

A comparison of hepatitis E and A in a teaching hospital in Northwestern Romania. Acute hepatitis E – a mild disease?

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Abstract

Background and aims. The incidence of locally acquired hepatitis E has increased in recent years across Europe. There are only few data on hepatitis E in Romania. The purpose of our research was to describe and compare hepatitis E and hepatitis A in adult patients.

Methods. We included all consecutive adult patients with hepatitis E and hepatitis A admitted to the Teaching Hospital of Infectious Diseases, Cluj-Napoca, Romania between January 2017 and August 2019.

Results. Hepatitis E incidence increased in 2018-2019 compared to 2017. The average age in hepatitis E (n=48) patients was 50.6 versus 39.1 years in hepatitis A (n=152, not including 262 minors) and two-thirds of the patients in both groups were men. Compared to hepatitis A, patients with hepatitis E presented significantly less modified AST and ALT, bilirubin, prothrombin index and INR levels. We found more comorbidities in hepatitis E patients adjusted for age and gender. Severe forms were found in 5 (3.3%) hepatitis A patients, compared to 12 (25%) of hepatitis E patients, of which 3 died. Ribavirin treatment was considered in 9 patients with acute-on-chronic hepatitis E, immunosuppression, cancers or neurological manifestations, showing good results.

Conclusions. We observed an increased number of hepatitis E cases. Although laboratory results were less modified compared to hepatitis A, we found a higher number of severe hepatitis E cases. Ribavirin treatment seems to be beneficial in patients with preexisting conditions.

Keywords: hepatitis E, hepatitis A, ribavirin

Background and aims

Hepatitis E is an anthroponosis with typically mild evolution caused by the Hepatitis E virus (HEV), the *Hepeviridae* family, whose members infect humans and other mammals [1,2]. Genotypes 3 and 4 are the most common in Europe, where the reservoir of infection is represented by asymptomatic but highly infectious pigs and wild boars (with reproductive index up to 8.8) [3–6].

Recent Romanian research found IgG HEV seroprevalence between 9.6% and 50% in farm, backyard pigs and wild boars in which PCR (Polymerase chain reaction) analysis in a small number of liver and spleen samples found

only genotype 3, similar to neighboring countries. Romanian data (in small samples) on IgG HEV seroprevalence in humans is scarce and variable: general population (5.9% - 28%, higher in older participants), students and medical staff (12.5-13.98%) and patients with hepatitis B or C (12%) [7–12].

In Europe, transmission occurs through consumption of contaminated and undercooked pork or other meat products [13,14] but other transmission routes have also been demonstrated (blood transfusions [15,16]). Vegetable products are rarely associated with HEV in Europe, probably due to tight regulation of pig manure use in farming [13,17]. Genotypes 3 and 4 may lead to

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chronic disease in immunocompromised patients [5,18] yet, the majority of individuals are asymptomatic [19].

Genotypes 1 and 2 of HEV are obligate human pathogens that only cause acute disease and are more common in developing countries: Asia (genotype 1), Africa (genotype 2) and Central America (both), being transmitted through fecal-oral route and contaminated water [5].

The number of laboratory-confirmed cases increased across Europe since 2006 to even more cases than hepatitis A in Germany, UK and France [3] with an estimated two million locally acquired cases each year in Europe [5].

HEV infects the liver but may be present in other organs (brain, kidney, placenta) [20,21] and HEV RNA (Ribonucleic acid) becomes detectable in blood and feces after 2-3 weeks post-exposure and lasting 3-6 weeks. After an incubation of 15-60 days, liver enzymes, anti-HEV IgM and then anti-HEV IgG levels increase marking the clinical onset. Anti-HEV IgM antibodies may persist up to 1 year, anti-HEV IgG are long-lasting and in immunosuppressed patients, HEV RNA may be detectable for more than 6 months being considered chronic infection [5]. Patients may develop HEV antibodies without any symptoms of hepatitis or high ALT (alanine aminotransferase) or AST (aspartate aminotransferase) values [22].

Risk factors for clinical manifestations include: male gender, age over 50 and preexisting liver disease [13,23]. Acute-on-chronic liver failure has considerable fatality and may benefit from antiviral treatment (ribavirin, interferon). Small studies have shown that ribavirin treatment (600-800 mg/day, short duration) in acute hepatitis E is safe and effective in patients with acute-on-chronic liver disease and transplant recipients [5,24–27]. Occasionally, neurologic lesions in acute hepatitis E patients were reported: neuralgic amyotrophy, Bell palsy, Guillain-Barré syndrome, encephalitis and myelitis [28,29]. Chronic cases (HEV RNA clearance failure after 6 months) have been reported in solid organ transplant recipients presenting long-lasting fatigue, elevated AST, ALT and γ -GT (Gamma-glutamyltransferase) and sometimes negative anti-HEV IgM and IgG [18,30,31]. EASL (European Association for the Study of the Liver) recommends HEV testing in patients with the aforementioned pathologies, regardless of liver enzyme levels [5].

According to Romanian regulations, all confirmed and suspected cases of acute viral hepatitis (A to E) are admitted and treated in infectious diseases wards. Our institution serves the Transylvania region, but most patients live in Cluj County.

