REVIEW

Systematic review of mixed cryoglobulinemia associated with hepatitis E virus infection: association or causation?

Fateh Bazerbachi¹,*, Michael D. Leise¹, Kymberly D. Watt¹, M. Hassan Murad², Larry J. Prokop³ and Samir Haffar⁴

¹Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA, ²Robert D. and Patricia E. Kern Center for the Science of Health Care Delivery, Mayo Clinic, Rochester, MN, USA, ³Library Public Services, Mayo Clinic, Rochester, MN, USA and ⁴Digestive Centre for Diagnosis and Treatment, Damascus, Syrian Arab Republic

*Corresponding author. Division of Gastroenterology and Hepatology, Mayo Clinic, 200 First Street S.W., Rochester, MN 55905, USA. Tel: +1 (507) 284–1825; Email: Bazerbachi.Fateh@mayo.edu

Abstract

Background and aim: Mixed cryoglobulinemia (MC) has been associated with several viral infections, and chronic hepatitis C is recognized as a major cause. MC associated with hepatitis E virus (HEV) has been described and little is known about this rare association. The aim of this study is to perform a systematic review of MC associated with HEV, and examine the presence of a causal relationship.

Methods: An experienced librarian conducted a search of databases from each database’s inception to 12 December 2016 based on a priori criteria. The risk of bias was assessed, and Hill’s criteria were applied to determine causality.

Results: Five publications met inclusion criteria, with a total of 15 cases. Three studies had low, one low to moderate and one moderate risk of bias. Median age was 43 years, and all patients came from Western Europe. Two patients were immunocompetent, while 13 were immunosuppressed, post solid organ transplant and had chronic hepatitis E. Renal involvement was observed in seven patients, mild to moderately severe cryoglobulinemic disease in one patient and severe cryoglobulinemic disease in three patients. One patient improved spontaneously, and another was treated with immunosuppressant reduction leading to viral clearance. Ten patients treated with peg-interferon or ribavirin for 3 months achieved loss of cryoglobulinemia and end-of-treatment response, but sustained virologic response was reported and achieved in two. Immunosuppressant achieved loss of cryoglobulinemia in three patients. One case of chronic renal failure, three cases of end-stage renal disease and one death were observed. Five of the nine Hill’s criteria were fulfilled.

Conclusion: MC has been described with HEV infection. A causal relationship between HEV infection and cryoglobulinemia is highly probable.

Key words: hepatitis E virus; hepatitis; virology; mixed cryoglobulinemia; systematic review

Submitted: 24 January 2017; Revised: 19 February 2017; Accepted: 15 March 2017
Mixed cryoglobulinemia and HEV infection

Introduction

Mixed cryoglobulinemia (MC) has been associated with several viral infections and at least nine viruses have been implicated [1]. Hepatitis C virus infection (HCV) is recognized as the major cause of MC reported in 90% of Italian patients in one series [2], although later studies found wide geographical variations [3]. Some cases of MC are related to human immunodeficiency virus (HIV) [4]. Hepatitis B virus infection (HBV) [5] and, less frequently, to hepatitis A virus infection (HAV) [6] as well as other viruses. Acute hepatitis E is reported mainly in immunocompetent patients, whereas chronic hepatitis E has been almost always limited to immunosuppression states such as malignancy, HIV infection and solid organ transplantation (SOT) [7,8]. MC associated with hepatitis E virus (HEV) infection in immunocompetent or immunosuppressed patients has been described and little is known about this rare association and whether causal inference could be made. To date, no systematic review addressing this association has been published. The aim of this study is to perform a systematic review of the association of hepatitis E in immunocompromised patients.

Methods

Literature search

A comprehensive search of several databases from each database’s inception to 12 December 2016, English, French and Spanish languages was conducted. The databases included Ovid MEDLINE In-Process & Other Non-Indexed Citations, Ovid MEDLINE, Ovid EMBASE, Ovid Cochrane Central Register of Controlled Trials, Ovid Cochrane Database of Systematic Reviews and Scopus. The search strategy was designed and conducted by an experienced librarian with input from the study’s principle investigator. Controlled vocabulary supplemented with keywords was used to search for cryoglobulinemia and hepatitis E. The search strategy is available in supplemental figure. In addition, we searched the first 100 entries of Google Scholar using the terms ‘hepatitis E’ and ‘cryoglobulinemia’ to look for articles not indexed in major databases. Reference lists were manually reviewed for additional cases.

