Microbiota Gut–Liver Axis during HCV Chronic Infection

Subjects: Microbiology

Contributor: Nadia Marascio , Carmen De Caro , Angela Quirino , Maria Mazzitelli , Emilio Russo , Carlo Torti , Giovanni Matera

Hepatitis C virus (HCV) still represents one of the most important worldwide health care problems. Since 2011, direct-acting antiviral (DAA) drugs have increased the number of people who have achieved a sustained virological response (SVR). Even if the program to eradicate HCV by 2030 is still ongoing, the SARS-CoV-2 pandemic has created a delay due to the reallocation of public health resources. HCV is characterized by high genetic variability and is responsible for hepatic and extra-hepatic diseases. Depending on the HCV genotype/subtype and comorbidities of patients, tailored treatment is necessary.

HCV microbiota gut–liver axis dysbiosis

1. HCV Infection: Epidemiology and Pathogenesis

To date, HCV infection remains a major global health issue. Estimates from the WHO count more than 58 million people having a chronic HCV infection, with a further 1.5 million new infections occurring every year [1]. The same estimates report almost 300,000 deaths due to HCV complications, such as liver cirrhosis (LC) or cancer [1]. Regions with the highest burden of disease are the Mediterranean areas, Southeast Asia, Africa, and some regions of the Americas [1].

HCV is a blood-borne RNA virus, lacking a proofreading activity during its replication, which thereby increases the likelihood of viral mutations and pathogenicity [2]. There are eight main genotypes and numerous subtypes, whose prevalence is largely geographically differentiated. HCV1 is very common across Europe and the United States, whereas HCV2, HCV4, and HCV5 genotypes are very common in African countries, and HCV2 and HCV6 are mainly predominant in Asia [3]. HCV7 is responsible for less than 1% of total HCV infections, and HCV8 was identified for the first time in patients living in Canada [4].

HCV transmission occurs by four main routes: blood transfusion, sharing of unsafe needles, syringes among intravenous drug users, unprotected sexual intercourse, and vertical transmission [5]. Although transmission by the vertical route and blood products significantly decreased over the last few decades, it is increasing among those who experience unprotected sex (especially among men who have sex with men) and intravenous drug abusers [6]. The risk of transmission is further increased by the presence of other viral or bacterial sexually transmitted infections, such as human immunodeficiency virus (HIV), syphilis, and gonorrhoea [6].

The possible evolution of HCV primary infection is related mainly to viral characteristics and host factors [7]. In most cases, primary infection is asymptomatic or has a non-specific onset symptomatology, whereas only a minority of acute cases are serious, leading, in the worst-case scenario, to fulminant hepatitis [8]. In 15–25% of cases the infection clears spontaneously, whereas in 75–85% it becomes chronic, especially in subjects who have some risk factors (e.g., HIV co-infection) for the inability of the immune system to clear it [8]. This also happens for the ability of HCV to counteract the retinoic acid-inducible gene-1 (RIG-1) pathway and to evade the immune challenge [9][10]. Liver disease progression is still being debated and seems to be related to specific host risk factors, such as age or alcohol abuse [11]. Although HCV has a major liver tropism, chronic infection may result in systemic disease involving several other systems [12]. It is often associated with weight loss, fatigue, nausea, abdominal pain, neuropsychiatric symptoms (such as depression), lympho-prolypherative disorders (e.g., B cell non-Hodgkin lymphoma), renal diseases, diabetes, cardiovascular and cerebrovascular diseases, and cryoglobulinaemic vasculitis [12]. HCV pathogenesis is associated with viral variability. The HCV3 genotype, inducing lipid accumulation, is significantly associated with steatosis compared to the HCV1 genotype [13]. The HCV1b subtype is capable of establishing chronic viral infection, while both HCV1 and HCV2 genotypes enhance the risk of kidney disease [4][14].