Our objective was to describe all cases of HEV infection admitted in our hospital during the study period in comparison to all hepatitis A adult patients. We focused

on patient characteristics that were available from our hospital electronic records.

Methods

The main part of the research was a retrospective case-case study of all available adult cases of acute hepatitis E and A admitted in the Teaching Hospital of Infectious Diseases of Cluj-Napoca, Romania, between 2017 January 1 and 2019 August 30.

The comparison group – Hepatitis A – was chosen due to similar (mainly enteral) transmission and usually self-limited evolution. Since hepatitis E is not common in children, we decided to include only adults.

Inclusion criteria were defined as: a diagnosis of acute hepatitis E or A at discharge (ICM10 (The 10th revision of the International Statistical Classification of Diseases and Related Health Problems) codes: B17.2 and B15.*, respectively), admission date between 2017 January 1 and 2019 August 30 and age > 18 years old. No specific exclusion criteria were used.

All clinical departments within our hospital were considered similar regarding diagnosis and management of the patients and the judgment of all doctors assigned to each patient was considered equivalent. We gathered information on laboratory parameters at admission (total and direct bilirubin, INR (International Normalized Ratio), prothrombin index, ALT, AST, γ -GT and ALP (Alkaline phosphatase)), final diagnosis and doctors' description of each patient's presentation and evolution. Environmental and alimentary exposure could not be reliably assessed.

The etiology of hepatitis A and E was established from blood samples by qualitative anti-HAV (Hepatitis A virus) and anti-HEV IgM respectively using *bioMérieux VIDAS® Hepatitis panel* electrochemiluminescence immunoassays (for HEV: positive concordance=97.65%, negative concordance=99.34%) [32]. According to our hospital protocol, valid since 2016, and during the whole study period, all suspected cases of acute viral hepatitis were tested simultaneously for hepatitis A – E from the same blood sample as a single laboratory request.

To put the main study into context, we counted all confirmed cases of acute viral hepatitis A-E ICM10 codes B15-B17.2), of all ages, registered in our hospital during the same period.

We further investigated the severe cases of both hepatitis E and A within the main study as case series. We defined severe cases of hepatitis E if INR >1.5, hepatic encephalopathy grades 2-4 and/or comorbidities (acute-on-chronic liver disease, confirmed immunosuppression) or neurological manifestations were found (according to EASL guideline 2018 and our hospital's protocol) [5]. The therapeutic approach for these patients was supportive treatment *plus* ribavirin (600-800 mg/day). We defined severe cases of hepatitis A if INR >1.5, hepatic encephalopathy grades 2-4 were present (according to

EASL 2017 guideline and our hospital’s protocol) [33]. The therapeutic approach for these patients was supportive treatment *plus* plasma products. Therefore, all patients received appropriate supportive treatment according to our hospital’s protocols and general recommendations, as needed: glycemic control, fluid balance correction, hepatoprotective agents, ammonia-reducing agents (L-arginine, rifaximin, lactulose) and, in cholestatic forms, ursodeoxycholic acid and prophylactic antibiotics [33]. We defined additional treatment as ribavirin in hepatitis E patients and plasma products in hepatitis A patients.

In assessing disease severity we gathered information on the common signs and symptoms of acute viral hepatitis, hepatic encephalopathy graded by West-Heaven criteria [34], chronic liver comorbidities (cirrhosis regardless of etiology, chronic hepatitis B and C, primary or secondary liver cancer, for whom MELD (Model for End-Stage Liver Disease) score was calculated [35]), other chronic comorbidities (chronic kidney disease, neurologic conditions, diabetes mellitus, immunosuppression of any cause). We presented a short description of deceased patients and hepatitis E cases with ribavirin treatment, highlighting comorbidities, evolution and possible causes of death.

All patients signed a general informed consent form at admission allowing anonymous research on data included in the electronic records. This study was approved by the ethics committee of our hospital.

Data were centralized in a spreadsheet, checked for consistency, anonymized and imported into R 3.6.1 [36] on Linux where all subsequent statistical analyses were performed. We used absolute and relative frequencies to describe categorical data and means with standard deviations or medians with IQR (Inter-quartile range) to describe numerical data. Comparisons between hepatitis A and hepatitis E groups were performed using both univariate methods (t-test for continuous variables with normal distribution according to the Shapiro-Wilk test, Mann-Whitney (MW) test for continuous variables with non-normal distribution, Fisher test for binary variables) as well as two multivariate logistic regression models adjusting for (model 1): age and gender and (model 2): all variables taken into account. Prior to logistic models, right-skewed data were transformed using the decimal logarithm. All statistical tests used a significance cut-off value at $p < .05$.

Results

A total of 48 hepatitis E adult patients and 152 hepatitis A adult patients were included. No pregnant women were found in either group. Hepatitis E cases represented 9.62% from all registered cases of acute viral hepatitis during the study period, including legal minors (Figure 1). No hepatitis E cases were registered in pediatric patients.

One hepatitis E patient had possible travel-related exposure (UK) and all other cases are believed to be autochthonous but alimentary and environmental exposure could not be reliably assessed.