Inclusion criteria based on the following definitions

Diagnosis of hepatitis E in immunocompetent patients was based on the detection of anti-HEV IgM with confirmation of acute case detected serologically by HEV RNA in blood or stool [9]. Diagnosis of hepatitis E in immunocompromised patients was based on detection of HEV RNA in blood or stool [10]. Diagnosis of chronic hepatitis E was defined by persistence of HEV replication for more than 6 months, or more than 3 months in the setting of SOT [11].

Diagnosis of cryoglobulinemia was defined by the presence of cryoglobulins in serum stored at 4 °C for several days in two fractions, and reversibility of the cryoprecipitation in one fraction replaced at 37 °C when a cryoprecipitate is formed [1,12]. The classification of cryoglobulinemia was established by immunofixation or immuno-electrophoresis, which confirms the presence of immunoglobulins, and enables classification into types I to III [12].

Diagnosis of cryoglobulinemic disease (CD) was established by the presence of circulating cryoglobulins and typical organ involvement, mainly skin, kidney or peripheral nervous system [1]. Severity of CD: in the absence of standardized disease severity of CD, experts classified the disease into mild to moderately severe, severe and life-threatening [1]. Mild to moderately severe CD is identified by the presence of purpura, arthritic manifestations, mild neuropathy or glomerulonephritis without renal failure. Severe CD is identified by the presence of cutaneous ulcers, ischemia, severe neuropathy, glomerulonephritis with renal failure and/or nephrotic syndrome or gastrointestinal involvement. Life-threatening CD is identified by the presence of rapidly progressive glomerulonephritis, central nervous system involvement, intestinal ischemia or alveolar hemorrhage.

Response to antiviral treatment was assessed by sustained virologic response (SVR) defined by absence of HEV RNA 24 weeks after the end of treatment [13].

Assessment of causal relation between MC and HEV infection was performed by applying the nine Hill’s criteria for causation on the documented cases [14].

We excluded duplicated studies and cases with the presence of concomitant acute or chronic liver disease.

Data extraction and assessment

Two reviewers (F.B., S.H.) assessed the quality of the studies and extracted the relevant data based on the inclusion/exclusion criteria.

Risk of bias assessment (methodological quality)

Given that there are no available validated tools to assess the risk of bias (i.e. methodological quality) of case reports and case-series, we derived items from the Newcastle-Ottawa Scale (NOS) that were appropriate for this systematic review. We removed from the NOS the items that related to comparability and adjustment (because the studies included were non-comparative). We retained for the purpose of bias assessment the items that focused on selection, representativeness of cases, and ascertainment of outcome and exposure. This resulted in five criteria in the form of questions with a binary response (yes/no), whether the item was suggestive of bias or not. These questions are listed in Table 1. We considered the quality of the report good (low risk of bias) when all five criteria were fulfilled, moderate when four were fulfilled and poor (high risk of bias) when three or fewer were fulfilled. This tool has been previously applied [15]. No disagreements were found between the reviewers.

Results

Study characteristics

The flow diagram of study selection is shown in Figure 1. We identified six publications between 2007 and 2016 that met the inclusion criteria [16–21]. One publication was excluded due to a potential co-presence of HCV and HEV [21]. Of the remaining five studies, four publications were full-text articles [16,17,20,22] and one was in the form of a letter to the editor [23]. There were four case reports [16,20,22,23] and one case-series [17]. Three studies were likely at low risk of bias and one at moderate risk. The case-series publication included 11 cases: 4 cases had low and 7 had moderate risk of bias (Table 1).

Patient characteristics

Demographics and disease features (Table 2)

Among the 15 cases, the median age was 43 years, the male-to-female ratio was 7 and all patients came from Western Europe. Two patients were immunocompetent without evidence of
chronic hepatitis E and 13 patients were immunosuppressed status post SOT, who also had chronic hepatitis E with persistent HEV replication (>6 months in 12 patients; >3 months in 1 patient) [19]. Genotype 3 testing failed in 1 patient, was not performed in 1 patient and was confirmed in 13 patients. All patients had type II or type III MC. Anti-HEV IgG, anti-HEV IgM and HEV RNA were detected in the cryoprecipitate in one patient.

