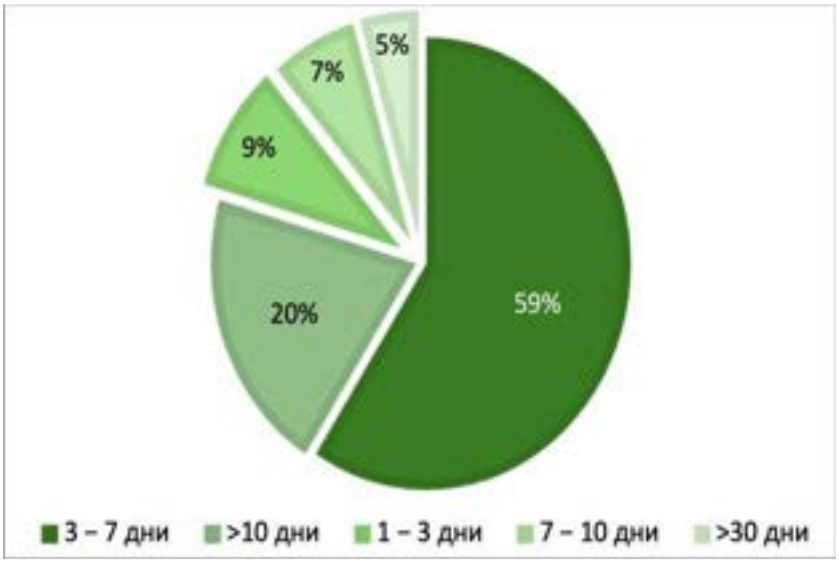


Figure 22. Duration of the prodromal period among individuals with hepatitis: percentage distribution.

Table 17. Duration of the prodromal period across the risk groups.

RISK GROUPS	PRE-ICTERIC PERIOD, DAYS	
	X̄	SD
Alcohol misuse	6,00	±1,41
CLD	7,00	±3,81
CLD and immunosuppressive condition	10,71	±7,11
Immunosuppressive condition	14,50	±10,85

ANOVA: F = 2,72; p = 0,057

The prodromal period was longer in immunocompromised individuals, and the difference is close to the statistical significance.

- **Clinical manifestations among individuals with hepatitis**

The subjective complaints reported by patients with hepatitis on admission are presented in Figure 23.

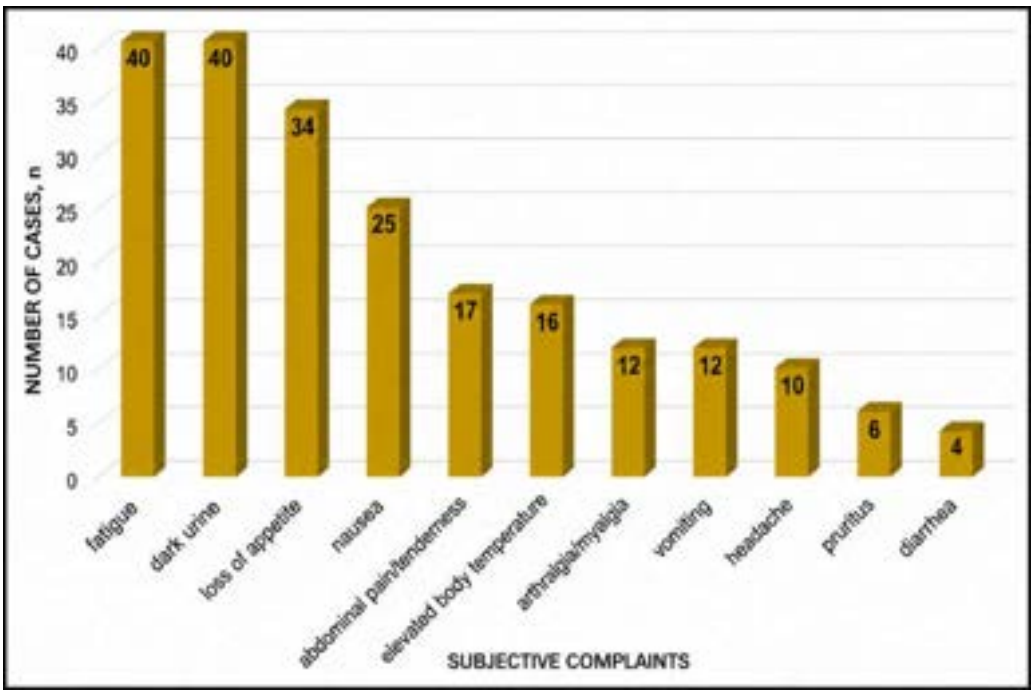


Figure 23. Subjective complaints among individuals with acute hepatitis E.

The most frequently reported complaints are fatigue, loss of appetite, and nausea. Fever was associated with concomitant conditions, including COVID-19, Mediterranean spotted fever, pneumonia, tonsillitis, urinary tract infection, and cholecystitis. Seventy-five per cent of the patients developed icteric hepatitis.
Abnormal findings on physical examination at admission are presented in Table 18.

Table 18. Proportion of objective clinical findings among patients with hepatitis E.

Abnormal findings on physical examination	Number	Frequency
Jaundice	35	80%
Hepatomegaly	42	95%
Splenomegaly	12	27%
Neurological abnormalities	4	9%
Rash	4	9%
Ascites and oedema	4	9%
Flapping tremor	3	7%
Dyscalculia	1	2%

Jaundice was the most common presentation among individuals with risk factors, with physical examination typically demonstrating hepatomegaly and jaundice of varying severity. Anicteric hepatitis was significantly more frequent among immunocompromised individuals (6/11; Fisher's exact test, $p = 0.0038$; OR = 12), whereas splenomegaly was significantly associated with liver cirrhosis (6/12; Fisher's exact test, $p = 0.015$; OR = 7.00).

Four patients presented with a rash, which was haemorrhagic in one case.

Ascites, flapping tremor, dyscalculia, and encephalopathy were observed in patients with liver cirrhosis and/or a history of alcohol misuse.

6.4 Analysis of Laboratory Parameters

- **Serum bilirubin**

Serum bilirubin concentrations varied widely over the course of the illness. In 34 of 46 patients (74%), peak bilirubin levels were recorded at admission (Figure 24).

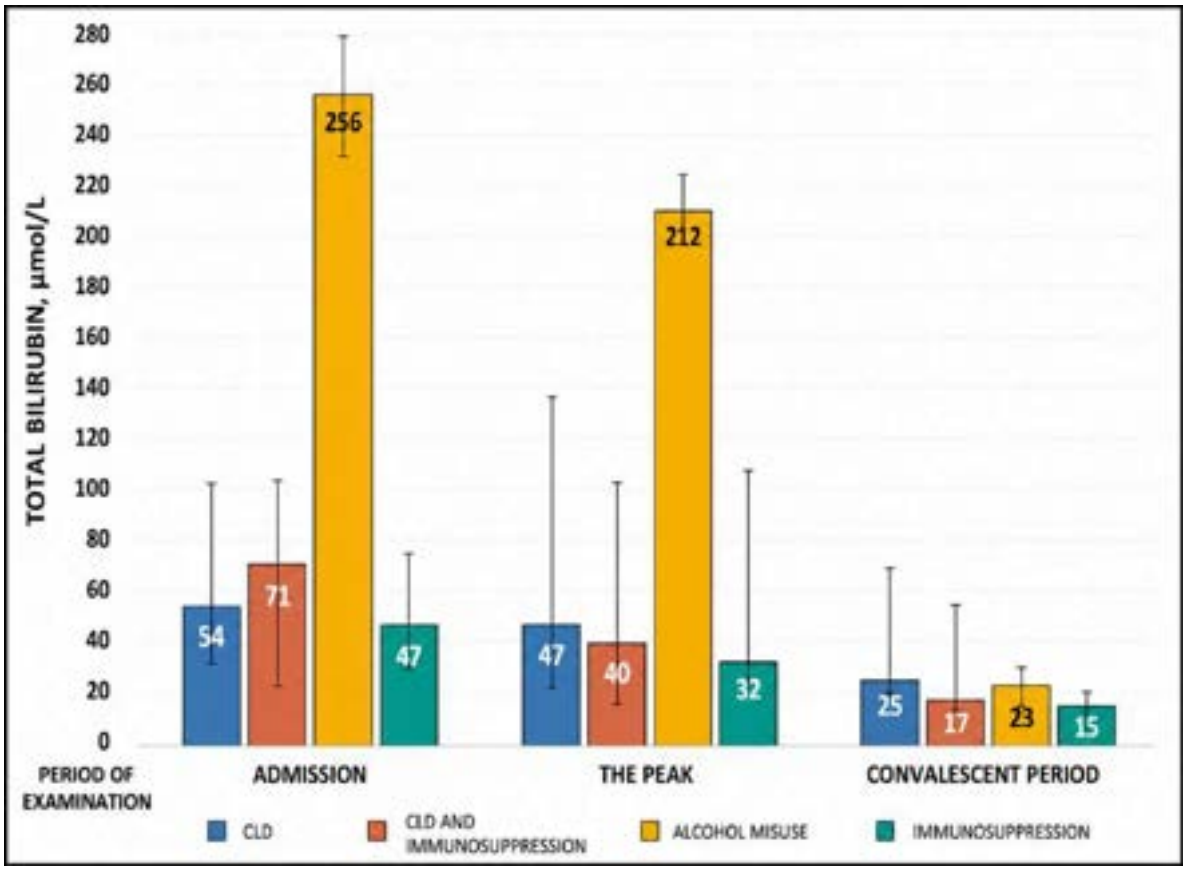


Figure 24. Changes in total bilirubin levels across risk groups and assessment time points (presented as median and interquartile range).

At the height of the illness, jaundice of varying severity was observed predominantly in patients with CLD (n = 18; Figure 25). The highest total bilirubin concentration recorded in the study cohort was 670.9 µmol/L.

Direct hyperbilirubinaemia predominated, occurring in 15 of 24 patients. Nine patients had predominantly unconjugated hyperbilirubinaemia; all had CLD.