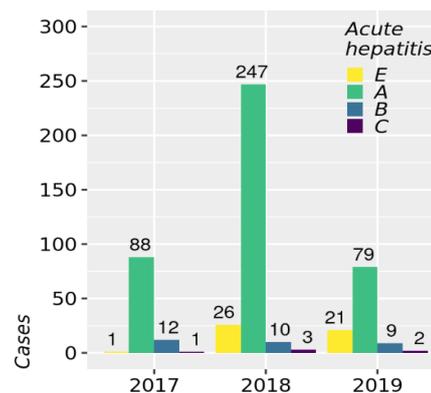


Figure 1. Distribution of acute viral hepatitis cases of all ages, during the study period (2017 Jan 1 to 2019 Aug 30).

Most cases in both groups were male (M: 119, F: 81, M/F ratio: 1.47) and hepatitis E patients were significantly older than hepatitis A patients (Table I).

Patients in both groups had similar median hospitalization length. The maximum duration was 43 days in a hepatitis E patient and 38 days in a hepatitis A patient (Table I).

Hepatitis E patients had significantly milder abnormalities in laboratory values at presentation for direct & total bilirubin, AST, ALT, ALP, INR and prothrombin index and γ -GT (if adjusted for age and gender) (Table I, Figure 2).

Table II summarizes chronic conditions associated with hepatitis E compared to hepatitis A: chronic liver disease, chronic kidney disease, neurologic disease, diabetes mellitus (univariate and adjusted for age & gender and other comorbidities).

Significantly more hepatitis E patients needed additional treatment (ribavirin, 9 patients, 18.8%) than hepatitis A patients (plasma, 5 patients, 3.3%) with OR=6.8 (OR=4.9 adjusted for age & gender) (Table II). All hepatitis E patients who received ribavirin had a favorable evolution and were either discharged at home or to another department for specialized treatment of their comorbidities (Table III). A short course of ribavirin treatment (up to 21 days), was considered for most of these patients and good results were observed (tendency towards normalization of laboratory parameters). Ribavirin treatment was stopped after 19 days in a patient due to severe thrombocytopenia. Two patients were transferred to other clinics after 10 and 21 days of ribavirin treatment, respectively and their treatment regimen was presumably continued. One severely immunosuppressed patient was recommended for 3 months (but showed good results after 12 days).

Table I. Hospitalization and laboratory parameters of the hepatitis E and A groups.

Hepatitis (Group)		E	A	Univariate statistics	Adjusted OR ¹ p, [95% CI]
		n (%)	n (%)		
Gender	M	31 (64.6)	88 (57.9)	ns.	
	F	17 (35.4)	64 (42.1)		
Age at presentation (years)	$\mu \pm SD$	50.62 \pm 15.6	39.06 \pm 15.0	MW: p<.001	
	50+	28 (58.3)	31 (20.4)		
	[40,50)	7 (14.6)	28 (18.4)		
	[30,40)	7 (14.6)	52 (34.2)		
	[18,30)	6 (12.5)	41 (27.0)		
Hospital stay duration (days)	Med (IQR)	9 (7-14)	11 (8-14)	MW: ns.	ns.
Direct bilirubin (mg/dL)	Med (IQR)	1.24 (0.34-5.02)	4.9 (2.66-6.99)	MW: p<.001	0.194, p<.001, [0.09, 0.38] *
Total bilirubin (mg/dL)	Med (IQR)	1.73 (0.68-5.76)	5.87 (3.38-8.2)	MW: p<.001	0.182, p<.001, [0.08, 0.39] *
ALP (IU/L)	Med (IQR)	154.5 (119.25-192.75)	205 (159.25-260.5)	MW: p<.001	0.046, p=.003 [0.01, 0.34] *
γ -GT (IU/L)	Med (IQR)	229 (123.5-327)	246 (154.75-355.5)	MW: ns.	0.343, p<.048, [0.12, 0.99] *
AST (IU/L)	Med (IQR)	145.5 (69-676.75)	870 (304.5-1666.75)	MW: p<.001	0.112, p<.001, [0.05, 0.23] *
ALT (IU/L)	> 350	17 (35.4)	99 (65.1)	OR=0.29, p<.001, [0.15, 0.58]	0.249, p<.001, [0.12, 0.51]
	Med (IQR)	401 (122.75-886.25)	1817.5 (919.25-2801.75)	MW: p<.001	0.045, p<.001, [0.02, 0.11] *
Prothrombin index (%)	> 350	26 (54.2)	132 (86.8)	OR=0.18, p<.001, [0.09, 0.37]	0.12, p<.001, [0.05, 0.27]
	Med (IQR)	88.25 (75.2-100.38)	72.7 (59.9-86.85)	T-test: p<.001	1.039, p<.001, [1.02, 1.06]
INR	< 70	9 (18.8)	60 (42.0)	OR=0.32, p=.005, [0.14, 0.71]	0.268, p=.002, [0.11, 0.61]
	Med (IQR)	1.06 (0.99-1.13)	1.16 (1.07-1.31)	MW: p<.001	0.036, p=.002, [0.0, 0.26]
	> 1.5	4 (8.3)	16 (11.2)	ns.	ns.