MC occurred during active viral infection in 14 patients and following HEV clearance obtained by reduction of immunosuppression medications in 1 patient [18]. Renal involvement was observed in seven patients: membranoproliferative glomerulonephritis (MPGN) in three patients, relapsing IgA nephropathy in two patients, nephroangiosclerosis in one patient, whereas renal biopsy was not performed in one patient. Rash, arthralgia and thrombocytopenia were noted in one patient who had renal disease [18]. CD was observed in four patients, was mild to moderately severe in one patient with self-limited severe arthritis and rash, and severe with MPGN in three patients. Data regarding the presence or absence of CD were lacking for the remaining seven patients.

**Patient management (Table 3 and Figure 2)**

One patient with self-limited arthritis required no treatment. Fourteen patients required intervention: immunosuppressant dose reduction (1 patient), antiviral monotherapy (10 patients) and immunosuppressant (3 patients). Interestingly, immunosuppressant dose reduction before occurrence of MC was undertaken in two patients: it was associated with viral clearance in one patient followed by occurrence of MC [18] and was

---

**Table 1. Risk of bias assessment of the included studies**

| First author/year | No. of cases | Question 1 | Question 2 | Question 3 | Question 4 | Question 5 | Risk of bias |
|-------------------|--------------|------------|------------|------------|------------|------------|-------------|
| Serratrice 2007 [16] | 1 | Yes | Yes | Yes | Yes | Yes | low |
| Kamar 2012 [17] | 4 | Yes | Yes | Yes | Yes | Yes | low |
| Fischke 2014 [18] | 7 | Yes | Yes | Yes | No | Yes | moderate |
| Del Bello 2015 [19] | 1 | Yes | Yes | Yes | Yes | Yes | low |
| Guinault 2016 [20] | 1 | Yes | Yes | Yes | Yes | Yes | low |

Questions 1–5 comprise the tool for risk of bias assessment of case reports and case-series:
1. Did the patient(s) represent the whole case(s) of the medical center? (The studies did not mention whether the reported patient(s) represented the whole case(s) of the medical center and we assumed that the authors have reported all the cases in their center giving the rarity of this association.)
2. Was the diagnosis correctly made?
3. Were other important diagnoses excluded?
4. Were all important data cited in the report?
5. Was the outcome correctly ascertained?

**Table 2. Cases of mixed cryoglobulinemia associated with HEV infection**

| First author/year | No. | Country | Age/sex | IC/IS | HEV infection | Mixed cryoglobulinemia |
|-------------------|-----|---------|---------|------|----------------|------------------------|
| Serratrice 2007 [16] | 1 | France | 51/Female | IC | (+) | Arthritis—rash | Mild/mod |
| Kamar 2012 [17] | 7 | France | 26/Male | IS/SOT | (+) | (+) | Arthritis—rash | Mild/mod |
| | 1 | | 40/Male | IS/KT | (+) | (+) | Arthritis—rash | Mild/mod |
| | 1 | | 24/Male | IS/KT | (+) | (+) | Arthritis—rash | Mild/mod |
| Fischke 2014 [18] | 1 | Germany | 35/Male | IS/LT | (+) | Not done | Rash—arthralgia—RF; Thrombocytopenia |
| Del Bello 2015 [19] | 1 | France | 46/Male | IS/KT | (+) | (+) | RF | MPGN | Severe |
| Guinault 2016 [20] | 1 | France | 48/Male | IC | Not possible* | (+) | RF—NS | MPGN | Severe |
| Total: 5 studies | 15 | France: 14 | Median: 43 | All | Genotype 3: | (+) | (+) | IgAN: 2 | Severe: 3 |

aGenotyping not possible due to failure to amplify sufficient HEV RNA.
NR: not report; IC: immunocompetent; IS: immunosuppressed; SOT: solid organ transplantation; KT: kidney transplantation; LT: liver transplantation; CD: cryoglobulinemic disease; RF: renal failure; NS: nephrotic syndrome; IgAN: IgA nephropathy; MPGN: membranoproliferative glomerulonephritis; NAS: nephroangiosclerosis; Mild/mod: mild to moderately severe.
unsuccessful in a second patient who also developed later MC [19]. IS dose reduction with the intention to treat MC associated with chronic hepatitis E was undertaken in one patient, leading to viral clearance. Ten patients were treated with antiviral monotherapy for 3 months (most of them receiving ribavirin). Loss of cryoglobulinemia and end-of-treatment response, defined by the absence of HEV RNA at the end of the treatment, were obtained in all of them but SVR was reported and obtained in two cases and not reported for the remaining cases. Three patients were treated with immunosuppressants (rituximab in one patient and steroids in two patients) with loss of cryoglobulinemia in two patients and two episodes of cryoglobulinemia responding to retreatment in the third patient.

