AP&T Alimentary Pharmacology and Therapeutics

Review article: alcohol and gut microbiota - the possible role of gut microbiota modulation in the treatment of alcoholic liver disease

G. Vassallo[a], A. Mirijello[a], A. Ferrulli, M. Antonelli, R. Landolfi, A. Gasbarrini & G. Addolorato

Department of Internal Medicine, Catholic University of Rome, Rome, Italy.

Correspondence to:
Dr G. Addolorato, Department of Internal Medicine, Catholic University of Rome, Gemelli Hospital, Largo Gemelli 8, Rome 00168, Italy.
E-mail: g.addolorato@rm.unicatt.it

[a]Both Authors have equally contributed to this work

Publication data
Submitted 3 July 2014
First decision 25 July 2014
Resubmitted 31 January 2015
Accepted 27 February 2015
EV Pub Online 23 March 2015

This uncommissioned review article was subject to full peer-review.

SUMMARY

Background
Alcohol abuse represents the most common cause of liver disease in the Western countries. Pre-clinical and clinical studies showed that alcohol consumption affects amount and composition of gut microbiota. Moreover, gut flora plays an important role in the pathogenesis of alcoholic liver injury.

Aim
To review the relationship between alcohol administration and changes on gut microbiota, its involvement in the pathogenesis of alcoholic liver disease, and how gut microbiota modulation could be a target for the treatment of alcoholic liver disease.

Methods
Articles were identified using the PubMed database with the search terms 'Alcohol', 'Gut Microbiota', 'Alcoholic liver disease', 'Probiotic', 'Prebiotic', 'Symbiotic' and 'Antibiotic'. English-language articles were screened for relevance. Full review of publications for the relevant studies was conducted, including additional publications that were identified from individual article reference lists.

Results
Alcohol abuse induces changes in the composition of gut microbiota, although the exact mechanism for this alteration is not well known. The translocation of bacterial products into the portal blood appears to play a key role in alcohol-induced liver damage. Several studies show that the modulation of gut microbiota seem to be a promising strategy to reduce alcohol-induced liver injury.

Conclusions
Further studies are needed to better understand the relationship between alcohol administration and changes in gut microbiota, and its involvement in alcoholic liver disease. Moreover larger studies are needed to confirm the preliminary results on the therapeutic effects of gut microbiota modulation.

Aliment Pharmacol Ther 2015; **41:** 917–927

doi:10.1111/apt.13164

INTRODUCTION

Alcohol abuse represents a risk factor for a number of diseases and a considerable contributor to the global burden of disease.[1] Harmful alcohol use is recognised to be the most common cause of alcoholic liver disease (ALD) and liver cirrhosis in the Western countries.[2] The progression of alcoholic liver disease is influenced by several factors (i.e. duration of alcohol abuse, drinking pattern and alcohol amount, nutritional status, gender, ethnicity, iron overload, co-existing metabolic syndrome, chronic hepatitis B and C virus co-infections and polymorphisms of genes involved in alcohol metabolism).[3] Among these, recent studies have suggested that gut flora could play an important role in the pathophysiology of alcoholic liver injury and other alcohol-related diseases.[4, 5]

It is well known that alcohol leads to mucosal damage, quantitative and qualitative alterations of gut flora (i.e. small intestinal bacterial overgrowth and dysbiosis), and increased gut permeability, resulting in the translocation of endotoxins and other bacterial products into portal blood flow.[6] Bacterial products stimulate the release of pro-inflammatory mediators such as reactive oxygen species (ROS), leukotrienes, chemokines and cytokines (e.g. TNF-α and IL-1β) exerting injurious effects to cells resulting in inflammatory infiltration and fibrosis in the liver, and possibly in other organs.[7]

The aim of this review was to discuss the effects of chronic alcohol consumption on gut microbiota composition and the relationship between gut bacterial-derived products and ALD. The modulation of gut flora as a target for the treatment of ALD and for other alcohol-related diseases will also be discussed.

Alcohol-induced changes on gut microbiota

The gut of normal human subjects is a perfect habitat for various types of bacteria. The term 'gut microbiota' refers to a complex mixture of diverse microbes present in the gastrointestinal lumen. It consists of approximately 10^{14} microbial cells,[8] which is 10 times the number of somatic cells in the human body.[9, 10] Their collective genome, named 'microbiome', contains at least 100 times as many genes as our own genome.[11] About 98% of the intestinal microbiota belongs to one of the four bacterial phyla, namely *Firmicutes, Bacteroidetes, Proteobacteria* and *Actinobacteria*,[12, 13] with little representation of other bacterial phyla.[8] Some of these bacteria, such as Lactobacilli and Bifidobacteria (>85% of total bacteria), live in a commensalism state, while others, such as *Clostridium* and *Fusobacterium*, could potentially become pathogens.[14] Fungi, protozoa, archaea and viral particles are also represented in microbiota.[12] These microorganisms start colonising the gut after birth, with a succession of different populations until a stable microbiota has been established.[15]

The exact role of microbiota is still largely unknown. Although some functions have already been demonstrated (i.e. catabolism of dietary fibre, maintenance of barrier functions, vitamin synthesis, drug and toxin metabolism and behavioural conditioning),[12, 16] this number is still growing. In particular, it seems that gut microbiota could be involved in inhibiting the growth of pathogenic bacteria, in stimulating the production of total and pathogen-specific mucosal IgA, in the production of nutrients for mucosal cells, development and modulation of immune system and immunological tolerance.[12, 17] Thus, gut microbiota seems to be involved in a bi-directional interaction with host, acting as a 'superorganism' in the maintenance of human homeostasis.[14] Moreover, several diseases seem to be associated to the alteration of this equilibrium.[12, 14]

Bacterial species and concentration vary along gastrointestinal tract. There are inter-individual variations related to age, dietary habits, geographical origin, stress and lifestyle and intra-individual variations related to periods of illness, medications or dietary changes.[16, 18] On this connection, both pre-clinical and clinical studies showed a relationship between alcohol administration and modification of gut microbiota.[6]