2. HCV Infection Effect on the Gut–Liver Axis

The gut–liver axis represents the link between the gut microbiota and the liver, where both communicate via the portal vein, systemic circulation, and biliary tract [15]. The portal vein provides about 70% of the blood to the liver, transporting nutrients and metabolites from the gut to the liver [16]. However, this route also transports toxic products such as: peptidoglycans, endotoxins or intact bacteria, which may disrupt the liver's metabolic functions [17]. Furthermore, the liver is responsible for bile acid (BAs) synthesis from cholesterol via 17 liver enzymes which are secreted in the biliary tract and reach the small intestine via the duodenum, combining with other components along the biliary tract and enabling the emulsification, digestion, and absorption of dietary fats. BAs are known to be significant regulators of lipid metabolism, glucose and energy homeostasis and are also involved in the regulation and communication of the gut–liver axis [18]. Approximately 95% of bile acids are reabsorbed at the terminal ileum level and return to the liver via the hepatic portal vein (the enterohepatic circulation) [19]. The residual 5% of BAs are deconjugated, dehydroxylated, and dehydrogenated by the colonic microbiota and progress to secondary bile acids (deoxycholic acid, lithocholic acid, and ursodeoxycholic acid) that arrive at the liver and subsequently the portal circulation through passive absorption [20]. This conversion is mediated by different gut bacteria, mainly *Clostridiales* [21]. Bile acids have several roles: food digestion, integrity of the gut mucosa, and antimicrobial activity against pathogens [22].

HCV alters the pathophysiology of the liver and decreases bile production, which is reflected in pro-inflammatory bacterial overgrowth and in the microbial community [23]. BA dysregulation plays an important role in the progression of cirrhosis to liver cancer [24]. HCV infection induces an unfavorable gut microenvironment and the reduction of *Ruminococcaceae* and *Lachnospiraceae*, which produce fecal short-chain fatty acids (SCFAs) crucial to maintaining metabolic homeostasis, integrity of the intestinal barrier and differentiation of Treg cells [25][26]. On

the other hand, HCV infects gut B-lymphocytes decreasing IgA levels and increasing intestinal permeability, thereby allowing bacterial translocation [27]. The increase in intestinal permeability and the transition of lipopolysaccharide (LPS)-generated liver inflammatory reactions through TLR4, promotes HCC progression, especially in subjects with chronic alcohol consumption [28]. High levels of cytokines, IgA and T cells during chronic HCV infection can control the gut community diversity; in particular, the abundance of *Prevotella* appears to be related to inflammatory mediator IL-17 [29].

3. Gut Microbiota and HCV Therapy

Until 2011, PEGylated-interferon (PEG-IFN) plus ribavirin (RBV) led to sustained virological response (SVR) with a success rate of about 54–56% in HCV patients. HCV genotype/subtype was a predictive parameter for SVR [30]. In the last nine years, anti-HCV therapy has improved due to the availability of direct-acting antiviral (DAA) drugs, which replaced in clinical practice the standard of care (SOC) treatment [31]. The NS3/4A, NS5A, and NS5B polymerase nonstructural proteins (NSs) are the direct targets of therapy [32]. NSs are important for viral replication, NS3 protease, and its cofactor NS4A, which catalyzes cleavage of viral polyprotein. NS5B is the RNA-dependent RNA polymerase (RdRp). NS5A contributes to viral replication and interacts with IFN-alpha protein kinase [33]. Approximately 5% of DAA treated patients do not achieve SVR due to resistance-associated substitutions (RASs) specific for each genotype/subtype. RASs can be selected by drug pressure on genomic target regions [34].

Recently, to avoid impact of treatment, the gut microbiota in a cohort of naïve patients was analyzed at the time of diagnosis. In contrast with previous literature, HCV infection in treatment-naive patients was associated with increased diversity of microbiota and the depletion of *Bacteroidetes* phyla and *Streptococcus* genus [35]. These contrasting results are probably due to the stages of disease analyzed, antiviral treatment, HCV genotypes, and demographic characteristics of the cohort. However, HCV-associated dysbiosis could be mitigated by modulating the gut microbial community to prevent a more severe illness [35].