Treatment side effects were reported in seven cases: ribavirin-induced anemia in one case necessitating dose reduction, recombinant erythropoietin administration and blood transfusion [19]; severe fatal intestinal mucositis following corticosteroids reduction in one case [18]; and absence of side effects in five cases. These data were not reported for the seven other treated cases.

**Final outcome**

The final outcome revealed spontaneous regression in one patient who had severe arthritis, improved kidney function in nine patients, chronic renal failure in one patient, end-stage renal disease 2–3 years after diagnosis in three patients and one death due to severe intestinal mucositis (Table 4).

**Generalizability of the results**

Given the good- and moderate-quality assessment of all included studies and the reports from three different centers, we believe that our results could be applied to all patients with cryoglobulinemia associated with HEV infection. However, we could not exclude a selection bias favoring the report of more severe cases.

**Application of Hill’s criteria**

We applied ‘Hill’s criteria for causation’ to the 15 documented cases (Table 5). Five of the nine criteria were fulfilled, which we consider as highly probable for a causal relationship.

**Discussion**

HEV is a rising threat in non-endemic regions, and there has been a renewed interest in its epidemiology, clinical manifestations and prevention [7,11,24,25]. HEV manifestations may range from acute, which may result in acute liver failure [26], to chronic in immunosuppressed individuals, and it may also masquerade through a myriad of extra-hepatic manifestations [24]. MC has been recognized as an extra-hepatic manifestation of HCV for a long time. The prevalence of HCV infection in MC ranges from 40 to 90%, whereas HCV-negative MC accounts for about 5–10% [27].

We identified 15 cases of MC associated with HEV. Of note, all patients originated from Western Europe, where HEV genotype 3 is prevalent, and all cases were reported by three major groups in France and Germany, which had extensive experience with HEV. In two studies of kidney- and liver-transplant patients with cryoglobulinemia, no cause of cryoglobulinemia was found in a significant number of patients [28,29]. HEV infection was not tested in these patients and it is unknown whether they had past or ongoing HEV infection. It is possible that MC associated with HEV infection is underreported.

| First author/year | No. | Treatment | Doses | Duration | Side effects | Management |
|-------------------|-----|-----------|-------|----------|--------------|------------|
| Kamar 2012 [17]  | 7   | Ribavirin (majority) | Not report | 3 months | Not report | –          |
|                   | 1   | Ribavirin | 600 mg | 3 months | No | –          |
|                   | 1   | Reduce immunosuppressant (tacrolimus) | – | – | No | –          |
|                   | 1   | Immunosuppressant (rituximab) | 375 mg/m²/week | 4 weeks | No | –          |
|                   | 1   | Pegylated interferon | 135 μg/week | 3 months | No | –          |
| Pischke 2014 [18]| 1a  | Immunosuppressant (steroids) | – | 2 courses | Mucositis/death | Supportive reduction ribavirin—erythropoietin transfusion– |
| Del Bello 2015 [19]| 1b  | Ribavirin | 1200 mg/d | 1 month | Anemia | No          |
|                   |     | Ribavirin | 600 mg/d | 2 months | No | –          |
| Guinault 2016 [20]| 1   | Plasmapheresis | – | 7 sessions | No | –          |
|                   |     | Immunosuppressant (steroid pulses) | 1 mg/kg/d | 18 days | No | –          |
| Total: 4 studies | 14  | Reduce immunosuppressant: 1 Antivirals: 10 Immunosuppressant: 3 | – | – | No side effects: 5 Not report: 7 | –          |

*aMixed cryoglobulinemia appeared after viral clearance.

*bImmunosuppressant reduction before appearance of mixed cryoglobulinemia.

Figure 2. Treatment of mixed cryoglobulinemia associated with HEV infection.