¹: odds-ratio adjusted on age and gender; *: marked variables were transformed to base 10 logarithm prior to logistic regression due to skewness, therefore odds-ratios show tenfold increases/decreases in the respective laboratory parameters; $\mu \pm SD$: mean \pm 1 standard deviation; Med (IQR): median (inter-quartile range); MW: Mann-Whitney test; OR [95% CI]: odds-ratio with p-value and 95% confidence interval; ns.: not statistically significant at $\alpha=0.05$; ALP: Alkaline phosphatase; γ -GT: Gamma-glutamyl transferase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; INR: International Normalized Ratio; IU: International units.

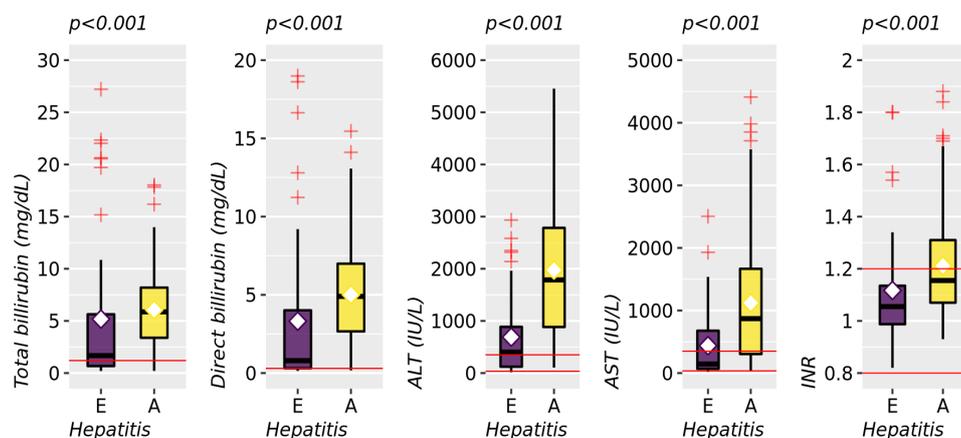


Figure 2. Several parameters differed significantly between hepatitis A and E groups. Laboratory reference ranges have been marked by horizontal red lines. Means are marked by diamonds. P-values from univariate Mann-Whitney tests.

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Table II. Preexisting conditions and severity factors for hepatitis E and A patients.

Hepatitis (Group)	E n (%)	A n (%)	OR (univariate) p, [95% CI]	Adjusted OR (univariate) ¹ p, [95% CI]	OR (multivariate) ² p, [95% CI]
Chronic liver disease	13 (27.1)	6 (3.9)	9.04, p<.001 [3.21, 25.45]	7.19, p<.001 [2.53, 22.73]	6.21, p=.002 [2.0, 20.5]
Liver cirrhosis	6 (12.5)	1 (0.7)	21.57, p<.001 [2.53, 184.16]	12.5, p=.026 [1.86, 250]	
Hepatitis B coinfection	6 (12.5)	4 (2.6)	5.29, p=.014 [1.43, 19.60]	6.71, p=.007 [1.7, 29.41]	
Neurologic disease	6 (12.5)	2 (1.3)	10.71, p=.003 [2.09, 55.04]	9.52, p=.011 [1.89, 71.43]	4.76, p=.098 [0.8, 38.3]
Chronic kidney disease	5 (10.4)	2 (1.3)	8.72, p=.009 [1.63, 46.54]	5.18, p=.065 [0.99, 38.46]	6.175, p=.056 [0.99, 50.0]
Diabetes mellitus	10 (20.8)	9 (5.9)	4.18, p=.004 [1.59, 11.02]	2.04, p=.190 [0.69, 5.99]	3.39, p=.029 [1.1, 10.1]
Additional treatment for severe disease *	9 (18.8)	5 (3.3)	6.78, p=.001 [2.15, 21.40]	4.93, p=.010 [1.49, 17.86]	4.47, p=.025 [1.2, 17.25]

¹: odds-ratio adjusted for age and gender; ²: odds-ratio in multiple logistic regression with all listed covariates (liver cirrhosis and hepatitis B coinfection included under chronic liver disease); * Additional treatment: hepatitis E – ribavirin, hepatitis A – plasma products. 95% CI: 95% confidence interval.

Table III. Hepatitis E patients who received ribavirin treatment. Summary of laboratory values (at admission / at discharge or transfer) and preexistent conditions.

