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

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Citation for published version (APA):

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Summary and General Discussion
SUMMARY

The described work comprises different clinical aspects of viral hepatitis B, C and E infection. First, the immune response during acute hepatitis B is investigated. Next, intrahepatic and plasma IP-10 levels are analysed as a marker of response to peginterferon-based therapy in chronic hepatitis B and C virus infection. Furthermore, various treatment options for chronic hepatitis B, C and E are discussed. Moreover, the hepatitis C-related disease burden in 2030 is predicted by using a modelling approach. Finally, the prevalence of chronic hepatitis E virus infection in allogeneic hematopoietic stem cell transplantation patients and its clinical relevance is studied.

Part I – Hepatitis B Virus Infection

Chapter 1 describes the immune responses of 9 patients with acute HBV-infection, in terms of NK-cell characteristics and HBV-specific T-cell function. Of these 9 patients, one became chronically infected and the other 8 cleared the virus within 6 months. Early time points after infection showed an increase in CD56bright NK-cells, and in the proportion of cells expressing markers of activation. Most of these normalised at week 24, while the proportion of TRAIL-positive CD56bright NK-cells remained high in the chronically infected patient. In patients that cleared HBV, functional HBV-specific CD8+ and CD4+ T-cell responses were observed, whereas in the patient who developed chronic infection, only low HBV-specific T-cell responses were found at all time points. This indicates that NK-cells are activated early during acute HBV-infection. In patients who clear acute HBV-infection, broad and multi-specific T-cell responses are observed. As exemplified by one patient who did not clear HBV-infection, failure of NK-cell normalisation as well as narrow T-cell responses may be indicative of chronic infection.

In Chapter 2, the role of pre- and on-treatment IP-10 levels in chronic hepatitis B (CHB) patients with high viral load, treated with a combination of peginterferon (pegIFN) and a nucleo(s)tid analogue (NA) is investigated. It was found that plasma IP-10 levels and IP-10 mRNA expression in the liver at baseline were correlated with one another, especially in HBeAg-positive patients. Higher pre-treatment IP-10 levels in plasma were associated with combined response (HBeAg-loss, ALT-normalization and HBV-DNA < 2,000 IU/mL) in HBeAg-positive, but not in HBeAg-negative CHB patients. Furthermore, there was a correlation between plasma and intrahepatic IP-10-levels and various markers of response such as ALT, HBV-DNA levels and HAI-score of the liver.

Chapter 3 describes a randomized controlled trial in CHB patients with a low viral load who received either pegIFN and adefovir, PegIFN and tenofovir for 48 weeks, or no treatment. The primary objective was HBsAg-loss. In this study, there was no significantly higher rate of HBsAg-loss at Week 72 in patients who were treated with combination therapy, compared to those who received no treatment. However, there was a strong decline in HBsAg of > 1log10 IU/mL observed in 21% of patients treated with combination therapy with pegIFN and adefovir or tenofovir, versus no change in patients who did not receive treatment.

Part II – Hepatitis C Virus Infection

Chapter 4 describes the dynamic changes of plasma IP-10 levels in chronic hepatitis C (CHC) patients treated several years ago, with a high-induction dose of standard
interferon (IFN) for 6 weeks, followed by treatment with pegIFN, ribavirin (RBV) and amantadine. There was no significant relation between pre- or on-treatment plasma IP-10 levels and sustained virological response (SVR). However, IP-10 levels at Day 1 of treatment increased significantly and this increase was related with a decline in HCV viral load at Day 1 of > 2.28log10. The increase of IP-10 levels from baseline to Day 1 was significantly greater in patients with a favourable IL28B genotype compared to patients with non-favourable IL28B genotypes. Furthermore, the rise in IP-10 from baseline to Day 1 was dependent on baseline IP-10 levels. In patients with a low baseline IP-10 of < 150 pg/mL, an almost 30-fold rise was seen at Day 1, whereas in patients with a high baseline IP-10 of > 600 pg/mL only a four-fold rise was observed.

Chapter 5 describes the results of a retrospective multicentre observational real-life cohort study of 53 patients with CHC genotype 4 and advanced liver fibrosis or compensated cirrhosis. The patients were treated with sofosbuvir and simeprevir with or without RBV. An SVR-rate of 92% was observed, which was in line with the data shown in large registration studies in CHC patients with HCV genotype 1. Whether or not the addition of RBV in cirrhotic patients leads to a higher SVR-rate in genotype 4 patients cannot be concluded from our study. In our cohort, all 12 patients who were treated with simeprevir, sofosbuvir and RBV achieved SVR whereas the four patients with a viral relapse did not receive RBV.

