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Investigation of underlying comorbidities as risk factors for symptomatic human hepatitis E virus infection

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SUMMARY

Background
Symptomatic Hepatitis E virus (HEV) infection occurs in few infected subjects, and the risk factors are not completely known.

Aims
To explore the risk factors for adverse clinical outcomes in acute HEV infections.

Methods
A large retrospective study was conducted. The baseline characteristics, clinical outcomes, and laboratory data of 512 acute HEV infection cases were analysed using logistic regression models.

Results
All patients exhibited autochthonous sporadic HEV infections, and most were elderly. Their symptoms varied from asymptomatic to severe liver diseases. In all, 215 patients (42.0%) had liver failure and/or decompensation, and 45 (8.2%) patients died within 3 months. Nearly 60% of patients had underlying chronic liver diseases (CLDs), 20% were cirrhotic, and various extrahepatic underlying comorbidities were common. The logistic regression analysis revealed that underlying CLDs, especially cirrhosis, were closely associated with disease severity (OR = 8.78, $P < 0.001$) but not with mortality in patients with severe liver diseases. In addition to the known factors, including an old age, the male gender and CLDs, we identified pre-existing extrahepatic tumours, diabetes, and chronic respiratory and renal diseases as novel independent predictors for adverse clinical outcomes. Importantly, patients without these four extrahepatic comorbidities showed a much lower mortality rate (4.2%, $P < 0.001$) than patients with one (18.5%) or more comorbidities (34.5%).

Conclusions
Previous comorbidities, including tumours, diabetes, and chronic liver, lung and kidney diseases, were independent risk factors for adverse outcomes, especially mortality, in acute HEV infections. This study provides valuable data for improving the prevention and control of HEV infection.

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INTRODUCTION

Hepatitis E virus (HEV) infections are not uncommon. Serological studies had revealed past infection rates ranging from 8% to 20% worldwide.[1, 2] HEV is usually transmitted via the faecal-oral route. With improvements in hygiene, the HEV outbreaks caused by genotypes 1 and 2 in developing countries, such as China, have diminished, while sporadic infections probably transmitted zoonotically by genotypes 3 and 4 have become more prevalent.[3] A few outbreak or large cohort studies revealed that most HEV-infected subjects were subclinical, with symptoms only occurring in a small proportion of all infections.[4, 5] However, HEV infections might cause severe liver diseases, for example, acute-on-chronic liver failure (ACLF), in cirrhotic patients.[6–8] Some patient-related factors, including an old age, the male gender, underlying CLDs and alcohol consumption, have been linked to severe diseases after HEV infection.[3, 9] However, previous studies were often limited by small sample sizes and incomplete datasets. Therefore, whether other factors affect symptomatic HEV infections remains largely unknown.

Recently, a prophylactic HEV vaccine was developed and approved by the Chinese FDA.[10] It showed great efficacy in the field by preventing new infections and severe diseases.[11] However, its wide use was hindered since such diseases only affect a small proportion of HEV-infected patients. Therefore, identifying the population at risk for symptomatic HEV infections is crucial and will be valuable for more effective and precise control of HEV infections in the future.

In this study, taking advantage of the unprecedented large number of patients with both mild and severe diseases caused by acute HEV infections, we explored the involvement of various host factors. For the first time, we identified underlying extrahepatic patient comorbidities as novel independent contributors to disease severity and mortality in HEV infections. We believe that these data will increase the awareness of this disease and guide the implementation of more effective measures to better control HEV infections in the future.

METHODS

Subjects

From September 2009 to September 2014, 635 acute HEV infection patients (anti-HEV immunoglobulin M positive, IgM+) were admitted to the Shanghai Public Health Clinical Centre, the regional tertiary hospital for infectious diseases in Shanghai, China. In all, 123 cases were excluded due to pregnancy or incomplete data; the remaining 512 cases were included in this retrospective study (Figure 1). The study was approved by the hospital's ethics committee.

Diagnosis of acute HEV infections

Suspected acute HEV-infected patients were initially screened by searching the hospital clinical database for two consecutive positive anti-HEV IgM test results (MP Biomedicals, Singapore). Nine cases were excluded by the initial screening due to the coexistence of other acute viral infection markers, including 2 HAV IgM+ individuals, 5 anti-HBc IgM+ individuals, 1 CMV IgM+ individual and 1 CMV/EBV IgM+ individual. The anti-HEV serological data of the 512 patients included in this study are summarised in Figure S1. All patients were seroconverted to serum HEV-IgG+, except 63 cases without enough follow-up data. In addition to serological data, the final diagnosis of acute hepatitis E was also based on the patients' clinical manifestations and the exclusion of other possible causes.

Definitions of patient clinical outcomes

In this study, liver failure and/or acute liver decompensation were regarded as severe disease forms. The definition of liver failure was a haemorrhagic tendency with an international normalised ratio (INR) ≥1.5, prothrombin activity (PTA) ≤40%, or rapidly increasing jaundice with either a total bilirubin (TBil) level 10 times greater than the upper normal limit or a daily increase ≥17.1 μmol/L. Liver decompensation was defined by the acute development of one or more major complications of the liver, such as ascites, hepatic encephalopathy, gastrointestinal haemorrhage or bacterial infection. Patient mortality was defined as death related to liver disease within 3 months of the disease onset.

Medical history and clinical data

Patient demographics, alcohol consumption, cigarette usage, family history of hepatitis, past medical history (previous comorbidities), and drug history were reviewed and recorded. The diagnosis of liver cirrhosis was confirmed by radiological or histological evidence. The drug history focused on prior exposures to potentially hepatotoxic medication before the onset of the current symptoms. The definition of CLDs was the presence of one or more of the following diseases: chronic hepatitis B, chronic hepatitis C, alcoholic liver disease, fatty liver disease, autoimmune liver disease, schistosomiasis, primary hepatic carcinoma and other