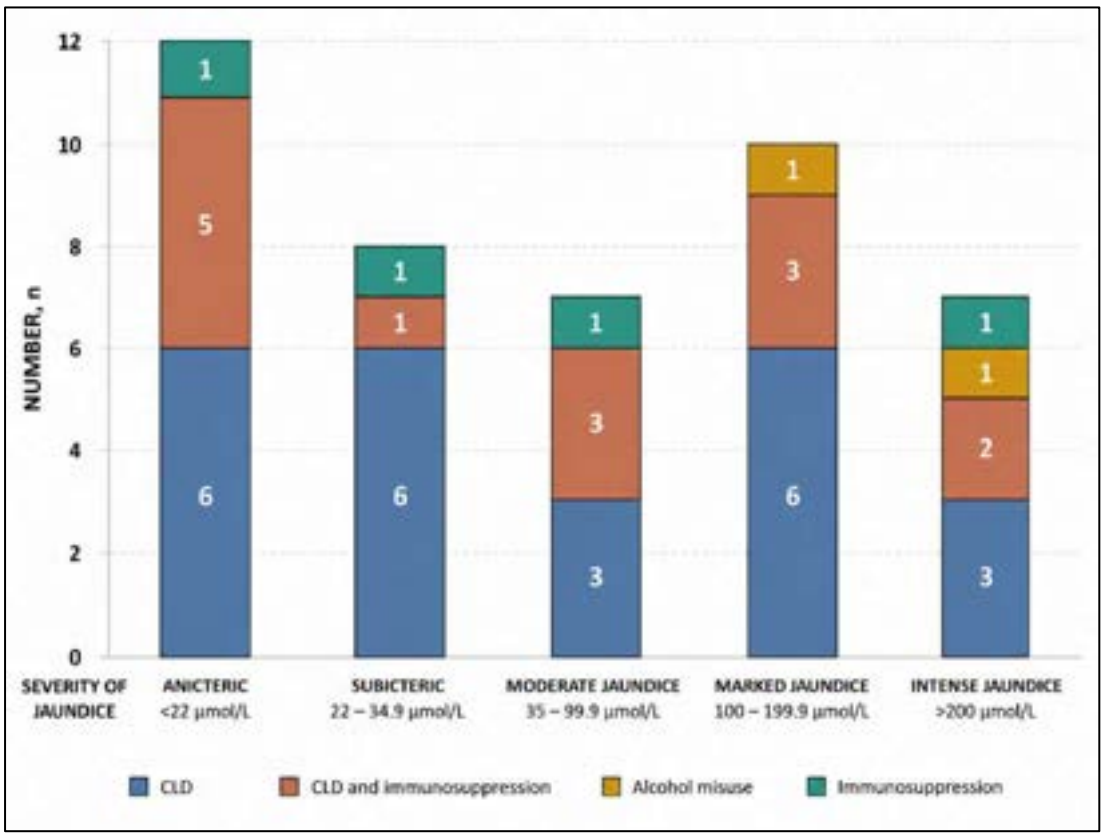


Figure 25. Severity of jaundice at the height of the illness among patients from risk groups.

Liver cirrhosis, coinfection with another hepatotropic virus, and immunosuppression were analysed separately to assess their associations with total bilirubin levels (Table 19).

Table 19. Total bilirubin levels at the peak of the illness according to individual concomitant risk factors.*

RISK FACTOR	Number n	Total bilirubin, µmol/l Min	Med	Max	p-value
Liver cirrhosis	10	8	96,7	670,9	p>0,05
Without cirrhosis	34	6	37,4	421	
		Mann-Whitney U-test: U=139, z= -0,87, p=,385 r=0,13			
Coinfection with another hepatitis virus	23	6	71	421	p>0,05
Without coinfection	21	10	27,6	670,9	
		Mann-Whitney U-test: U=191, z= -1,19, p=,235, r=0,18			
Concomitant immunosuppression	18	*6*	37,4	*670,9*	p>0,05
Without coinfection	*26*	*8*	*76,65*	*421*	
		Mann-Whitney U-test: U=206, z= -0,67, p=,504, r=0,10			

*For each of the three analyses, patients with risk factors and clinical manifestations of hepatitis were stratified according to the presence or absence of the respective factor.

Higher total bilirubin levels were observed in patients with liver cirrhosis and in those coinfected with another hepatotropic virus, whereas lower levels were recorded in immunocompromised patients. During convalescence, the number of anicteric patients increased to 39. In two patients with liver cirrhosis, greenish jaundice persisted for a prolonged period despite preservation of their general condition.

- **Hepatocellular injury enzymes – alanine aminotransferase and aspartate aminotransferase (ALT and AST)**

ALT levels were highest at admission and declined significantly at the peak of the illness and during convalescence (Figure 26). At the peak of the illness, ALT levels differed significantly between patients with CLD and immunosuppression and those with CLD alone (p = 0.016).

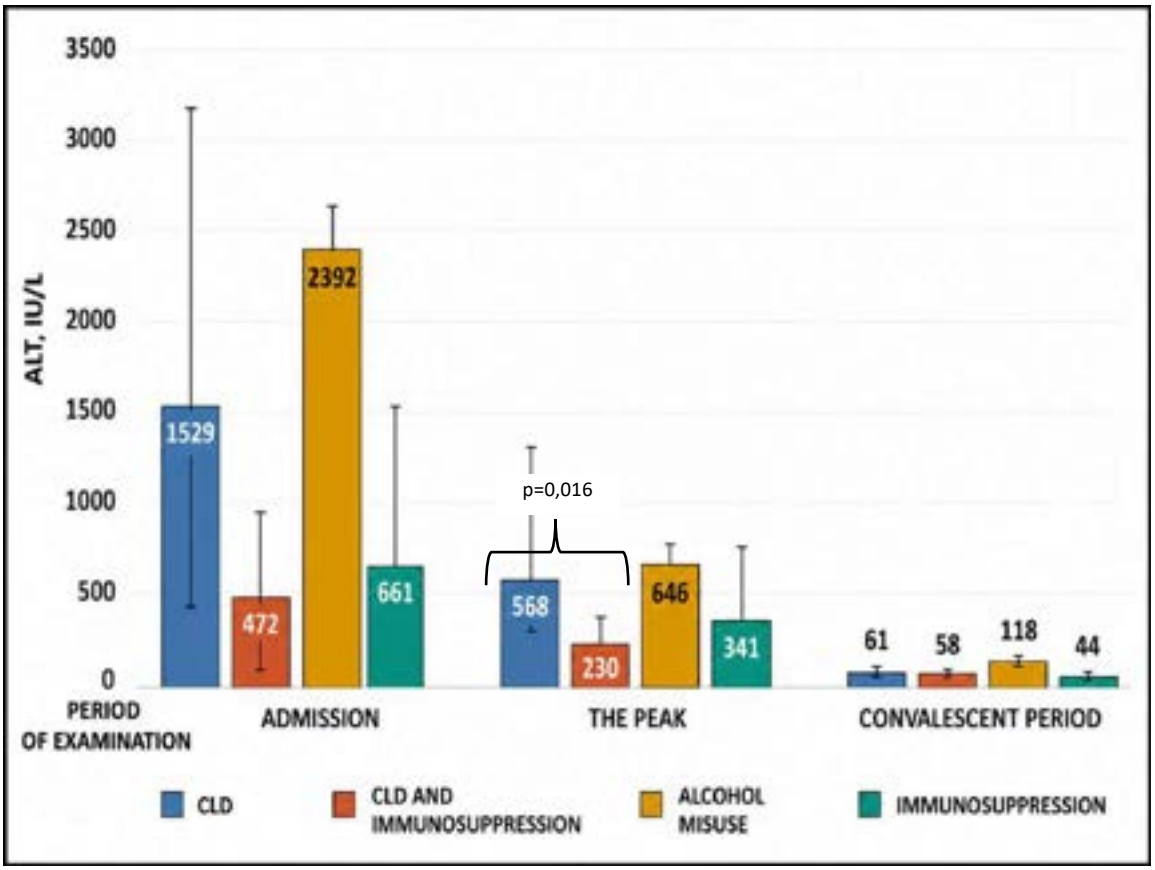


Figure 26. ALT levels across risk groups during the different stages of the illness (presented as median and interquartile range).

When analysed separately, both liver cirrhosis and immunosuppression were associated with significantly lower ALT levels (p < 0.05; Figures 27 and 28).

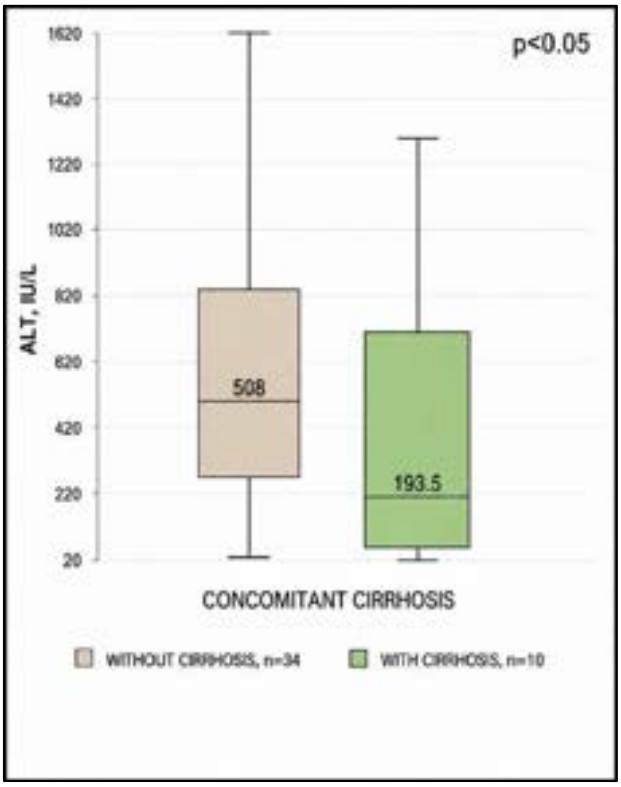


Figure 27. ALT levels at the peak of the illness according to the presence of concomitant liver cirrhosis (p = 0.046).

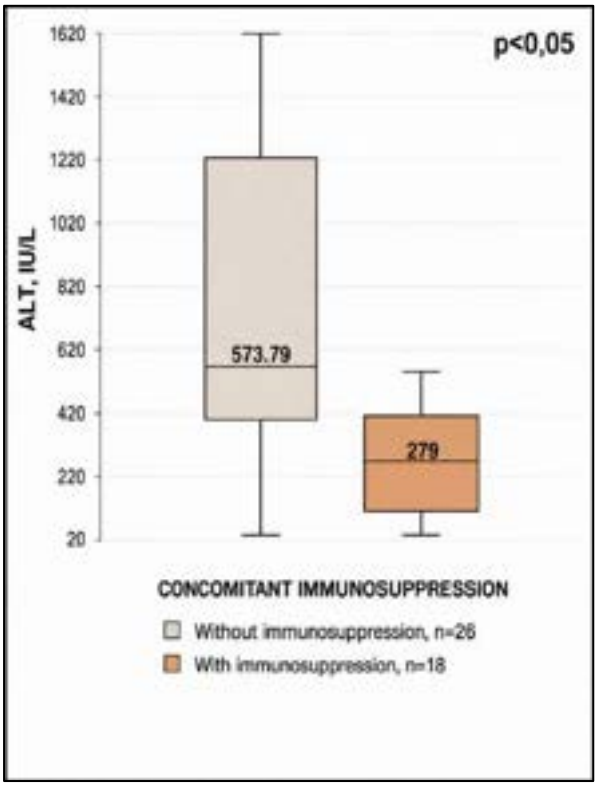


Figure 28. ALT levels at the peak of the illness according to the presence of concomitant immunosuppression (p = 0.02).