Chapter 6 describes a case of a patient with CHC genotype 1a and advanced liver fibrosis, who failed previous therapy with pegIFN and RBV, and subsequently relapsed after therapy with a combination of a NS5A and NS3 inhibitor during 24 weeks in a clinical trial. NS5A as well as NS3 resistance-associated substitutions (RAS) were present and explained the failure of the treatment. After 18 months, NS5A RAS remained abundantly present, but NS3 RAS had disappeared. Whilst waiting for registration and reimbursement of DAAs, the patient underwent gastric bypass surgery for morbid obesity. This patient achieved SVR after treatment with sofosbuvir, simeprevir and RBV for 24 weeks. The dose of simeprevir was adjusted based on therapeutic drug monitoring (TDM), as simeprevir levels were lower than described in literature due to the gastric bypass surgery.

Chapter 7 predicts the effect of different treatment strategies (using a modelling approach) on the HCV-viremic population and related disease burden (defined as liver-related mortality and incidence of hepatocellular carcinoma (HCC)) in 2030. The treatment strategies used comprise various scenarios: starting from a “base scenario”, the amount of CHC patients treated with antiviral therapy is gradually increased based on different criteria. The most important scenario’s that were analysed to predict the HCV-viremic population and disease burden in 2030 were:

A. “base-scenario”: CHC patients with severe liver fibrosis or cirrhosis (META VIR ≥ F3/F4) are treated with IFN-based therapy;
B. “increased efficacy”: the same criteria as the base scenario, but treatment with DAAs instead of IFN-based therapy;
C. “all patients”: all patients are treated with DAAs, regardless of fibrosis-stadium;
D. “screening and elimination”: all patients are treated with DAAs, with an increased amount of patients with new HCV-diagnoses as input, and a lower amount of acute HCV-infections due to preventive measures.
The HCV-viraemia prevalence in The Netherlands used to calculate the base scenario is 0.12%, which is the most recently established prevalence at the time of conducting the study (2013/2014). According to the base scenario, the HCV-viremic population would decrease by 45% by 2030. This would decrease by 85% if the number of treated patients would be increased to all patients (scenario C). Furthermore, the number of individuals with HCC and liver-related deaths are estimated to decrease by 19% and 27% respectively in the base scenario; both patient groups are expected to further decreased by 60–68% when extending treatment to all CHC-patients (C). With all analysed strategies, a significant reduction in the burden of HCV-viremic infections, liver-related deaths and HCC was predicted.

**Part III – Hepatitis E Virus Infection**

Chapter 8 describes the prevalence of chronic HEV-infection in patients who underwent allogeneic hematopoietic stem cell transplantation (alloHSCT) in The Netherlands. Chronic HEV-infection was established in 5 out of 130 patients (4%), which is higher than was reported in other cohorts of immunocompromised patients, such as solid organ transplant recipients and HIV-patients (0.12–3%) in The Netherlands. There was a possible relation between chronic HEV-infection and hepatic graft versus host disease (GvHD), as in three of the five patients with chronic HEV-infection hepatic GvHD was suspected. In two of these patients, treatment with RBV led to rapid clearance of the virus and resolution of GvHD. In the third patient, the HEV-infection was (retrospectively) diagnosed after the patient had died and therefore left untreated. GvHD in this patient was therapy-refractory. In conclusion, a higher prevalence of chronic HEV-infection in alloHSCT patients than was reported earlier in immunocompromised patients in The Netherlands was observed, and these findings may suggest a relation between hepatic GvHD and chronic HEV-infection.
GENERAL DISCUSSION

Immunological phenomena in HBV-infection

The mechanism of developing chronic HBV-infection, rather than clearing the infection, is not fully understood but is likely multifactorial. The ability to control (and clear) an acute HBV-infection is thought to be related to the balance between the quantity of infected hepatocytes and the efficiency of the T-cell response. This T-cell response may be attenuated by several factors that could be divided into self-save\textsuperscript{1–3} and viral escape mechanisms.\textsuperscript{4–10} Development of chronic hepatitis B (CHB) is associated with impairment of the innate and adaptive immune responses and with narrow and weak T-cell responses.\textsuperscript{1–3,6,8,9,11} It remains a question of contention whether the impairment of T-cell response is a cause or consequence of persistent infection.