Alteration of gut microbiota in pre-clinical studies

Recent advances in the understanding of the effects of alcohol administration on the amount and composition of gut microbiota have emerged from animal studies (Table 1). Mutlu and colleagues studied the composition of gut microbiota in two groups of rats after a period of 10 weeks intragastric administration of alcohol or dextrose. Ileal and colonic mucosal microbiota composition was analysed with Length Heterogeneity PCR (LH-PCR) fingerprinting technique. A significant alteration of colonic microbiota (dysbiosis) was found in alcohol-fed rats with respect to dextrose-fed rats. Moreover alcohol-induced colonic dysbiosis was prevented by the administration of lactobacillus GG or oats.[19] Using an interesting design based on the genetic analysis of gut microbiome, Yan and colleagues investigated the changes in the intestinal microbial community in a group of alcohol-fed rats compared to controls after 3 weeks of alcohol consumption. They observed a bacterial overgrowth in the proximal small intestine, dysbiosis and suppression of Reg3b and Reg3g anti-microbial

Aliment Pharmacol Ther 2015; 41: 917–927

Table 1 | Pre-clinical studies evaluating the relationship between alcohol consumption and gut microbiota alterations

Authors	Number of animals enrolled	Type of sample	Method	Results
Mutlu *et al.*[19]	18 alcohol-fed rats 15 control rats	Ileal and colonic sample	Ileal and colonic mucosal-attached microbiota composition were interrogated by Length Heterogeneity PCR (LH-PCR) fingerprinting	Altered mucosa-associated microbiota composition in the colon at 10 weeks of daily alcohol gavage Both Lactobacillus GG and oats prevented alcohol-induced dysbiosis up to 10 weeks of alcohol treatment
Yan *et al.*[20]	Not available	Small and large intestine	Quantitative changes in the intestinal microflora of these animals were assessed first using conventional culture techniques and pyrosequencing of the intestinal contents	Intestinal bacterial overgrowth was observed in the gastrointestinal tract of mice fed alcohol for 3 weeks compared with control mice fed an isocaloric liquid diet Sequencing of 16S ribosomal RNA genes revealed a relative abundance of Bacteroidetes and Verrucomicrobia bacteria in mice fed alcohol compared with a relative predominance of Firmicutes bacteria in control mice Alcohol feeding was associated with down-regulation in gene and protein expression of bactericidal c-type lectins Reg3b and Reg3g in the small intestine Treatment with prebiotics partially restored Reg3g protein levels, reduced bacterial overgrowth and lessened alcoholic steatohepatitis
Bull-Otterson *et al.*[25]	Eight alcohol-fed mice eight control mice	Faecal samples	Metagenomic analysis of the gut microbiome was performed by analysing the faecal DNA by amplification of the V3-V5 regions of the 16S rRNA gene and large-scale parallel pyrosequencing on the 454 FLX Titanium platform	Chronic ethanol feeding caused a decline in the abundance of both Bacteriodetes and Firmicutes phyla, with an increase in the gram-negative Proteobacteria and gram-positive Actinobacteria phyla Ethanol caused an increase in plasma endotoxin, faecal pH, hepatic inflammation and injury. The ethanol-induced pathogenic changes in the microbiome and the liver were prevented by Lactobacillus GG supplementation
Campos Canesso *et al.*[26]	10–14 alcohol-fed mice 10–14 control mice	Contents of the small intestines	Contents of the small intestines were assessed collecting the contents of the small intestines and using conventional culture techniques	Alcohol consumption increased the number of cultivable bacteria observed based on the CFUs counted There was no liver injury after alcohol consumption, and there was less neutrophil infiltration and lower pro-inflammatory cytokine levels in the liver in germ-free mice compared with alcohol-fed conventional mice Conventionalisation of germ-free mice with intestinal contents from alcohol-fed conventional mice induced injury and inflammation in both the liver and the intestine Previous treatment with a high-fibre diet decreased liver injury and gut permeability in alcohol-fed conventional mice

molecules.[20] The reduction in anti-microbial proteins Reg3b and Reg3g following chronic alcohol exposure might contribute to the enteric dysbiosis, observed after alcohol consumption. The same authors observed a relative abundance of *Bacteroidetes* and *Verrucomicrobia* in alcohol-fed mice compared with a relative predominance of *Firmicutes* in control mice.[20] Moreover, an overgrowth of *Akkermansia muciniphila* was observed in alcohol-fed mice. Since this microbe is able to degrade mucin,[21, 22] some authors hypothesised that it could promote bacterial translocation (one of the factors involved in alcohol liver injury).[23] Moreover, Yan and colleagues in the same study showed that the population of *Lactobacilli* was depleted in alcohol-treated mice.[20] This last evidence could explain the beneficial effect of probiotics in the prevention of increased gut permeability, endotoxinemia and liver injury reported in alcohol-fed mice.[24] In contrast with previous data a study based on metagenomic analysis of the gut microbiome showed a decline of both *Bacteriodetes* and *Firmicutes* phyla in alcohol-fed mice, with an increase in the Gram-negative *Proteobacteria* and Gram-positive *Actinobacteria* phyla.[25] The bacterial genera that showed the biggest expansion were the Gram-negative *Alcaligenes* and Gram-positive *Corynebacterium.*[25]

Recently, Campos Canesso and co-workers compared germ-free mice with conventional mice in an acute alcohol intake model (administration of ethanol in their drinking water for 7 days, with a higher dose of alcohol administered on day 7). Alcohol consumption produced intestinal bacterial overgrowth and dysbiosis in conventional mice with a prevalence of *Enterobacteriaceae*. Moreover, germ-free mice showed a reduced liver pathology (lipid content) after alcohol administration compared with conventional mice. Interestingly, the administration of intestinal contents from conventional mice to germ-free mice induced inflammation in the small intestine and the liver. Finally, the administration of fibres reduced gut permeability and protected conventional mice from liver injury after acute alcohol consumption.[26]