Similar findings were observed for AST. Compared with patients with other risk factors, those with liver cirrhosis and immunocompromised patients had significantly lower AST levels both at admission and at the peak of the illness.
Most patients had a De Ritis ratio <1 (n = 39). A De Ritis ratio >1 was observed more frequently among patients with liver cirrhosis (3/5).

- **Cholestatic enzymes — gamma-glutamyl transferase and alkaline phosphatase (GGT and ALP)**

Changes in GGT and ALP levels over the course of the illness are presented in Figures 29 and 30.

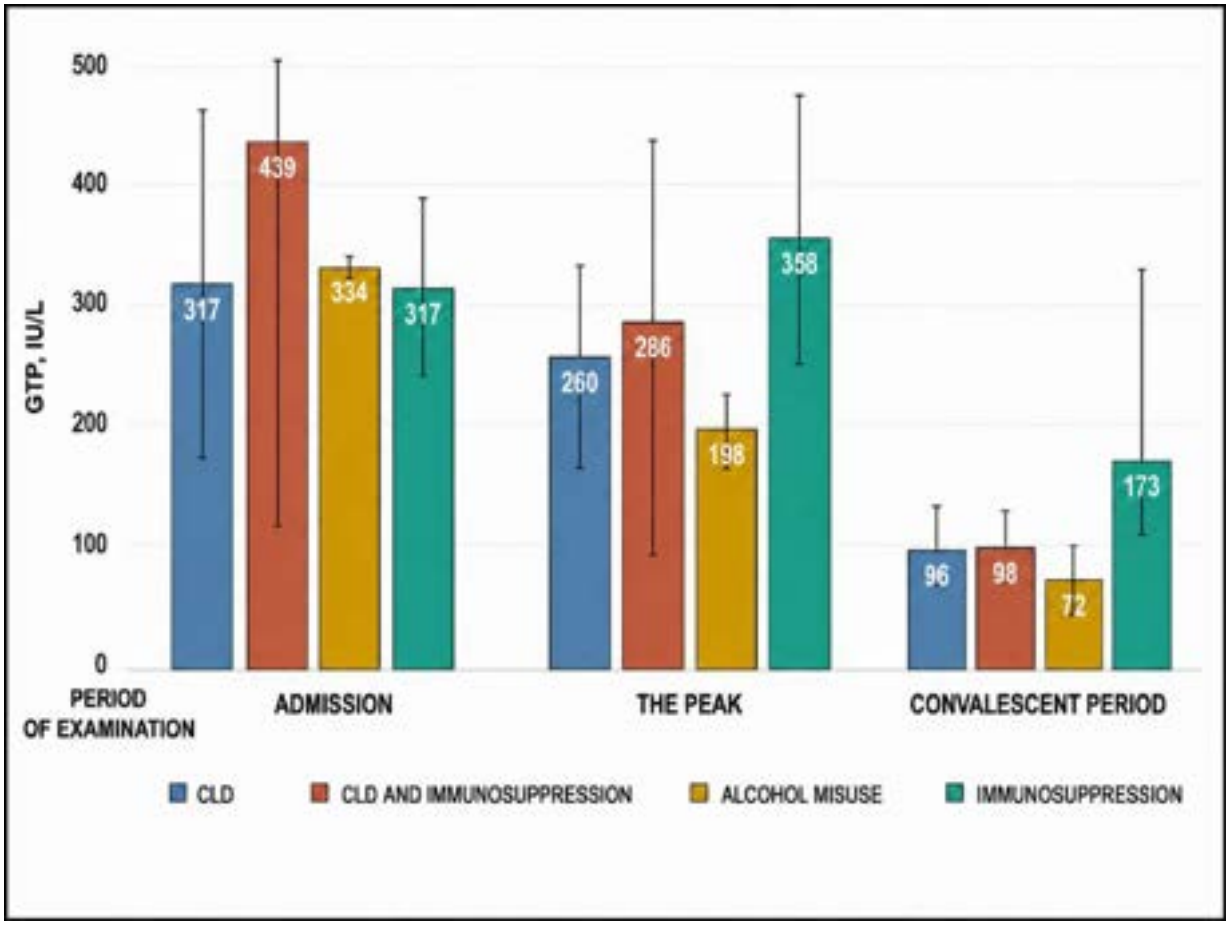


Figure 29. GGT levels across the risk groups during the different stages of the illness (presented as median and interquartile range), $p > 0.05$.

No statistically significant differences in cholestatic enzyme levels were observed between the risk groups.

Patients with concomitant immunosuppression had higher GGT levels, whereas those coinfected with another hepatotropic virus had lower levels.

During convalescence, ALP levels were significantly higher in patients with liver cirrhosis than in those without cirrhosis.

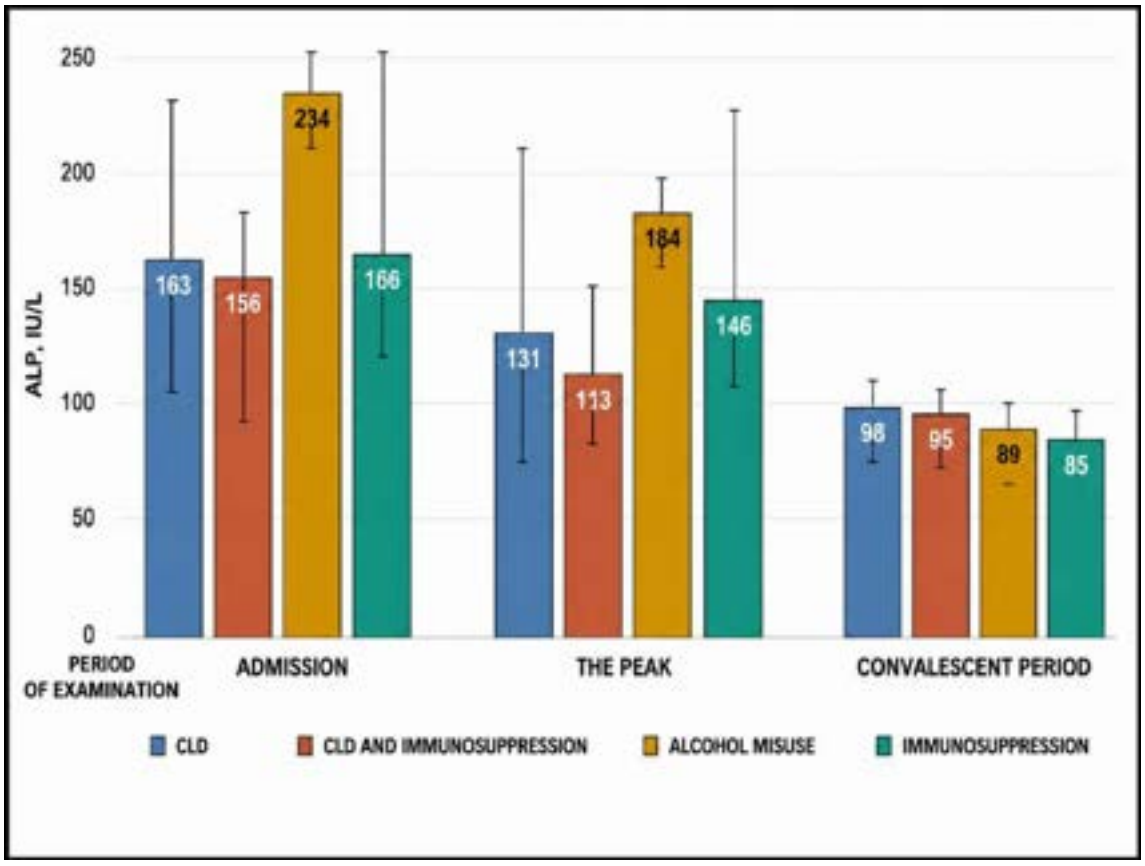


Figure 30. ALP levels across the risk groups during the different stages of the illness (presented as median and interquartile range), $p > 0.05$.

- **Haemostatic parameters — prothrombin index (PI) and fibrinogen**

The haemostatic parameters in patients with different risk factors are presented in Figures 31, 32 and 33.

At the peak of the illness, patients with CLD and immunosuppression had significantly lower fibrinogen levels than those with CLD alone (Figure 31).

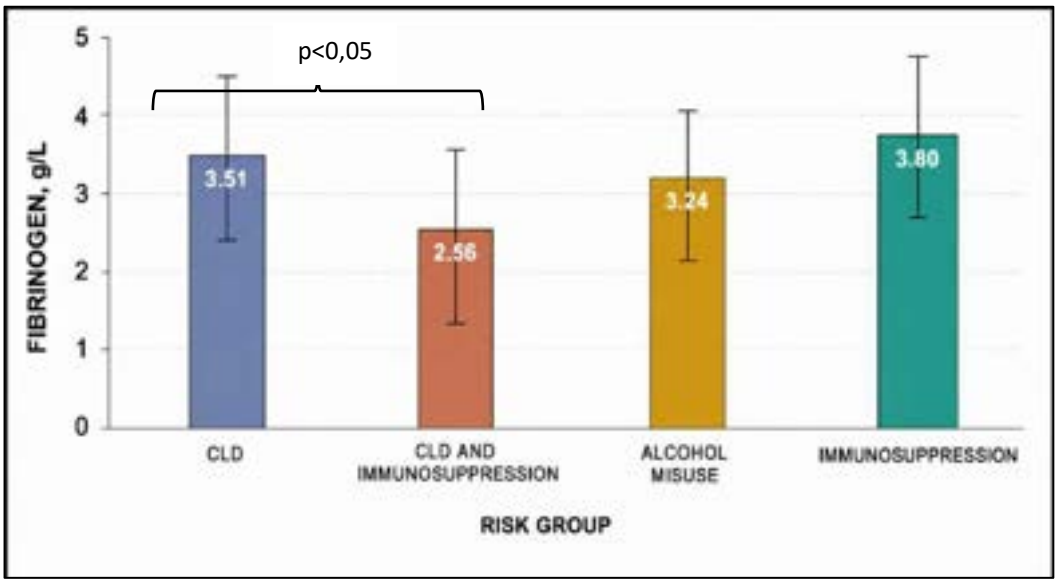


Figure 31. Fibrinogen levels at the peak of the illness across the risk groups, $p < 0.05$.

Patients with liver cirrhosis had significantly lower prothrombin index and fibrinogen levels than those without cirrhosis (Figures 32 and 33).