It was earlier shown that HBV-specific T-cell responses in CHB patients with low viral load are relatively narrow and only directed towards the HBV core antigen (HBcAg). These T-cells have a strong proliferative ability by which they supposedly keep HBV replication at a low level. In CHB patients with high viral load, HBV-specific T-cell responses are low. A partial recovery of the HBV-specific T-cell response was observed in patients with HBsAg-loss or long-term viral suppression (with NA's).\textsuperscript{11}

NK-cells play a pivotal role in the clearance of HBsAg during interferon (IFN) - based therapy. Their phenotype and function are significantly altered during and after treatment with interferon.\textsuperscript{12} The findings (Chapter 1) that NK-cells are highly activated during acute HBV-infection, and that HBV-specific CD8+ and CD4+ responses in patients who clear the virus, are broad and functional, confirms the suggestion that these immune reactions are very significant for clearance of the virus. Whether or not these findings are cause or consequence of the HBV-infection remains an open question and a topic for further research.

In Chapter 2 it is described, that plasma IP-10 levels and IP-10 mRNA expression in the liver are correlated with each other in HBeAg-positive CHB patients. Higher pre-treatment IP-10 levels in plasma are associated with combined response (HBeAg-loss, low HBV-DNA (< 2,000 IU/mL) and normal ALT) in HBeAg-positive, though not in HBeAg-negative CHB patients. This suggests that IP-10 reflects intrahepatic immune activation. The fact that IP-10 mRNA expression in the liver was also associated with plasma ALT-levels supports this hypothesis. Given the function of IP-10 being a chemo-attractant for inflammatory cells, high intrahepatic IP-10 expression is essential for migration of leukocytes into the liver in response to a viral infection. In HBeAg-positive patients, pre-treatment immune activation is important for further induction of the innate immune system, thus inducing a virological response. IP-10 kinetics shortly after start of peginterferon (pegIFN)-based treatment, showed a significant two- to threefold rise in IP-10 plasma levels at Day 3 after start of treatment, irrespective of HBeAg-status or response to therapy. This indicates that early after start of pegIFN-based treatment, the immune activation of responders and non-responders was similar. In conclusion, our findings suggest that the increase in IP-10 levels early after start of pegIFN-based treatment is the result of a non-specific stimulation of the IFN type I immune response. This causes the induction of multiple interferon-stimulated genes (ISGs) which provoke in turn response to therapy.
HBV treatment and future perspectives

Presently, the closest to a cure for CHB is loss of HBV-DNA and HBsAg with or without formation of anti-HBs antibodies, also named functional cure. However with a functional cure HBV is still integrated in the nucleus of the hepatocyte as cccDNA. Therefore in the future also removal of cccDNA in the cell, also named total or chemical cure, is the ultimate goal for treatment of CHB patients.

Combined response to current therapy is defined as HBeAg-loss, low HBV-DNA (< 2,000 IU/mL) and normal ALT in HBeAg-positive CHB patients; or low HBV-DNA (< 2,000 IU/mL) and normal ALT in HBeAg-negative CHB patients. Current guidelines for treatment of CHB recommend treatment with either a finite course of pegIFN or long-standing nucleos(t)ide analogue (NA) therapy. The aim of these treatment modalities is maintained viral suppression. The decision to treat a patient who has a high HBV-DNA load and active liver inflammation is obvious, as the risk to develop liver cirrhosis and possibly hepatocellular carcinoma (HCC) if untreated is substantial. For HBeAg-negative CHB patients with low viral load (<2,000 IU/mL) and low inflammatory activity (normal ALT), treatment is not indicated, although these patients are at increased risk of liver cirrhosis and development of CHB. However, there are no treatment intervention studies available on this topic in CHB patients with low viral load. An earlier study in HBeAg-negative CHB patients with high viral load (>2,000 IU/mL) treated with a combination of pegIFN and lamivudine showed disappointing results regarding HBsAg-loss. In a recent randomised controlled study 740 HBeAg-positive and -negative CHB patients with high viral load were randomly assigned to treatment with a combination of pegIFN and tenofovir, or pegIFN, or tenofovir alone. This study showed promising results for the combination therapy with HBsAg-loss of 9.1 % compared to 2.8 % with either monotherapy (tenofovir or pegIFN alone). A previously conducted study showed a relatively high rate of HBsAg-loss among CHB patients with high viral load (HBeAg-positive and HBeAg-negative) treated with a combination of pegIFN and adefovir. The results of those studies suggest that combination therapy in patients with high viral load is superior to pegIFN monotherapy, with respect to HBsAg-loss. Response to combination therapy was associated with low baseline HBsAg-levels, a feature that is not useful for patients with low viral load, as they characteristically already have a low HBsAg-level. Apparently, the antiviral immune response in those patients keeps HBV-DNA and HBsAg at low levels as T-cell exhaustion is less severe when HBV-DNA levels are low. This suggests, that up-regulating the innate and adaptive immune response by antiviral treatment in CHB patients with low viral load could be successful in terms of viral clearance. As there were no studies available assessing treatment in HBeAg-negative CHB patients with low viral load, we decided to set-up a randomised controlled study with the aim to assess HBsAg-loss during or following therapy with combination therapy (pegIFN plus adefovir or tenofovir versus no treatment), and to establish markers of response to therapy. The results were that we did not find a higher rate of HBsAg-loss in the group of HBeAg-negative CHB patients with low viral load who were treated with combination of pegIFN and a NA (adefovir or tenofovir) compared to patients who received no treatment (Chapter 3). However, we did observe a strong decline in HBsAg of >1log10 IU/mL in 21 % of patients treated with combination therapy with pegIFN and adefovir or tenofovir, versus no change in HBsAg-levels in patients who did not
receive treatment. Possibly a longer duration of follow-up of these patients may show whether the strong decline in HBsAg-levels in the treatment arms eventually may lead to higher rates of HBsAg-loss. In patients who had a strong HBsAg decline, a marked rise in ALT-level after start of therapy was observed, and a rebound in HBsAg-level after stopping treatment. This indicates a possible enhancement of the antiviral immune response due to the treatment. These patients are an interesting group to study further, enabling the selection of CHB patients with a low viral load who could benefit from pegIFN-based therapy.