4. Gut Microbiota and Therapeutic Manipulation

HCV eradication achieving SVR improves inflammation and intestinal dysbiosis in the majority of treated patients. Current DAA therapy could be potentiated in order to improve control of extrahepatic and liver-associated complications, by using probiotics, prebiotics, or an appropriate diet [35][36]. Dietary food intake is the first cause of changes in the intestinal flora. Gut microbiota can rapidly change its composition under specific dietary pressure. An animal-based diet, for instance, decreases the abundance of *Firmicutes* and increases the prevalence of bile-tolerant microorganisms [37].

The effects of probiotics and prebiotics are mainly reported in animal models. Probiotics have a beneficial effect on liver disease, *Lactobacillus casei* reduces plasma levels of LPS-binding protein (LBP). *Bifidobacterium* decreases fat accumulation in the liver. In patients with LC, a combination of *Lactobacillus* spp., *Bifidobacterium* spp., and *Streptococcus* spp. is effective in preventing secondary hepatic encephalopathy [38]. The prebiotic fructo-

oligosaccharides (FOSs) restore gut microbiota composition and intestinal barrier function. Lactulose increases the growth of *Bifidobacterium* and decreases LPS in serum [38]. Fecal microbiota transplantation (FMT) is able to improve gut dysbiosis and reduce hospitalization in patients with LC [39]. In addition, FMT following rifaximin antibiotic therapy could remove *S. salivarius* and increase abundance of healthy microbiome for patients in broad clinical stages. Rifaximin reduces endotoxemia, secondary bile acids, and harmful metabolite levels [40]. To cure dysbiosis by using classical approaches, next-generation phage therapy has been proposed. Clinical trials are underway to reduce pathogenic bacterial species in the gut community [41].

References

1. World Health Organization (WHO). Available online: https://www.who.int (accessed on 5 August 2022).
2. Yamane, D.; McGivern, D.R.; Masaki, T.; Lemon, S.M. Liver injury and disease pathogenesis in chronic hepatitis C. Curr. Top. Microbiol. Immunol. 2013, 369, 263–288.
3. Gower, E.; Estes, C.; Blach, S.; Razavi-Shearer, K.; Razavi, H. Global epidemiology and genotype distribution of the hepatitis C virus infection. J. Hepatol. 2014, 61 (Suppl. 1), S45–S57.
4. Marascio, N.; Rotundo, S.; Quirino, A.; Matera, G.; Liberto, M.C.; Costa, C.; Russo, A.; Trecarichi, E.M.; Torti, C. Similarities, differences, and possible interactions between hepatitis E and hepatitis C viruses: Relevance for research and clinical practice. World J. Gastroenterol. 2022, 28, 1226–1238.
5. Soza, A.; Riquelme, A.; Arrese, M. Routes of transmission of hepatitis C virus. Ann Hepatol. 2010, 9 (Suppl. 1), 33.
6. Nijmeijer, B.M.; Koopsen, J.; Schinkel, J.; Prins, M.; Geijtenbeek, T.B. Sexually transmitted hepatitis C virus infections: Current trends, and recent advances in understanding the spread in men who have sex with men. J. Int. AIDS Soc. 2019, 22 (Suppl. 6), e25348.
7. Negro, F. Natural History of Hepatic and Extrahepatic Hepatitis C Virus Diseases and Impact of Interferon-Free HCV Therapy. Cold Spring Harb. Perspect. Med. 2020, 10, a036921.
8. Jackowiak, P.; Figlerowicz, M.; Kurzyńska-Kokorniak, A.; Figlerowicz, M. Mechanisms involved in the development of chronic hepatitis C as potential targets of antiviral therapy. Curr Pharm. Biotechnol. 2011, 12, 1774–1780.
9. Schoggins, J.W.; Rice, C.M. Innate immune responses to hepatitis C virus. Curr. Top. Microbiol. Immunol. 2013, 369, 219–242.
10. Irshad, M.; Mankotia, D.S.; Irshad, K. An insight into the diagnosis and pathogenesis of hepatitis C virus infection. World J. Gastroenterol. 2013, 19, 7896–7909.