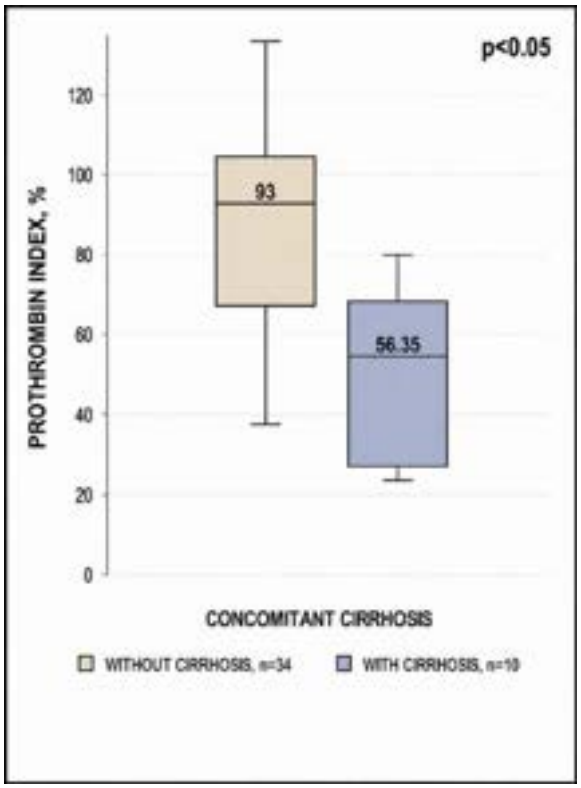


Figure 32. Prothrombin index levels at the peak of the illness according to the presence of concomitant liver cirrhosis, $p < 0.05$.

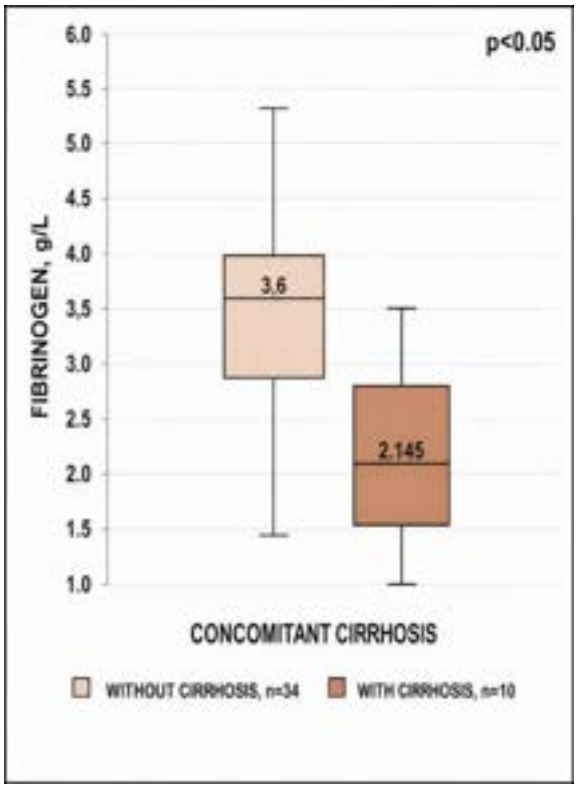


Figure 33. Fibrinogen levels at the peak of the illness according to the presence of concomitant liver cirrhosis, $p < 0.05$.

- **Parameters reflecting hepatic synthetic function — proteins, cholesterol, triglycerides and serum cholinesterase**

Reduced serum protein levels were observed in 32% of the patients studied, most commonly among those with CLD. In 43% of these patients, hypoproteinaemia was accompanied by hypoalbuminaemia (Figure 34).

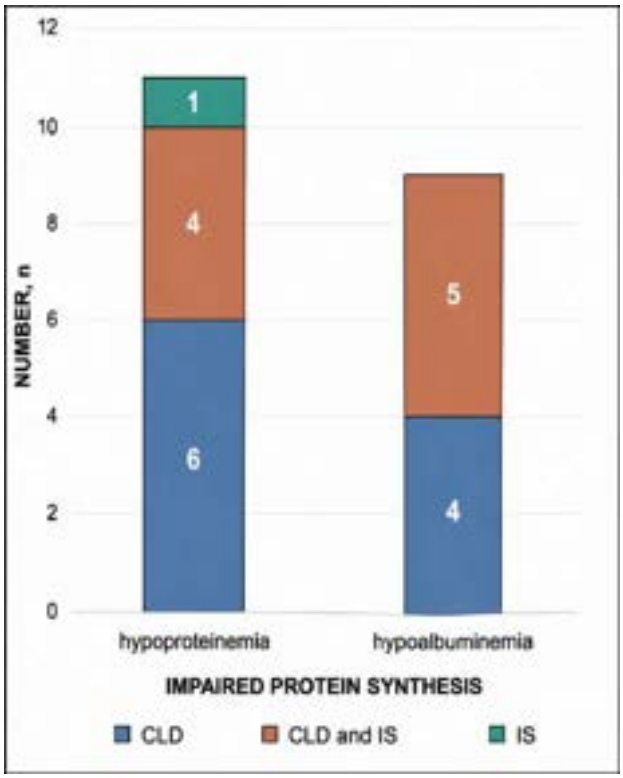


Figure 34. Hypoproteinaemia and hypoalbuminaemia across the risk groups.

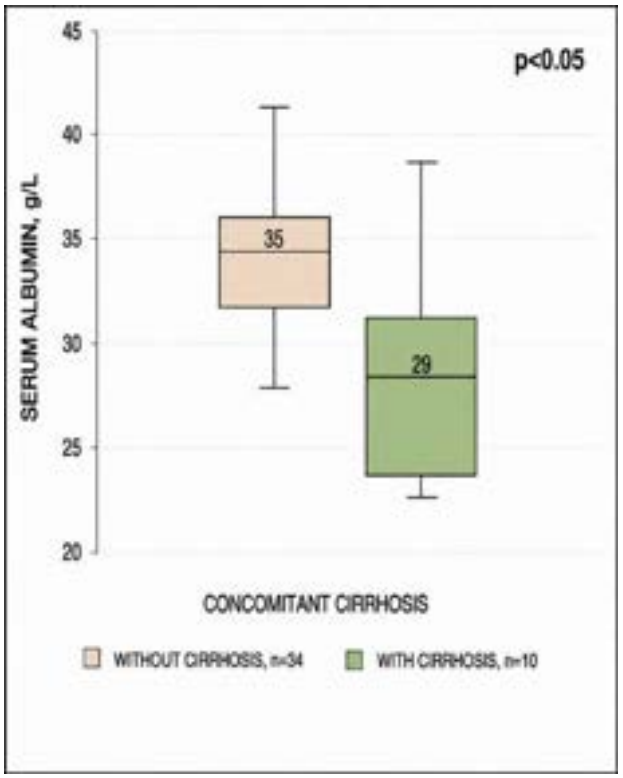


Figure 35. Serum bilirubin levels at the peak of the illness according to the presence of concomitant liver cirrhosis, $p < 0.05$.

Patients with liver cirrhosis had significantly lower serum albumin (Figure 35), total cholesterol and serum cholinesterase levels than those without cirrhosis ($p < 0.05$).

- **Abnormalities in other laboratory parameters**

Table 20 presents the abnormalities observed in selected additional laboratory parameters at the peak of the illness.

Table 20. Abnormalities in the complete blood count, nitrogenous waste products and serum electrolytes

Laboratory abnormality	Number	Frequency
Leucocytosis (>10,5G/l)	8	18%
Anaemia (<120 g/l)	5	11%
Thromocytopenia (<150G/l)	5	11%
Elevated urea (>7mmol/l)	11	25%
Elevated creatinine (>120μmol/l)	8	18%
Hyponatraemia (<135mmol/l)	4	9%
Hypochloraemia (<98mmol/l)	2	5%

Leucocytosis with neutrophilia (7/44) was associated with concomitant conditions, including cholecystitis, Mediterranean spotted fever and pneumonia. Marked leucocytosis of 123.1 G/L with lymphocytosis (85% lymphocytes) was recorded in a polymorbid patient with CLD and lymphocytic leukaemia.

In patients with *HEV/HBV* coinfection, thrombocytopenia was observed in all five cases, while anaemia was recorded more frequently (4/9).

Elevated urea and creatinine levels were observed in 15/44 patients and were significantly more frequent among those with diabetes mellitus (Fisher's exact test, $n = 8$, $p = 0.042$). Of these, three developed hepatorenal syndrome in the course of the ascitic-oedematous form of acute-on-chronic liver failure, while two developed transient acute kidney injury.

Dilutional hyponatraemia was observed more frequently in patients with liver cirrhosis (3/4), acute-on-chronic liver failure and ascites.

6.5 Observed extrahepatic manifestations

Extrahepatic manifestations were observed in 17/44 patients. The most frequent were haematological abnormalities or the presence of more than one type of extrahepatic manifestation (Table 21). Haematological abnormalities were more frequent in patients coinfected with another hepatotropic virus.

Table 21. Extrahepatic manifestations observed in the study patients

EXTRAHEPATIC MANIFESTATIONS		NUMBER
NEUROLOGICAL, n=4	Vertigo	1
	Ataxia	2
	Altered consciousness	1
HAEMATOLOGICAL, n= 9	Thrombocytopenia (Thr<150G/l)	5
	Anaemia (Hb<120g/l)	5
TRANSIENT ACUTE KIDNEY INJURY		2
EXACERBATION OF CVD, n=5	Arrhythmia	3
	Congestive heart failure	2
PANCREATITIS		1
>1 EXTRAHEPATIC MANIFESTATIONS		6

Selected patients with extrahepatic manifestations are presented below.

Medical record No. 9869/2018 — a 45-year-old man with alcohol misuse was admitted with toxoinfectious, icteric and haemorrhagic syndromes, accompanied by qualitative disturbances of consciousness, including psychomotor agitation and aggressive behaviour, which resolved within 24 hours. Anaemia (Hb 102 g/L) and thrombocytopenia (platelet count 118 G/L) were also observed. The diagnosis was confirmed by the detection of high antibody titres of both classes.

Medical record No. 9078/2019 — a 58-year-old man with clinical, laboratory and CT findings consistent with acute pancreatitis, who made a full recovery.

Medical record No. 5407/2018 — a 58-year-old man with liver cirrhosis, diabetes mellitus and acute-on-chronic liver failure. Anaemia (Hb 92 g/L) and thrombocytopenia (platelet count 20 G/L) were observed. The patient also developed euthyroid sick syndrome (ESS), characterised by reduced thyroid hormone levels despite normal TSH values (Table 22).

Table 22. Serial fT3, fT4 and TSH levels in a patient with euthyroid sick syndrome

Laboratory parameter (референтни стойности) / Day of the hospital stay	TSH (0,27 – 4,20 mIU/l)	fT_3 (3,10 – 6,80 pmol/l)	fT_4 (12,0 – 22,0 pmol/l)
15. day	1,04	2,2	11,78
25. day	1,74	1,9	7,46

6.6 Clinical forms of hepatitis E in patients with risk factors

Severe disease and complications were observed in 52% of the patients (23/44). These patients had significantly higher bilirubin, ALT and AST levels ($p < 0.05$), as well as significantly lower protein levels and values of certain haemostatic parameters ($p < 0.05$).

