

Update on Alcohol and Viral Hepatitis

Stefano Gitto¹, Giovanni Vitale², Erica Villa¹ and Pietro Andreone*²

¹Dipartimento di Gastroenterologia, Azienda Ospedaliero-Universitaria & University of Modena and Reggio Emilia, Modena, Italy;

²Dipartimento di Scienze Mediche e Chirurgiche, University of Bologna and Dipartimento dell'Apparato Digerente, Azienda Ospedaliero-Universitaria di Bologna, Policlinico Sant'Orsola Malpighi, Bologna, Italy

Abstract

Alcohol consumption is often associated with viral hepatitis. Although alcohol is known to worsen viral liver disease, the interactions between alcohol and viral hepatitis are not fully understood. Molecular alterations in the liver due to alcohol and viral hepatitis include effects on viral replication, increased oxidative stress, cytotoxicity, and a weakened immune response. Clinically, alcohol enhances disease progression and favors induction of primitive liver neoplasm. The use of new antivirals for hepatitis C and well-established drugs for hepatitis B will determine how viral hepatitis can be controlled in a large percentage of these patients. However, alcohol-related liver disease continues to represent a barrier for access to antivirals, and it remains an unresolved health issue.

© 2014 The Second Affiliated Hospital of Chongqing Medical University. Published by XIA & HE Publishing Ltd. All rights reserved.

Introduction

Worldwide, alcohol-related morbidity and mortality represent a major public health issue. Given that low or moderate use of alcohol reduces anxiety and inhibition, alcoholic beverages are often consumed during normal recreational activities. The United States (US) National Institute on Alcohol Abuse and Alcoholism defines "heavy drinking" as consuming more than four drinks/day or fourteen drinks/week for males, and three drinks/day or seven drinks/week for females. Approximately 25% of heavy drinkers will develop alcohol-related health problems.¹ The risk threshold for developing alcohol-related liver disease is 20–30 g of ethanol intake per day, cirrhosis developing in 10–20% of those consuming more than 80 g of ethanol daily. However, light or moderate alcohol consumption (70–140 g/week, 140–280 g/week, respectively) may protect subjects, in the absence of any other liver condition, against the development of hypertransaminasemia.²

Keywords: Alcohol; HBV; HCV.

Abbreviations: DC, dendritic cell; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, Interferon; MHC, major histocompatibility complex; SVR, sustained virological response; US, United States.

Received: 29 September 2014; Revised: 03 November 2014; Accepted: 03 November 2014

* DOI: 10.14218/JCTH.2014.00030.

***Correspondence to:** Pietro Andreone, Dipartimento di Scienze Mediche e Chirurgiche, University of Bologna and Dipartimento dell'Apparato Digerente, Azienda Ospedaliero-Universitaria di Bologna, Policlinico Sant'Orsola Malpighi, Padiglione 11, Via Massarenti 9, 40138 Bologna, Italy. Tel: +39-051-6363618, Fax: +39-051-345-806, E-mail: pietro.andreone@unibo.it

The hepatitis B (HBV) and C (HCV) viruses are leading causes of chronic liver disease and are responsible for 1.2–1.5 million deaths annually.³ Regarding HCV infection, the vast majority of patients respond well to treatment, and with the use of emerging direct acting antivirals, virological response rates are expected to increase to 90–95%.⁴ For HBV, patients undergoing treatment can achieve at least virological remission.⁵ Given the high prevalence of hepatitis virus infection in the general population, pathological alcohol consumption is often found in patients who test positive for hepatitis virus.⁶

Here, we review and analyze the epidemiology of alcohol use and viral hepatitis, the mechanisms underlying the interaction between alcohol and hepatitis viruses, and the impact of both alcohol consumption and hepatitis viruses on liver disease. The literature search included published articles (peer reviewed original, review and meta-analyses) and we used the following search terms: alcohol and HCV; alcohol and HBV; alcohol and hepatitis C; alcohol and hepatitis B; interaction alcohol and HCV; and interaction alcohol and HBV.

Epidemiology

In the US, alcohol is linked to 50% of liver-related deaths, accounting for \$3 billion annually.⁷ In recent years, alcohol consumption has grown rapidly in the People's Republic of China, with an annual increase of 400%.⁸ In Europe, more than 18% of the population >18 years of age in Germany drink more than 20–30 g of alcohol per day, and 5% show high-risk drinking behavior (>80 g/d).⁹ Factors that have been linked to hepatitis virus infections include alcohol use, smoking, and being overweight.¹⁰ The prevalence of HCV in drinkers varies, ranging from 5% to 56%, and it is high in patients with evidence of liver disease (33–50% versus 2–10%).¹¹ Many alcoholics are polysubstance abusers, and intravenous drug use is the main risk factor for HCV infection (60%).^{12,13} Since HBV can also be transmitted in this manner, it is reasonable to suspect that the prevalence of HBsAg in alcohol consumers would be greater than the general population. Other risk factors for alcohol drinkers include increased likelihood for trauma, hospitalization, blood transfusions and risky sexual behavior.¹¹ Rosman *et al.*¹⁴ studied the prevalence of HBV and HCV in a cohort of 150 alcoholics and 166 non-drinkers. Although there was no difference in the prevalence of HBV in drinkers and non-drinkers, the prevalence of anti-HCV was significantly higher in alcoholics relative to non-drinkers, suggesting that alcohol abuse is an independent risk factor for HCV but not HBV infection.