Alteration of gut microbiota in human studies

Few studies investigated the relationship between alcohol abuse and changes in human gut microbiota (Table 2). Bode and colleagues evaluated types and numbers of bacteria examined in aspirates from the jejunum of alcoholic and hospitalised control patients. An increased number of microorganisms was found in chronic alcoholic patients, suggesting that bacterial overgrowth might contribute to functional and/or morphological abnormalities of the small intestine, commonly found in alcoholic patients.[27] In a subsequent study, the same authors evaluated, by using hydrogen breath test, small intestine bacterial overgrowth (SIBO) in chronic alcoholics ($n = 45$) and in subjects without history of alcohol abuse ($n = 60$).[28] A significant higher prevalence of SIBO in alcoholics with respect to controls was found. No differences were found in the prevalence of SIBO in alcoholic patients with liver cirrhosis compared to alcoholic patients without liver cirrhosis.[28] These results have been confirmed by Hauge and colleagues in alcoholic patients admitted to hospital for detoxification who underwent upper gastrointestinal endoscopy with gastric and duodenal biopsies for bacteriological culture. A high prevalence of bacterial overgrowth (Gram-positive aerobic cocci) in the upper gastrointestinal tract was found in alcoholic patients.[29] According to the authors, bacterial overgrowth could explain, at least in part, the gastrointestinal symptoms frequently complained by alcoholic patients (i.e. diarrhoea, nausea and abdominal pain).[29] The same group of researchers have already demonstrated that the prevalence of *Helicobacter pylori* infection did not differ between alcoholic patients and controls.[30] This result was confirmed by Buzás in a larger sample study [31]

Casafont Morencos and colleagues compared 89 patients with alcoholic cirrhosis and 40 healthy subjects to assess the prevalence of intestinal bacterial overgrowth by H2-glucose breath test. Intestinal bacterial overgrowth was documented approximately in one-third of patients with alcoholic cirrhosis while in none of the healthy subjects.[32] Moreover the prevalence of intestinal bacterial overgrowth was significantly higher in decompensated patients. The prevalence of spontaneous bacterial peritonitis was significantly higher in patients who had intestinal bacterial overgrowth than in patients who did not.[32] In these patients, bacterial overgrowth may be a condition pre-disposing to the infection of the ascitic fluid.[32] Some authors speculated that bacterial overgrowth in the upper small intestine might contribute to mucosal damage and affect the absorption of macro- and micronutrients in alcoholic patients.[6]

In a recent retrospective chart review, Gabbard and colleagues confirmed a higher prevalence of SIBO in patients with moderate alcohol consumption with respect to teetotaler patients, using H2- lactulose breath test.[33]

Besides quantitative changes of gut microbiota (SIBO), chronic alcohol consumption has been also associated to qualitative microbiota changes, such as dysbiosis, a con-

Table 2 | Clinical studies evaluating the relationship between alcohol consumption and gut microbiota alterations

Authors	Number of patients enrolled	Type of sample	Method	Results
Bode *et al.*[27]	27 alcoholic patients 13 control patients	Jejunal juice	Samples of jejunal juice were aspirated in the fasting state and were analysed using conventional culture techniques	The mean number of aerobic and anaerobic microorganisms was higher in alcoholic patients Significant counts of bacteria obtained during anaerobic incubation were more frequent in the alcoholics than in the controls Coliform microorganisms were cultured much more frequently from the jejunal fluid of alcoholic patients The incidence of Gram-negative anaerobic bacteria and endospore-forming rods was higher in the aspirates from alcoholic patients In both groups, the number of microorganisms in jejunal fluid correlated closely with the pH found in the gastric juice No correlation was found between the numbers or types of microorganisms in the jejunum and the type or degree of liver disease in alcoholic patients
Bode *et al.*[28]	45 alcoholic patients 60 control patients	Expired air	The hydrogen breath test has been used to investigate the incidence of small-bowel bacterial overgrowth	The percentage of cases with bacterial overgrowth in the group of patients with alcoholic liver disease was almost three times that of controls not abusing alcohol No significant difference in the incidence of bacterial overgrowth among alcoholic patients with cirrhosis and alcoholic patients without cirrhosis Among patients with alcoholic liver disease, the mouth-to-caecum transit time was prolonged by 21.5% in comparison with controls not abusing alcohol
Hauge *et al.*[30]	24 alcoholic patients 12 control patients	Gastric biopsies	Samples were collected using upper gastrointestinal endoscopy. Histological examination, microbial culture and direct microscopy were used to detect *Helicobacter pylori*	*Helicobater pylori* infection was not more frequent in alcoholic patients than in controls
Casafont Morencos *et al.*[32]	89 alcoholic patients with alcoholic cirrhosis 40 control patients	Expired air	Bacterial overgrowth was measured by breath test after ingestion of glucose	Intestinal bacterial overgrowth was documented in the 30.3% of patients with alcoholic cirrhosis and in none of healthy subjects The prevalence of intestinal bacterial overgrowth was significantly higher in cirrhotic patients with ascites than in those with no evidence of ascites and among patients with Child–Pugh class C than in patients with a class A or B The prevalence of spontaneous bacterial peritonitis was significantly higher in patients who had intestinal bacterial overgrowth than in patients who did not

Table 2 | (Continued)