Age	Sex	Direct bilirubin (mg/dL)	AST (IU/L)	ALT (IU/L)	INR	Recommendation for ribavirin treatment according to EASL 2018 guideline and hospital protocol	Duration of ribavirin treatment (days) In hospital / total duration	Evolution	MELD score (3-months risk of death)
51	F	28.51 / 21.51	36 / 59	15 / 24	1.17 / 1.02	Breast cancer, liver, lung and bone metastases	10 / presumably continued	Transfer to gastroenterology dept.	
21	M	0.25 / 0.19	19 / 20	75 / 35	0.92 / 0.99	Sagittal sinus thrombosis, bilateral facial palsy, one episode of seizures	12 / 21	Transfer to neurology dept.	
36	M	25.02 / 19.17	191 / 149	322 / 214	1.27 / 1.41	Chronic hepatitis B with advanced fibrosis	21 / presumably continued	Transfer to gastroenterology dept.	23 (19.6%)
38	M	1.67 / 1.98	1010 / 147	1750 / 607	1.12 / 1.02	Hodgkin lymphoma with chemotherapy, bone marrow transplantation	12 / up to 3 months	Discharged at home	
63	M	16.64 / 3.9	270 / 53	865 / 80	1.34 / 1.36	Coagulation deficiency factors VIII & IX, autoimmune hepatitis	7 / 7	Discharged at home	
64	M	0.27 / 0.32	321 / 74	1014 / 332	0.99 / 0.96	Retroperitoneal liposarcoma	14 / 21	Discharged at home	
69	M	1.31 / 0.81	570 / 85	436 / 141	1.12 / 1.01	Newly diagnosed colon cancer, diabetes mellitus	2 / 15	Discharged at home	
74	M	0.67 / 1.16	460 / 37	1013 / 219	1.06 / 0.96	Ethanol liver cirrhosis, Alzheimer and vascular and dementia, diabetes mellitus	21 / 21	Discharged at home	7 (1.9%)
75	M	24.1 / 7.11	645 / 44	374 / 23	1.57 / 1.38	Newly diagnosed ethanol liver cirrhosis and hepatocarcinoma	19 / stopped due to thrombocytopenia	Transfer to gastroenterology dept.	24 (19.6%)

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; EASL: European Association for the Study of the Liver; INR: International Normalized Ratio; IU: International units; MELD: Model for End-Stage Liver Disease.

Table IV. Summary of laboratory values (at admission / last before death) and preexisting conditions in patients who died with acute hepatitis E.

Age	Sex	Direct bilirubin (mg/dL)	AST (IU/L)	ALT (IU/L)	INR	Preexisting conditions interpreted as causes of death	MELD score (3-months risk of death)
59	M	18.99 / 28.3	618 / 73	262 / 16.6	1.8 / 4.15	Hemorrhagic shock from esophageal varices, alcoholic liver cirrhosis (Child-Pugh C)	35 (52.6%)
61	M	12.8 / 23.71	1537 / 253	526 / 39.9	1.8 / 1.81	Hemorrhagic shock from esophageal varices, alcoholic liver cirrhosis (Child-Pugh C)	27 (19.6%)
65	M	4.92 / 14.51	157 / 23	50 / 56.4	1.54 / 1.37	Multiple system organ failure, alcoholic and hepatitis B viral cirrhosis (Child-Pugh B), endocarditis, cerebral embolism	31 (52.6%)

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; INR: International Normalized Ratio; IU: International units; MELD: Model for End-Stage Liver Disease.

The three deceased patients (6.25% of all hepatitis E patients and 23.1% of hepatitis E patients with preexisting liver disease) had hepatitis E infection superimposed on end-stage alcoholic liver disease and none of them received ribavirin because of severe thrombocytopenia. Two of them died because of bleeding from esophageal varices. The third patient, with chronic hepatitis B infection and *Streptococcus tholarensis* endocarditis died because of cerebral hemorrhage, septic cerebral embolism and multiple system organ failure (Table IV).

Elevated MELD scores were found in severe cases of hepatitis E with acute-on-chronic liver failure, including the three deceased patients (Tables III-IV).

Discussion

Our study showed not only an increased number of hepatitis E cases in recent years but also a high number of severe and/or lethal cases as an emerging source of morbidity and a new healthcare challenge [37]. This increase cannot be explained by better detection as the same protocol was used since before this study started (2016). Indeed, other European countries have experienced a similar trend with no definitive explanation. In several countries, hepatitis E turned into the most frequent cause of acute viral hepatitis [3,38] though, in our hospital, a great number of hepatitis A cases were admitted in 2018, mainly in children and young adults.

The diagnosis and/or follow-up of hepatitis E include serological and PCR-based assays [5,39]. In our patients, diagnosis was based on clinical criteria (suspected acute viral hepatitis or elevated liver enzymes) and serological testing, ready within 1-2 days. All new acute cases were tested for viral hepatitis A-E on the same laboratory request according to the hospital protocol. Follow-up or genotyping was not performed in any patient.

We found a higher proportion of hospitalized men compared to women, similar to another study [13], yet HEV seroprevalence research in Europe reported no

gender difference [40,41]. No conclusive explanation has been provided but behavioral factors, food preferences and comorbidities (such as alcohol use disorder and chronic liver disease, more prevalent in men [42,43]) may have contributed to it.

In our study, hepatitis E affected only adults, with a median age of 52, similar to acute hepatitis B and C [42,44,45], while hepatitis A is found mainly in children and young adults [42,44]. This imbalance may be attributed to both preexisting liver conditions as well as alimentary exposure.

Since low infecting doses tend to cause asymptomatic infection [5,46], clinical manifestations may be associated with preexisting conditions, larger meals or highly contaminated food items.

Acute hepatitis E may develop as acute-on-chronic liver disease with high fatality rates [5,19]. Diabetes mellitus may slow-down liver regeneration and may cause immunosuppression [47]. We found more chronic conditions in hepatitis E patients compared to hepatitis A which may explain the higher prevalence of severe clinical course and a higher fatality of hepatitis E irrespective of age and gender.