Virus-alcohol interaction

It is well known that alcohol consumption negatively affects virus-related liver disease, but the complex interactions between alcohol and hepatitis virus infection are not fully understood. Possible explanations include effects on viral replication, increased oxidative stress, cytotoxicity, and a weakened immune response (Table 1).

Molecular aspects

HCV can modify major histocompatibility complex (MHC) class II-restricted antigen presentation and dendritic cell (DC) function, leading to the persistence of virally infected cells. According to Osna *et al.*,¹⁵ ethanol enhances the action of HCV by further suppressing both MHC class I- and class II-restricted antigen presentation. Interferon (IFN), which usually supports antigen presentation, exhibits decreased efficacy in the presence of both HCV and alcohol. More recently, it was shown that betaine or s-adenosylmethionine treatment can reverse the ethanol-induced change in methylation potential by reversing proteasome inhibition and interferon signaling deficits.¹⁶ In a mouse model of HCV, analysis of ethanol-induced hepatic modifications revealed that phosphorylation of serine 99 in the HCV core gene had a significant role in the progression of hepatic damage.¹⁷

Effects on viral replication

The role of alcohol metabolism on HCV replication *in vitro* remains controversial. Zhang *et al.*¹⁸ reported that alcohol increased HCV replication and decreased the antiviral action of IFN- α by activating the nuclear factor-kappa B pathway and the endogenous opioid system. Similarly, using an HCV sub-genomic replicon cell system, it was demonstrated that alcohol increased HCV replication.¹⁹ Alcohol and HCV also have an additive effect on cyclooxygenase-2 enzyme activity. McCartney *et al.*²⁰ used HCV replicon cell lines expressing cytochrome P450 2E1 to show that physiological

concentrations of alcohol (0-100 mmol/L) caused a cytochrome P450 2E1-dependent increase in HCV-RNA levels. Seronello *et al.*²¹ proposed another explanation for alcohol-induced increases in HCV replication, suggesting that the metabolism of alcohol modulates host lipid metabolism. The physiological levels of ethanol, acetaldehyde, and acetone stimulated HCV replication by modulating the host's lipid metabolism and this required elevations in nicotinamide adenine dinucleotide levels. However, Plumlee *et al.*²² reported that ethanol inhibited HCV replication in several independent human hepatoma lines. This discrepancy may be explained by acute alcohol metabolism mediated rapid increases in oxidative stress and subsequent inhibition of viral replication.²³ Recently, in an animal model of HCV using chimeric mice transplanted with HCV-infected human hepatocytes, alcohol exposure was shown to promote persistent HCV infection and liver injury.²⁴

The influence of alcohol consumption on HCV replication in humans is also controversial. For example, it has been reported that serum HCV-RNA levels in drinkers were either increased^{25,26} or the same as abstinent subjects.^{27,28} Moreover, in previous drinkers, HCV-RNA levels either had no effect²⁹ or decreased viral load.³⁰ In a meta-analysis, Anand *et al.*³¹ suggested that variation in results were due primarily to differences in the classification of alcohol consumption. Based on the available data, the authors were unable to confirm the positive or negative effect of alcohol consumption on serum HCV-RNA levels.

The SCID transgenic mouse model of HBV has sustained reliable levels of virus replication,³² and HBV replication in these mice was enhanced by alcohol consumption. The levels of hepatitis B surface antigen (HBsAg) and viral DNA were increased 7-fold in animals treated with alcohol relative to control. Moreover, ethanol treatment increased HBV-RNA levels and improved expression of surface, core, and X antigens. These data may account in part for the increased occurrence of HBV markers among drinkers. In contrast, an earlier study found that alcohol consumption decreased serum levels of surface antigens and increased liver

Table 1. Possible mechanisms by which alcohol worsens virus-related liver disease

Alcohol actions	Sharing of HCV	Sharing of HBV	Effects	Ref.
Suppression of MHC class I-II	Yes	N.A.	Decrease action of interferon	15
Enhance endogenous opioid system	N.A.	N.A.	Decrease action of interferon; boost of HCV replication	18
Additive effect in cyclooxygenase-2	Yes	N.A.	Improvement of liver damage	19
Modulation of host lipid metabolism	N.A.	N.A.	Enhance HCV replication	21
Trigger oxidative stress	Yes	N.A.	Liver damage; disease progression; enhance HBV replication	34-37
Induction of pro-fibrogenic cytokine	Yes	N.A.	Disease progression	35
Activation of hepatic stellate cells	Yes	N.A.	Disease progression	35
Break of immune self-tolerance	N.A.	N.A.	Reduction of cellular immune response versus HCV	38,40
Development of adaptive immune responses	N.A.	N.A.	Reduction of cellular immune response versus HCV	38,40

N.A.: data not available; Ref.: reference number

accumulation of HBsAg in an HBsAg transgenic mouse model.³³ This study was triggered by the finding that alcoholics with hepatitis B have low circulating levels of viral particles but test positive for HBV-DNA. This may be due to alcohol-induced impairment of protein secretion, particularly HBsAg particles.