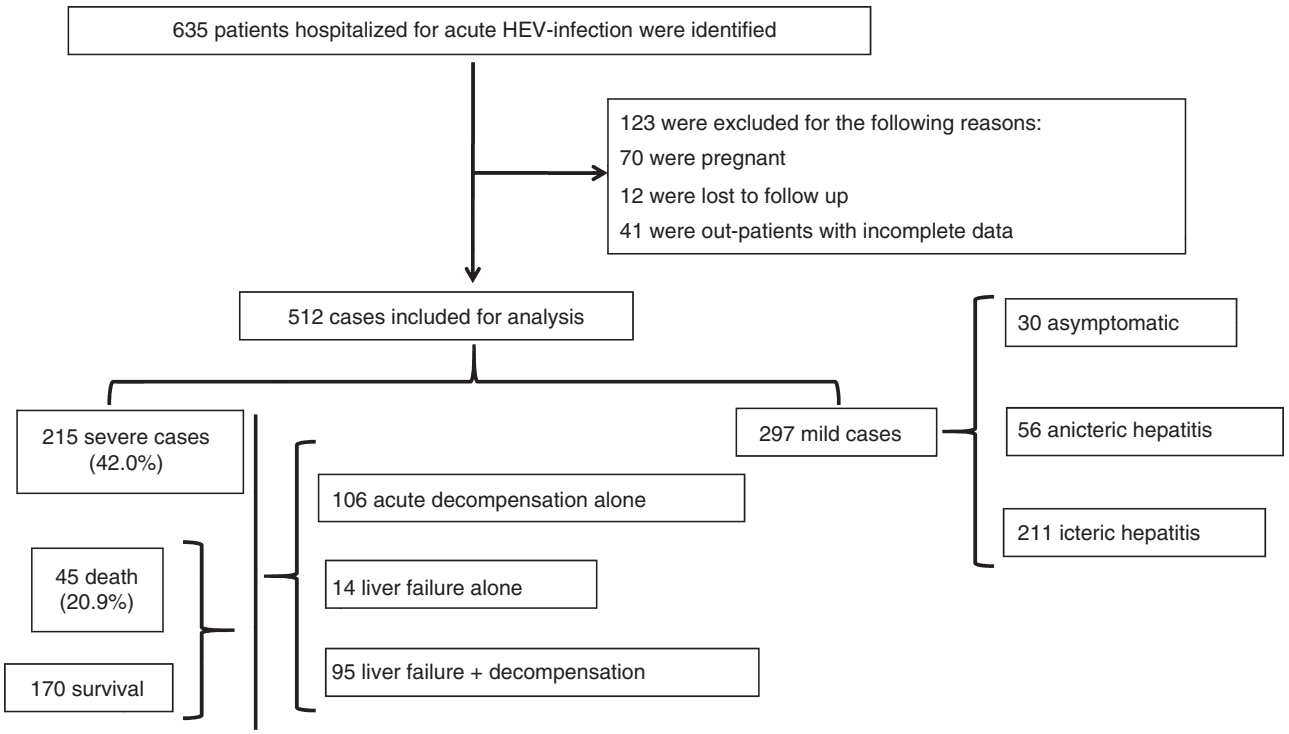

Figure 1 | Patient screening, enrolment and flow chart according to symptoms and disease severity. Among the 512 patients in the final analysis, 215 patients (42.0%) had severe liver diseases, manifested as acute liver decompensation and/or liver failure. In all, 45 (20.9%) of 215 patients with severe liver diseases died within 3 months of the disease onset. This study focused on identifying the risk factors for these adverse clinical outcomes.

liver diseases, such as hepatic cysts and hepatic haemangioma. The definition of other extrahepatic underlying diseases was the presence of one or more pre-existing comorbidities affecting various major organ systems, including chronic digestive disorders (e.g. chronic peptic ulcers, chronic gastritis, cholecystitis, gallstones), chronic respiratory diseases (e.g. chronic obstructive pulmonary disease, bronchial asthma, bronchiectasis, tuberculosis, phthisis), chronic renal diseases (e.g. kidney stones, renal cysts, chronic renal insufficiency, chronic glomerulonephritis), cardio-cerebrovascular diseases (e.g. apoplexy, cerebral thrombosis, stroke, encephalic angioma, infarction of the brain, coronary heart disease, obsolete myocardial infarction), autoimmune diseases (e.g. rheumatoid arthritis, systemic lupus erythaematosus), endocrine diseases (e.g. hyperthyroidism, hypothyroidism, Cushing's syndrome, hypophysoma), extrahepatic tumours, diabetes and hypertension. Available laboratory test data at different time points were recorded starting from the time of admission to the hospital. Laboratory data on day one and at the peak disease state are reported in this study.

Statistical analysis

Normal continuous variables are presented as the mean ± s.d., non-normal variables are presented as the median and range (Q1–Q3), and categorical variables are presented as the number (%). The Chi-square test and Fisher's exact test were used for comparing categorical variables. The Mann–Whitney *U* test or ANOVA were used for comparing non-normal or normal continuous variables. A multivariate logistic analysis was used for examining the risk factors for severe liver diseases and patient mortality. Analyses were performed using PASW Statistics 18 (Quarry Bay, HK). The statistical tests were two-sided, and significance was defined as $P < 0.05$.

RESULTS

Study subjects and their clinical outcomes (Figure 1)

Among the 512 included patients, 215 (42.0%) had severe liver diseases, including 106 (20.7%) with liver decompensation, 14 (2.7%) with liver failure and 95 (18.6%) with both liver decompensation and liver failure. In all, 45 patients (8.8%) with severe diseases died within 3 months. The remaining 297 (58%) patients had

relatively mild diseases, including 211 (41.2%) with icteric hepatitis, 56 (10.9%) with anicteric hepatitis and 30 (5.9%) with no symptoms.

Demographic and medical profiles of the HEV-infected subjects (Table 1)

Consistent with previous reports, the majority of this cohort of symptomatic HEV-infected patients was found to be elderly males. Their average age was 53.2 years; approximately 42% of the males were drinkers, and approximately 44% were smokers. The high rate of liver failure (21.3%) or decompensation (39.3%) among the acute HEV-infected patients indicated that their underlying conditions might pre-dispose them to more severe diseases. Indeed, further investigation revealed an unusually high proportion of underlying CLDs (approximately 60%) and various other extrahepatic comorbidities in both male and female patients (Table 1). The aetiologies of their previous CLDs included the following: having liver cirrhosis (20.9%), being an HBsAg carrier (37.9%), and having various other liver diseases, for example, previously having schistosomiasis (6.1%). More males used alcohol and cigarettes, while more females took potentially hepatoxic medicines ($P < 0.05$). A comparison of male and female patients revealed no significant differences in their pre-existing comorbidities, except for slightly more hypertension ($P < 0.05$) and cardio-cerebrovascular ($P < 0.01$) and autoimmune diseases in females ($P < 0.05$). Additional differences included the dominance of alcohol-related and autoimmune liver diseases in males (8.7%) and females (7.3%) respectively (both $P < 0.001$).