Generally, hepatitis E is a mild disease needing only supportive treatment. Severe, immunocompromised patients and patients with acute-on-chronic liver disease are candidates for etiologic treatment with ribavirin and PEGylated interferon-alpha, with expected favorable results [5,48].

In other European studies performed on patients with decompensated chronic liver disease, hepatitis E was found in a small proportion of cases (3.2%) with similar mortality to patients without hepatitis E (27% vs. 26%), most likely due to decompensated liver disease [27,49].

Although chronic liver disease (cirrhosis, hepatitis B and C, alcohol use and other causes) has a high prevalence (1100 / 100000, age-adjusted) in Romania [50], hepatitis E probably occurs in a small number of patients compared to

other European countries [49].

Among the 9 patients who received ribavirin, significant improvement was found in all cases; patients were either discharged at home or transferred to other departments for further care of their comorbidities. Acute-on-chronic liver failure was demonstrated in 3 of the treated cases, 5 other cases had immunologic deficiencies and another one presented with neurologic manifestations that triggered the search for hepatitis E infection [24–26].

In a small study, Pischke et al. showed that a short ribavirin treatment (3–6 weeks) demonstrated rapid clinical improvement and HEV RNA clearance in 9/11 solid organ transplant recipients [27].

The three deceased patients with acute-on-chronic end-stage liver disease with fulminant evolution and/or severe comorbidities did not receive etiologic treatment because of severe thrombocytopenia. Therefore, of the 6 cases with acute-on-chronic hepatitis, 3 cases who received ribavirin had a favorable outcome and the other 3 did not receive ribavirin and died, more likely due to decompensated liver disease than to hepatitis E as suggested by other studies in developed countries [49]. Other studies also reported high mortality rates in patients with chronic liver disease [19,26,27,51].

Only 5 hepatitis A cases (3.3%) developed severe disease with coagulation abnormalities and received plasma products, all with a favorable outcome. Overall, more hepatitis E cases required additional treatment compared to hepatitis A despite less modified laboratory parameters.

Our study had several limitations: acute and recent HEV infections might be difficult to discriminate solely by IgM antibodies, but in the presence of clinical manifestations, we might assume that all cases were acute and locally acquired, except for one case. Genotyping was not possible, we presumed that genotypes 3 and 4 are involved, as showed the studies performed in Romania and elsewhere in Europe [5,7]. No reliable data on our patients' alimentary habits were available, but the assumption is that pork products are responsible for most cases, in a similar manner to other European countries [13]. Follow-up was not ensured in all cases, which may have been valuable in measuring the rate of chronic HEV infection.

Conclusions

An increased number of hepatitis E cases were admitted to our hospital during the last two years. Hepatitis E patients have less modified laboratory parameters compared to hepatitis A. Preexisting conditions were more frequent in hepatitis E patients, which seems to lead to a higher number of severe and fatal cases. Short-term ribavirin treatment could be an effective therapeutic approach for selected patients with acute hepatitis E (older age, acute-on-chronic liver disease and / or immunosuppression) but further studies are needed.

References

1. Purdy MA, Harrison TJ, Jameel S, Meng XJ, Okamoto H, Van der Poel WHM, et al. ICTV Virus Taxonomy Profile: Hepeviridae. *J Gen Virol.* 2017;98:2645–2646.
2. Smith DB, Simmonds P; International Committee on Taxonomy of Viruses Hepeviridae Study Group, Jameel S, Emerson SU, Harrison TJ, et al. Consensus proposals for classification of the family Hepeviridae. *J Gen Virol.* 2014;95(Pt 10):2223–2232.
3. Adlhoc C, Avellon A, Baylis SA, Ciccaglione AR, Couturier E, de Sousa R, et al. Hepatitis E virus: Assessment of the epidemiological situation in humans in Europe, 2014/15. *J Clin Virol.* 2016;82:9–16.
4. Bouwknecht M, Frankena K, Rutjes SA, Wellenberg GJ, de Roda Husman AM, van der Poel WH, et al. Estimation of hepatitis E virus transmission among pigs due to contact-exposure. *Vet Res.* 2008;39:40.
5. European Association for the Study of the Liver. EASL Clinical Practice Guidelines on hepatitis E virus infection. *J Hepatol.* 2018;68:1256–1271.
6. Rutjes SA, Lodder WJ, Lodder-Verschoor F, van den Berg HH, Vennema H, Duizer E, et al. Sources of hepatitis E virus genotype 3 in the Netherlands. *Emerg Infect Dis.* 2009;15:381–387.
7. Mrzljak A, Dinjar-Kujundzic P, Jemersic L, Prpic J, Barbic L, Savic V, et al. Epidemiology of hepatitis E in South-East Europe in the “One Health” concept. *World J Gastroenterol.* 2019;25:3168–3182.
8. Porea D, Anita A, Demange A, Raileanu C, Oslobanu Ludu L, Anita D, et al. Molecular detection of hepatitis E virus in wild boar population in eastern Romania. *Transbound Emerg Dis.* 2018;65:527–533.
9. Porea D, Anita A, Paslaru A, Savuta G. Wild Boar Hepatitis E Seroprevalence in Hunting Funds from Buzău and Galați Counties. 2016;5.
10. Voiculescu M, Iliescu L, Ionescu C, Micu L, Ismail G, Zilisteanu D, et al. A cross-sectional epidemiological study of HBV, HCV, HDV and HEV prevalence in the SubCarpathian and South-Eastern regions of Romania. *J Gastrointest Liver Dis.* 2010;19:43–48.
11. Anita A, Anita D, Ludu L, Savuta G. Seroepidemiological Investigation of Human and Swine Hepatitis in Botoșani County. 2010;4.
12. Aniță A, Gorgan L, Aniță D, Oșlobanu L, Pavio N, Savuța G. Evidence of hepatitis E infection in swine and humans in the East Region of Romania. *Int J Infect Dis.* 2014;29:232–237.
13. Faber M, Askar M, Stark K. Case-control study on risk factors for acute hepatitis E in Germany, 2012 to 2014. *Euro Surveill.* 2018;23. doi:10.2807/1560-7917.ES.2018.23.19.17-00469
14. Renou C, Roque-Afonso AM, Pavio N. Foodborne transmission of hepatitis E virus from raw pork liver sausage, France. *Emerg Infect Dis.* 2014;20:1945–1947.
15. Boxall E, Herborn A, Kochethu G, Pratt G, Adams D, Ijaz S, et al. Transfusion-transmitted hepatitis E in a “nonhyperendemic” country. *Transfus Med.* 2006;16:79–83.
16. Matsubayashi K, Kang JH, Sakata H, Takahashi K, Shindo