11. Vento, S.; Nobili, V.; Cainelli, F. Clinical course of infection with hepatitis C is still poorly understood. BMJ 2006, 332, 374–375.
12. Tang, L.; Marcell, L.; Kottilil, S. Systemic manifestations of hepatitis C Infection. Infect Agent Cancer. 2016, 11, 29.
13. Janiak, M.; Perlejewski, K.; Grabarczyk, P.; Kubicka-Russel, D.; Zagordi, O.; Berak, H.; Osuch, S.; Pawełczyk, A.; Bukowska-Ośko, I.; Płoski, R.; et al. Hepatitis C virus (HCV) genotype 1b displays higher genetic variability of hypervariable region 1 (HVR1) than genotype 3. Sci. Rep. 2019, 9, 12846.
14. Goel, A.; Bhadauria, D.S.; Aggarwal, R. Hepatitis C virus infection and chronic renal disease: A review. Indian J. Gastroenterol. 2018, 37, 492–503.
15. Tremaroli, V.; Bäckhed, F. Functional interactions between the gut microbiota and host metabolism. Nature 2012, 489, 242–249.
16. Cremer, J.; Arnoldini, M.; Hwa, T. Effect of water flow and chemical environment on microbiota growth and composition in the human colon. Proc. Natl. Acad. Sci. USA 2017, 114, 6438–6443.
17. Li, H.; Gao, Z.; Zhang, J.; Ye, X.; Xu, A.; Ye, J.; Jia, W. Sodium butyrate stimulates expression of fibroblast growth factor 21 in liver by inhibition of histone deacetylase 3. Diabetes 2012, 61, 797–806.
18. Brandl, K.; Kumar, V.; Eckmann, L. Gut-liver axis at the frontier of host-microbial interactions. Am. J. Physiol. Gastrointest. Liver Physiol. 2017, 312, G413–G419.
19. Chiang, J.Y.L.; Ferrell, J.M. Bile Acid Metabolism in Liver Pathobiology. Gene Expr. 2018, 18, 71–87.
20. Wahlström, A.; Kovatcheva-Datchary, P.; Ståhlman, M.; Bäckhed, F.; Marschall, H.-U. Crosstalk between Bile Acids and Gut Microbiota and Its Impact on Farnesoid X Receptor Signalling. Dig. Dis. 2017, 35, 246–250.
21. Ridlon, J.M.; Kang, D.-J.; Hylemon, P.B. Bile salt biotransformations by human intestinal bacteria. J. Lipid Res. 2006, 47, 241–259.
22. Swann, J.R.; Want, E.J.; Geier, F.M.; Spagou, K.; Wilson, I.D.; Sidaway, J.E.; Nicholson, J.K.; Holmes, E. Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. Proc. Natl. Acad. Sci. USA 2011, 108 (Suppl. 1), 4523–4530.
23. Duboc, H.; Rajca, S.; Rainteau, D.; Benarous, D.; Maubert, M.A.; Quervain, E.; Thomas, G.; Barbu, V.; Humbert, L.; Despras, G.; et al. Connecting dysbiosis, bile-acid dysmetabolism and gut inflammation in inflammatory bowel diseases. Gut 2013, 62, 531–539.
24. Paul, B.; Lewinska, M.; Andersen, J.B. Lipid alterations in chronic liver disease and liver cancer. JHEP Rep. 2022, 4, 100479.

25. El-Mowafy, M.; Elgaml, A.; El-Mesery, M.; Sultan, S.; Ahmed, T.A.E.; Gomaa, A.I.; Aly, M.; Mottawea, W. Changes of Gut-Microbiota-Liver Axis in Hepatitis C Virus Infection. Biology 2021, 10, 55.

26. Inoue, T.; Nakayama, J.; Moriya, K.; Kawaratani, H.; Momoda, R.; Ito, K.; Iio, E.; Nojiri, S.; Fujiwara, K.; Yonedaet, M.; et al. Gut Dysbiosis associated with hepatitis C virus infection. Clin. Infect. Dis. 2018, 67, 869–877.

