Clinical studies on hepatitis B, C, and E virus infection
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CHAPTER 8

Hepatitis E Virus Infection and Hepatic Graft versus Host Disease in Allogeneic Hematopoietic Stem Cell Transplantation Recipients

* contributed equally

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ABSTRACT

Hepatitis E virus (HEV) genotype 3 infection has become important for immunocompromised patients, because of their propensity to develop chronic HEV-infection and liver cirrhosis. We retrospectively investigated the incidence of HEV-infection in patients with elevated ALT levels, in a cohort of 130 allogeneic hematopoietic stem cell (alloHSCT) recipients. Of a total of 130 patients, 123 had one or more episodes of elevated ALT-levels. Five out of these 123 patients had HEV-infection (4%). Interestingly, 3 of these patients had signs of concomitant graft versus host disease (GvHD) of the liver. These data demonstrate that HEV-infection is prevalent among alloHSCT recipients and may be related to the presence of GvHD. HEV-infection should be considered in all alloHSCT recipients with elevated ALT-levels, particularly in patients with GvHD of the liver.
INTRODUCTION

Hepatitis E virus (HEV) is a non-enveloped, single stranded RNA virus, which can be subdivided into at least four genotypes. Genotypes 1 and 2 are human viruses causing acute hepatitis, mainly in young adults in tropical countries. Genotypes 3 and 4 are zoonotic, with pigs as the main reservoir in Europe and parts of Asia. The most prevalent HEV genotype in Europe is genotype 3, which is normally asymptomatic and self-limiting. It does, however, pose a threat to immunocompromised patients who may develop chronic HEV-infection (58-93%) and liver cirrhosis. In a Dutch cohort of 328 allogeneic hematopoietic stem cell transplantation (alloHSCT) recipients, 8 cases of HEV-infection (2.4%) were found of which 5 developed chronic HEV-infection. This suggests that there is a considerable risk of post-transplant HEV-infection for alloHSCT recipients. Most patients with HEV-infection have increased ALT-levels and most alloHSCT patients experience one or more episodes of elevated transaminase levels post-transplantation. The differential diagnosis of elevated liver enzymes including transaminases following alloHSCT is however extensive and includes medication toxicity, pre-existing liver conditions such as fatty liver disease and graft versus host disease (GvHD) of the liver. Moreover, as infections may incite GvHD it can be hypothesized that HEV can provoke GvHD of the liver. Our aim was to identify the prevalence of HEV-infection among alloHSCT patients with elevated ALT-levels.

METHODS

We performed a retrospective analysis of ALT-levels in all 130 patients who received an allogeneic HSCT between January 1st 2005 and April 1st 2015 at our institution. Elevated ALT-levels were defined as: ALT > 50 U/L for at least four consecutive weeks, recurrent elevated ALT-levels > 50 U/L for a shorter period of time with normal ALT-levels in between, or an episode of peaking ALT of > 100 U/L during a period of less than 4 weeks. HEV-RNA was measured at times of ALT-elevation in stored PCR-grade plasma samples using a real-time quantitative PCR amplifying the open reading frame (ORF) 3 region of HEV. For patients with HEV-infection, additional serial plasma samples were retrieved and tested to follow HEV-infection over time.

RESULTS

Patient characteristics are summarized in Table 1. Of 130 alloHSCT recipients (70 men and 60 women), 123 showed one or more episodes of elevated ALT-levels (total: 147 episodes). Hepatic GvHD was diagnosed or strongly suspected (based on elevated cholestatic liver enzymes in combination with biopsy-proven GvHD of skin or intestine, that responded to steroid-therapy) in 19 patients (16% of the 130 alloHSCT patients). For 141/147 episodes of ALT-elevation a plasma sample was available for HEV-RNA testing. Five samples belonging to 5 different patients were HEV-RNA positive, resulting
### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Allogeneic HSCT patients (n=130)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age – yr</strong></td>
</tr>
<tr>
<td>Mean (range)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
</tr>
<tr>
<td>Male/ Female</td>
</tr>
<tr>
<td><strong>Diagnosis – no. (%)</strong></td>
</tr>
<tr>
<td>AML</td>
</tr>
<tr>
<td>ALL</td>
</tr>
<tr>
<td>CML</td>
</tr>
<tr>
<td>CLL</td>
</tr>
<tr>
<td>Non Hodgkin Lymphoma</td>
</tr>
<tr>
<td>Hodgkin Lymphoma</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td><strong>Type of allogeneic HSCT – no. (%)</strong></td>
</tr>
<tr>
<td>RIST-sib</td>
</tr>
<tr>
<td>RIST-MUD</td>
</tr>
<tr>
<td>MA-sib</td>
</tr>
<tr>
<td>MA-MUD</td>
</tr>
<tr>
<td>CB</td>
</tr>
</tbody>
</table>