Role of oxidative stress

Both alcohol and HCV increase hepatic oxidative stress. Otani *et al.*³⁴ tested Huh-7 cells expressing the HCV core protein and cytochrome P450 2E1 after alcohol exposure and showed that HCV and alcohol reduced mitochondrial glutathione depletion, improved mitochondrial reactive oxygen species, depolarized mitochondria, and promoted cell death. In fact, mitochondrial reactive oxygen species induced by the HCV core and cytochrome P450 2E1 resulted in a decrease of mitochondrial antioxidant ability and sensitivity to oxidants and tumor necrosis factor- α . In HCV core transgenic mice treated chronically with progressive amounts of ethanol (up to 20%), there was elevated hepatic lipid peroxidation and a synergistic induction of the profibrogenic cytokine transforming growth factor-1 and hepatic stellate cells.³⁵ There was no effect on fatty acid oxidation and inhibition of very-low density lipoprotein secretion. Importantly, ethanol consumption and virus together significantly enhanced lipid peroxidation and increased hepatic transforming growth factor- β and tumor necrosis factor- α gene expression. These findings suggested a possible role of ethanol in the accelerated development of liver fibrosis and cirrhosis in HCV infected patients who also consume alcohol. In another study, 145 consecutive patients with chronic hepatitis C were subdivided into three groups: abstainers, moderate drinkers, and heavy drinkers.³⁶ It was shown that stimulation of oxidative stress by alcohol may negatively impact the evolution of chronic hepatitis C. Consistent with this hypothesis, heavy drinkers had a high prevalence of lipid peroxidation-related antibodies and a more severe level of liver damage relative to abstainers. Moreover, infected patients consuming alcohol had significantly greater diffuse piecemeal necrosis than abstainers. In a cellular model of HBV,³⁷ HepAD38 cells infected with HBV, ethanol treatment activated transcription of HBV promoters by increasing the expression of nuclear receptors and transcription factors. In addition, cytochrome P450 2E1-induced oxidative stress enhanced ethanol-related transactivation of HBV. Taken together, these findings provide a molecular explanation for the enhanced progression of disease in HBV patients who abuse alcohol.

Effect on immune system

Gene-expression profiling of liver tissue samples from patients with HCV or alcohol-related cirrhosis revealed that immune and viral gene responses were downregulated in patients with both HCV infection and alcohol intake.³⁸ Alcohol-induced oxidative modifications of liver constituents enhance specific immune responses that break self-tolerance in the hepatic tissue. The development of adaptive immune responses results in alcohol-mediated stimulation of innate immunity, contributing to hepatic inflammation during alcoholic liver disease. However, the progression of alcoholic disease to cirrhosis and hepatocellular carcinoma (HCC) is likely related to interactions with both the innate and adaptive immune systems. In support of this hypothesis, mice

chronically exposed to ethanol exhibited reduced cellular immune response to HCV core and non-structural proteins, and this alteration tended to lead to insufficient dendritic cell maturation and a predominant Th2-immune response.³⁹ According to another study, alcohol promoted an immune impairment, and this may explain the high rate of chronicity of viral infection in alcohol abusers.⁴⁰

Progression of liver disease

Alcohol is a significant comorbid factor for the progression of liver disease. The oxidation of ethanol to water and carbon dioxide is mediated by the following hepatic enzymes: alcohol-dehydrogenase, microsomal ethanol oxidizing system and catalase in peroxisomal membrane, and all creating acetaldehyde. Acetaldehyde is oxidized to acetate by aldehyde-dehydrogenase and forms hybrid-adducts with reactive residues. Consequently, acetaldehyde negatively impacts proteins and small molecules via lipid peroxidation and nucleic acid oxidation.⁴¹ Rigamonti *et al.*³⁶ suggested that oxidative stress may be one of the main mechanisms by which alcohol leads to the progression of chronic hepatitis. In HCV patients, not only alcohol use accelerates the progression of fibrosis, but it also favors decompensation and worsens survival.⁴² In a retrospective study, 6,354 patients managed for alcohol dependence or abuse were evaluated on the influence of HCV infection.⁴³ The mortality rate in patients with HCV (15%) during hospitalization was doubled relative to drinkers without infection. These data were confirmed in a subsequent study that reported HCV as an independent predictor of mortality among drinkers.⁴⁴ In another study examining the progression of HCV-related liver disease and the risk of HCC onset in moderate (<80 g/day) and heavy (>80 g/day) Japanese drinkers, the authors found a 1.5-2.5-fold increased risk for the development of cirrhosis and HCC in the alcohol group compared to the abstinent group.²⁷ Moreover, clinical manifestations, such as variceal bleeding, ascites, and encephalopathy, were more frequent in drinkers. Recently, Fuster *et al.*⁴⁵ demonstrated that HCV-positive alcoholic patients died at a younger age relative to those without infection. In a meta-analysis involving 15,000 patients with chronic HCV infection, heavy drinking (210 to 560 g/week) was shown to increase the risk of advanced fibrosis and compensated and decompensated cirrhosis.⁴⁶ In addition, this study highlighted the following points: 1) the negative effect of alcohol consumption may be underestimated in liver biopsy studies because heavy drinkers, who are typically not fully compliant, may avoid this invasive examination; 2) although it was clearly demonstrated that alcohol and HCV together worsened patient outcome, the exact threshold above which alcohol significantly accelerated disease progression was unclear.