Patients with liver cirrhosis were more likely to develop severe disease and complications (8/10; $p = 0.046$), including acute-on-chronic liver failure (n = 4), gastrointestinal bleeding (n = 4), hepatic encephalopathy (n = 2), or more than one complication (n = 6).

The anicteric form (n = 9) occurred predominantly in immunocompromised patients (6/9), whereas the cholestatic form (n = 4) was observed mainly among patients with alcohol misuse (3/4), in whom jaundice was prolonged. These patients also had significantly higher total bilirubin and ALP levels.

Ascitic-oedematous syndrome (n = 2) was observed in a woman with newly diagnosed liver cirrhosis and a man with heart failure. Acute liver failure (n = 2) developed in a woman with HEV/HBV coinfection and in a man with *HEV* infection and acute alcoholic hepatitis.

6.7 Disease outcome

In 37/44 cases, the disease resulted in recovery after a convalescent period of varying duration, or in clinical improvement and stabilisation of the underlying liver disease. The mean length of hospital stay was 9 days (minimum 5, maximum 48 days). Hospitalisation was significantly longer in patients with severe disease and complications than in those with mild disease, with a mean duration of 12 days in the former group ($p < 0.05$).

A relapse within one month was observed in two patients with *HAV/HEV* coinfection.

Two patients with liver cirrhosis died within one year after recovering from acute hepatitis E.

The case-fatality rate was 6.8%. Three patients died: two with acute exacerbation of chronic liver failure and one with acute liver failure in the setting of *HEV/HBV* coinfection.

6.8 Serological and molecular biological tests

The results of the diagnostic serological and molecular biological investigations are presented in Table 23.

Table 23. Distribution of patients with hepatitis according to positive markers of *HEV* infection.

Diagnostic test	**anti-HEV IgM(+)**, IgG(–)	**anti-HEV IgM(+), IgG(+)**	**anti-HEV** IgM(–), **IgG(+)***	**HEV RNA**
NUMBER of INDIVIDUALS	2	37	5	1

*high anti-HEV IgG level

In all patients, the diagnosis was confirmed serologically by the detection of specific *anti-HEV IgM* and/or high levels of *anti-HEV IgG*. In one patient with liver cirrhosis and leukaemia, the diagnosis was confirmed by the detection of *anti-HEV IgM* and *HEV RNA* at a level of 4,927,500 IU/μL.

7. Comparative analysis of clinical manifestations and laboratory abnormalities in anti-HEV-positive individuals from risk groups and those without concomitant risk factors

7.1 Clinical manifestations and laboratory abnormalities in anti-HEV-positive individuals without concomitant risk factors

Fifteen *anti-HEV(+)* individuals without concomitant risk factors were studied. *Anti-HEV IgM* and *IgG* were detected in 6/15, while high *anti-HEV IgG* levels were found in 9/15. Eight of the 15 individuals were also tested for serum *HEV RNA*, which was not detected.

At the time of the positive serological results, 2/6 *anti-HEV IgM*(+) and IgG(+) men, aged 19 and 37 years, presented with non-specific complaints and icteric syndrome. Laboratory findings showed direct hyperbilirubinaemia, with a maximum total bilirubin level of 88.7 μmol/L, and hepatocellular injury, with a maximum ALT level of 2,547 IU/L. In both patients, the disease followed an uncomplicated course and resulted in recovery after a five-day hospital stay.

Three of the nine *anti-HEV IgG(+)* men reported episodes of dyspeptic symptoms during the preceding 12 months, which they associated with exacerbations of concomitant chronic gastritis or cholelithiasis. Their hepatocellular injury enzyme levels were within the reference range.

Five of the 15 individuals were recovering after hospital treatment for COVID-19 and reported asthenia, fatigue and variable appetite, which they associated with recovery from coronavirus infection and the prolonged use of various medications.

7.2 Comparative analysis of clinical manifestations and laboratory abnormalities in anti-HEV-positive individuals with and without concomitant risk factors

The frequency of subjective complaints and jaundice among individuals with and without concomitant risk factors is presented in Table 24. Individuals with risk factors were more likely to report subjective complaints and to develop the icteric form of hepatitis.

Table 24. Frequency of subjective complaints and jaundice in the two groups compared

SYMPTOM/SIGN	Total, N	Individuals with complaints / jaundice				p-value*
		n	%	OR	95% CI	
SUBJECTIVE COMPLAINTS						**<0,001**
Individuals without risk factor	15	5	33	27,3	5,58 – 133,98	
Individuals from risk factor	44	41	93			
JAUNDICE						**<0,001**
Individuals without risk factor	15	2	13	25,3	4,81 – 132,84	
Individuals from risk factor	44	35	79			

* p-value from the two-sided Fisher's exact test; statistical significance was also confirmed using the chi-square test with Yates' correction.

Significant differences were also observed in total bilirubin, ALT and AST levels between the two groups (Table 25).

Table 25. Comparison of selected laboratory parameters between the two groups

PARAMETER	Total,N	VALUE		U	z	p-value
		X̄ ± SD	Med			
TOTAL BILIRUBIN				27,0	–4,42	**<0,001**
Individuals without risk factor	15	20,5 ± 24,7	12,0			
Individuals from risk factor	44	93,8 ± 99,8	58,8			
ALT				42,0	–4,11	**<0,001**
Individuals without risk factor	15	316,7 ± 707,7	36			
Individuals from risk factor	44	1609,1 ± 2108,7	845			
AST				29,0	–4,36	**<0,001**
Individuals without risk factor	15	152,6 ± 287,2	45			
Individuals from risk factor	44	1036,4 ± 1423,2	489			

Concomitant risk factors were associated with higher levels of these laboratory parameters.

DISCUSSION

Since the discovery of *HEV* at the end of the twentieth century, hepatitis E has become a global health problem. Research continues to clarify the significance of risk factors associated with high morbidity and mortality, vaccines are being developed, and preventive strategies for risk groups are being investigated.

The present study examines the prevalence and clinical manifestations of *HEV* infection among 616 individuals from the Pazardzhik region, including both individuals with risk factors for acquiring *HEV* infection and developing severe disease and those without concomitant risk factors.

Globally, *anti-HEV IgG* seroprevalence was estimated at 12.47% in 2019. In Europe, it varies widely, from 0.5% to 52.5%, and increases with age (Hartl et al., 2016; Li et al., 2020). Our results are close to the high level reported in France – 32%, and exceed the rates previously reported in Bulgaria. According to Golkocheva-Markova E. et al. (2023), the frequency of *anti-HEV* seropositive individuals in different subpopulations ranges from 5.9% to 24.5% and increases significantly with age (Golkocheva-Markova et al., 2023). Data from Switzerland indicate that this association is most pronounced in individuals over 60 years of age (Niederhauser et al., 2018; Takahashi et al., 2010), which is consistent with our findings.

The frequency of *anti-HEV IgM* positivity among adults may vary between regions and depends on the epidemiological situation, including the occurrence of outbreaks and waterborne epidemics (Alhatlani et al., 2020). The specificity and sensitivity of the assay used, the testing methodology and the timing of testing are also important (Jang et al., 2011). Detection of *anti-HEV IgM* without confirmation by *anti-HEV IgG* may represent a false-positive result due to cross-reactivity with other viruses, such as EBV and CMV, which are more common in younger individuals (Hyams et al., 2014).

Our finding of a higher frequency of *anti-HEV* positivity among men is consistent with observations reported by other authors (Пишмишева & Баймакова, 2014; Пишмишева-Пелева, 2018, 2022, 2022; Aggarwal & Sanjay, 2010; Anderson, 2010; Dalton et al., 2018; Kamar et al., 2014). Leblebicioglu H. and Bansal M. explain this finding by the more frequent involvement of men in high-risk occupations or activities (Bansal et al., 2017; Leblebicioglu & Ozaras, 2018). Monini M. studied forestry workers in Italy and associated the higher frequency of seropositivity both with occupational exposure and with more frequent and/or prolonged exposure to *HEV* (Monini et al., 2020).
In our study group, the number of individuals engaged in a high-risk occupation or activity was too small to allow definitive conclusions regarding the influence of these risk factors. Further research in this area is required.

Dietary habits influenced by religion may also affect the prevalence of *HEV* infection. *Anti-HEV*(+) individuals were predominantly of Bulgarian ethnicity, whereas most participants belonging to other ethnic groups were Muslim and excluded or substantially restricted the consumption of pork and pork products. This may reduce the risk of *HEV* acquisition through the main foodborne route. Similar observations were reported by Пишмишева М. in the first study of *HEV* infection conducted in the Pazardzhik region between 2014 and 2017 (Пишмишева-Пелева, 2018, 2022).

The higher proportion of seropositive individuals from risk groups observed in our study is consistent with the reports of Dalton H. and Memon M. They described a higher frequency of *anti-HEV* positivity among patients with liver cirrhosis, chronic hepatitis and non-alcoholic steatohepatitis (Dalton, Stableforth, et al., 2008; Memon et al., 2024). In addition to liver disease, excessive alcohol consumption is associated with an increased risk of infection and severe disease (Balaban et al., 2022;

A. C. Fantilli et al., 2019; Parfieniuk-Kowerda et al., 2021a). Patients with CLD and those with alcohol misuse were the risk groups in which *anti-HEV* positivity was detected in at least 50%.

According to our findings, seropositive individuals with immunosuppression predominantly had diabetes mellitus and CLD. Impaired glucose metabolism and poor glycaemic control compromise immune defence and may increase susceptibility to *HEV* infection. Patients with diabetes may also develop more severe hepatitis E because of metabolic dysfunction, impaired hepatic function and reduced hepatocyte regenerative capacity (M. Faber, Askar, et al., 2018; Geerlings & Hoepelman, 1999; Pas et al., 2012).
Other immunosuppressive conditions, including solid tumours, haematological malignancies and treatment with cytostatic or immunomodulatory agents, may also impair viral clearance, reduce immunoglobulin production and weaken the T-cell response (Chinen & Shearer, 2010). When concomitant liver disease is present, T-cell differentiation and the production of signalling and effector molecules are also impaired, together with reduced clearance by activated T lymphocytes and diminished phagocytosis by Kupffer cells (Chovancová, 2019). These mechanisms may explain the higher proportion of *anti-HEV*(+) individuals with both immunosuppression and CLD compared with those with immunosuppression alone. The observed proportions were higher than those reported by Buescher G., according to whom *anti-HEV* seroprevalence among immunocompromised individuals ranges from 6% to 29.6%, and among HIV-positive individuals from 3.5% to 19.4% (Buescher et al., 2021).
We examined two HIV-positive men, both of whom were anti-HEV-negative. This number is insufficient to draw conclusions regarding the significance of HIV infection, and further studies in this group are required.