Authors	Number of patients enrolled	Type of sample	Method	Results
Hauge *et al.*[29]	22 alcoholic patients 12 control patients	Gastric and duodenal biopsies	Gastric and duodenal biopsies were taken for tissue pathology, quantitative and qualitative anaerobic and aerobic bacteriological culture and for culture of *Helicobacter pylori* (antral biopsies)	There were signs of gastritis by endoscopy in 64% of alcoholic patients and in 58% of the controls Significantly more bacteria, dominated by Gram-positive aerobic cocci, were present in the gastric biopsies of alcoholic patients than in those of controls There were 2.6 times more bacteria in the duodenal biopsies of alcoholic patients than in those of the controls Bacterial overgrowth was found in the stomach in 90% of alcoholic patients and in 50% of controls
Buzás[31]	73 alcoholic patients 40 control patients	Gastric biopsies	Samples were collected using upper gastrointestinal endoscopy. Histological examination, microbial culture and direct miscoscopy were used to detect *Helicobacter pylori*	There was no correlation between severity of drinking and *Helicobater pylori* infection
Bhonchal *et al.*[37]	Not available	Duodenal biopsies	Duodenal (D2) biopsies were obtained by upper gastrointestinal endoscopy and processed immediately for microbiological analysis	Marked qualitative and quantitative alterations of small intestinal microflora was documented in alcoholic patients There was increased bacterial growth of both Gram-positive cocci and Gram-negative bacilli in the patients with alcoholic liver disease
Chen *et al.*[35]	12 alcoholic patients with alcoholic cirrhosis 24 healthy subjects	Faecal samples	The faecal microbial communities was analysed by way of 454 pyrosequencing of the 16S ribosomal RNA V3 region followed by real-time quantitative polymerase chain reaction	Community-wide changes of faecal microbiota in alcoholic patients with liver cirrhosis were observed compared with healthy controls The proportion of phylum Bacteroidetes was significantly reduced, whereas Proteobacteria and Fusobacteria were highly enriched in the cirrhosis group. Enterobacteriaceae, Veillonellaceae and Streptococcaceae were prevalent in patients with liver cirrhosis at the family level A positive correlation was observed between Child–Turcotte–Pugh score and Streptococcaceae. Lachnospiraceae decreased significantly in patients with liver cirrhosis and correlated negatively with Child–Turcotte –Pugh score
Mutlu *et al.*[34]	48 alcoholic patients 18 healthy subjects	Colonic biopsies	Colonic biopsy samples from subjects were analysed for microbiota composition using length heterogeneity PCR fingerprinting and multitag pyrosequencing	Altered colonic microbiome (dysbiosis) in alcoholic patients Alcoholic patients with dysbiosis had lower median abundances of Bacteroidetes and higher ones of Proteobacteria Correlation with high levels of serum endotoxin
Gabbard *et al.*[33]	196 patients	Expired air	Patients were underwent to lactulose breath test	Higher prevalence of bacterial overgrowth in alcohol consumers than in teetotalers Significantly lower rates of a positive lactulose breath in patients with a history of cholecystectomy Neither proton pump inhibitor use nor tobacco use was associated with a positive lactulose breath test

dition in which the symbiotic relationship between gut microbioma and host is lost.

In a recent study, Mutlu and colleagues evaluated the mucosa-associated colonic microbiome of 48 alcoholic patients, with and without liver disease, compared to 18 healthy subjects. Colonic biopsy samples were analysed for microbiota composition using LH-PCR fingerprinting and multitag pyrosequencing. Confirming pre-clinical data [19] an alteration in function and composition of colonic microbiome was found in alcoholic patients. In particular, alcoholic patients with dysbiosis showed a reduction in *Bacteroidetes* and an increase in *Proteobacteria*.[34] Moreover there was no correlation between the duration of sobriety and the presence of dysbiosis, suggesting that the effects of chronic alcohol consumption are not temporary but rather long-lasting.[34]

The higher abundances of *Proteobacteria* is in line with Chen and colleagues who also pointed out an increased prevalence of *Fusobacteria* in HBV- and alcohol-related cirrhotic patients.[35] On the contrary, *Prevotellaceae* seem to be more represented in alcohol-related cirrhosis than in HBV-related cirrhosis and controls, probably due to ethanol metabolism in human gut.[35] In contrast to the pre-clinical data by Yan in experimental fed alcohol mice,[20] clinical studies by Mutlu and Chen showed a reduction in intestinal *Bacteroides* in alcoholic patients.[34, 35] This difference could be explained by the increased population's heterogeneity and different study methods.[36] However, further studies are needed to confirm these preliminary results.

Bhonchal and colleagues confirmed the presence of qualitative and quantitative alterations of small intestinal microflora in chronic alcoholics with ALD using microbiological analysis of duodenal (D2) biopsies obtained during upper gastrointestinal endoscopy. Although conducted with other methods, this study is in line with previous studies showing bacterial overgrowth, both of Gram-positive cocci and Gram-negative bacilli, in alcoholic patients with ALD.[37]

Based on these available studies, it is difficult to draft a definitive position on the effects of alcohol on gut microbiota. The major limitations are related to the small sample size of the studies, heterogeneity of analytical methods, differences in alcohol abuse parameters and in the degree of ALD. However, it seems that alcohol consumption and abuse is able to produce quantitative and qualitative alterations of gut micriobiota. To date, there are ongoing studies about metagenomics, transcriptomics and metabolomics in alcoholic patients. These studies will help to explain microbe-microbe and microbe-host interactions, and may suggest new strategies to manipulate commensal microflora.[20]

Mechanisms of alcohol-induced changes on gut microbiota

The reason for bacterial overgrowth and dysbiosis in alcoholic patients is not completely known.[23] Since alcohol can reduce gastrointestinal motility, this has been proposed as the key mechanism for faecal stasis and bacterial overgrowth, resulting in a higher number of luminal bacteria.[6, 38] Alcohol-induced suppression of innate immune response (i.e. Reg family proteins suppression)[20] and adaptive immune response (GALT lymphoid cells depletion) represent another proposed mechanism.[39, 40] These data suggested that alcohol consumption could suppress the Th I-type cellular immune response leading to increased susceptibility to oral infections.[39] Kavanaugh and colleagues showed a substantial loss of both T-cells and dendritic cells in intestine of rats exposed both to alcohol and burn injury compared with intestine of rats receiving either burn or sham injury.[41] Pre-clinical studies also showed that alcohol administration in mice is able to suppress bactericidal protein expression, regenerating islet derived (Reg)-3b and Reg 3g. The treatment with prebiotics partially restored Reg3g protein levels, while it mitigated bacterial overgrowth.[20, 42] Finally, it is not yet well known if alcohol exerts a direct effect on the intestinal microbiota.