In HBsAg-positive patients, alcohol intake is linked with increased hepatic necroinflammatory activity and fibrosis progression. In addition alcohol abuse in patients with chronic hepatitis B is associated with an increased risk of cirrhosis and the development of HCC.⁴⁷ However, in a study analyzing the relationship between alcohol consumption and liver stiffness in chronic HBV-infected patients, it was found that the prevalence of advanced fibrosis in HBV-positive patients with mild to moderate alcohol intake was comparable to that of nondrinkers.⁴⁸ In an observational, nationwide study, a significant association between alcohol consumption and mortality in patients with chronic viral hepatitis was shown,

where excessive alcohol consumption was significantly connected with death at young age for both HBV- and HCV-infected individuals.⁴⁹ In addition, when examining the role of prior HBV infection in patients with advanced alcoholic liver disease, it was demonstrated that anti-HBc-positive alcoholic patients with cirrhosis exhibited more severe liver disease than anti-HBc-negative cirrhosis patients.⁵⁰

Hepatocellular carcinoma

It is well-documented that HCV-positive drinkers are more likely to develop HCC than abstinent individuals.^{51,52} Tsutsumi *et al.*⁵³ reported that alcohol use accelerated the onset of HCC in HCV-positive subjects. Moreover, HCC developed at a younger age in patients with both alcohol abuse and HCV infection than those with a single etiologic agent. Loomba *et al.*⁵⁴ demonstrated in HBsAg-positive patients that the probability of HCC onset was higher among alcohol users and overweight, obese, and extremely obese patients relative to other patients, showing a synergistic effect between weight and alcohol. In a retrospective analysis of 716 Japanese patients affected by cirrhosis, the following three groups were compared: drinkers, HCV-positive, and patients with both characteristics.⁵⁵ The results indicated the following: 1) alcohol abuse was associated with decreased survival in HCV-positive cirrhosis patients without early-stage HCC; 2) HCC development in alcoholics without HCV infection was lower relative to the other two groups; and 3) HCC occurred at a younger age in HCV-positive patients with an alcohol habit relative to the other two groups. Similarly, HCC was more aggressive, with a worse outcome after surgery, in HCV-positive alcohol-consuming subjects compared to abstinent patients.⁵⁶ Another study demonstrated that alcoholics with HCV infection and HCC had a shorter disease-free period after curative resection and a poorer 3 year survival relative to HCV-positive non-drinkers (12.6 versus 25.4 months and 67% versus 95%, respectively).⁵⁷

Concerning HBV, several reports have indicated that a past infection can be a risk factor for developing HCC in patients with alcoholic cirrhosis. Uetake *et al.*⁵⁸ reported that heavy cumulative alcohol intake and a past HBV-infection are independent risk factors for HCC development in patients with alcoholic cirrhosis. In contrast, in 123 HCV-positive and HBsAg-negative patients, habitual drinking was not a relevant risk factor for HCC.⁵⁹

Antivirals in drinkers

Alcohol abuse appears to decrease responsiveness to interferon therapy, reducing both sensitivity and compliance.⁶⁰ The molecular mechanisms underlying this negative influence involve the synergistic inhibitory action of virus and alcohol on the cellular response to interferon.⁶¹ Indeed, excessive alcohol use was linked to a lower HCV response in patients treated with an interferon-based regimen.⁶² However, when the relevant amount of alcohol consumption was quantified in this context, it was found that the sustained virological response (SVR) rates of drinkers with levels of alcohol consumption up to 24 g in 24 hours were similar to those of nondrinkers.⁶³ In addition, it was reported that adherence to treatment and rates of SVR were similar in non-drinkers and past drinkers, whereas patients with a short-term abstinence were more likely to discontinue treatment and exhibited a reduced rate of SVR.⁶⁴ The rate of SVR was not impaired in

alcohol users who were able to complete the treatment, suggesting that compliance is the only significant factor influencing the probability of SVR with interferon therapy.⁶⁴ In general, a multidisciplinary approach seems best to guarantee a similar SVR between treated patients with hepatitis C with or without alcohol dependence.⁶⁵ This was recently confirmed by Lin *et al.*,⁶⁶ who demonstrated that HCV patients with hazardous alcohol consumption can be treated effectively, regardless of whether they were followed by a dedicated reference nurse. It seems that at least a year of abstinence is necessary for HCV-positive patients to achieve adequate adherence to the antiviral treatment.⁶⁷ Other authors, confirming the importance of patient attitude, proposed "motivational enhancement therapy" for HCV subjects with alcohol use disorders, and this protocol was significantly effective in maintaining abstinence.⁶⁸

With respect to HBV, neither slight nor hazardous alcohol consumption levels negatively affect antiviral efficacy. Similar findings were reported for SVR in patients affected by chronic hepatitis B treated with Entecavir.⁶⁹ Nevertheless, alcohol consumption may lead to the overtreatment of patients with HBV infection. It was recently reported that elevated aminotransferase activity in only half of patients with HBV was due to an immune active chronic hepatitis B, whereas non-alcoholic fatty liver disease or alcohol consumption were considered other possible causes for enzyme elevation.⁷⁰

Alcohol can hinder antiviral treatment, and a substantial proportion of patients with hepatitis B and the majority of those with hepatitis C remain untreated. While in Eastern European countries a lack of financial resources is the most frequent cause of nontreatment; in nonEastern countries, parenteral drug and alcohol abuse are the main barriers to therapy.⁷¹ Regarding the treatment of HBV infection, the rates of nontreatment in Western Europe range from 37 to 66%. The reasons for nontreatment varied among the studies.⁷¹ In one Italian study of more than 3,000 patients, immigrant status and alcohol abuse were independently associated with non-treatment.⁷²

Conclusions and future perspectives

Today, alcohol-related liver disease is a major social and health problem, especially for industrialized and emerging countries. Since many alcoholics are polysubstance abusers, it follows that there is a link between alcohol abuse and parenteral virus infection. It is important to note, however, that drinkers exhibit a high prevalence of viral infection in the absence of polysubstance use and abuse. Drinkers are at additional risk of trauma, hospitalization, blood transfusions, and risky sexual behavior and this may explain the elevated prevalence of virus in ethanol consumers. Alcohol negatively affects viral liver disease by suppressing MHC-I and II and by direct and indirect negative effects on viral replication, increasing both oxidative stress and cytotoxicity, and weakening immune response. These actions together negatively affect prognosis of these patients, leading to accelerated progression of liver damage, a greater incidence of HCC, and, definitively, higher mortality. In addition, noncompliance with antiviral therapy is a problem with alcoholic patients, further contributing to poor prognosis.