- M, Kato M, et al. A case of transfusion-transmitted hepatitis E caused by blood from a donor infected with hepatitis E virus via zoonotic food-borne route. *Transfusion*. 2008;48:1368–1375.
17. Loyon L. Overview of Animal Manure Management for Beef, Pig, and Poultry Farms in France. *Front Sustain Food Syst*. 2018;2:36. doi: 10.3389/fsufs.2018.00036.
 18. Kamar N, Garrouste C, Haagsma EB, Garrigue V, Pischke S, Chauvet C, et al. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. *Gastroenterology*. 2011;140:1481–1489.
 19. Zhang S, Chen C, Peng J, Li X, Zhang D, Yan J, et al. Investigation of underlying comorbidities as risk factors for symptomatic human hepatitis E virus infection. *Aliment Pharmacol Ther*. 2017;45:701–713.
 20. Debing Y, Moradpour D, Neyts J, Gouttenoire J. Update on hepatitis E virology: Implications for clinical practice. *J Hepatol*. 2016;65:200–212.
 21. Pischke S, Hartl J, Pas SD, Lohse AW, Jacobs BC, Van der Eijk AA. Hepatitis E virus: Infection beyond the liver? *J Hepatol*. 2017;66:1082–1095.
 22. Horvatits T, Schulze Zur Wiesch J, Lütgehetmann M, Lohse AW, Pischke S. The Clinical Perspective on Hepatitis E. *Viruses*. 2019;11. doi: 10.3390/v11070617.
 23. Dalton HR, Stableforth W, Thurairajah P, Hazeldine S, Remnarace R, Usama W, et al. Autochthonous hepatitis E in Southwest England: natural history, complications and seasonal variation, and hepatitis E virus IgG seroprevalence in blood donors, the elderly and patients with chronic liver disease. *Eur J Gastroenterol Hepatol*. 2008;20:784–790.
 24. Goyal R, Kumar A, Panda SK, Paul SB, Acharya SK. Ribavirin therapy for hepatitis E virus-induced acute on chronic liver failure: a preliminary report. *Antivir Ther*. 2012;17:1091–1096.
 25. Péron JM, Dalton H, Izopet J, Kamar N. Acute autochthonous hepatitis E in western patients with underlying chronic liver disease: A role for ribavirin? *J Hepatol*. 2011;54:1323–1324; author reply 1324–5.
 26. Péron JM, Abravanel F, Guillaume M, Gérolami R, Nana J, Anty R, et al. Treatment of autochthonous acute hepatitis E with short-term ribavirin: a multicenter retrospective study. *Liver Int*. 2016;36:328–333.
 27. Pischke S, Hardtke S, Bode U, Birkner S, Chatzikyrkou C, Kauffmann W, et al. Ribavirin treatment of acute and chronic hepatitis E: a single-centre experience. *Liver Int*. 2013;33:722–726.
 28. Dalton HR, Kamar N, van Eijk JJJ, Mclean BN, Cintas P, Bendall RP, et al. Hepatitis E virus and neurological injury. *Nat Rev Neurol*. 2016;12:77–85.
 29. Jha AK, Nijhawan S, Nepalia S, Suchismita A. Association of Bell's Palsy with Hepatitis E Virus Infection: A Rare Entity. *J Clin Exp Hepatol*. 2012;2:88–90.
 30. Gérolami R, Moal V, Colson P. Chronic hepatitis E with cirrhosis in a kidney-transplant recipient. *N Engl J Med*. 2008;358:859–860.
 31. Legrand-Abravanel F, Kamar N, Sandres-Saune K, Garrouste C, Dubois M, Mansuy JM, et al. Characteristics of autochthonous hepatitis E virus infection in solid-organ transplant recipients in France. *J Infect Dis*. 2010;202:835–844.
 32. VIDAS® Hepatitis panel. Available from: <https://www.biomerieux-diagnostics.com/vidasr-hepatitis-panel-0>. BioMérieux Clin. Diagn. .
 33. European Association for the Study of the Liver. EASL Clinical Practical Guidelines on the management of acute (fulminant) liver failure. *J Hepatol*. 2017;66:1047–1081.
 34. Vilstrup H, Amodio P, Bajaj J, Cordoba J, Ferenci P, Mullen KD, et al. Hepatic encephalopathy in chronic liver disease: 2014 Practice Guideline by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver. *Hepatology*. 2014;60:715–735.
 35. Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, et al. A model to predict survival in patients with end-stage liver disease. *Hepatology*. 2001;33:464–470.
 36. The R language and environment for statistical computing. R Core Team, Foundation for Statistical Computing, Vienna, Austria; Available from: <https://www.r-project.org/>
 37. Hartard C, Gantzer C, Bronowicki JP, Schvoerer E. Emerging hepatitis E virus compared with hepatitis A virus: A new sanitary challenge. *Rev Med Virol*. 2019;29:e2078. doi: 10.1002/rmv.2078
 38. Doting MHE, Weel J, Niesters HGM, Riezebos-Brilman A, Brandenburg A. The added value of hepatitis E diagnostics in determining causes of hepatitis in routine diagnostic settings in the Netherlands. *Clin Microbiol Infect*. 2017;23:667–671.
 39. Vollmer T, Diekmann J, Eberhardt M, Knabbe C, Dreier J. Monitoring of Anti-Hepatitis E Virus Antibody Seroconversion in Asymptomatically Infected Blood Donors: Systematic Comparison of Nine Commercial Anti-HEV IgM and IgG Assays. *Viruses*. 2016;8:232. doi: 10.3390/v8080232.
 40. Faber MS, Wenzel JJ, Jilg W, Thamm M, Höhle M, Stark K. Hepatitis E virus seroprevalence among adults, Germany. *Emerg Infect Dis*. 2012;18:1654–1657.
 41. Alberts CJ, Schim van der Loeff MF, Sadik S, Zuure FR, Beune EJAJ, Prins M, et al. Hepatitis E virus seroprevalence and determinants in various study populations in the Netherlands. *PLoS One*. 2018;13:e0208522.
 42. Analiza evoluției bolilor transmisibile aflate în supraveghere, Raport pentru anul 2016. [Analysis of the communicable diseases under surveillance. Report for 2016]. Institutul Național de Sănătate Publică, Centrul Național de Supraveghere și Control al Bolilor Transmisibile; 2017. Available from: <http://cnscbt.ro/index.php/rapoarteanuale/779-analiza-evolutiei-bolilor-transmisibile-aflate-in-supraveghere-raport-pentru-anul-2016/file>
 43. Flemming JA, Dewit Y, Mah JM, Saperia J, Groome PA, Booth CM. Incidence of cirrhosis in young birth cohorts in Canada from 1997 to 2016: a retrospective population-based study. *Lancet Gastroenterol Hepatol*. 2019;4:217–226.
 44. Drositis I, Bertias A, Lionis C, Kouroumalis E. Epidemiology and molecular analysis of hepatitis A, B and