Table 3. Modality and site-effects of treatment of mixed cryoglobulinemia associated with HEV infection.

| First author/year | No. | Treatment | Doses | Duration | Side effects | Management |
|-------------------|-----|-----------|-------|----------|--------------|------------|
| Kamar 2012 [17]  | 7   | Ribavirin (majority) | Not report | 3 months | Not report | –          |
|                   | 1   | Ribavirin | 600 mg | 3 months | No | –          |
|                   | 1   | Reduce immunosuppressant (tacrolimus) | – | – | No | –          |
|                   | 1   | Immunosuppressant (rituximab) | 375 mg/m²/week | 4 weeks | No | –          |
|                   | 1   | Pegylated interferon | 135 μg/week | 3 months | No | –          |
| Pischke 2014 [18]| 1a  | Immunosuppressant (steroids) | – | 2 courses | Mucositis/death | Supportive reduction ribavirin—erythropoietin transfusion– |
| Del Bello 2015 [19]| 1b  | Ribavirin | 1200 mg/d | 1 month | Anemia | No          |
|                   |     | Ribavirin | 600 mg/d | 2 months | No | –          |
| Guinault 2016 [20]| 1   | Plasmapheresis | – | 7 sessions | No | –          |
|                   |     | Immunosuppressant (steroid pulses) | 1 mg/kg/d | 18 days | No | –          |
| Total: 4 studies | 14  | Reduce immunosuppressant: 1 Antivirals: 10 Immunosuppressant: 3 | – | – | No side effects: 5 Not report: 7 | –          |
Table 4. Results of treatment of mixed cryoglobulinemia associated with HEV infection

| First author/year | No. | Treatment | Before treatment | Results of treatment | Outcome |
|-------------------|-----|-----------|------------------|----------------------|---------|
| RNA eGFR | Cryo | ETR | SVR | eGFR |
| Kamar 2012 [17] | 7 | Ribavirin (majority) | (+) | NR | Yes | NR | Improved<sup>a</sup> Improvement |
| | 1 | Ribavirin | (+) | eGFR 35 | (-) | Yes | Yes | eGFR 35 Chronic renal failure |
| | 1 | IS reduction | (+) | eGFR 39 | (-) | – | – | eGFR 35 ES RD 2 years after diagnosis |
| | 1 | IS (rituximab) | (+) | eGFR 37 | (-) | – | – | Dialysis dependent ES RD 3 years after diagnosis |
| | 1 | Pegylated interferon | (+) | eGFR 35 | (-) | Yes | NR | eGFR 27 ES RD 2 years after diagnosis |
| Pischke 2014 [18] | 1 | IS (steroids)<sup>b</sup> | (-) | eGFR 28 | Recurrent | Negative before treatment | eGFR 87 Death (mucositis) |
| Del Bello 2015 [19] | 1 | Ribavirin<sup>c</sup> | (-) | eGFR 41 | (-) | Yes | Yes | eGFR 60 Improved |
| Guinault 2016 [20] | 1 | IS reduction − IS (steroids) | (-) | eGFR 19 | (-) | – | – | eGFR 38 Improved |
| Total: 4 studies | 14 | IS reduction: 1 | (-):13 | | Recurrent: 1 | Yes: 10 | Yes: 2 | | Improved: 9 Chronic renal failure: 1 ES RD: 3 | |
| Antivirals: 10 | | IS: 3 | |

<sup>a</sup>Outcome not reported in details but significant amelioration of serum creatinine at the end of antiviral treatment was observed.

<sup>b</sup>Cryoglobulinemia appeared after viral clearance following reduction of immunosuppressive drug.

<sup>c</sup>Immunosuppressant reduction before occurrence of mixed cryoglobulinemia.

NR: not reported; IS: immunosuppressant; eGFR: estimated glomerular filtration rate given in mL/min/m²; Cryo: cryoglobulinemia; ETR: end-of-treatment response; SVR: sustained virologic response; ESRD: end-stage renal disease.

Table 5. Application of Hill’s criteria on 15 cases of mixed cryoglobulinemia (MC) associated with HEV infection