ROLE OF GUT MICROBIOTA IN ALCOHOLIC LIVER DISEASE

ALD represents a wide-spectrum of liver injury, ranging from simple steatosis to steatohepatitis, cirrhosis and its complications (e.g. hepatocellular carcinoma), developing in patients with chronic alcohol abuse.[2]

Besides the effects of environmental and host factors on the pathogenesis of ALD, in the last years growing evidences suggested the importance of alcohol-induced changes on gut microbiota.[43, 44] The dysbiosis related to alcohol abuse, in particular the increase in Proteobacteria,[34] may induce an intestinal mucosal inflammation.[8, 45] Moreover, chronic alcohol abuse could result in intestinal bacterial overgrowth and increase gut permeability.[36, 38] Both alterations could increase bacterial translocation from intestinal lumen to portal blood with a massive exposure of liver parenchyma to Lipopolysaccharides (LPS). LPS are able to stimulate innate immune receptors, such as Toll-like receptors and CD14, which activate hepatic stellate and Kupffer cells,[7] with the consequent release of pro-inflammatory mediators, such as

ROS, leukotrienes, chemokines and cytokines (e.g. TNFα and IL-1β), that ultimately contribute to liver damage and prolong inflammation in patients affected by chronic alcohol abuse.[7] Microbiota quantitative and qualitative alterations leading to high LPS concentrations in the portal blood represent an injury for the hepatocytes, already damaged by the chronic alcohol exposure. In particular, according to some authors, the effect of gut microbiota seems to play a major role in the pathogenesis of ALD.[36]

First clinical data on the involvement of gut microbiota in the pathogenesis of ALD were showed by Fukui and colleagues, demonstrating a significantly higher blood endotoxin concentration in patients affected by alcoholic cirrhosis with respect to patients affected by non-alcoholic cirrhosis.[46] This evidence was supported by a pre-clinical study conducted by Adachi in alcohol feeding rats: antibiotic treatment was able to prevent the onset of alcohol-induced liver injury.[47] Moreover, Campos Canesso and co-workers showed that the administration of alcohol to germ-free mices is associated to the absence of liver inflammation and injury.[26] These preliminary results indicate that alcohol alone is not sufficient for the development of liver disease, and that the presence of microbiota alterations is also necessary. More recently, Parlesak and colleagues showed that chronic alcohol abuse impairs the function of the intestinal barrier, which might enhance the translocation of bacterial toxins, thereby contributing to inflammatory processes in ALD.[48] A significant increase in endotoxemia was also found after acute alcohol intoxication.[49] The endotoxemia seems to be correlated with haemodynamic derangement in cirrhotic portal hypertension, and with levels of soluble TNFα-receptors.[50]

These studies showed as the alteration of gut microbiota is involved in pathogenesis of ALD and may also influence the risk of development of severe complications (i.e. spontaneous bacterial peritonitis) that have poor prognosis.[8] Although further studies are needed to understand and clarify this relationship, these evidences suggest that gut microbiota could be a therapeutic target for the treatment of ALD.

Microbiota as target for the treatment of alcoholic liver disease

Pre-clinical studies have shown that the pre-treatment with antibiotics to cleanse the gut flora, or with probiotics (lactobacilli) to repopulate gut flora, is able to reduce LPS endotoxin induced by alcohol and fat infusion and to prevent alcoholic liver injury.[24, 47, 51] In the clinical practice, the cornerstone of the treatment of ALD is to achieve and maintain long-term total alcohol abstinence.[52] However, there is a subgroup of ALD patients (about 5–15%) that shows progression to fibrosis and cirrhosis despite total alcohol abstinence.[3] Therapeutic modulation of gut microbiota in addition to total alcohol abstinence might be an adjunctive strategy for the treatment of ALD with the aim to prevent or delay hepatic damage.[53–55] At present, some preliminary human evidences indicate that antibiotics and probiotics are effective to reduce Gram-negative bacteria population and to prevent alcohol-induced liver injury and progression of liver disease.[36, 54–56] Human studies have also shown that the prophylactic administration of antibiotics in patients with liver cirrhosis is effective in preventing infections in upper gastrointestinal haemorrhage,[57] in preventing first episode[58] and recurrence of spontaneous bacterial peritonitis[59] and in maintaining remission of hepatic encephalopathy.[60]

Despite these data, there are few human studies investigating the role of antibiotics in patients with ALD. A preliminary study on a small sample of patients, affected by ALD and treated with antibiotics (norfloxacin and neomycin), showed an improvement of Child–Pugh score of these patients after 3 and 6 months of treatment.[56]

The fear of complications related to long-term antibiotic administration (i.e. antibiotic resistance and hepatic side effects) are the main reasons of the lack of studies concerning antibiotic treatment in patients with ALD.[61, 62] On this connection, rifaximin, a nonabsorbable oral antibiotic with broad-spectrum anti-microbial activity, has been used in ALD[63] and approved for the long-term treatment of hepatic encephalopathy in patients with liver cirrhosis.[60] Starting from this background, three studies conducted in patients with alcoholic cirrhosis showed that the treatment with rifaximin improves systemic haemodynamics and renal function,[64] cirrhosis-related thrombocytopenia[65] and survival, reducing risk of developing complications of portal hypertension.[66] According to the conclusions of these studies, considering the minimal intestinal absorption of rifaximin, it can be hypothesised that the effect of rifaximin in patients with ALD are related to intestinal decontamination. However to confirm these preliminary results, randomised clinical trials with large sample size are needed to clarify the role of antibiotics and, in particular, of rifaximin in the treatment of ALD.