27. Preveden, T.; Scarpellini, E.; Milic, N.; Luzza, F.; Abenavoli, L. Gut microbiota changes and chronic hepatitis C virus infection. Expert Rev. Gastroenterol. Hepatol. 2017, 11, 813–819.

28. Gupta, H.; Youn, G.S.; Shin, M.J.; Suk, K.T. Role of Gut Microbiota in Hepatocarcinogenesis. Microorganisms 2019, 7, 121.

29. Aly, A.M.; Adel, A.; El-Gendy, A.O.; Essam, T.M.; Aziz, R.K. Gut microbiome alterations in patients with stage 4 hepatitis C. Gut Pathog. 2016, 8, 42.

30. Manns, M.P.; McHutchison, J.G.; Gordon, S.C.; Rustgi, V.K.; Shiffman, M.; Reindollar, R.; Goodman, Z.D.; Koury, K.; Ling, M.; Albrecht, J.K. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: A randomised trial. Lancet 2001, 358, 958–965.

31. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2016. J. Hepatol. 2017, 66, 153–194.

32. Marascio, N.; Quirino, A.; Barreca, G.S.; Galati, L.; Costa, C.; Pisani, V.; Mazzitelli, M.; Matera, G.; Liberto, M.C.; Focà, A.; et al. Discussion on critical points for a tailored therapy to cure hepatitis C virus infection. Clin. Mol. Hepatol. 2019, 25, 30–36.

33. Bartenschlager, R.; Penin, F.; Lohmann, V.; André, P. Assembly of infectious hepatitis C virus particles. Trends Microbiol. 2011, 19, 95–103.

34. Pawlotsky, J.M. Hepatitis C virus resistance to direct-acting antiviral drugs in interferon-free regimens. Gastroenterology 2016, 151, 70–86.

35. Sultan, S.; El-Mowafy, M.; Elgaml, A.; El-Mesery, M.; El Shabrawi, A.; Elegezy, M.; Hammami, R.; Mottawea, W. Alterations of the Treatment-Naive Gut Microbiome in Newly Diagnosed Hepatitis C Virus Infection. ACS Infect. Dis. 2021, 7, 1059–1068.

36. Pérez-Matute, P.; Íñiguez, M.; Villanueva-Millán, M.J.; Recio-Fernández, E.; Vázquez, A.M.; Sánchez, S.C.; Morano, L.E.; Oteo, J.A. Short-term effects of direct-acting antiviral agents on inflammation and gut microbiota in hepatitis C-infected patients. Eur. J. Intern. Med. 2019, 67, 47–58.

37. David, L.A.; Maurice, C.F.; Carmody, R.N.; Gootenberg, D.B.; Button, J.E.; Wolfe, B.E.; Ling, A.V.; Devlin, A.S.; Varma, Y.; Fischbach, M.A.; et al. Diet rapidly and reproducibly alters the human gut

microbiome. Nature 2014, 505, 559–563.

38. Wang, L.; Wan, Y.Y. The role of gut microbiota in liver disease development and treatment. Liver Res. 2019, 3, 3–18.
39. Bajaj, J.S.; Kassam, Z.; Fagan, A.; Gavis, E.A.; Liu, E.; Cox, I.J.; Kheradman, R.; Heuman, D.; Wang, J.; Gurry, T.; et al. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: A randomized clinical trial. Hepatology 2017, 66, 1727–1738.
40. Bajaj, J.S.; Heuman, D.M.; Sanyal, A.J.; Hylemon, P.B.; Sterling, R.K.; Stravitz, R.T.; Fuchs, M.; Ridlon, J.M.; Daita, K.; Monteith, P.; et al. Modulation of the metabiome by rifaximin in patients with cirrhosis and minimal hepatic encephalopathy. PLoS ONE 2013, 8, e60042.
41. Stern, J.; Miller, G.; Li, X.; Saxena, D. Virome and bacteriome: Two sides of the same coin. Curr. Opin. Virol. 2019, 37, 37–43.