| Hill’s criteria | Hill’s definition | Application | Result |
|-----------------|-------------------|-------------|--------|
| 1. Strength (effect size) | 'A small association does not mean there is not a causal effect, though the larger the association, the more likely it is causal' | Low number of reported cases | Not fulfilled |
| 2. Consistency (reproducibility) | 'Consistent findings observed by different persons in different places with different samples strengthens the likelihood of an effect' | Reports from three groups interested in HEV infection in 2 countries; Possible unawareness bias for other groups | Not fulfilled |
| 3. Specificity | 'Causation is likely if there is very specific population at specific site and disease with no other likely explanation' | Specific population with no other explanation; 13 immunosuppressed patients with chronic hepatitis E genotype 3; 2 immunocompetent patients with acute hepatitis E genotype 3 | Fulfilled |
| 4. Temporality | 'The effect has to occur after the cause' | Simultaneous presence of HEV & MC (14 patients); Occurrence after viral clearance (1 patient) but HEV may trigger autoimmunity | Fulfilled |
| 5. Biological gradient (dose-response) | 'Greater exposure should generally lead to greater incidence of the effect' | No study correlates viral load & MC occurrence; Low viral load in one patient at assessment’s time | Not fulfilled |
| 6. Plausibility | 'Plausible mechanism between cause and effect is helpful' | Immunopathological mechanism; Presence of anti-HEV IgG, anti-HEV IgM and HEV RNA in the only tested patient | Fulfilled |
| 7. Coherence | 'Coherence between epidemiological & laboratory findings increases likelihood of effect' | Low number of reported cases | Not fulfilled |
| 8. Experiment | 'Occasionally it is possible to appeal to experimental evidence' | Loss of MC after HEV eradication by antiviral treatment in 10 patients | Fulfilled |
| 9. Analogy | 'Effect of similar factors may be considered' | Several other viruses cause MC (especially HCV) | Fulfilled |
To the best of our knowledge, Guinault et al. were the first to isolate anti-HEV IgG, IgM and HEV RNA from the precipitate in a patient with severe CD [20]. This finding favors a causal relationship between HEV infection and MC. HEV RNA was not assessed in the cryoprecipitate in any of the previous reports. Moreover, a recent retrospective cross-sectional study examined 68 German patients with cryoglobulinemia [30] and revealed a statistically significant difference in the presence of anti-HEV IgG antibodies in patients with essential cryoglobulinemia when compared with cryoglobulinemia of other defined causes (p = 0.043). This study suggests that previous HEV infection might play a role in some cases of cryoglobulinemia that are currently classified as essential.

HEV could trigger an autoimmunity response that may account for the development of extra-hepatic manifestations after viral clearance, as MC was observed in a patient following viral clearance [24,31]. Reports of chronic hepatitis E have been almost all limited to immunosuppressed patients infected with genotype 3. All 13 patients with chronic hepatitis E in this review had immunosuppressed status post SOT and genotype 3 was confirmed in 12 patients and was not done in 1 patient.

Renal involvement was reported in nearly half of patients in this review. Although it is difficult to implicate HEV infection in the two cases of relapsing IgA nephropathy, it is possible that HEV may have triggered this relapse given that, in one patient, proteinuria returned to its baseline, and cryoglobulinemia had become undetectable when viral clearance was achieved following ribavirin monotherapy.

Similarly to MC associated with chronic hepatitis C, the treatment of MC associated with chronic hepatitis E could also depend on the severity of CD [1]. In the absence of life-threatening CD, the treatment could be directed toward eradication of viral replication with or without immunosuppressant.

The first-line therapy for HEV in chronic hepatitis E is to reduce the immunosuppressant dose when possible, especially that of agents targeting T-cells. This treatment may achieve viral clearance in one-third of patients [32] and this was the method of treatment in one patient in our review, leading to viral clearance [17].

The second line of treatment to eradicate HEV in chronic hepatitis E is the use of antivirals monotherapy. Peg-interferon could induce rejection in SOT patients and should be avoided in that setting, while ribavirin is considered the antiviral treatment of choice, especially in the SOT recipient, according to a recent systematic review of the literature [33]. A 3-month course of ribavirin 600 mg/day is appropriate in SOT recipients with chronic hepatitis E genotype 3 according to a large multicenter retrospective study published recently [13]. In MC associated with HCV infection, SVR was achieved in more than half of the patients treated with pegylated interferon plus ribavirin in a previous meta-analysis [34] and in most patients treated with sofosbuvir-based direct-acting antiviral regimens in a recent case-series study (83%) [35]. In our review, loss of cryoglobulinemia and end-of-treatment response were achieved in 10 patients treated with antiviral monotherapy (pegylated interferon or ribavirin). Further studies are needed to confirm these initial results and to document the rate of SVR in this setting.

Immunosuppressant may be indicated for the treatment of CD in the absence of viral replication or in severe CD. In our review, immunosuppressant was given despite viral replication in two patients: in one patient, no antiviral therapy at that time had been given to HEV-positive kidney transplant patients [17,19]; and, in a second patient, a low viral load 1 week after admission was present [20].