### Table 2. Characteristics of HEV-infected patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex, age§ (yr)</th>
<th>Underlying disease</th>
<th>Transplantation type (yr)</th>
<th>Hepatic GvHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M, 60</td>
<td>CLL</td>
<td>MUD-RIST (2006)</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>M, 37</td>
<td>ALL</td>
<td>MA-MUD (2010)</td>
<td>Yes*</td>
</tr>
<tr>
<td>3</td>
<td>F, 70</td>
<td>CLL</td>
<td>MUD-RIST (2011)</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>M, 41</td>
<td>CML</td>
<td>MA-MUD (2012)</td>
<td>Yes*</td>
</tr>
<tr>
<td>5</td>
<td>F, 54</td>
<td>Hodgkin Lymphoma</td>
<td>MUD-RIST (2014)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

§Age at time of HEV diagnosis; *Elevated transaminases and cholestatic liver enzymes have been attributed to hepatic GvHD because they occurred at the time of biopsy-proven chronic GvHD of the gastro-intestinal tract and/or skin. A liver biopsy was not taken.

Abbreviations: CLL: chronic lymphoid leukemia; ALL: acute lymphatic leukemia; CML: chronic myeloid leukemia; MUD: matched-unrelated donor; RIST: reduced intensity stem cell transplantation; MA: myeloablative.

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in an HEV-infection prevalence of 4% among alloHSCT recipients with ALT-elevations. All these 5 patients had persistent or recurrent ALT-elevations. Of the five patients with HEV-infection, three had suspicion of concomitant hepatic GvHD; two patients (patient 2 and 4) had biopsy-proven GvHD of the skin and intestine, and suspected GvHD of the liver at the time of HEV infection, in one patient (patient 5) hepatic GvHD was confirmed by a liver biopsy. We describe the case histories of the five HEV-infected patients below (Table 2. and Figure 1).

Patient 1 underwent a reduced intensity alloHSCT (RIST) of a matched unrelated donor (MUD) because of chronic lymphatic leukemia (CLL) in 2006. He developed acute and chronic GvHD of the skin, for which he received prednisolone that could be tapered and finally stopped in 2009. In 2014 a relapse of the CLL occurred which was treated with fludarabine/cyclophosphamide/rituximab (FCR) chemotherapy. This was complicated by chronic varicella zoster infection and herpes simplex infection, for which he received maintenance treatment with valaciclovir and ganciclovir. He was diagnosed with HEV-infection 9 years after alloHSCT, a few months after the last cycle of FCR. Treatment of HEV-infection with ribavirin was initially successful, but after stopping therapy a relapse occurred. Also a second course of therapy did not result in clearance of the virus.

Patient 2 received a myeloablative (MA) MUD alloHSCT because of acute lymphatic leukemia (ALL) in 2010. Three months after alloHST he developed cytomegalovirus (CMV) reactivation, which was treated with valganciclovir, and acute, biopsy-proven GvHD of the skin and intestine which was treated with prednisolone. During this time, ALT-levels were elevated, but they normalized upon treatment for CMV-reactivation and GvHD. Three years later, during an exacerbation of chronic GvHD of the skin and intestine

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**Figure 1.** Graphic representation of the three patients with HEV-infection and suspected or diagnosed hepatic GvHD (patient 2, 4 and 5). See for detailed description of patient histories the main text.
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(biopsy-proven), he was diagnosed with HEV-infection (Figure 1a). GvHD of the liver was considered because of elevated transaminases, however, this was not confirmed by a liver biopsy. HEV-infection was treated successfully with ribavirin and the transaminases normalized subsequently.

Patient 3 received a MUD-RIST because of CLL in 2011. The transplantation was complicated by chronic GvHD of the skin and oropharynx, which was treated with prednisolone, cyclosporin, imatinib and eventually, in 2014, rituximab. She also had disseminated varicella zoster and herpes simplex virus infections for which she received valaciclovir. HEV-infection occurred after rituximab therapy, and was successfully treated with ribavirin. The patient eventually died from recurrent opportunistic respiratory tract infections with *Hae- mophilus influenzae*.