Probiotics are nonpathogenic microorganisms, able to modify host gut microbiota. Some human studies have

demonstrated that administration of probiotics can improve liver function by improving immune response to enteric pathogens,[67] decreasing oxidative damage/stress [55] and reducing endotoxin levels.[68] In a recent open-label study, *Lactobacillus casei Shirota* was administered three times daily for 4 weeks to a group of 12 patients affected by alcoholic cirrhosis (n = 12), compared to a control group who did not receive probiotics. Probiotics administration restored neutrophil phagocytic activity. Further studies are needed to better understand this effect.[67] Kirpich and colleagues randomised 66 alcoholic patients to receive standard therapy (abstinence plus vitamins) plus probiotics (*Bifidobacterium bifidum* and *Lactobacillus plantarum* 8PA3) vs. standard therapy alone for 5 days. The short-term oral supplementation with probiotics was associated to greater improvement in terms of liver function test than standard therapy alone and to the restoration of the bowel flora, although this data were evaluated by analysing bacterial culture of faecal samples.[54] In addition, the study conducted by Loguercio and colleagues, evaluating the effects of administration of probiotics for 3 months in patients with ALD, showed significantly reduced plasma levels of oxidative stress parameters, an improvement of liver function test and cytokine levels.[55] According to these preliminary studies, probiotics treatment may improve the prognosis of ALD. Moreover the effects of probiotics are strain-dependent, therefore, further clinical studies are warranted to determine which probiotic strain should be used and which patient population should be treated.[23] Finally, further studies are warranted to assess the safety of administration of probiotics, in particular in patients with gut barrier leakage for the possible higher risk of systemic infection.[69, 70]

Another group of molecules able to modify gut microflora is represented by prebiotics. Prebiotics are complex carbohydrates that cannot be digested in the intestinal lumen and are metabolised by gut microflora, such as lactulose. The latter is used in patients with liver disease for the treatment and prevention of hepatic encephalopathy.[71] Some pre-clinical studies showed that the administration of prebiotics could attenuate liver damage in rats.[72] However, to date, there are no clinical studies in patients with ALD.

Finally, synbiotics are combinations of prebiotics and probiotics. Starting by a pre-clinical study showing that synbiotics could prevent liver damage during heavy alcohol consumption,[73] Riordan and colleagues randomised 30 cirrhotic patients to receive symbiotic preparations for 7 days. The study showed that modulation of gut flora with synbiotics could improve liver function in these patients.[74] However, further confirmatory studies are needed to speculate a possible role in the treatment of ALD.

CONCLUSIONS

Alcohol abuse produces qualitative and quantitative changes in gut microbiota. The exact mechanism at the basis of bacterial overgrowth and dysbiosis in alcoholic patients is still poorly understood. The translocation of bacterial products into the portal blood, due to alcohol's toxic effect on gut barrier and to alcohol-induced changes in gut microbiota, seems to play a key role in alcohol-induced liver damage.

To date, several studies have been conducted in pre-clinical and clinical settings to understand pathophysiological mechanisms and relationships between alcohol, gut microbiota and liver damage. The heterogeneity of methods led to inconclusive results. Thus, this is still an open research field. However, the modulation of gut flora seems to be a promising strategy to reduce alcohol-induced liver injury and to prevent disease progression. Further studies are needed to draft definitive conclusions.

AUTHORSHIP

Guarantor of the article: Giovanni Addolorato.
Author contributions: G. Vassallo, A. Mirijello, A. Ferrulli, M. Antonelli: interpreted literature data. G. Vassallo, A. Mirijello and Giovanni Addolorato thought the scientific rationale, wrote and revised the paper. A. Gasbarrini, R. Landolfi, G. Addolorato: revised the final version of the paper. Each one of the authors has contributed to the writing and reviewing of the paper and approved the final version.

ACKNOWLEDGEMENT

We thank Ms. Caterina Mirijello for her expert revision of the English language. The present review was supported by "Fondazione Ricerca in Medicina ONLUS" and by the Italian Ministry for University, Scientific and Technological research (MURST).
Declaration of personal and funding interests: None.