The negative synergism between alcohol use and viral infection is clear, and defining a specific threshold for safe alcohol consumption is difficult and perhaps impossible.

Therefore, in the presence of acute or chronic liver disease, complete abstinence is mandatory.

Although data regarding treatment of HCV positive alcoholics is limited, the use of new antivirals for HCV treatment is promising. It is well known that the available drugs for HBV can control the infection in a large percentage of patients. However, alcohol-related diseases are and will continue to be major clinical problems, also because alcohol constitutes a barrier for antiviral treatment. The cost of HCV treatment will increase in the coming years as the availability of direct antiviral agents for HCV infection will become more widespread. Indeed, to use or not these therapies in patients with alcohol disorder, will be a very complex choice.

In conclusion, given the complexity of this matter, major scientific efforts should focus on the following points of interest:

- increased knowledge of the relationship between HBV and alcohol;
- identification, if possible, of a safe threshold for controlled alcohol consumption;
- consolidation of a multidisciplinary approach for patients with chronic liver disease and alcohol habit;
- major expansion of antiviral access for subjects with alcohol-related health problems;
- overcoming the moral and social stigma of alcoholism, as it is simply a disease.

Conflict of interest

None

Author contributions

Review design (SG, PA), manuscript writing (SG, PA), critical revision (GV, EV, PA).

References

[1] www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/standard-drink. Accessed September 2014.

[2] Suzuki A, Angulo P, Sauver J, Muto A, Okada T, Lindor K. Light to moderate alcohol consumption is associated with lower frequency of hypertransaminasemia. *Am J Gastroenterol* 2007;102:1912–1919. doi: 10.1111/j.1572-0241.2007.01274.x.

[3] World Health Organization. Prevention and control of viral hepatitis infection: framework for global action. Geneva: WHO, 2012.

[4] European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2014;60:392–420. doi: 10.1016/j.jhep.2013.11.003.

[5] European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of chronic hepatitis B virus infection. *J Hepatol* 2012;57:167–185.

[6] Gramenzi A, Caputo F, Biselli M, Kuria F, Loggi E, Andreone P, *et al*. Alcoholic liver disease—pathophysiological aspects and risk factors. *Aliment Pharmacol Ther* 2006;24:1151–1161. doi: 10.1111/j.1365-2036.2006.03110.x.

[7] Maher JJ. Alcoholic liver disease. In: Feldman M, Friedman LS, Sleisenger MH, editors. *Gastrointestinal and Liver Disease Vol II*. Philadelphia: Saunders, 2002:1375–1391. doi: 10.1016/j.jhep.2012.02.010.

[8] Cochrane J, Chen H, Conigrave KM, Hao W. Alcohol use in China. *Alcohol* 2003;38:537–542. doi: 10.1093/alcalc/agg111.

[9] Mueller S, Millonig G, Seitz HK. Alcoholic liver disease and hepatitis C: a frequently underestimated combination. *World J Gastroenterol* 2009;15:3462–3471. doi: 10.3748/wjg.15.3462.

[10] Fan JY, Huang TJ, Jane SW, Chen MY. Prevention of Liver Cancer Through the Early Detection of Risk-Related Behavior Among Hepatitis B or C Carriers. *Cancer Nurs* 2014 May 15.

[11] Singal AK, Anand BS. Mechanisms of synergy between alcohol and hepatitis C virus. *J Clin Gastroenterology* 2007;41:761–772. doi: 10.1097/MCG.0b013e3180381584.

[12] Alter MJ. Epidemiology of hepatitis C virus infection. *World J Gastroenterol* 2007;13:2436–2441. doi: 10.3748/wjg.v13.i17.2436.

[13] Galperim B, Cheinquer H, Stein A, Fonseca A, Lunge V, Ikuta N. Prevalence of hepatitis C virus in alcoholic: patients: role of parenteral risk factors. *Arq Gastroenterol* 2006;43:81–84. doi: 10.1590/S0004-28032006000200004.

[14] Rosman AS, Waraich A, Galvin K, Casiano J, Paronetto F, Lieber CS. Alcoholism is associated with hepatitis C but not hepatitis B in an urban population. *Am J Gastroenterol* 1996; 91:498–505.

[15] Osna NA. Hepatitis C virus and ethanol alter antigen presentation in liver cells. *World J Gastroenterol* 2009;15:1201–1208. doi: 10.3748/wjg.15.1201.

[16] Kharbanda KK, Bardag-Gorce F, Barve S, Molina PE, Osna NA. Impact of altered methylation in cytokine signaling and proteasome function in alcohol and viral mediated diseases. *Alcohol Clin Exp Res* 2013;37:1–7. doi: 10.1111/j.1530-0277.2012.01840.x.

[17] Noh DH, Lee EJ, Kim AY, Lee EM, Min CW, Kang KK, *et al*. Alcohol Induced Hepatic Degeneration in a Hepatitis C Virus Core Protein Transgenic Mouse Model. *Int J Mol Sci* 2014;15:4126–4141. doi: 10.3390/ijms15034126.

[18] Zhang T, Li Y, Lai JP, Douglas SD, Metzger TS, O'Brien CP, *et al*. Alcohol potentiates hepatitis C virus replicon expression. *Hepatology* 2003;38:57–65. doi: 10.1053/jhep.2003.50295.