Association does not entail causation, and the most important, and perhaps most difficult, question to answer is whether MC is simply associated with HEV infection or caused by this infection. The British medical statistician and father of modern randomized controlled trials, Sir Austin Bradford Hill, published in 1965 nine ‘viewpoints’ to establish a causal relationship between a putative cause and an effect (Table 5) [14]. Since then, these ‘viewpoints’, known in the literature as ‘Hill’ criteria, have become a frequently cited framework for causal inference in epidemiological studies. We cautiously apply Hill’s criteria to examine the relation between MC and HEV, and we do not attempt to give definitive conclusions. In our review, four of the Hill’s criteria were not fulfilled by the documented cases. The non-fulfillment of the strength and coherence effects is due to the low number of reported cases and this is subject to change if more cases are recognized. Second, the lack of consistency criteria could be due to a possible unawareness bias. Lastly, the non-fulfillment of biological effect criteria is due to the lack of evidence. Lack of known evidence does not signify absence of true evidence, as it is impossible to determine its validity without intentional experimentation. Moreover, we are aware that the way each criterion should be applied, interpreted and weighted must be carefully measured against the novel types of data available in each unique situation [36]. Given the absence of a scoring system when Hill’s criteria are applied, we consider the fulfillment of five criteria as highly probable for a causal link.

We acknowledge that our review entails several shortcomings. First, the methods of diagnosis and classification of cryoglobulinemia were not mentioned in published reports. We presumed that when the diagnosis of MC and its type was made, the validated laboratory methods were implemented. Second, for seven patients, it was not possible to establish whether CD was present or absent, what were the doses of antiviral monotherapy and what were the ensuing side effects of treatment. Third, SVR, which is the most important index for the efficacy of antiviral treatment, was not reported in 8 of 10 patients treated with antiviral monotherapy, although loss of cryoglobulinemia and end-of-treatment response were obtained in all of them. Fourth, our proposed tool for risk of bias assessment of case reports and case-series has not been validated, although it was derived from a commonly used instrument. We derived simple and reproducible questions that included important parameters to fit the question at hand. Lastly, the number of published cases is too small to allow precise characterization of this possible extra-hepatic manifestation of HEV and could limit the evaluation of a causal relationship. Further studies are needed to delineate the frequency of MC associated with HEV, its pathophysiology, its relationship with the viral load and to establish the rate of SVR of antiviral monotherapy.

In conclusion, MC has been described with HEV infection. A causal relationship between HEV infection and cryoglobulinemia is highly probable.

Conflict of interest statement: none declared.

References
1. Ramos-Casals M, Stone JH, Cid MC et al. The cryoglobulinemias. Lancet 2012;379:348–60.
2. Ferri C, Greco F, Longombardo G et al. Association between hepatitis C virus and mixed cryoglobulinemia [see comment]. Clin Exp Rheumatol 1991;9:621–4.
3. Sansonno D, Carbone A, De Re V et al. Hepatitis C virus infection, cryoglobulinaemia, and beyond. Rheumatology 2007;46:572–8.

4. Dimitrakopoulos AN, Kordossis T, Hatzakis A et al. Mixed cryoglobulinemia in HIV-1 infection: the role of HIV-1. Ann Intern Med 1999;130:226–30.

5. Yadav YK, Aggarwal R, Gupta O et al. Hepatitis-B associated cryoglobulinemia presenting as pseudoeucaryctosis. J Lab Physicians 2011;3:133–5.

6. Murat G, Bernon H, Zenone T et al. [A case of hepatitis A-associated cryoglobulinemia]. Ann Biol Clin (Paris) 1999;57:218–20.

7. Haffar S, Bazerbachi F, Lake JR. Making the case for the development of a vaccine against hepatitis E virus. Liver International 2015;35:311–16.

8. Miyamura T. Hepatitis E virus infection in developed countries. Virus Res 2011;161:40–6.

9. Kamar N, Bendall R, Legrand-Abravanel F et al. Hepatitis E. Lancet 2012;379:2477–88.

10. Behrendt P, Steinmann E, Manns MP et al. The impact of hepatitis E in the liver transplant setting. J Hepatol 2014;61:1418–29.

11. Haffar S, Bazerbachi F, Lake JR. HEV-associated cryoglobulinaemia and extrahepatic manifestations of hepatitis E. Lancet Infect Dis 2015;15:268.

12. Danoisesexu J. The diagnosis and classification of the cryoglobulinemic syndrome. Autoimmun Rev 2014;13:359–62.

13. Kamar N, Izopet J, Tripon S et al. Ribavirin for chronic hepatitis E virus infection in transplant recipients. N Engl J Med 2014;370:1111–20.