Clinical outcomes in HEV-infected patients with or without CLDs (Figure 2)

HEV superinfection has been reported to cause more severe CLDs, especially cirrhosis, and other problems, such as ACLF.[1, 6, 7] Thus, we next assessed the clinical

Table 1 | Basic demographic characteristics and underlying comorbidities in symptomatic HEV-infected patients

Characteristics	Male (n = 347)	Female (n = 165)	P value	Total (n = 512)
Age, years (Q1–Q3)	55 (42–64)	55 (40–63)	0.93	55 (41–64)
Alcohol user, *n* (%)	145 (41.8)	8 (4.8)	<0.001	153 (29.9)
Smoker, *n* (%)	154 (44.4)	6 (3.6)	<0.001	160 (31.3)
Seasons (Spring:Summer:Autumn:Winter)	103:80:68:96	51:35:31:48		154:115:99:144
Admission duration, days (Q1–Q3)	24 (14–36)	21 (15–31)	0.14	23 (14–34)
Family history of liver disease, *n* (%)	62 (17.9)	25 (15.2)	0.44	87 (17)
Potentially hepatoxic medication, *n* (%)	53 (15.3)	38 (23.0)	<0.05	91 (17.8)
Chronic liver diseases, *n* (%)	210 (60.5)	91 (55.2)	0.25	301 (58.8)
Liver cirrhosis	76 (21.9)	31 (18.8)	0.42	107 (20.9)
HBsAg carrier	139 (40.1)	55 (33.3)	0.14	194 (37.9)
Fatty liver disease	30 (8.7)	9 (5.5)	0.20	39 (7.6)
Alcoholic liver disease	30 (8.7)	0	<0.001	30 (5.9)
Autoimmune liver disease	3 (0.9)	12 (7.3)	<0.001	15 (2.9)
Past schistosomiasis	21 (6.1)	10 (6.1)	0.36	31 (6.1)
Chronic hepatitis C	7 (2.0)	4 (2.4)	0.75	11 (2.1)
Drug-induced liver disease	7 (2.0)	6 (3.6)	0.37	13 (2.5)
Hepatic carcinoma	13 (3.8)	2 (1.2)	0.16	15 (2.9)
Other miscellaneous liver diseases	14 (4.0)	6 (3.6)	0.83	20 (3.9)
Underlying comorbidities, *n* (%)				
Cardio-cerebrovascular diseases	15 (4.3)	18 (10.9)	<0.01	33 (6.4)
Hypertension	51 (14.7)	37 (22.4)	<0.05	88 (17.2)
Diabetes	35 (10.1)	20 (12.1)	0.49	55 (10.7)
Respiratory disease	28 (8.1)	12 (7.3)	0.75	40 (7.8)
Digestive disease	33 (9.5)	14 (8.5)	0.71	47 (9.2)
Renal disease	49 (14.1)	19 (11.5)	0.42	68 (13.3)
Autoimmune disease	5 (1.4)	7 (4.2)	<0.05	12 (2.3)
Endocrine disease	3 (0.9)	5 (3.0)	0.12	8 (1.6)
Extrahepatic tumours	13 (3.8)	9 (5.5)	0.37	22 (4.3)

Data are presented as the median (Q1–Q3) or the number of patients (%). P values are derived from comparisons between males and females using the chi-squared or Mann–Whitney U test. Other miscellaneous liver diseases included mainly hepatic cysts or haemangiomas.