Patient 4 received a MA-MUD HSCT because of blast crisis chronic myeloid leukemia (CML-BC) in 2012. The transplantation was complicated by acute and chronic steroid-refractory biopsy-proven GvHD of the intestine, which was treated with mesenchymal stem cell transplantation resulting in partial remission. He had peaking ALT-elevations during this period which was attributed to GvHD, iron deposition and medication toxicity. A liver biopsy was not performed. Retrospective analysis of stored plasma samples revealed that he acquired HEV-infection before alloHSCT. HEV-infection and GvHD remained active until he succumbed to systemic yeast infection 16 months after alloHSCT (Figure 1b). In this patient, HEV-infection was diagnosed after his death, and therefore it was left untreated.

Patient 5 received a MUD-RIST because of relapsing Hodgkin’s lymphoma in 2011, which was complicated by GvHD of the skin and liver. At the time of diagnosis of GvHD, the patient was also diagnosed with HEV-infection (Figure 1c). A liver biopsy taken at that time showed an irregular morphology of the bile duct epithelium, consistent with GvHD. Moreover perportal infiltrates were seen together with some lobular inflammation and

![Figure 1. Liver biopsy of patient 5: combination of GvHD and HEV-infection.](image)

(a) Enlarged 40x, PAS-D-staining. The arrow shows a damaged bile duct as a sign of GvHD.

(b) Enlarged 20x, H&E-staining. Lobular inflammatory infiltrate (arrowhead) and Councilman bodies (apoptosis, arrow).

(c) Enlarged 20x, H&E-staining. Portal tract with portal vein (+), hepatic artery and bile duct (circle), surrounded by mixed periportal inflammatory infiltrate with some eosinophils (arrow), as can be seen in HEV-infection.

(d) Enlarged 40x, H&E staining, detail of 1b. apoptotic bodies (arrow).
Councilman bodies (apoptotic bodies), the latter fitting with both hepatic GvHD and HEV-infection (Figure 2). Analysis of stored plasma samples revealed that HEV-infection preceded GvHD of the liver, suggesting that HEV-infection may have provoked this allo-immune response. The patient responded well to treatment with ribavirin but eventually succumbed to relapsed Hodgkin’s lymphoma.

DISCUSSION

The prevalence of HEV-infection was 4% in this cohort which is higher than was reported for the general population (0.13%)\(^1\), for solid organ transplant patients (1–3%)\(^5\), for HIV-patients (0.12%)\(^13\), and for another Dutch alloHSCT cohort (2.4%)\(^8\). The latter study was performed between 2006 and 2011, while our study was conducted between 2005 and 2015. All HEV-infections in our cohort occurred after 2012, which fits with the observation that HEV-prevalence in Europe is rising\(^12\) and offers an explanation for the higher incidence we observed. As is recommended in recently published guidelines for treatment of viral hepatitis in patients with hematological disorders\(^13\), infection with HEV should be considered in these immunocompromised patients, especially those with chronic lymphopenia, who are at-risk for chronic HEV-infection with rapid advancement to liver cirrhosis.

We observed that HEV-infection coincided with (suspected) hepatic GvHD in three of five HEV-RNA positive patients. In this study population of 130 patients, 19 patients had (suspected) hepatic GvHD, of which 3 (16%) had concomitant HEV-infection. Of the 111 patients without hepatic GvHD only 2 (2%) had HEV-infection. GvHD is characterized by an immune response of donor lymphocytes against host tissues. Intense conditioning procedures or local damage, for example caused by infection, may induce such allo-immune responses and GvHD\(^14\). Therefore, one can hypothesize that HEV-infection can also initiate or maintain (hepatic) GvHD. In two out of the three patients (patients 2 and 5) with concomitant HEV-infection and suspicion or diagnosis of hepatic GvHD, treatment with ribavirin led to rapid clearance of the virus in parallel with resolution of GvHD. In one patient (patient 4) with concomitant HEV-infection and (suspected) hepatic GvHD, HEV-infection was not diagnosed and therefore left untreated. GvHD in this patient was therapy-refractory.

AlloHSCT recipients often demonstrate elevated ALT-levels, a sign of liver tissue damage that is usually ascribed to drug toxicity, iron deposition, infection, sinusoidal obstruction syndrome (SOS) or GvHD\(^15\). Hepatic GvHD is difficult to distinguish from other liver diseases, and our observation that 16% of patients with (probably or proven) hepatic GvHD had concomitant HEV infection underlines the importance of serologic testing of these patients.

In conclusion, allogeneic HSCT recipients are at high risk of liver disease, including HEV-infection. It may cause chronic liver inflammation ultimately leading to cirrhosis, and our observations led us to hypothesize that HEV can provoke or sustain hepatic GvHD. While our results need further study and confirmation in larger alloHSCT cohorts, data from our and another group\(^8\) demonstrate that HEV-infection should be considered in all alloHSCT recipients with persistently elevated ALT-levels, particularly in those with concomitant hepatic GvHD.
REFERENCES