An inadequate antibody response in immunosuppressed individuals limits the diagnostic reliability of serological methods alone (Kamar et al., 2008). Molecular testing for *HEV RNA* provides reliable confirmation of infection. When hepatitis E is suspected in immunocompromised individuals, nucleic acid amplification testing is recommended even when serological results are negative (Dalton et al., 2018; Kar & Karna, 2020).
HEV RNA was not detected in the immunocompromised individuals examined in our study. In a Danish study involving 4,023 immunosuppressed patients, *HEV RNA* was detected in 0.15% (Buescher et al., 2021; Harritshøj et al., 2020). Detection of *HEV* antigen in faeces may also have diagnostic value in these groups (Dalton et al., 2018). At present, this test is not available in Bulgaria.

Immunocompromised patients are at risk of developing chronic hepatitis E, particularly following blood-borne transmission. This route of infection is also possible in patients undergoing maintenance haemodialysis, who frequently receive blood-derived products (Ismail et al., 2020). In our study, the proportion of *anti-HEV*(+) individuals with chronic kidney disease undergoing haemodialysis was 14%. This was lower than the rates reported in England (36.8%), Turkey (20.6%) and Lebanon (21.63%), but higher than those reported in Greece (4.8%), Spain (6.3%) and Tunisia (10.2%) (Ben-Ayed et al., 2015; Harrison et al., 2013; Ismail et al., 2020; Mateos et al., 1998; Stefanidis et al., 2004; Uçar et al., 2009). Lower rates have also been reported by studies conducted in haemodialysis centres in Bulgaria: 8.75% in Plovdiv (М. Атанасова et al., 2020) (Атанасова et al., 2020) and 10.7% in a larger study from Southern Bulgaria (Кеворкян А. et al., 2020) (Kevorkyan et al., 2023). According to the literature, *anti-HEV* seroprevalence among haemodialysis patients worldwide varies widely, from 0% in Brazil (Parana R, 1997) to 68.6% in southern Iran (Ouji M, 2021). Differences have been observed between endemic and non-endemic regions and between individual dialysis centres (Kevorkyan et al., 2023; Ouji et al., 2021; Parana et al., 1997).
Patients with renal failure are commonly polymorbid and have impaired immune responses due to multiple factors, including uraemia, metabolic abnormalities, anaemia, hypoproteinaemia, vitamin deficiencies and electrolyte disturbances (Chinen & Shearer, 2010; Kato et al., 2008; Parana et al., 1997). They are generally more susceptible to infections (Harrison et al., 2013). Nevertheless, in our

study, individuals with chronic kidney disease undergoing haemodialysis had the lowest proportion of *anti-HEV* positivity among the risk groups. One possible explanation is the dietary regimen followed by patients with renal failure, which involves restricted protein intake, including reduced meat consumption, which may therefore decrease the risk of foodborne transmission. Because of their compromised immune response, serological testing alone may be insufficient to detect *HEV* infection in this population.
A limitation of our study is that haemodialysis patients were not tested for HEV RNA.

The comparative analysis also demonstrated significant differences according to the number of concomitant risk factors. The higher proportion of seropositive individuals with only one risk factor may be explained by the predominance of patients with CLD and individuals with alcohol misuse in this group.
The frequency of concomitant risk factors for acquiring *HEV* infection and developing severe disease increases with age (Guo et al., 2023). Comparison of the risk groups by age showed that patients with CLD and those with alcohol misuse were the youngest, while the proportion of seropositive individuals in these groups was the highest. *Anti-HEV IgG* positivity was detected in 48% and 45%, respectively. By comparison, anti-HEV IgG seroprevalence among patients with liver disease in Europe and Asia ranges from 10% to 40%, while a rate of 8.9% has been reported among individuals with alcohol misuse in Croatia (A. C. Fantilli et al., 2019; Vilibic-Cavlek et al., 2016; Yang et al., 2019).
CLD and alcohol misuse therefore emerged as the leading risk factors associated with *anti-HEV* positivity.

Among the individuals without concomitant risk factors, we observed a higher proportion of anti-*HEV IgG* positivity than that reported by other authors. In Germany, *anti-HEV IgG* seroprevalence is 16.8%, with a pronounced increase with advancing age (M. Faber, Willrich, et al., 2018). Population-based studies from the Balkan countries remain scarce. According to Vilibic-Cavlek T. (2023), *anti-HEV* seroprevalence reaches 9.7% in Albania and Greece, 15% in Serbia and 17.1% in Romania, while the highest rate has been reported in Bulgaria – up to 20.9% (Vilibic-Cavlek et al., 2023).

The higher frequency of *anti-HEV IgG* positivity among individuals from risk groups compared with those without concomitant risk factors may also be partly attributable to the statistically significant age difference between the groups. Our results are consistent with data from Bulgaria and other countries and confirm the significance of risk factors for acquiring *HEV* infection (Bai et al., 2018; Balaban et al., 2022; Dalton et al., 2011; M. Faber, Askar, et al., 2018; Golkocheva-Markova et al., 2023; Haffar et al., 2017; Kmush et al., 2015; Mariya et al., 2020; Mrzljak et al., 2020; Riveiro-Barciela et al., 2014; Tulen et al., 2019; Zhang et al., 2017).

The statistically significant findings from the regression analysis support the leading role of CLD as a risk factor for *anti-HEV* positivity, as also reported by authors from Bulgaria and other countries (Atiq et al., 2009; A. C. Fantilli et al., 2019; Hamid et al., 2002; KONDILI et al., 2006; Memon et al., 2024; RAMACHANDRAN et al., 2004; Riveiro-Barciela et al., 2014; Wong et al., 2021; Yang et al., 2019). The lower frequency of seropositivity among patients with chronic kidney disease undergoing haemodialysis may be related to their dietary habits, as discussed above. Кеворкян А. emphasises that the accurate assessment of *anti-HEV* seroprevalence is also influenced by the study period, antibody kinetics and participant selection criteria (Kevorkyan et al., 2023).
It is possible that some of the individuals classified as having no risk factors provided inaccurate or incomplete information regarding their dietary habits, alcohol consumption or activities.

This study provides the first data on the prevalence of *HEV* infection among blood donors from the Pazardzhik region. Our findings are consistent with those reported by Faber M., who observed relatively stable *anti-HEV* levels in individuals aged 18–34 years and a marked increase in

seropositivity with advancing age (M. S. Faber et al., 2012). In China, Liu reported a low frequency of *anti-HEV* positivity among individuals aged under 25 years and an increase among those aged over 55 years (H. Liu et al., 2024), which also supports our results. Blood donors with an *anti-HEV IgM*(+), *IgG*(–) profile were predominantly under 40 years of age. EBV and CMV infections are common in younger individuals, and cross-reactivity may result in false-positive *anti-HEV IgM* findings (Hyams et al., 2014). Blood donors with evidence of past *HEV* infection *IgG(+)* and *IgM(–)* were predominantly over 40 years of age, which is consistent with findings reported by other authors (Mansuy et al., 2016).
The proportion of blood donors in whom *anti-HEV IgG* was detected was close to that reported in other European countries. Seroprevalence among blood donors ranges between 10% and 20% in Austria, Denmark, Norway, Spain and the United Kingdom; it reaches 22.4% in France, exceeds 50% in southern France, and is reported at 43.5% in Poland, 30% in the Netherlands and 20.4% in Switzerland (Capai et al., 2019; Grabarczyk et al., 2018; Mansuy et al., 2016; Mooij et al., 2018; Niederhauser et al., 2018; Slot et al., 2013).
In 2021, Баймакова M. and colleagues studied blood donors in Bulgaria and detected *anti-HEV IgG* in 25.9%, predominantly in men, which is consistent with our observations (Baymakova et al., 2021). The cited study did not include blood donors from the Pazardzhik region. Our result of 27.65% was close to the rates reported in the Stara Zagora region (27.1%) and Plovdiv (27.5%) (Baymakova et al., 2021).
For the first time in Bulgaria, results from molecular testing for *HEV RNA* in donated blood are presented. The *HEV RNA*(–) results among the blood donors examined may be attributable to delayed testing, the short duration of viraemia in *HEV* infection or past infection. According to meta-analysis data, the global frequency of *HEV RNA*(+) blood donors ranges from 0.01% to 0.14%, with the highest frequency in Asia and the lowest in North America and Australia (Webb & Dalton, 2019). In Europe, the frequency reaches 0.10%, with variation between countries: 0.28% in Germany and 0.31% in Serbia (Wolski et al., 2023).

Our analysis of the clinical manifestations of *HEV* infection showed that the profile of patients who developed clinically manifested hepatitis E was similar to that reported by other authors. Clinically apparent hepatitis E occurred more frequently among *anti-HEV*(+) individuals from risk groups, men, individuals of Bulgarian ethnicity and those aged over 55 years (Пишмишева-Пелева, 2018, 2022; Dalton, Bendall, et al., 2008). The prevalence of *HEV* infection among other ethnic groups remains unclear. Пишмишева M. suggested that Roma individuals may acquire the infection in early childhood and more often have a subclinical course, similar to hepatitis A, or may not seek medical care because they lack health insurance or because the disease is mild (Пишмишева & Баймакова, 2014; Пишмишева-Пелева, 2018, 2022).

The lower number of hepatitis E cases registered after 2018 and during the COVID-19 pandemic reflects a trend also observed in Germany, France, the United Kingdom and Spain after the beginning of the pandemic (Курчатова A. et al., 2018; Н. Владимирова et al., 2019, 2020, 2021, 2022; Izopet et al., 2019). This has mainly been attributed to reduced healthcare-seeking among patients with non-respiratory symptoms and the substantially less frequent use of diagnostic tests other than those for SARS-CoV-2 infection (Young et al., 2025). In Spain, for example, the number of hospitalisations for acute viral hepatitis declined overall (Ramos-Rincon et al., 2023). Epidemiological analyses confirm that the reduced reported incidence of hepatitis E during the pandemic was more likely attributable to limitations in diagnosis, case detection and reporting than to a true reduction in the number of cases or changes in the clinical course (D. Huang et al., 2025; Javanmardi et al., 2025; Young et al., 2025).

The autumn–winter seasonality of registered hepatitis E cases observed in our study is consistent with reports of a higher frequency of cases during the colder months (Пишмишева-Пелева, 2018, 2022). Seasonality in hepatitis E is observed predominantly in endemic regions where *HEV1* and *HEV2*

circulate and transmission is mainly waterborne. In South Asia and Africa, the number of cases increases during or shortly after the monsoon season because of heavy rainfall and contamination of water sources. In China, by contrast, the number of cases is highest during winter and spring around the period of traditional festivals, when an increased risk of foodborne *HEV* transmission has been suggested (Aggarwal & Naik, 2009; Kamar et al., 2012; K. Liu et al., 2016; Nelson et al., 2019).
Unlike our observations, developed countries in which *HEV3* is also prevalent do not show a clearly defined seasonal pattern in hepatitis E incidence, although a slight increase has been reported during the second and third quarters of the year (Guerrero-Vadillo et al., 2024; Oeser et al., 2019).