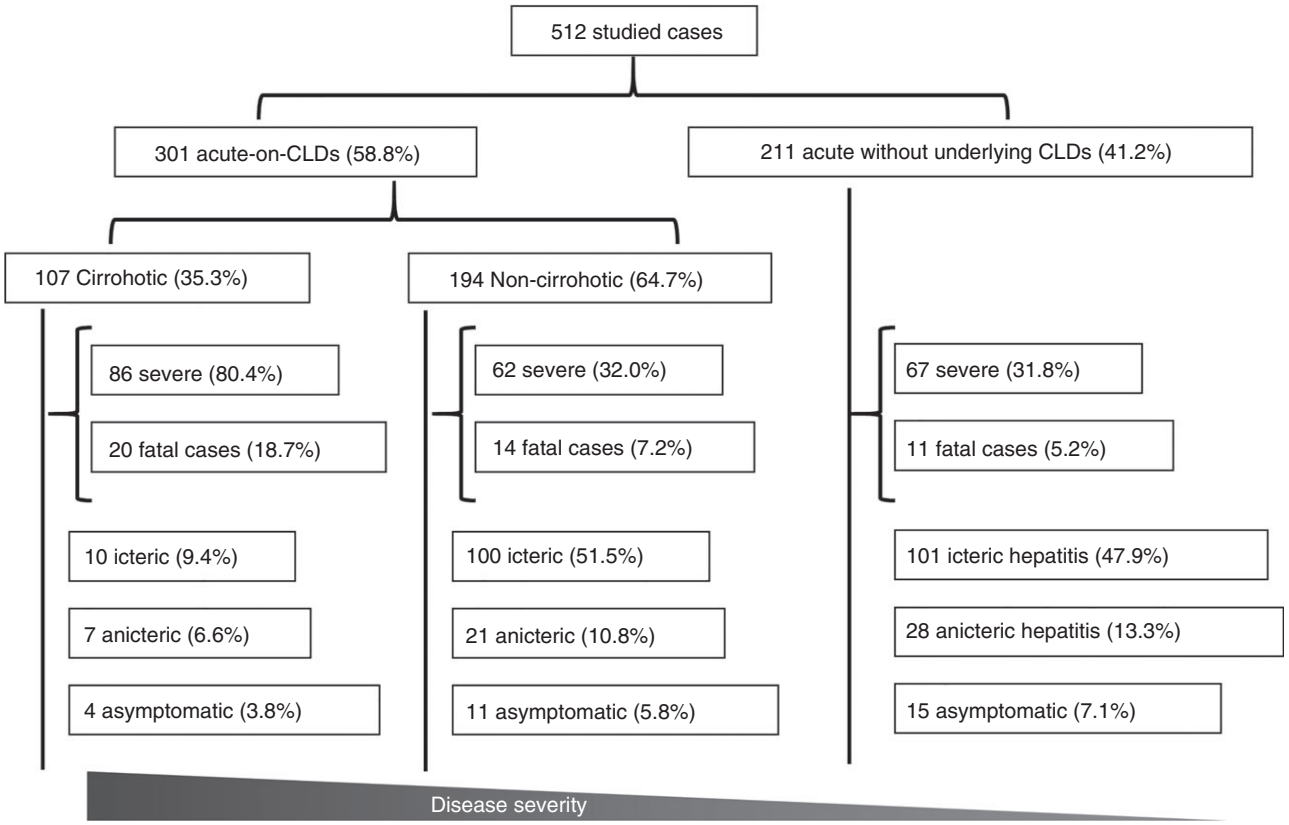

Figure 2 | Clinical features of 512 admitted acute HEV-infected patients. Severe cases included patients with liver decompensation and/or liver failure. Fatal cases were defined as death related to liver disease within 3 months of the disease onset, and they were part of the severe cases. Mild cases included patients with icteric, anicteric and asymptomatic forms of hepatitis. The number in each set of parentheses refers to its corresponding percentage in each patient subgroup. CLDs, chronic liver diseases.

outcomes in patients with or without underlying CLDs. Among the 107 previously cirrhotic patients, 86 (80.4%) manifested a severe form of liver disease (liver failure and/or liver decompensation), and their short-term mortality reached nearly 20%. In patients with noncirrhotic CLDs, 32.0% had severe liver diseases, and their mortality rate was 7.2%, while 51.5% of patients exhibited icteric hepatitis. Noticeably, these were also the youngest of all the patients, with an average age of 50.3 years (Table S1). For the non-CLDs group, the rates of severe diseases and mortality were 31.8% and 5.2% respectively. Patients in this group also exhibited a relatively higher proportion of mild diseases, including 47.9% with icteric hepatitis, 13.3% with anicteric hepatitis and 7.1% with no symptoms (Figure 2).

Risk factors for severe clinical symptoms and mortality in acute HEV-infected patients

Risk factors for symptomatic HEV infections other than an old age, the male gender and previous CLDs remain poorly understood. Through comparisons, we found that acute HEV-infected patients with severe liver diseases were comparable to those with mild liver diseases in terms of gender, alcohol consumption, smoking and use of potentially hepatoxic medications; however, those with severe liver diseases did show many more pre-existing CLDs than those with mild liver diseases – the proportions of CLDs ($P < 0.001$), cirrhosis ($P < 0.001$) and HBsAg positivity ($P < 0.05$) were all significantly higher (Table 2). Regarding underlying extrahepatic comorbidities, the two groups were comparable in their histories of hypertension and digestive, endocrine and autoimmune diseases. In contrast, the severe cases had significantly more underlying respiratory ($P < 0.01$) and renal diseases ($P < 0.001$) and extrahepatic tumours ($P < 0.05$), and they also showed a trend for more cardio-cerebrovascular diseases ($P = 0.06$) and diabetes ($P = 0.09$). Further multivariate analysis assessing independent predictors for disease severity confirmed that the underlying lung and

Table 2 | Previous comorbidities and disease severity in symptomatic HEV infections

Characteristics	Mild HEV (n = 297)	Severe HEV (n = 215)	P value
Male gender, *n* (%)	196 (66.0)	151 (70.2)	0.31
Age, years (Q1–Q3)	52 (39–61)	58 (47–67)	<0.001
Alcohol user, *n* (%)	80 (26.94)	73 (34.0)	0.09
Smoker, *n* (%)	91 (30.64)	69 (32.1)	0.73
Seasons (Spring:Summer:Autumn:Winter)	93:66:50:88	61:49:49:56	
Admission duration, days (Q1–Q3)	21 (14–29)	29 (15–44)	<0.001
Family history of liver disease, *n* (%)	43 (14.8)	44 (20.5)	0.08
Potentially hepatoxic medication, *n* (%)	50 (16.8)	41 (16.1)	0.77
Chronic liver diseases, *n* (%)	153 (51.5)	148 (68.8)	<0.001
Liver cirrhosis	21 (7.1)	86 (40)	<0.001
HBsAg carrier	102 (34.3)	92 (42.8)	<0.05
Fatty liver disease	22 (7.4)	17 (7.9)	0.83
Alcoholic liver disease	18 (6.1)	12 (5.6)	0.82
Autoimmune liver disease	4 (1.4)	11 (5.1)	<0.05
Past schistosomiasis	13 (4.4)	18 (8.4)	0.06
Chronic hepatitis C	8 (2.7)	3 (1.4)	0.38
Drug-induced liver disease	5 (1.7)	8 (3.7)	0.15
Hepatic carcinoma	5 (1.7)	10 (4.7)	<0.05
Other miscellaneous liver diseases	9 (3.0)	11 (5.1)	0.23
Underlying comorbidities, *n* (%)			
Cardio-cerebrovascular diseases	14 (4.7)	19 (8.8)	0.06
Hypertension	52 (17.5)	36 (16.7)	0.82
Diabetes	25 (8.4)	30 (14.0)	0.09
Respiratory disease	14 (4.7)	26 (12.1)	<0.01
Digestive disease	36 (12.1)	32 (14.9)	0.36
Renal disease	13 (4.4)	34 (15.8)	<0.001
Autoimmune disease	4 (1.4)	4 (1.9)	0.64
Endocrine disease	8 (2.7)	4 (1.9)	0.50
Extrahepatic tumours	8 (2.7)	14 (4.7)	<0.05

Data are presented as the median (Q1–Q3) or the number of patients (%). *P* values are derived from comparisons between mild and severe cases using the chi-squared or Mann–Whitney *U* test. The severe cases included patients with liver failure and/or decompensation. The remaining was regarded as mild cases. Other miscellaneous liver diseases included mainly hepatic cysts or haemangiomas.

kidney diseases were indeed independently associated with the development of severe liver diseases, in addition to the two know factors, an old age and underlying CLDs (Table S2).