14. Hill AB. The environment and disease: association or causation? Proc R Soc Med 1965;58:295–300.

15. Haffar S, Bazerbachi F, Prokop L et al. Frequency and prognosis of acute pancreatitis associated with fulminant or non-fulminant acute hepatitis A: a systematic review. Pancreatology 2017;17:166–75.

16. Serratrice J, Didier P, Colson P et al. Acute polyarthritis revealing hepatitis A. Clin Rheumatol 2007;26:1973–5.

17. Kamar N, Weclawiak H, Guilbeau-Frugier C et al. Hepatitis E virus and the kidney in solid-organ transplant patients. Transplantation 2012;93:617–23.

18. Pischke S, Behrendt P, Manns MP et al. HEV-associated cryoglobulinaemia and extrahepatic manifestations of hepatitis E. Lancet Infect Dis 2014;14:678–9.

19. Del Bello A, Guilbeau-Frugier C, Josse AG et al. Successful treatment of hepatitis E virus-associated cryoglobulinnemic membranoproliferative glomerulonephritis with ribavirin. Transpl Infect Dis 2015;17:279–83.

20. Guinauld D, Ribes D, Delas A et al. Hepatitis E virus-induced cryoglobulinnemic glomerulonephritis in a nonimmunocompromised person. Am J Kidney Dis 2016;67:660–3.

21. Marson P, Donadel C, Vicarioto M et al. Low prevalence of hepatitis E virus in type II mixed cryoglobulinemia. Haematologica 1995;80:574–5.

22. Meisner S, Polywka S, Memmler M et al. Definition of chronic hepatitis E after liver transplant conforms to convention. Am J Transplant 2015;15:3011–12.

23. Pischke S, Behrendt P, Manns MP et al. HEV-associated cryoglobulinaemia and extrahepatic manifestations of hepatitis E. Lancet Infect Dis 2014;14:678.

24. Bazerbachi F, Haffar S, Garg SK et al. Extra-hepatic manifestations associated with hepatitis E virus infection: a comprehensive review of the literature. Gastroenterol Rep (Oxf) 2016;4:1–15.

25. Haffar S, Bazerbachi F, Garg S et al. Frequency and prognosis of acute pancreatitis associated with acute hepatitis E: a systematic review. Pancreatology 2015;15:321–6.

26. Bazerbachi F, Haffar S. Acute fulminant vs. acute-on-chronic liver failure in hepatitis E: diagnostic implications. Infect Dis Soc 2015;47:112.

27. Sansonno D, Dammacco F. Hepatitis C virus, cryoglobulinaemia, and vasculitis: immune complex relations. Lancet Infect Dis 2005;5:227–36.

28. Faguere S, Kamar N, Boulestin A et al. Prevalence of cryoglobulinaemia and autoimmunity markers in renal-transplant patients. Clin Nephrol 2008;69:239–43.

29. Garrouste C, Kamar N, Boulestin A et al. Prevalence of cryoglobulinaemia and autoimmune markers in liver transplant patients. Exp Clin Transplant 2008;6:184–9.

30. Pischke S, Polywka S, Haag F et al. Association of hepatitis E virus and essential cryoglobulinaemia? J Clin Virol 2015;67:23–4.

31. Wedemeyer H, Rybczynska J, Pischke S et al. Immunopathogenesis of hepatitis E virus infection. Semin Liver Dis 2013;33:71–8.

32. Kamar N, Garrouste C, Haagsma EB et al. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. Gastroenterology 2011;140:1481–9.

33. Peters van Ton A, Gevers T, Drenth J. Antiviral therapy in chronic hepatitis E: a systematic review. J Viral Hepat 2015;22:965–73.

34. Fabrizi F, Dixit V, Messa P. Antiviral therapy of symptomatic HCV-associated mixed cryoglobulinemia: meta-analysis of clinical studies. J Med Virol 2013;85:1019–27.

35. Sise ME, Bloom AK, Wisocky J et al. Treatment of hepatitis C virus-associated mixed cryoglobulinemia with direct-acting antiviral agents. Hepatology 2016;63:408–17.

36. Fedak KM, Bernal A, Capshaw ZA et al. Applying the Bradford Hill criteria in the 21st century: how data integration has changed causal inference in molecular epidemiology. Emerg Themes Epidemiol 2015;12:14.