Different treatment strategies might also be considered, such as add-on therapy of pegIFN to NAs, switch from NA therapy to pegIFN, or therapeutic vaccination added to either pegIFN or NAs. A recent study in HBe-negative CHB patients, who were treated with NAs for at least one year and who had undetectable HBV-DNA, showed that addition of a 48 week course of pegIFN was tolerated poorly and did not result in a significant increase of HBsAg clearance. An additional analysis of this study showed that HBsAg decline at week 24 might be useful to identify patients who may benefit from add-on treatment with pegIFN eventually leading to HBsAg-loss.

In the study described in Chapter 3, CHB patients who have no or little inflammatory activity, and a limited increased health risk, are treated with a very toxic treatment with only a small chance of success. To apply this to clinical practice, is in the writer’s opinion, a bridge too far. However, it is of great importance to find a finite curative treatment for CHB, also for the group of CHB patients with low virus activity, to minimize health care costs, risk of reactivation, and overcoming possible stigmata.

New compounds, currently under development, are directed against the virus itself (cccDNA targeted therapy, inhibition of capsid assembly, polymerase inhibitors, RNA interference and HBsAg production targeted therapy), the host proteins involved (entry inhibitors, microRNA’s, FXR-agonists) or the host immune response (TLR agonists). Combining different treatment modalities that act against the replication of the virus and enhance immune response would hopefully result in higher rates of functional cure than with current therapy. The ultimate goal is to achieve a total cure, including the elimination of cccDNA from the nucleus of the hepatocyte. However, this therapy is not expected in the next decade.

**Response markers in chronic HCV-infection**

Multiple inflammatory chemokines and cytokines have been suggested as markers for treatment outcome because of their regulatory function in the HCV-specific immune response. Most of these cytokines are modulated by exogenous IFN and play a critical role in viral clearance. After an infection with HCV, the innate immune system initiates a non-specific immune response through type I IFN, leading to the activation of the intracellular pathway resulting in the induction of multiple ISGs, among which the gene encoding IP-10. This cytokine, and especially its relation to IL28B polymorphisms, viral clearance and response to antiviral therapy, has been described in CHC.

In Chapter 4 a high rise in plasma IP-10 levels shortly after administration of high-dose interferon is described in CHC patients. This might in part be caused by a dose-dependent effect of IFN. However, the fact that the amount of this rise is dependent of baseline IP-10 levels, suggests an important role of pre-treatment activation of the innate immune system in CHC, which is in turn closely related to IL28B genotype.
To conclude, in CHC, low pre-treatment activity of the innate immune system appears to predispose to a higher educability of activity due to IFN-based treatment. These observations on IP-10 in CHC have eventually led to a better understanding of the mechanisms of clearance of the viral infection. However, for predicting the treatment outcome for CHC, measurement of plasma IP-10 level has no relevance today.