In contrast, the risk factors for mortality in severe HEV infections differed from the above results. Surprisingly, our data indicated no significant associations between underlying CLDs ($P = 0.27$) and liver cirrhosis ($P = 0.49$) with patient mortality. In contrast, we discovered that older males ($P < 0.05$), HBsAg carriers ($P < 0.05$), and those with previous diabetes ($P < 0.05$) and kidney diseases ($P < 0.001$) were more frequent in the mortality group (Table 3). In addition, there was also a trend for more hepatoxic medication usage in the mortality group ($P = 0.06$). Further statistical analysis assessing independent risk factors for mortality confirmed that the male gender, an old age and pre-existing diabetes and renal diseases were independently associated with mortality in HEV-infected patients (Tables S3 and S4).

Since patients with or without previous CLDs may manifest different clinical symptoms, we further analysed the risk factors for disease severity and mortality in four patient subgroups according to their status of previous CLDs: subgroup 1 included patients with underlying cirrhosis; subgroup 2 included noncirrhotic patients; subgroup 3 included patients with underlying noncirrhotic CLDs; and subgroup 4 included patients without underlying CLDs. The data are shown in Tables S5–S8. Indeed, pre-existing diabetes, lung and kidney diseases and extrahepatic tumours were repetitively identified as risk factors for severe HEV infection or mortality in different subgroups, suggesting their involvement in symptomatic HEV infections.

Table 3 | Previous comorbidities and mortality in acute HEV-infected patients with severe liver diseases

Characteristics	Survival (n = 170)	Mortality (n = 45)	P value
Male gender, n (%)	112 (65.9)	39 (86.7)	<0.01
Age, years (Q1–Q3)	57.5 (44.8–66)	63 (53–72)	<0.05
Alcohol user, n (%)	56 (32.9)	17 (37.8)	0.78
Smoker, n (%)	51 (30.0)	18 (40.0)	0.70
Seasons (Spring:Summer:Autumn:Winter)	51:37:39:43	10:12:10:13	
Admission duration, days (Q1–Q3)	30 (17–44)	19 (6.5–41)	<0.05
Family history of liver disease, n (%)	34 (20.0)	10 (22.2)	0.74
Potentially hepatoxic medication, n (%)	28 (16.5)	13 (28.9)	0.06
Chronic liver diseases, n (%)	114 (67.1)	34 (75.6)	0.27
Liver cirrhosis	66 (38.8)	20 (44.4)	0.49
HBsAg carrier	66 (38.8)	26 (57.8)	<0.05
Underlying comorbidities, n (%)			
Cardio-cerebrovascular disease	15 (8.8)	4 (8.9)	0.99
Hypertension	25 (14.7)	11 (24.4)	0.12
Diabetes	19 (11.2)	11 (24.4)	<0.05
Respiratory disease	19 (11.2)	7 (15.6)	0.42
Digestive disease	24 (14.1)	8 (17.8)	0.54
Renal disease	16 (9.4)	18 (40.0)	<0.001
Autoimmune disease	4 (2.4)	0 (0)	0.17
Endocrine disease	3 (1.8)	1 (2.2)	0.66
Extrahepatic tumours	9 (5.3)	5 (11.1)	0.18

Data are presented as the median (Q1–Q3) or the number of patients (%). P values are derived from comparisons between cases of survival and mortality using the Chi-squared or Mann–Whitney U test. The severe liver diseases included liver failure and/or decompensation. Mortality was defined as death related to liver disease within 3 months of the disease onset. The remaining cases were regarded as survival.

Previous comorbidities led to increased clinical complications and mortality

To further understand the contributions of underlying diabetes, tumours and chronic kidney and lung conditions to the disease severity caused by acute HEV infections, we next divided patients into the following three distinct categories according to their comorbidity status: the no comorbidities (n = 380), one comorbidity (n = 103) and ≥2 comorbidities (n = 29) groups. As shown in Table 4, the three groups differed significantly in many laboratory markers, liver complications, disease severity and mortality. In general, along with increasing comorbidities, the bilirubin (both Tbil and Dbil) and systemic inflammation levels were elevated (indicated by higher neutrophil and lower lymphocyte counts), while liver function decreased (manifested as coagulopathy and lower serum protein levels). More importantly, the mortality rate of patients without these four types of comorbidities was only 4.2%; in comparison, the mortality rate was 18.5% and 34.5% in patients with one or more underlying comorbidities, respectively. Consistently, the occurrences of liver complications and other adverse outcomes, including ascites, bacterial infections, and hepatic encephalopathy, were all nearly two- to threefold higher in those with comorbidities than in those with no comorbidities (P values were all less than 0.001, Table 4).

Finally, to elucidate the roles of hepatic and extrahepatic risk factors, we incorporated both the well-known and other known factors, including age and gender, in the multivariate logistic regression models and compared their effects on disease severity and mortality in all HEV-infected patients and patient subgroups (Table 5). Including all the patients, our analysis revealed that liver cirrhosis was indeed the most significant factor for severe liver diseases (OR = 8.78, P < 0.001), followed by other extrahepatic underlying comorbidities (OR = 2.65 and 4.15 for one or more comorbidities, respectively), while noncirrhotic CLDs were not significant contributors to either disease severity or mortality. However, for mortality, the extrahepatic comorbidities were the leading factors rather than the cirrhosis, as indicated by their unusually high ORs and small P values (ORs for mortality = 14.04 and 10.37 for ≥2 comorbidities in the mild and severe cases, respectively, P < 0.001, as shown in Table 5). Moreover, the associations between underlying extrahepatic comorbidities and disease severity or

Table 4 | Clinical features and laboratory data in symptomatic HEV-infected patients