Hepatitis E is generally a self-limiting disease, with asymptomatic and mild clinical forms predominating among immunocompetent individuals. This may explain the low frequency of clinically apparent cases observed among individuals without risk factors. In contrast, individuals with concomitant risk factors are more likely to develop clinically apparent disease, severe illness and complications, and have a higher risk of a fatal outcome (Balaban et al., 2022; Dalton et al., 2011; M. Faber, Askar, et al., 2018; Kmush et al., 2015; Qiu et al., 2023; Tulen et al., 2019; Zhang et al., 2017). In our study, *anti-HEV(+)* individuals, predominantly those with CLD, developed clinically apparent hepatitis E more frequently than immunocompromised individuals. Hepatic abnormalities and immune and systemic dysregulation predispose patients both to infection and to a severe course of *HEV* infection. *HEV* may also trigger decompensation of CLD and the development of acute-on-chronic liver failure (Atiq et al., 2009; A. C. Fantilli et al., 2019, 2019; Haim-Boukobza et al., 2015; Kanda et al., 2024, 2024; Parfieniuk-Kowerda et al., 2021b; Qiu et al., 2023, 2023; Riveiro-Barciela et al., 2014, 2014; Yang et al., 2023, 2023). The weakened immune response in immunocompromised individuals predisposes them to persistent viraemia and chronic hepatitis E, with progressive fibrosis and the development of cirrhosis (Damiris et al., 2022). Chronic hepatitis E was not identified in the patients included in our study.
Following an extensive review of the accessible literature, we found no published reports of registered cases of chronic hepatitis E in Bulgaria.

The mean duration of the pre-icteric period in our patients was nine days, which was longer than the mean of six days reported by Пишмишева М. (Пишмишева-Пелева, 2018, 2022). An impaired immune response contributes to a longer pre-icteric period in patients with immunosuppression, while the delayed serological response complicates early diagnosis in this risk group (Damiris et al., 2022; Ghandili et al., 2022; Hoofnagle et al., 2012).

The epidemiological history of the patients examined suggested foodborne transmission. Asthenia, fatigue, dark urine and dyspeptic complaints of varying severity are also among the leading symptoms reported by other authors (Пишмишева-Пелева, 2022). Compared with the previous study conducted in the Pazardzhik region, our patients more frequently reported musculoskeletal pain (27% versus 7.91%) and fever (36% versus 22%), which may also have been related to another concomitant illness. Less frequently reported symptoms included pruritus (14% versus 30.9%) and diarrhoea (9% versus 10.1%) (Пишмишева-Пелева, 2022).

According to the literature, *HEV* infection in patients with CLD is more frequently accompanied by jaundice and hepatic decompensation (Aggarwal, 2011; Hoofnagle et al., 2012; Horvatits et al., 2019). We observed clinical manifestations of hepatic decompensation among patients with liver cirrhosis and among individuals with alcohol misuse.

Analysis of the laboratory abnormalities revealed higher total bilirubin levels among patients with liver cirrhosis and those with alcohol misuse. This is supported by published evidence associating chronic alcohol use and CLD with hyperbilirubinaemia of varying severity (>100 μmol/L) (A. Fantilli et al., 2021; Hoofnagle et al., 2012). Пишмишева М. reported maximum serum bilirubin levels at the peak of the illness, whereas most of our patients had their highest values at admission

(Пишмишева-Пелева, 2018). Indirect hyperbilirubinaemia was less frequent, and impaired conjugation was attributable to liver injury of varying severity.

The lower total bilirubin levels observed in immunocompromised patients are consistent with reports from other authors. In the setting of immunosuppression, *HEV* infection may follow an atypical course with mild to moderate laboratory abnormalities, including total bilirubin levels below 100 μmol/L (A. Fantilli et al., 2021; Hoofnagle et al., 2012; Mechnik et al., 2001). Immunosuppression is associated with a greater risk of chronic hepatitis E, although no evidence of chronic infection was identified among the immunocompromised individuals in our study.
The significantly higher ALT levels observed among patients with CLD, including those with chronic hepatitis B, and individuals with alcohol misuse reflect greater hepatocellular injury, probably caused by the combined effects of *HEV/HBV* coinfection and *HEV* infection with a toxic insult from alcohol (A. C. Fantilli et al., 2019; Wong et al., 2021). The lower ALT levels observed in patients with liver cirrhosis or immunosuppression may be attributable to reduced hepatic metabolic and synthetic activity and a weakened immune response, respectively. Liver cirrhosis is associated with progressive disruption of hepatic architecture, including fibrosis and a reduction in the functional hepatocyte mass (Ginès et al., 2021; Sullivan et al., 2022). The weakened immune response associated with immunosuppression results in less pronounced inflammation and hepatocyte necrosis and, consequently, lower serum ALT levels (Damiris et al., 2022; Péron et al., 2006). It is possible that the combination of multiple factors, such as CLD and immunosuppression, exerts a stronger effect on ALT levels. The De Ritis ratio >1 and reduced haemostatic parameters observed in patients with liver cirrhosis reflect the reduced functional parenchymal mass and advanced impairment of hepatic function (Giannini et al., 1999).
Marked hepatocellular injury supports the diagnosis of acute hepatitis, whereas a rapid decline in ALT and AST levels at the peak of the illness may delay and complicate diagnosis in risk groups (Пишмишева-Пелева, 2018). Patients with CLD are more likely to seek gastroenterological care and may be treated for decompensated liver disease without being tested for hepatitis E.

Haematological abnormalities were the most frequent extrahepatic manifestations observed in our study. Leucocytosis is not characteristic of viral hepatitis and, in all affected patients, was associated with another condition. Anaemia and thrombocytopenia in liver cirrhosis result from multiple factors, including bleeding, splenomegaly and hypersplenism, bone marrow suppression, reduced thrombopoietin levels, B-vitamin deficiencies, iron deficiency, toxin- or antibody-mediated platelet destruction and haemolysis (Bihari et al., 2016; Fierro-Angulo et al., 2024; Peck-Radosavljevic, 2017; Simonetto et al., 2020). In HEV infection, destruction of erythrocytes and platelets may also be immune-mediated (Dalton et al., 2018; Pischke et al., 2017). Autoimmune anaemia with detectable anti-erythrocyte antibodies was observed in only one patient. Haematological abnormalities were more frequent among patients with *HEV/HBV* coinfection. *HBV* is known to infect bone marrow, suppress thrombopoiesis and erythropoiesis, and enhance eryptosis (Mei et al., 2022). In *HEV/HBV* coinfection, different mechanisms may act in combination, resulting in more severe liver injury and coagulation and haematological abnormalities (Shi et al., 2025).

The elevated urea and creatinine levels recorded among patients with concomitant diabetes mellitus reflected diabetic nephropathy of varying severity. Cases of membranoproliferative and membranous glomerulonephritis have been reported in immunocompetent patients infected with *HEV1* and *HEV3*. No such cases were identified in our study. These renal abnormalities are transient, and kidney function recovers following resolution of the infection (Dalton et al., 2018).
Apart from Guillain–Barré syndrome and amyotrophic sclerosis, the other neurological manifestations observed in our study have rarely been reported in the literature. We identified a report from China of a patient similar to one of our cases, who developed persistent vertigo during an icteric form of hepatitis E that resolved as bilirubin levels declined (Deng et al., 2025). Transient ataxia has

rarely been reported; it is more commonly described as part of an encephalitic or meningoencephalitic syndrome during chronic *HEV* infection (Hoofnagle et al., 2012; Kamar et al., 2011, 2012).
Loggini A. et al. also reported a case of reversible encephalopathy in 2019 (posterior reversible encephalopathy syndrome, or PRES) in a woman with serological evidence of acute hepatitis E (Loggini et al., 2019). Neurological manifestations also resolve after *HEV* clearance, and most patients recover completely (Kamar et al., 2011).

Cardiovascular involvement is uncommon during *HEV* infection. Extrahepatic manifestations such as arrhythmia and myocarditis have been described in patients with hepatitis E (Dalton et al., 2018; Woolson et al., 2014). Tachyarrhythmia and exacerbation of congestive heart failure prompted the admission of some of our patients to the Department of Cardiology, where hepatitis E was subsequently diagnosed.
Acute pancreatitis is a documented extrahepatic manifestation in regions endemic for *HEV1*. Although a direct association between *HEV3* infection and pancreatic injury has not been established, pancreatitis is considered possible with all *HEV* genotypes (Dalton et al., 2018; Kamar et al., 2012; Pischke et al., 2017). Our literature review identified European reports of acute pancreatitis in young men who had travelled to India or Pakistan, either during acute hepatitis E or as the sole manifestation of *HEV* infection (Deniel et al., 2011; Haffar et al., 2015; Hoofnagle et al., 2012; Jaroszewicz et al., 2005; Pischke et al., 2017). In our study, the clinical manifestations of acute pancreatitis predominated in one man, in whom *HEV* infection was confirmed serologically.

Reports of thyroiditis during *HEV* infection have also been published (Dalton et al., 2018). We did not observe thyroiditis; however, one patient with liver cirrhosis and acute-on-chronic liver failure developed euthyroid sick syndrome. Euthyroid sick syndrome is an adaptive mechanism that prevents excessive catabolism in severely ill and depleted patients (Меков & Боянов, 2022). It has been described in patients admitted to intensive care units and in association with cardiovascular disease, sepsis, decompensated liver cirrhosis, acute-on-chronic liver failure and other conditions (de Vries et al., 2015; Golombek, 2008; Langer et al., 2023).

Among the patients with diabetes in our study, elevated blood glucose levels required transition to an intensified insulin regimen and adjustment of insulin doses. It has been suggested that the infective dose may be lower in patients with diabetes and that *HEV* infection may contribute to the decompensation of diabetes mellitus (Пишмишева-Пелева, 2018, 2022).

Our analysis of the clinical course showed that anicteric disease occurred predominantly among immunocompromised individuals and more frequently in men. This differs from the findings of Пишмишева М., according to whom the anicteric form was more frequent in women. Cholestatic syndrome occurred predominantly among individuals with alcohol misuse, which is consistent with the findings of the cited study conducted in the Pazardzhik region during a different period (Пишмишева-Пелева, 2018).

Most patients with liver cirrhosis developed severe disease and complications, which is consistent with publications by other authors (Пишмишева-Пелева, 2018; An et al., 2025; Kumar Acharya et al., 2007; Riveiro-Barciela et al., 2014; Zhao et al., 2023). Liver cirrhosis is also a risk factor for the development of more than one complication: *HEV* may trigger acute-on-chronic liver failure, hepatic decompensation and associated complications (Джурков В. & Кръстев Н., 2015). Fatal acute liver failure was associated with fulminant hepatitis in a woman with diabetes mellitus and *HBV* infection of unknown duration. According to the literature, *HEV* may reactivate chronic HBV infection by disrupting immune control of *HBV* replication, inducing an inflammatory response and causing liver injury of varying severity (Chen et al., 2016; Horvatits et al., 2019; Shi et al., 2025). *HEV/HBV* coinfection may present as fulminant hepatitis, particularly in older and immunocompromised patients, including those with concomitant diabetes mellitus (Lai et al., 2018; Tseng et al., 2020).