**HCV treatment and future perspectives**

Since the introduction and registration of DAAs for the treatment of CHC in 2014, the perspective of HCV treatment has totally changed. DAAs are highly effective in eradicating HCV-infection, and have little side effects. Shortly after registration in 2014, DAAs were not widely reimbursed because of their high costs, but were only available for patients with advanced liver fibrosis (Metavir F3 score) or liver cirrhosis (Metavir F4 score). Based on those reimbursement criteria and the fact that limited literature was available on the treatment with DAA’s of genotype 4 CHC patients, patients with genotype 4 CHC and advanced fibrosis or cirrhosis were selected for treatment with sofosbuvir and simeprevir, with or without ribavirin (RBV) for 12 weeks. In the EASL HCV treatment guidelines these patients are not excluded from the recommendation for treatment with sofosbuvir or simeprevir despite the lack of studies showing the efficacy of such treatment. Chapter 5 describes the results of a retrospective multicentre observational study in a real-life cohort of 53 patients showing sustained virological response (SVR) in 92% of these patients. This was the first study to show efficacy of the combination treatment with sofosbuvir and simeprevir in patients with CHC genotype 4 and advanced liver fibrosis or cirrhosis. Whether or not the addition of RBV in cirrhotic CHC patients leads to a higher SVR-rate in genotype 4 patients remains unknown. In the described study cohort, all 12 patients who were treated with simeprevir, sofosbuvir and RBV achieved SVR, whereas the four patients with a viral relapse did not receive RBV. Although the numbers were relatively small to see statistically significant differences, the addition of RBV to the 12-week regime of sofosbuvir and simeprevir could be considered in treatment-experienced genotype 4 CHC patients with advanced fibrosis or cirrhosis. At present, there are more all-oral treatment options with DAAs available for genotype 4 CHC, mainly based on the combination of sofosbuvir and NS5A inhibitors. Although there seems to be no relevant difference in terms of efficacy between the different available treatment options for genotype 4 CHC, it is worthwhile having the combination of simeprevir and sofosbuvir (+/-RBV) ready as an efficacious and registered treatment option, especially in case of NS5A resistance, as all other DAA combinations contain NS5A inhibitors.

Some scenarios described in Chapter 7 (e.g. offering treatment to all HCV-infected individuals) are close to the current situation in The Netherlands, as all CHC patients are now treated without restrictions. This means, that should the predictions made in Chapter 7 be correct, 85% of all CHC patients in The Netherlands would soon be cured. The remaining 15% would then consist of those who failed DAA therapy (due to viral resistance or non-compliance), those who are unable to be treated with DAAs (unwillingness to be treated, or due to comorbidity), and those who have not yet been diagnosed with CHC. To eradicate CHC in The Netherlands, the undiagnosed HCV viremic population should be found, which is easiest achieved by screening the whole population. Based on cost-effectiveness-analyses, the Dutch Health Council advised
in 2016 to not screen the whole population but only certain risk groups such as first generation migrants from HCV endemic countries, PWID and MSM.\textsuperscript{36–39} It was shown earlier in Western and Asian countries that treatment of CHC lowers all-cause mortality and hepatocellular carcinoma (HCC) incidence\textsuperscript{40–42} and is cost-effective for all CHC patients.\textsuperscript{40–45} Therefore, population-based screening should be considered, since treatment has dramatically improved.

With the further development of even newer pan-genotypic regimens with higher genetic barriers to develop resistance, one may hypothesize that in CHC, HCV genotyping and quantification of HCV load may not be necessary anymore.

The case history described in Chapter 6 illustrates the usefulness of resistance-associated substitutions (RAS) measurement in patients with previously failed DAA therapy. Chapter 6 also underlines that although the relatively new DAAs have been well-studied, information about pharmacokinetics or treatment success in different special (rare) populations are still not well known. Therapeutic drug monitoring (TDM) may be helpful in those special cases.

There are still several issues that require future studies, such as optimal timing of treatment of acute HCV-infection, and pre- or post-exposure prophylaxis in high-risk groups such as MSM.

Another issue is the question whether or not patients with an SVR should be further medically controlled and, if so, for how long. Liver stiffness measured using Fibroscan seems to decrease after successful treatment of CHC, with both IFN-based and DAA therapy. This also seems the case for some patients who pre-treatment have liver stiffness measurements in the cirrhotic range, dropping post-treatment to a value below the threshold of cirrhosis.\textsuperscript{46} What effect this drop in liver stiffness has on HCC-risk is unknown and until that time, those patients should still be controlled in the clinic as cirrhotics.