Characteristics	No comorbidities (n = 380)	One comorbidity (n = 103)	≥2 comorbidities (n = 29)	P value
Male gender, n (%)	256 (67.4)	74 (71.8)	17 (58.6)	0.38
Age, years	51 ± 15.4	58.7 ± 15.5	63.6 ± 13.2	<0.001
Admission duration, days	23 (15–33)	26.7 ± 16.6	27.8 ± 23.4	0.98
Clinical features, n (%)				
Jaundice	293 (77.1)	89 (86.4)	24 (82.8)	0.11
Ascites	62 (16.3)	41 (39.8)	13 (44.8)	<0.001
Infection	101 (26.6)	49 (47.6)	17 (58.6)	<0.001
HE	40 (10.5)	28 (27.2)	9 (31.0)	<0.001
Gastrointestinal haemorrhage	6 (1.6)	4 (3.9)	0	0.24
Outcomes of mild disease, n (%)				
Asymptomatic	26 (6.8)	2 (1.9)	2 (6.9)	0.17
Anicteric	50 (13.2)	5 (4.9)	1 (3.5)	<0.05
Icteric	171 (45.0)	33 (32.0)	7 (24.1)	<0.01
Outcomes of severe disease, n (%)				
Liver failure	10 (2.6)	4 (3.9)	0	0.51
Liver decompensation	66 (17.4)	31 (30.1)	9 (31.0)	<0.01
Liver failure and liver decompensation	57 (15.0)	28 (27.2)	10 (34.5)	<0.01
Death	16 (4.2)	19 (18.5)	10 (34.5)	<0.001
Laboratory parameters on day one				
ALT, IU/L	411 (91.25–1032)	355 (94–901)	199 (68.5–1004.5)	0.66
AST, IU/L	167 (62.3–563.3)	184 (83–622)	196 (66.5–367)	0.75
TBil, μmol/L	78.45 (18.9–209)	185.3 (47.1–392.1)	194 (26.35–367.5)	<0.001
ALB, g/dL	37.6 ± 6.2	35.4 ± 5.6	34.6 ± 5.6	<0.001
INR	1.1 (1–1.3)	1.2 (1–1.5)	1.1 (0.9–2)	<0.05
PTA	90 (68–107)	76.9 ± 28.6	86 (40.5–112)	<0.05
AFP, ng/mL	7.8 (3–48.3)	6.8 (2.8–27)	16.1 (4.6–31.3)	0.71
Leucocyte count, 10^9/L	5.4 (4.2–6.9)	6.1 (4.2–8.3)	6 (4.8–7.3)	0.08
Platelet count, 10^9/L	146 (103–194)	130 (88.25–190.5)	144 (104.5–204.5)	<0.001
Neutrophil count, 10^9/L	3.1 (2.2–4.4)	3.8 (2.4–5.9)	4 (2.7–5.5)	<0.01
Laboratory parameters at the peak				
ALT, IU/L	328.5 (89–929.8)	362 (82–832)	140 (53.5–555)	<0.05
AST, IU/L	143 (60.3–483.3)	163 (76–575)	132 (61–249.5)	0.46
TBil, μmol/L	84.9 (19.4–240.2)	189 (50.2–402.2)	194 (30.4–440.3)	<0.001
ALB, g/dL	37.2 ± 6.2	35.2 ± 6.0	35.3 ± 6.5	<0.01
INR	1.1 (1.0–1.3)	1.2 (1–1.6)	1.1 (0.9–2.3)	<0.05
PTA	91 (66–107)	80 (52–100)	86 (35.5–116)	<0.05
AFP, ng/mL	9.4 (3.1–53.3)	7.47 (3.3–26.9)	16.1 (4.9–24.8))	0.78
Leucocyte count, 10^9/L	5.4 (4.3–6.9)	6.2 (4.5–8.3)	7.2 (4.9–9.4)	<0.01
Platelet count, 10^9/L	147 (100–193.5)	130 (80.75–189.25)	144 (84–204)	0.26
Neutrophil count, 10^9/L	3.2 (2.2–4.4)	3.8 (2.5–5.8)	4.5 (2.8–7.4)	<0.001

HE, hepatic encephalophagy; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBil, total bilirubin; ALB, albumin; INR, international normalised ratio; PTA, prothrombin activity; AFP, alpha fetoprotein.

Data are presented as the mean ± s.d., the median (Q1–Q3) or the number of patients (%). Patients were grouped as have no, one or ≥2 comorbidities according to their previous conditions of underlying diabetes, extrahepatic tumours, or chronic renal or respiratory diseases. Comparisons among these groups were performed using the appropriate statistical tests, including the chi-square test, ANOVA or the Mann–Whitney U test, depending on the type of dataset.

mortality were further corroborated by the subgroups analysis (Table 5), since the influence of underlying CLDs would be eliminated in these analyses. Noticeably, after adjusting for both age and sex, the extrahepatic comorbidities were still identified as independent predictors for disease severity, and especially mortality, in symptomatic HEV-infected patients, regardless of their underlying CLD status.

DISCUSSION

In this study, we examined data from 512 HEV infection cases; in comparison, the largest sample size of previous

Table 5 | Risk factors for disease severity and mortality in symptomatic HEV-infected patients