REFERENCES

1. *World Health Organization Global Status Report On Alcohol*. Geneva, Switzerland: World Health Organization, 2004.
2. Tilg H, Day CP. Management strategies in alcoholic liver disease. *Nat Clin Pract Gastroenterol Hepatol* 2007; **4**: 24–34.
3. O'Shea RS, Dasarathy S, McCullough AJ. Alcoholic liver disease. *Am J Gastroenterol* 2010; **105**: 14–32.
4. Bode JC, Parlesak A, Bode C. Gut-derived bacterial toxins (endotoxin) and alcoholic liver disease. In: Agarwal DP, Seitz HK, eds. *Alcohol in Health and Disease*. New York, Basel: Marcel Dekker, 2001; 369–86.
5. Salaspuro M. Alcohol metabolism by gut flora. Metabolic, pathogenetic, and clinical implications. In: Agarwal DP, Seitz HK, eds. *Alcohol in Health and Disease*. New York, Basel: Marcel Dekker, 2001; 67–85.
6. Bode C, Bode JC. Effect of alcohol consumption on the gut. *Best Pract Res Clin Gastroenterol* 2003; **17**: 575–92.
7. Lucey MR, Mathurin P, Morgan TR. Alcoholic hepatitis. *N Engl J Med* 2009; **360**: 2758–69.
8. Goel A, Gupta M, Aggarwal R. Gut microbiota and liver disease. *J Gastroenterol Hepatol* 2014; **29**: 1139–48.
9. Guarner F, Malagelada JR. Gut flora in health and disease. *Lancet* 2003; **361**: 512–9.
10. O'Keefe SJ. Nutrition and colonic health: the critical role of the microbiota. *Curr Opin Gastroenterol* 2008; **24**: 51–8.
11. Gill SR, Pop M, Deboy RT, *et al.* Metagenomic analysis of the human distal gut microbiome. *Science* 2006; **312**: 1355–9.
12. Lopetuso LR, Scaldaferri F, Petito V, Gasbarrini A. Commensal Clostridia: leading players in the maintenance of gut homeostasis. *Gut Pathog* 2013; **5**: 23.
13. Frank DN, St Amand AL, Feldman RA, *et al.* Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA* 2007; **104**: 13780–5.
14. Purchiaroni F, Tortora A, Gabrielli M, *et al.* The role of intestinal microbiota and the immune system. *Eur Rev Med Pharmacol Sci* 2013; **17**: 323–33.
15. Garcovich M, Zocco MA, Roccarina D, Ponziani FR, Gasbarrini A. Prevention and treatment of hepatic encephalopathy: focusing on gut microbiota. *World J Gastroenterol* 2012; **18**: 6693–700.
16. Angelakis E, Armougom F, Million M, Raoult D. The relationship between gut microbiota and weight gain in humans. *Future Microbiol* 2012; **7**: 91–109.
17. Allen CA, Torres AG. Host-microbe communication within the GI tract. *Adv Exp Med Biol* 2008; **635**: 93–101.
18. Hill DA, Hoffmann C, Abt MC, *et al.* Metagenomic analyses reveal antibiotic-induced temporal and spatial changes in intestinal microbiota with associated alterations in immune cell homeostasis. *Mucosal Immunol* 2010; **3**: 148–58.
19. Mutlu E, Keshavarzian A, Engen P, *et al.* Intestinal dysbiosis: a possible mechanism of alcohol-induced endotoxemia and alcoholic steatohepatitis in rats. *Alcohol Clin Exp Res* 2009; **33**: 1836–46.
20. Yan AW, Fouts DE, Brandl J, *et al.* Enteric dysbiosis associated with a mouse model of alcoholic liver disease. *Hepatology* 2011; **53**: 96–105.
21. Derrien M, Collado MC, Ben-Amor K, Salminen S, de Vos WM. The Mucin degrader Akkermansia muciniphila is an abundant resident of the human intestinal tract. *Appl Environ Microbiol* 2008; **74**: 1646–8.
22. Collado MC, Derrien M, Isolauri E, de Vos WM, Salminen S. Intestinal integrity and Akkermansia muciniphila, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. *Appl Environ Microbiol* 2007; **73**: 7767–70.
23. Yan AW, Schnabl B. Bacterial translocation and changes in the intestinal microbiome associated with alcoholic liver disease. *World J Hepatol* 2012; **4**: 110–8.
24. Nanji AA, Khettry U, Sadrzadeh SM. Lactobacillus feeding reduces endotoxemia and severity of experimental alcoholic liver (disease). *Proc Soc Exp Biol Med* 1994; **205**: 243–7.
25. Bull-Otterson L, Feng W, Kirpich I, *et al.* Metagenomic analyses of alcohol induced pathogenic alterations in the intestinal microbiome and the effect of Lactobacillus rhamnosus GG treatment. *PLoS ONE* 2013; **8**: e53028.
26. Campos Canesso M., Lacerda Queiroz N., Marcantonio C., *et al.* Comparing the effects of acute alcohol consumption in germ-free and conventional mice: the role of the gut microbiota. *BMC Microbiol* 2014; **14**: 240.
27. Bode JC, Bode C, Heidelbach R, Dürr HK, Martini GA. Jejunal microflora in patients with chronic alcohol abuse. *Hepatogastroenterology* 1984; **31**: 30–4.
28. Bode C, Kolepke R, Schäfer K, Bode JC. Breath hydrogen excretion in patients with alcoholic liver disease evidence of small intestinal bacterial overgrowth. *Z Gastroenterol* 1993; **31**: 3–7.
29. Hauge T, Persson J, Danielsson D. Mucosal bacterial growth in the upper gastrointestinal tract in alcoholics (heavy drinkers). *Digestion* 1997; **58**: 591–5.
30. Hauge T, Persson J, Kjerstadius T. *Helicobacter pylori*, active chronic antral gastritis, and gastrointestinal symptoms in alcoholics. *Alcohol Clin Exp Res* 1994; **18**: 886–8.
31. Buzás GM. Prevalence of *Helicobacter pylori*, correlations between alcohol consumption and gastroduodenal damage. *Orv Hetil* 1997; **138**: 2791–5.
32. Casafont Morencos F, de las Heras Castaño G, Martín Ramos L, *et al.* Small bowel bacterial overgrowth in patients with alcoholic cirrhosis. *Dig Dis Sci* 1996; **41**: 552–6.
33. Gabbard SL, Lacy BE, Levine GM, *et al.* The impact of alcohol consumption and cholecystectomy on small intestinal bacterial overgrowth. *Dig Dis Sci* 2014; **59**: 638–44.
34. Mutlu EA, Gillevet PM, Rangwala H, *et al.* Colonic microbiome is altered in alcoholism. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G966–78.
35. Chen Y, Yang F, Lu H, *et al.* Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology* 2011; **54**: 562–72.
36. Hartmann P, Chen WC, Schnabl B. The intestinal microbiome and the leaky gut as therapeutic targets in alcoholic liver disease. *Front Physiol* 2012; **3**: 402.
37. Bhonchal S, Nain CK, Taneja N, *et al.* Modification of small bowel microflora in chronic alcoholics with alcoholic liver disease. *Trop Gastroenterol* 2007; **28**: 64–6.
38. Addolorato G, Montalto M, Capristo E, *et al.* Influence of alcohol on gastrointestinal motility: lactulose breath hydrogen testing in orocecal transit time in chronic alcoholics, social drinkers and teetotaler subjects. *Hepatogastroenterology* 1997; **44**: 1076–81.
39. Sibley D, Jerrells TR. Alcohol consumption by C57BL/6 mice is associated with depletion of lymphoid cells from the gut-associated lymphoid tissues and altered resistance to oral infections with Salmonella typhimurium. *J Infect Dis* 2000; **182**: 482–9.
40. Choudhry MA, Fazal N, Goto M, Gamelli RL, Sayeed MM. Gut-associated lymphoid T cell suppression