A recent point of discussion is the occurrence and prognosis of HCC in CHC patients after treatment with DAAs. Treatment with IFN-based therapy ameliorates prognosis of HCC.\textsuperscript{47,48} Various studies in CHC patients treated with DAAs showed either a higher recurrence rate of earlier curatively treated HCC,\textsuperscript{49,50} or no difference in incidence of HCC.\textsuperscript{51,52} One may hypothesize that by taking away inflammation by successful treatment of CHC, (whether this is with IFN-based therapy or with DAAs) prognosis of HCC ameliorates. However, this hypothesis has not been studied for treatment with DAAs. Future research is needed in large patient groups with long-term follow-up after successful treatment of CHC with DAAs to clarify these issues.

**HEV-Infection**

It is common knowledge that HEV-infection with genotype 3 is asymptomatic and self-limiting in immunocompetent individuals. However, this poses a threat to immunocompromised patients who are at risk to develop chronic HEV-infection (58–93\%)\textsuperscript{53–56} and liver cirrhosis.\textsuperscript{54,57,58} A higher prevalence of chronic HEV-infection was reported of 4\% (Chapter 8) in allogeneic hematopoietic stem cell transplantation (alloHSCT) recipients than was earlier described (0.12–3\%).\textsuperscript{56,59,60} There is a possible relation between chronic HEV-infection and hepatic graft versus host disease (GvHD) as in three out of the five patients with chronic HEV-infection hepatic GvHD was suspected. However, hepatic GvHD is difficult to distinguish from other liver diseases, particularly in the
absence of a liver biopsy. This possibly has led to an overestimation of hepatic GvHD prevalence in our cohort. Nevertheless, this leads to the hypothesis that HEV-infection may in two ways affect alloHSCT recipients: by causing chronic liver inflammation ultimately leading to cirrhosis, and by provoking or sustaining hepatic GvHD. With the increasing prevalence of HEV-infection in Europe,\textsuperscript{61, 62} this infection should be considered in all alloHSCT recipients and other immunocompromised patients with persistently elevated ALT-levels, particularly in those with concomitant hepatic GvHD.

Treatment with RBV was an effective therapy for most of the patients we describe in Chapter 8 with chronic HEV-infection. Possible treatment options described in literature are lowering the immunosuppressant therapy,\textsuperscript{63} which is often dangerous and therefore ill advised, or treatment with pegIFN\textsuperscript{64} or RBV.\textsuperscript{65} Due to the toxicity profile, RBV is the most frequently chosen treatment option. RBV is often dosed as 600 mg per day, based on a case series described in 2014. This dosage seems to be too low, as from the writer’s experience, frequent relapses after stopping treatment with this dose are observed. The duration of therapy is another point of discussion. Most cases describe a 3-6 month duration of treatment. A recent study showed an SVR of 63% after treatment for 3 months. This study also showed that a decreased HEV-RNA of 0.5 log copies/mL 1 week after start of treatment had an 88% positive predictive value in predicting SVR.\textsuperscript{66} Another recent study showed that protracted fecal shedding of HEV-RNA may predict treatment failure. Patients with chronic HEV-infection treated for 3 months with RBV who had still HEV-RNA detectable in their stools (but not in plasma) at end of treatment all had a relapse after stopping therapy.\textsuperscript{67} Treatment of chronic HEV-infection with RBV should be high-dose (1000–1200 mg daily if tolerated), and response-guided, based on HEV-PCR in stool, with continuation of treatment at least 2 months after HEV-RNA is first negative in stools. For patients who are still refractory to treatment with RBV, new treatment options with sofosbuvir,\textsuperscript{68–70} nucleoside analogues,\textsuperscript{71} or possibly monoclonal antibodies against the ORF3 protein of HEV\textsuperscript{72} are of interest for further research.

In conclusion, as the prevalence of HEV-infection in Europe is increasing,\textsuperscript{61, 62} prevalence of chronic HEV-infection among immunocompromised patients is expected to be significant as well. As untreated chronic HEV-infection may rapidly result in liver cirrhosis, awareness among treating physicians of immunocompromised patients is essential, especially when ALT-levels are elevated. Future research will be focused on treatment of chronic HEV-infection, such as dosage, duration, and response guidance of therapy with RBV, direct-acting antiviral agents, or nucleoside analogues. Immunocompromised patients should be advised to avoid consumption of undercooked meat products, especially porcine.
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