Predictors	Severe diseases		Mortality		Mortality in severe diseases	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Total patients (*n* = 512)						
Male gender	1.18 (0.77–1.81)	0.45	3.66 (1.43–9.37)	<0.01	3.67 (1.39–9.12)	<0.05
Age, years (continuous)	1.01 (1–1.02)	0.11	1.02 (1–1.05)	0.11	1.01 (0.99–1.04)	0.35
CLD levels						
No CLDs	Reference		Reference		Reference	
Noncirrhotic CLDs	1.01 (0.65–1.57)	0.97	1.44 (0.6–3.47)	0.42	1.65 (0.62–4.4)	0.32
Cirrhosis	8.78 (4.94–15.58)	<0.001	4.16 (1.78–9.74)	<0.01	1.94 (0.77–4.89)	0.16
Comorbidity levels						
No comorbidities	Reference		Reference		Reference	
One comorbidity	2.65 (1.61–4.36)	<0.001	3.97 (1.88–8.36)	<0.001	2.69 (1.23–5.9)	<0.05
≥2 comorbidities	4.15 (1.78–9.64)	<0.01	14.04 (5–39.44)	<0.001	10.37 (3.28–32.82)	<0.001
Cirrhotic patients (*n* = 107)						
Male gender	0.71 (0.23–2.21)	0.56	8.57 (1.05–70.05)	<0.05	11.11 (1.36–90.98)	<0.05
Age, years (continuous)	1.03 (0.99–1.07)	0.18	1.02 (0.98–1.06)	0.38	1.02 (0.98–1.06)	0.4
Comorbidity levels:						
No comorbidities	Reference		Reference		Reference	
One comorbidity	9.01 (1.11–73.23)	<0.05	2.49 (0.79–7.80)	0.09	1.82 (0.6–5.56)	0.29
≥2 comorbidities	NA	0.99	3.29 (0.22–49.49)	0.13	4.05 (0.38–42.72)	0.24
Patients without cirrhosis (*n* = 405)						
Male gender	1.25 (0.78–1.99)	0.35	2.53 (0.86–7.44)	0.09	2.26 (0.67–7.68)	0.19
Age, years (continuous)	1.01 (1–1.02)	0.16	1.018 (0.99–1.05)	0.24	1.01 (0.98–1.04)	0.54
Comorbidity levels						
No comorbidities	Reference		Reference		Reference	
One comorbidity	2.29 (1.33–3.92)	<0.01	5.86 (2.07–16.57)	<0.01	3.89 (1.31–12.12)	<0.05
≥2 comorbidities	3.89 (1.64–9.19)	<.01	19.02 (5.72–63.29)	<.001	12.82 (3.32–49.5)	<0.001
Patients with noncirrhotic CLDs (*n* = 194)						
Male gender	1.44 (0.711–2.90)	0.31	1.20 (0.33–4.41)	0.78	0.97 (0.17–5.50)	0.98
Age, years (continuous)	1.01 (0.99–1.04)	0.22	1 (0.96–1.04)	0.94	1.01 (0.96–1.06)	0.74
Comorbidity levels						
No comorbidities	Reference		Reference		Reference	
One comorbidity	3.05 (1.42–6.54)	<0.01	10.52 (2.85–44.46)	<0.01	5.63 (1.23–25.87)	<0.05
≥2 Comorbidities	2.7 (0.8–9.13)	0.11	23.33 (4.3–127.73)	<0.001	22.6 (2.74–186.17)	<0.01
Patients without underlying CLDs (*n* = 211)						
Male gender	1.16 (0.62–2.19)	0.64	10.87 (1.08–109.87)	<0.05	6.63 (0.70–63.29)	0.1
Age, years (continuous)	1.01 (1–1.03)	0.48	1.06 (1–1.12)	0.05	1.04 (0.98–1.09)	0.17
Comorbidity levels:						
No comorbidities	Reference		Reference		Reference	
One comorbidity	1.82 (0.85–3.9)	0.13	2.27 (0.45–11.49)	0.32	1.86 (0.33–10.57)	0.46
≥2 comorbidities	5.69 (1.61–20.19)	<0.05	18.05 (2.91–112.05)	<0.01	6.54 (1.03–41.41)	<0.05

CLDs, chronic liver diseases; NA, not available.

According to the severity of underlying CLDs or comorbidities, the CLDs and comorbidities were both categorised into three levels, with no CLDs or comorbidities as the reference category, respectively. No, one and ≥2 comorbidities were determined according to previous patient conditions, including underlying diabetes, extrahepatic tumours, or chronic renal or respiratory diseases. Disease severity included liver failure and/or decompensation. Mortality was defined as death related to liver disease within 3 months of the disease onset. Multivariate logistic regression models were then used to assess the associations between various risk factors and different disease outcomes, as indicated.

studies was less than 300.[9, 12–18] This particularly large dataset enabled us to comprehensively investigate the risk factors for symptomatic HEV infections, disease severity and mortality, and for the first time, we are able to answer several important questions.

First, what is the likely disease status of HEV infection in China? Recently, an epidemiological study of a large community cohort in China indicated a high prevalence of past HEV infection (20–40% anti-HEV IgG positivity) and nearly a 1% annual new infection rate in the overall

population.[5, 19, 20] In most cases, the infection was subclinical, with only less than 1% of new infections considered symptomatic, suggesting a benign nature of acute HEV infections.[3–5, 20–22] However, previous studies on symptomatic infections in Chinese tertiary hospital settings often revealed completely opposite results.[12–18] In this study, we found that the HEV-induced symptoms varied greatly from anicteric hepatitis to severe illnesses, including liver failure and decompensation, with the proportions of severe diseases and mortality reaching nearly 40% and 10%, respectively. These figures were quite similar to those of several previous reports from China, suggesting that these values likely reflect the reality in large Chinese hospitals.[12–18] For this reason, we can certainly conclude that HEV is indeed one of the most important contributors to both severe diseases and mortality caused by acute hepatotropic viral infections in China.

Second, how many symptomatic HEV infections are acute-on-chronic or on-cirrhotic, and do they differ in disease severity? Previous studies revealed that CLDs, especially liver cirrhosis, could pre-dispose HEV-superinfected individuals to a greater risk for developing severe diseases.[1, 6, 7, 23, 24] In agreement with these studies, we found that nearly 60% of our HEV-infected subjects had underlying liver diseases, including nearly 40% HBsAg-positive individuals and 20% cirrhotic individuals, and various other CLDs, for example, alcoholic liver disease, autoimmune liver disease and nonalcoholic steatohepatitis. These results show a much higher prevalence of CLDs in symptomatic HEV-infected patients than in the general population.[25] We also noticed that as much as 80% of cirrhotic individuals superinfected by HEV developed severe liver diseases, confirming cirrhosis as the leading contributor to disease severity. Noticeably, of the symptomatic HEV-infected patients in our study, 38% had noncirrhotic CLDs, and more than 40% were without pre-existing CLDs. These two subgroups were relatively uncharacterised in previous studies; interestingly, we noticed that their disease severity and mortality were quite similar, at approximately 30% and 5–7%, respectively. These data indicated two novel facts. First, severe liver diseases did occur in quite a few HEV-infected patients with no prior liver cirrhosis. Second, underlying noncirrhotic CLDs may not significantly impact the disease outcomes. Therefore, the presence of risk factors other than underlying CLDs for HEV-induced symptoms was strongly suggested.