- C in a semi-urban and rural area of Crete. *Eur J Intern Med.* 2013;24:839–845.
45. Hofstraat S, Falla A, Veldhuijzen I, Hahné S, Benthem BHB van, Tivoschi L, et al. Systematic review on hepatitis B and C prevalence in the EU/EEA; 2016. Available from: <https://doi.org/10.2900/24396>
 46. Hewitt PE, Ijaz S, Brailsford SR, Brett R, Dicks S, Haywood B, et al. Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. *Lancet.* 2014;384:1766–1773.
 47. Singh KK, Panda SK, Shalimar, Acharya SK. Patients with Diabetes Mellitus are Prone to Develop Severe Hepatitis and Liver Failure due to Hepatitis Virus Infection. *J Clin Exp Hepatol.* 2013;3:275–280.
 48. Kamar N, Pischke S. Acute and Persistent Hepatitis E Virus Genotype 3 and 4 Infection: Clinical Features, Pathogenesis, and Treatment. *Cold Spring Harb Perspect Med.* 2019;9. doi: 10.1101/cshperspect.a031872.
 49. Blasco-Perrin H, Madden RG, Stanley A, Crossan C, Hunter JG, Vine L, et al. Hepatitis E virus in patients with decompensated chronic liver disease: a prospective UK/French study. *Aliment Pharmacol Ther.* 2015;42:574–581.
 50. Pimpin L, Cortez-Pinto H, Negro F, Corbould E, Lazarus JV, Webber L, et al. Burden of liver disease in Europe: Epidemiology and analysis of risk factors to identify prevention policies. *J Hepatol.* 2018;69:718–735.
 51. Kamar N, Bendall R, Legrand-Abravanel F, Xia NS, Ijaz S, Izopet J, et al. Hepatitis E. *Lancet.* 2012;379:2477–2488.