Our observation of a predominantly favourable outcome among patients who developed hepatitis E is also consistent with the published evidence. Only isolated reports were identified regarding relapse in patients with *HEV/HAV* coinfection. According to these reports, relapses have a milder clinical course, and their frequency is similar to that observed in *HAV* monoinfection (Gabrielli et al., 2023; Sayed, 2023).

The case-fatality rate in our study was 6.8%, which is higher than the rate reported worldwide, ranging from 0.5% to 3%. By comparison, a case-fatality rate of 5.75% was reported in the Pazardzhik region between 2014 and 2017 (Пишмишева М., 2018). All deaths in our study occurred among patients with CLD who developed acute liver failure or acute-on-chronic liver failure. International studies identify CLD as a major risk factor for severe disease and death in HEV infection. The case-fatality rate in this risk group ranges from 22% to 43% (Iqbal et al., 2023; Meng XJ, 2023).

The diagnosis of acute hepatitis E in the patients included in our study was based on the detection of specific *anti-HEV IgM* and/or *IgG*; *HEV RNA* was also demonstrated in one patient. In patients with only high and increasing *anti-HEV IgG* levels and clinical and laboratory evidence of hepatitis, acute *HEV* infection without an *IgM* response or possible reinfection was considered. According to the literature, 10–20% of patients with acute hepatitis E do not develop *anti-HEV IgM* or have a false-negative result. In such cases, confirmation by detection of *HEV RNA* in blood or serum and/or an increase in *anti-HEV IgG* to ≥4 times the cut-off value is recommended. Reinfection in *anti-HEV IgG*(+) individuals also results in a rapid rise in *IgG* levels (S. Huang et al., 2010; Rivero-Juarez et al., 2021; Tang et al., 2023). Interpretation of these findings is not always straightforward, particularly when distinguishing acute hepatitis from reinfection.
A limitation of our study is that not all patients were tested for *HEV RNA*; however, antibody levels were monitored over time in all patients.

Our investigation of the clinical manifestations of *HEV* infection in seropositive individuals without concomitant risk factors showed that the infection more frequently followed a subclinical or mild course with rapid recovery. On this basis, we consider that hepatitis E in individuals without risk factors generally has a mild course and may remain undiagnosed, which is also supported by observations reported by other authors (Hoofnagle et al., 2012; Kamar et al., 2012, 2014).

CONCLUSION

Hepatitis E virus infection is prevalent among individuals from risk groups and in the general population, including blood donors. Serological evidence of HEV infection was detected in 34% of all individuals tested, predominantly in men of Bulgarian ethnicity aged over 55 years. Individuals from risk groups were affected more frequently, particularly those with concomitant liver disease.
Clinically apparent HEV infection developed significantly more often in individuals with risk factors. Concomitant liver diseases, including liver cirrhosis, chronic viral hepatitis and hepatic steatosis, were associated with more severe disease, complications and extrahepatic manifestations. The case-fatality rate reached 6.8%, with deaths occurring in the setting of acute liver failure or acute-on-chronic liver failure.
HEV infection in risk groups requires targeted testing, careful interpretation of diagnostic findings and heightened clinical awareness.

Proposed algorithm for testing donated blood for HEV

HEV infection can also be transmitted through blood. Individuals at increased risk of acquiring HEV infection and developing severe disease frequently require blood products. To reduce the risk of transfusion-transmitted infection, we propose that donated blood should also be tested for anti-HEV IgM and IgG.
When serological testing yields a positive result, the following algorithm may be applied in accordance with EMA and EASL recommendations:

1. Universal screening of donated blood for HEV, involving the testing of samples from every blood donor:

1.1 Testing of minipools comprising samples from several blood donors using RT-PCR for the detection of HEV RNA, with an appropriate assay having a lower limit of detection of 2,000 IU/mL;

1.2 If the minipool result is positive, individual testing of all samples included in the positive minipool;

1.3 Preparation of blood components from donors who test negative for HEV RNA on individual testing;

1.4 Updating the blood donor's record. The donor may donate blood again six months after the positive result or following recovery if clinically apparent hepatitis E develops.

CONCLUSIONS, CONTRIBUTIONS AND RECOMMENDATIONS

1. Conclusions

1.1 Hepatitis E virus infection is prevalent in the populations studied and affects men of Bulgarian ethnicity more frequently. Individuals with *anti-HEV IgG* constitute the largest proportion, at 29.57%, and their frequency increases with age. *Anti-HEV IgM* is detected predominantly in younger individuals.

1.2 Seropositive individuals are more frequently found in risk groups than in the general population. Individuals with concomitant liver disease or immunosuppression and those with alcohol misuse more frequently have detectable *anti-HEV IgG*. Concomitant liver disease, including liver cirrhosis, chronic hepatitis and hepatic steatosis, is the risk factor associated with the highest frequency of *anti-HEV* positivity, whereas this risk is significantly lower among patients undergoing maintenance haemodialysis.

1.3 The proportion of blood donors with specific *anti-HEV* antibodies is 40%. It is lowest among the youngest blood donors and highest among those aged over 50 years. *HEV RNA* was not detected in the seropositive blood donors tested.

1.4 Clinically apparent hepatitis develops more frequently in men from risk groups, of Bulgarian ethnicity and aged over 55 years. Immunosuppression is associated with a longer pre-icteric period and anicteric hepatitis.

1.5 Concomitant liver disease is the leading risk factor for clinically apparent icteric hepatitis E. Liver cirrhosis, irrespective of its aetiology, is associated with severe disease and complications, the development of acute-on-chronic liver failure and a greater risk of a fatal outcome.

2. Contributions

2.1 Contributions of original scientific significance

2.1.1 For the first time in the Pazardzhik region, the prevalence of hepatitis E virus infection has been investigated in groups at risk of acquiring infection and developing severe disease, including individuals with concomitant liver disease, immunosuppression, alcohol misuse and end-stage renal disease.

2.1.2 For the first time in Bulgaria, the clinical course of hepatitis E in individuals from risk groups has been investigated, including clinical symptoms, complications, extrahepatic manifestations, laboratory abnormalities and outcomes.

2.1.3 For the first time, the prevalence of hepatitis E virus infection among blood donors from the Pazardzhik region has been investigated using both serological and molecular testing of donated blood.

2.2 Contributions of scientific and applied significance

2.2.1 For the first time, a comparative analysis of the prevalence of hepatitis E virus infection in the Pazardzhik region has been conducted between the general population and risk groups for acquiring HEV infection and developing severe disease.

2.2.2 For the first time in Bulgaria, a comparative analysis of the clinical manifestations and laboratory abnormalities of hepatitis E virus infection has been conducted between individuals from risk groups and the general population.

2.2.3 An adapted algorithm for testing donated blood for *HEV* has been proposed.

2.3 Confirmatory contributions

2.3.1 The importance of demographic characteristics has been confirmed: male sex and age over 50 years are associated with a higher prevalence of HEV infection and more frequent development of clinically apparent hepatitis E.

2.3.2 Concomitant liver disease is a leading risk factor for acquiring HEV infection and developing severe disease. Liver cirrhosis is associated with the development of acute-on-chronic liver failure, complications and a fatal outcome.

3. Recommendations

To improve the diagnosis and prevention of HEV infection, the following measures are recommended:

- Access to HEV testing should be improved by ensuring the availability of diagnostic assays in virology laboratories, allowing timely testing and reporting of results.

- Donated blood should be screened for *HEV*. This recommendation is addressed to the Ministry of Health and transfusion haematology centres.

- Diagnostic testing for *HEV* infection should be performed in patients from risk groups and in those with suspected toxic or drug-induced hepatitis. This recommendation is addressed to specialists in gastroenterology and internal medicine.

- The diagnosis of acute hepatitis E should be based on the detection of antibodies of both classes or on the detection of *anti-HEV IgM* and/or *IgG* together with *HEV RNA*. Isolated anti-*HEV IgM* positivity should be interpreted with caution and requires additional confirmatory testing.

PUBLICATIONS AND PARTICIPATION IN SCIENTIFIC FORUMS RELATED TO THE DISSERTATION

1. PUBLICATIONS

1.1 Pishmisheva M., Shikov P., Golkocheva-Markova E., Kotsev St., Naseva E., Vatev N., Argirova R. Spread of Hepatitis E Viral Infection among Haemodialysis Patients in Pazardzhik District, Bulgaria. *International Journal of Current Microbiology and Applied Sciences (IJCMAS)*; 2020; 9(1): 1086–1092.

1.2 Pishmisheva-Peleva M., Kotsev St., Golkocheva-Markova E., Argirova R. Extrahepatic Manifestations in Patients with Acute Hepatitis E, Pazardzhik, Bulgaria, 2014–2022. *Problems of Infectious and Parasitic Diseases*; 2024; 52(2): 5–10. (Scopus Q4; SJR 2024: 0.118).

1.3 Kotsev St., Pishmisheva-Peleva M. Hepatitis E—the Vulnerable. *MedInfo*; 2023; XXII(9): 38–41. ISSN: 2603-4158.

1.4 Kotsev St., Golkocheva-Markova E., Shopova M., Naseva E., Pishmisheva-Peleva M. Distribution of Hepatitis E Virus Infection among Alcohol Abusers from Pazardzhik Region. *Preventive Medicine X*; 2022; 1(21): 13–14. ISSN: 1314-5681.

2. PARTICIPATION IN SCIENTIFIC FORUMS

2.1 Kotsev St., Pishmisheva-Peleva M., Stoeva V., Golkocheva-Markova E., Ismailova Ch., Naseva E., Vatev N. Prevalence of Hepatitis E Virus Infection among Blood Donors (oral presentation). 10th Jubilee National Conference “HIV and Exotic Parasitic and Infectious Diseases in the Post-COVID-19 Pandemic Period”, Tsigov Chark, 25–28 May 2023.

2.2 Kotsev St., Golkocheva-Markova E., Ismailova Ch., Argirova R., Pishmisheva-Peleva M. The Different Faces of Hepatitis E (oral presentation). 16th National Conference on Infectious Diseases of the Bulgarian Society of Infectious Diseases, Pravets, 5–7 October 2023.

2.3 Kotsev St., Golkocheva-Markova E., Ismailova Ch., Vatev N., Pishmisheva-Peleva M. Clinical Manifestations of Hepatitis E Virus Infection among Individuals with Concomitant Risk Factors (oral presentation). 12th National Scientific Conference “HIV and Exotic Parasitic and Infectious Diseases”, Tsigov Chark, 29 May–1 June 2025.

2.5 Kotsev St., Golkocheva-Markova E., Pishmisheva-Peleva M. Prevalence of Hepatitis E Virus Infection among Individuals from the General Population in the Pazardzhik Region (oral presentation). Scientific Conference “Systemic Infectious Diseases in Society”, Sofia, 15–17 November 2024.

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