[19] Trujillo-Murillo K, Alvarez-Martínez O, Garza Rodríguez L, Martínez-Rodríguez H, Bosques-Padilla F, Ramos-Jimenez J, *et al*. Additive effect of ethanol and HCV subgenomic replicon expression on COX-2 protein levels and activity. *J Viral Hepat* 2007;14:608–617. doi: 10.1111/j.1365-2893.2006.00837.x.

[20] McCartney EM, Semendric L, Helbig KJ, Hinze S, Jones B, Weinman SA, *et al*. Alcohol metabolism increases the replication of hepatitis C virus and attenuates the antiviral action of interferon. *J Infect Dis* 2008;198:1766–1775. doi: 10.1086/593216.

[21] Seronello S, Ito C, Wakita T, Choi J. Ethanol enhances hepatitis C virus replication through lipid metabolism and elevated NADH/NAD⁺. *J Biol Chem* 2010;285:845–854. doi: 10.1074/jbc.M109.045740.

[22] Plumlee CR, Lazaro CA, Fausto N, Polyak SJ. Effect of ethanol on innate antiviral pathways and HCV replication in human liver cells. *Virology* 2005;2:89. doi: 10.1186/1743-422X-2-89.

[23] Choi J, Forman HJ, Ou JH, Lai MM, Seronello S, Nandipati A. Redox modulation of the hepatitis C virus replication complex is calcium dependent. *Free Radic Biol Med* 2006;41:1488–1498. doi: 10.1016/j.freeradbiomed.2006.08.008.

[24] Osna NA, Kharbanda KK, Sun Y, Simpson RL, Poluektova LE, Ganesan M, *et al*. Ethanol affects hepatitis C pathogenesis: humanized scid Alb-uPA mouse model. *Biochem Biophys Res Commun* 2014;450:773–776. doi: 10.1016/j.bbrc.2014.06.048.

[25] Yoshihara H, Noda K, Kamada T. Interrelationship between alcohol intake, hepatitis C, liver cirrhosis, and hepatocellular carcinoma. *Recent Dev Alcohol* 1998;14:457–469.

[26] Loguercio C, Di Pierro M, Di Marino MP, Federico A, Disalvo D, Crafa E, *et al*. Drinking habits of subjects with hepatitis C virus-related chronic liver disease: prevalence and effect on clinical, virological and pathological aspects. *Alcohol Alcohol* 2000;35:296–301. doi: 10.1093/alcalc/35.3.296.

[27] Khan KN, Yatsushashi H. Effect of alcohol consumption on the progression of hepatitis C virus infection and risk of hepatocellular carcinoma in Japanese patients. *Alcohol Alcohol* 2000;35:286–295. doi: 10.1093/alcalc/35.3.286.

[28] Romero-Gomez M, Grande L, Nogales MC, Fernandez M, Chavez M, Castro M. Intrahepatic hepatitis C virus replication is increased in patients with regular alcohol consumption. *Dig Liver Dis* 2001;33:698–702. doi: 10.1016/S1590-8658(01)80048-7.

[29] Anand BS, Velez M. Influence of chronic alcohol abuse on hepatitis C virus replication. *Dig Dis* 2000;18:168–171. doi: 10.1159/000051390.

[30] Cromie SL, Jenkins PJ, Bowden DS, Dudley FJ. Chronic hepatitis C: effect of alcohol on hepatic activity and viral titre. *J Hepatol* 1996;25:821–826. doi: 10.1016/S0168-8278(96)80284-4.

[31] Anand BS, Thornby J. Alcohol has no effect on hepatitis C virus replication: a meta-analysis. *Gut* 2005;54:1468–1472. doi: 10.1136/gut.2004.056697.

[32] Larkin J, Clayton MM, Liu J, Feitelson MA. Chronic ethanol consumption stimulates hepatitis B virus gene expression and replication in transgenic mice. *Hepatology* 2001;34:792–797. doi: 10.1053/jhep.2001.27565.

[33] Nalpas B, Pourcel C, Feldmann G, Housset C, Tiollais P, Brechot C, *et al*. Chronic alcohol intoxication decreases the serum level of hepatitis B surface antigen in transgenic mice. *J Hepatol* 1992;15:118–124. doi: 10.1016/0168-8278(92)90022-H.

[34] Otani K, Korenaga M, Beard MR, Li K, Qian T, Showalter LA, *et al*. Hepatitis C virus core protein, cytochrome P450 2E1, and alcohol produce combined mitochondrial injury and cytotoxicity in hepatoma cells. *Gastroenterology* 2005;128:96–107. doi: 10.1053/j.gastro.2004.10.045.

[35] Perlemuter G, Letteron P, Carnot F, Zavala F, Pessayre D, Nalpas B, *et al*. Alcohol and hepatitis C virus core protein additively increase lipid peroxidation and synergistically trigger hepatic cytokine expression in a transgenic