Third, what are the unknown risk factors for symptomatic HEV infections and disease severity? Previous studies showed that an old age, the male gender, alcohol use and CLDs are risk factors.[3, 9] Underlying comorbidities have also been proposed to contribute to the development of severe diseases[23]; however, the small sample sizes in past studies and the complicated underlying comorbidities in the elderly made this question difficult to answer. Therefore, it remains unclear whether, which and how underlying diseases pre-dispose patients to severe HEV infections. Here, the large dataset of 512 symptomatic HEV-infected patients enabled us to explore the potential roles of various common comorbidities in HEV-induced diseases. Indeed, in addition to CLDs, convincing evidence emerged that diabetes, extrahepatic tumours, and chronic lung and kidney diseases contributed to both disease severity and mortality, while some other common comorbidities, such as hypertension, digestive disorders and endocrine-immune diseases, did not. In hindsight, we think that the data fit very well with the pathophysiology of these diseases. The functions of the kidneys and lungs are known to potentially suffer from liver failure.[26–28] Accordingly, underlying lung and kidney diseases might pre-dispose HEV-infected patients to subsequent lung or kidney failure in the context of severe liver injury, which may lead to multiorgan failure and certainly increase the risk of death. For cancer patients, their dampened immunity and subhealthy status may pre-dispose them to more severe HEV infections.[29] In addition, in agreement with a previous report on its role in severe viral hepatitis,[30] we found that diabetes was another independent risk factor. One possible explanation may be the impaired capacity of liver regeneration in the presence of insulin resistance, or the impaired anti-viral immunity of the patients.[31]

Our data also supported a two-hit model for mortality development during HEV infection with differential involvements of underlying comorbidities: the model starts with hit I, 'severe liver injury', and then proceeds hit II, 'multiorgan failure and death' (Figure S2). This hypothesis was derived from our findings of underlying comorbidities as risk factors for disease severity and mortality. We found that liver cirrhosis was associated with disease severity but not mortality, while four other extrahepatic comorbidities, such as chronic lung and kidney diseases were associated with mortality. Therefore, we propose that the underlying liver diseases only affect the first hit by causing severe liver injury, leading to liver failure and decompensation; this could explain why HEV superinfection caused much more severe liver complications in cirrhotic patients. However, after the first hit, extrahepatic comorbidities execute the second hit and cause fatal diseases. In this second phase, underlying cirrhosis or other CLDs may not play significant roles.

Finally, our work has important implications for the effective control of HEV-induced diseases through future prophylactic vaccinations. Although an HEV vaccine is currently licensed in China and the phase III clinical trial demonstrated the vaccine to be 100% effective against the disease,[10, 32] universal vaccination will not be cost-effective because symptomatic cases are rare, occurring in less than one of 10 000 cases per year.[4, 5, 11, 20] Therefore, information regarding the risk factors of symptomatic infections will be critical for targeting the disease more precisely. Here, several previously unknown risk factors were identified, including diabetes, tumours, and chronic lung and kidney diseases. In addition, due to the large sample size, we were able to estimate and compare the effects of each risk factor. On the basis of these data, we propose a hierarchal view of the different risks for disease severity and mortality in symptomatic HEV infections (Figure S3). Indeed, in agreement with previous reports, cirrhotic individuals carry the highest risk and represent the most vulnerable population to severe HEV infections.[6, 7] Meanwhile, the noncirrhotic elderly population, the risk level could vary from low to high depending on how many underlying comorbidities are present. The data also prompt us to foresee a steady increase in HEV-induced diseases in China in the future if no public measures are taken. The reasons for this prediction include the following: first, the Chinese population is ageing; second, more than 10% of elderly Chinese individuals are chronically infected with HBV[33, 34]; third, the prevalence of underlying comorbidities is increasing in China.[25, 35–38] In this context, we think that the more precise and effective anti-HEV vaccination of at-risk populations is urgent and necessary to reduce the negative impact of HEV infections on public health in the future.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Basic characteristics and underlying extra-hepatic comorbidities in-patients' subgroups with or without CLDs.

Table S2. Independent predictors for development of liver failure and/or liver decompensation during acute HEV infection.

Table S3. Independent predictors for mortality in acute HEV-infected patients with severe liver diseases.

Table S4. Previous comorbidities and mortality in acute HEV-infected patients.

Table S5. Patients with underlying cirrhosis.

Table S5.1. Previous comorbidities and the disease severity in acute HEV-infected patients with underlying cirrhosis.

Table S5.2. Previous comorbidities and mortality in severe acute HEV-infected patients with underlying cirrhosis.

Table S5.3. Previous comorbidities and mortality in acute HEV-infected patients with underlying cirrhosis.

Table S5.4. Clinical features and disease outcomes in acute HEV-infected patients with underlying cirrhosis.

Table S6. Noncirrhotic patients.

Table S6.1. Previous comorbidities and the disease severity in noncirrhotic acute HEV-infected patients.

Table S6.2. Previous comorbidities and mortality in noncirrhotic patients with severe liver diseases caused by acute HEV infections.

Table S6.3. Previous comorbidities and mortality in noncirrhotic patients with acute HEV infections.

Table S6.4. Clinical features and disease outcomes in noncirrhotic patients with acute HEV infections.

Table S7. Patients with underlying noncirrhotic CLDs.

Table S7.1. Previous comorbidities and the disease severity in acute HEV-infected patients with underlying noncirrhotic CLDs.

Table S7.2. Previous comorbidities and mortality in severe acute HEV-infected patients with noncirrhotic CLDs.

Table S7.3. Previous comorbidities and mortality in acute HEV-infected patients with noncirrhotic CLDs.

Table S7.4. Clinical features and disease outcomes in acute HEV-infected patients with noncirrhotic CLDs.

Table S8. Patients without underlying CLDS.**Table S8.1.** Previous comorbidities and the disease severity in acute HEV-infected patients without underlying CLDs.

Table S8.2. Previous comorbidities and mortality in severe acute HEV-infected patients without underlying CLDs.

Table S8.3. Previous comorbidities and mortality in acute HEV-infected patients without underlying CLDs.

Table S8.4. Clinical features and disease outcomes in acute HEV-infected patients without underlying CLDs.

Figure S1. The anti-HEV serological status of all the patients.

Figure S2. The two-hit model of mortality development during symptomatic HEV infection.

Figure S3. The hierarchal view of population subgroups carrying different risks for disease severity and mortality after HEV infections.

AUTHORSHIP

Guarantor of the article: Drs Shuye Zhang and Liang Chen, take responsibility for the integrity of the work as a whole, from inception to published article.
Author contributions: Shuye Zhang: study concept and design; data interpretation; manuscript drafting; study supervision; funding acquisition; Chong Chen: data acquisition, analysis and interpretation; manuscript drafting; Jinbiao Peng, Xinyan Li, Dandan Zhang, Jingjing Yan, Yunling Zhang, Chuan Lu, Jinna Xun, Weixia Li, Yun Ling, and Yuxian Huang: technical support and data acquisition; Liang Chen: study concept and design; critical manuscript revisions for important intellectual content; administrative, technical, or material support; study supervision; funding acquisition.
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