- mouse model. *J Hepatol* 2003;39:1020–1027. doi: 10.1016/S0168-8278(03)00414-8.
- [36] Rigamonti C, Mottaran E, Reale E, Rolla R, Cipriani V, Capelli F, *et al*. Moderate alcohol consumption increases oxidative stress in patients with chronic hepatitis C. *Hepatology* 2003;38:42–49. doi: 10.1053/jhep.2003.50275.
- [37] Min BY, Kim NY, Jang ES, Shin CM, Lee SH, Park YS, *et al*. Ethanol potentiates hepatitis B virus replication through oxidative stress-dependent and -independent transcriptional activation. *Biochem Biophys Res Commun* 2013;431:92–97. doi: 10.1016/j.bbrc.2012.12.081.
- [38] Mas VR, Fassnacht R, Archer KJ, Maluf D. Molecular mechanisms involved in the interaction effects of alcohol and hepatitis C virus in liver cirrhosis. *Mol Med* 2010;16:287–297. doi: 10.2119/molmed.2009.00165.
- [39] Aloman C, Gehring S, Wintermeyer P, Kuzushita N, Wands JR. Chronic ethanol consumption impairs cellular immune responses against HCV NS5 protein due to dendritic cell dysfunction. *Gastroenterology* 2007;132:698–708. doi: 10.1053/j.gastro.2006.11.016.
- [40] Piasecki BA, Lewis JD, Reddy KR, Bellamy SL, Porter SB, Weinrieb RM, *et al*. Influence of alcohol use, race, and viral coinfections on spontaneous HCV clearance in a US veteran population. *Hepatology* 2004;40:892–899. doi: 10.1002/hep.1840400419.
- [41] Gramenzi A, Caputo F, Biselli M, Kuria F, Loggi E, Andreone P, *et al*. Alcoholic liver disease – pathophysiological aspects and risk factors. *Aliment Pharmacol Ther* 2006;24:1151–1161. doi: 10.1111/j.1365-2036.2006.03110.x.
- [42] Zarski JP, Mc Hutchison J, Bronowicki JP, Sturm N, Garcia-Kennedy R, Hodaj E, *et al*. Rate of natural disease progression in patients with chronic hepatitis C. *J Hepatol* 2003;38:307–314. doi: 10.1016/S0168-8278(02)00387-2.
- [43] Tsui JJ, Pletcher MJ, Vittinghoff E, Seal K, Gonzales R. Hepatitis C and hospital outcomes in patients admitted with alcohol-related problems. *J Hepatol* 2006;44:262–266. doi: 10.1016/j.jhep.2005.07.027.
- [44] Singal AK, Kuo YF, Anand BS. Hepatitis C virus infection in alcoholic hepatitis: prevalence patterns and impact on in-hospital mortality. *Eur J Gastroenterol Hepatol* 2012;24:1178–1184. doi: 10.1097/MEG.0b013e328355c0e0.
- [45] Fuster D, Sanvisens A, Bolao F, Serra I, Rivas I, Tor J, *et al*. Impact of hepatitis C virus infection on the risk of death of alcohol-dependent patients. *J Viral Hepat* 2014 Aug 18. doi: 10.1111/jvh.12290.
- [46] Hutchinson SJ, Bird SM, Goldberg DJ. Influence of alcohol on the progression of hepatitis C virus infection: a meta-analysis. *Clin Gastroenterol Hepatol* 2005;3:1150–1159. doi: 10.1016/S1542-3565(05)00407-6.
- [47] Larkin J, Clayton MM, Liu J, Feitelson MA. Chronic ethanol consumption stimulates hepatitis B virus gene expression and replication in transgenic mice. *Hepatology* 2001;34:792–797. doi: 10.1053/jhep.2001.27565.
- [48] Ong A, Wong VW, Wong GL, Chan HL. The effect of caffeine and alcohol consumption on liver fibrosis - a study of 1045 Asian hepatitis B patients using transient elastography. *Liver Int* 2011;31:1047–1053. doi: 10.1111/j.1478-3231.2011.02555.x.
- [49] Marcellin P, Pequinot F, Delarocque-Astagneau E, Zarski JP, Ganne N, Hillon P, *et al*. Mortality related to chronic hepatitis B and chronic hepatitis C in France: evidence for the role of HIV coinfection and alcohol consumption. *J Hepatol* 2008;48:200–207. doi: 10.1016/j.jhep.2007.09.010.
- [50] Zhang M. The presence of hepatitis B core antibody is associated with more advanced liver disease in alcoholic patients with cirrhosis. *Alcohol* 2013;47:553–558. doi: 10.1016/j.alcohol.2013.07.003.
- [51] Fukushima W, Tanaka T, Ohfuji S, Habu D, Tamori A, Kawada N, *et al*. Does alcohol increase the risk of hepatocellular carcinoma among patients with hepatitis C virus infection? *Hepatol Res* 2006;34:141–149. doi: 10.1016/j.hepres.2005.12.003.
- [52] Hassan MM, Hwang LY, Hatten CJ, Swaim M, Li D, Abbruzzese JL, *et al*. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. *Hepatology* 2002;36:1206–1213. doi: 10.1053/jhep.2002.36780.
- [53] Tsutsumi M, Ishizaki M, Takada A. Relative risk for the development of hepatocellular carcinoma in alcoholic patients with cirrhosis: A multiple logistic-regression coefficient analysis. *Alcohol Clin Exp Res* 1996;20:758–762. doi: 10.1111/j.1530-0277.1996.tb01683.x.
- [54] Loomba R, Yang HI, Su J, Brenner D, Illoeje U, Chen CJ. Obesity and alcohol synergize to increase the risk of incident hepatocellular carcinoma in men. *Clin Gastroenterol Hepatol* 2010;8:891–898. doi: 10.1016/j.cgh.2010.06.027.
- [55] Yamada M, Shiroeda H, Hayashi R, Yano H, Sato K, Tsutsumi M, *et al*. Survival rates of early-stage HCV-related liver cirrhosis patients without hepatocellular carcinoma are decreased by alcohol. *J Clin Biochem Nutr* 2011;48:167–169. doi: 10.3164/jcbn.09-119GFR.
- [56] Kubo S, Kinoshita H, Hirohashi K, Tanaka H, Tsukamoto T, Shuto T, *et al*. High malignancy of hepatocellular carcinoma in alcoholic patients with hepatitis C virus. *Surgery* 1997;121:425–429. doi: 10.1016/S0039-6060(97)90313-5.
- [57] Okada S, Ishii H, Nose H, Okusaka T, Kyogoku A, Yoshimori M, *et al*. Effect of heavy alcohol intake on long-term results after curative resection of hepatitis C virus related hepatocellular carcinoma. *Jpn J Cancer Res* 1996;87:867–873.
- [58] Uetake S, Yamauchi M, Itoh S, Kawashima O, Takeda K, Ohata M. Analysis of risk factors for hepatocellular carcinoma in patients with HBs antigen- and anti-HCV antibody-negative alcoholic cirrhosis: clinical significance of prior hepatitis B virus infection. *Alcohol Clin Exp Res* 2003;27(8 Suppl):475–515. doi: 10.1097/01.ALC.0000079449.47468.80.
- [59] Adachi S, Shibuya A, Miura Y, Takeuchi A, Nakazawa T, Saigenji K. Impact of occult hepatitis B virus infection and prior hepatitis B virus infection on development of hepatocellular carcinoma in patients with liver cirrhosis due to hepatitis C virus. *Scand J Gastroenterol* 2008;43:849–856.
- [60] Mochida S, Ohnishi K, Matsuo S, Kakiyama K, Fujiwara K. Effect of alcohol intake on the efficacy of interferon therapy in patients with chronic hepatitis C as evaluated by multivariate logistic regression analysis. *Alcohol Clin Exp Res* 1996;20:371–377. doi: 10.1111/j.1530-0277.1996.tb01811.x.
- [61] Di Bona D, Cippitelli M, Fionda C, Cammà C, Licata A, Santoni A, *et al*. Oxidative stress inhibits IFN-alpha-induced antiviral gene expression by blocking the JAK-STAT pathway. *J Hepatol* 2006;45:271–279. doi: 10.1016/j.jhep.2006.01.037.
- [62] Ohnishi K, Matsuo S, Matsutani K, Itahashi M, Kakiyama K, Suzuki K, *et al*. Interferon therapy for chronic hepatitis C in habitual drinkers: comparison with chronic hepatitis C in infrequent drinkers. *Am J Gastroenterol* 1996;91:1374–1379.
- [63] Bruggmann P, Dampz M, Gerlach T, Kravec Z, Falcato L. Treatment outcome in relation to alcohol consumption during hepatitis C therapy: an analysis of the Swiss Hepatitis C Cohort Study. *Drug Alcohol Depend* 2010;110:167–171. doi: 10.1016/j.drugalcdep.2010.02.016.
- [64] Anand BS, Currie S, Dieperink E, Bini EJ, Shen H, Ho SB, *et al*. Alcohol use and treatment of hepatitis C virus: results of a national multicenter study. *Gastroenterology* 2006; 130:1607–1616. doi: 10.1053/j.gastro.2006.02.023.
- [65] Le Lan C, Guillygomarch A, Danielou H, Le Dreau G, Lainé F, Vedeilhé C, *et al*. A multi-disciplinary approach to treating hepatitis C with interferon and ribavirin in alcohol-dependent patients with ongoing abuse. *J Hepatol* 2012; 56:334–340. doi: 10.1016/j.jhep.2011.05.021.
- [66] Lin CC. Hepatitis C treatment outcome in relation to alcohol consumption and racial differences in southeastern Taiwan. *J Formos Med Assoc* 2014 Apr 21. doi: 10.1016/j.jfma.2014.03.001.
- [67] North CS, Sims O, Hong BA, Jain MK, Brown G, Lisker-Melman M, *et al*. An empirical study of alcohol consumption by patients considering HCV treatment. *Am J Drug Alcohol Abuse* 2014;40:484–489. doi: 10.3109/00952990.2014.945592.
- [68] Dieperink E, Fuller B, Isenhardt C, McMaken K, Lenox R, Pocha C, *et al*. Efficacy of motivational enhancement therapy on alcohol use disorders in patients with chronic hepatitis C: a randomized controlled trial. *Addiction* 2014;109:1869–1877. doi: 10.1111/add.12679.
- [69] Chung WG, Kim HJ, Choe YG, Seok HS, Chon CW, Cho YK, *et al*. Effect of alcohol and obesity on entecavir therapy. *Clin Mol Hepatol* 2012;18:195–202. doi: 10.3350/cmh.2012.18.2.195.
- [70] Spradling PR, Bulkow L, Teshale EH, Negus S, Homan C, Simons B, *et al*. Prevalence and causes of elevated serum aminotransferase levels in a population-based cohort of persons with chronic hepatitis B virus infection. *J Hepatol* 2014 Jun 6.
- [71] Papatheodoridis GV, Tsochatzis E, Hardtke S, Wedemeyer H. Barriers to care and treatment for patients with chronic viral hepatitis in Europe: a systematic review. *Liver Int* 2014;34:1452–1463. doi: 10.1111/liv.12565.
- [72] Antonucci G, Mazzotta F, Puoti M, Angeletti C, Girardi E, Santantonio T, *et al*. Factors associated with access to antiviral treatment in a multicentre cross-sectional study of patients with chronic hepatitis B in Italy. *J Viral Hepat* 2012;19:881–889. doi: 10.1111/j.1365-2893.2012.01615.x.