enhances bacterial translocation in alcohol and burn injury. *Am J Physiol Gastrointest Liver Physiol* 2002; **282**: G937–47.
41. Kavanaugh MJ, Clark C, Goto M, *et al.* Effect of acute alcohol ingestion prior to burn injury on intestinal bacterial growth and barrier function. *Burns* 2005; **31**: 290–6.
42. Kolida S, Gibson GR. Prebiotic capacity of inulin-type fructans. *J Nutr* 2007; **137**: 2503S–6S.
43. Curtis BJ, Zahs A, Kovacs EJ. Epigenetic targets for reversing immune defects caused by alcohol exposure. *Alcohol Res* 2013; **35**: 97–113.
44. Park BJ, Lee YJ, Lee HR. Chronic liver inflammation: clinical implications beyond alcoholic liver disease. *World J Gastroenterol* 2014; **20**: 2168–75.
45. Mukhopadhya I, Hansen R, El-Omar EM, Hold GL. IBD-what role do Proteobacteria play? *Nat Rev Gastroenterol Hepatol* 2012; **9**: 219–3.
46. Fukui H, Brauner B, Bode JC, Bode C. Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: reevaluation with an improved chromogenic assay. *J Hepatol* 1991; **12**: 162–9.
47. Adachi Y, Moore LE, Bradford BU, Gao W, Thurman RG. Antibiotics prevent liver injury in rats following long-term exposure to ethanol. *Gastroenterology* 1995; **108**: 218–24.
48. Parlesak A, Schafer C, Schutz T, Bode JC, Bode C. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. *J Hepatol* 2000; **32**: 742–7.
49. Urbaschek R, McCuskey RS, Rudi V, *et al.* Endotoxin, endotoxin-neutralizing-capacity, sCD14, sICAM-1, and cytokines in patients with various degrees of alcoholic liver disease. *Alcohol Clin Exp Res* 2001; **25**: 261–8.
50. Trebicka J, Krag A, Gansweid S, *et al.* Endotoxin and tumor necrosis factor-receptor levels in portal and hepatic vein of patients with alcoholic liver cirrhosis receiving elective transjugular intrahepatic portosystemic shunt. *Eur J Gastroenterol Hepatol* 2011; **23**: 1218–25.
51. Uesugi T, Froh M, Arteel GE, Bradford BU, Thurman RG. Toll-like receptor 4 is involved in the mechanism of early alcohol- induced liver injury in mice. *Hepatology* 2001; **34**: 101–8.
52. Addolorato G, Mirijello A, Leggio L, Ferrulli A, Landolfi R. Management of alcohol dependence in patients with liver disease. *CNS Drugs* 2013; **27**: 287–99.
53. Gao B, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology* 2011; **141**: 1572–85.
54. Kirpich IA, Solovieva NV, Leikhter SN, *et al.* Probiotics restore bowel flora and improve liver enzymes in human alcohol-induced liver injury: a pilot study. *Alcohol* 2008; **42**: 675–82.
55. Loguercio C, Federico A, Tuccillo C, *et al.* Beneficial effects of a probiotic VSL#3 on parameters of liver dysfunction in chronic liver diseases. *J Clin Gastroenterol* 2005; **39**: 540–3.
56. Madrid AM, Hurtado C, Venegas M, Cumsille F, Defilippi C. Long-term treatment with cisapride and antibiotics in liver cirrhosis: effect on small intestinal motility, bacterial overgrowth, and liver function. *Am J Gastroenterol* 2001; **96**: 1251–5.
57. Leber B, Spindelboeck W, Stadlbauer V. Infectious complications of acute and chronic liver disease. *Semin Respir Crit Care Med* 2012; **33**: 80–95.
58. Terg R, Fassio E, Guevara M, *et al.* Ciprofloxacin in primary prophylaxis of spontaneous bacterial peritonitis: a randomized, placebo-controlled study. *J Hepatol* 2008; **48**: 774–9.
59. Ginés P, Rimola A, Planas R, *et al.* Norfloxacin prevents spontaneous bacterial peritonitis recurrence in cirrhosis: results of a double-blind, placebo-controlled trial. *Hepatology* 1990; **12**(4 Pt 1): 716–24.
60. Bass NM, Mullen KD, Sanyal AG, *et al.* Rifaximin treatment in hepatic encephalopathy. *N Engl J Med* 2010; **362**: 1071–81.
61. Campillo B, Dupeyron C, Richardet JP, Mangeney N, Leluan G. Epidemiology of severe hospital-acquired infections in patients with liver cirrhosis: effect of long-term administration of norfloxacin. *Clin Infect Dis* 1998; **26**: 1066–70.
62. Fernández J, Navasa M, Gómez J, *et al.* Bacterial infections in cirrhosis: epidemiological changes with invasive procedures and norfloxacin prophylaxis. *Hepatology* 2002; **35**: 140–8.
63. Rivkin A, Gim S. Rifaximin: new therapeutic indication and future directions. *Clin Ther* 2001; **33**: 812–27.
64. Kalambokis GN, Mouzaki A, Rodi M, *et al.* Rifaximin improves systemic hemodynamics and renal function in patients with alcohol-related cirrhosis and ascites. *Clin Gastroenterol Hepatol* 2012; **10**: 815–8.
65. Kalambokis GN, Mouzaki A, Rodi M, Tsianos EV. Rifaximin improves thrombocytopenia in patients with alcoholic cirrhosis in association with reduction of endotoxaemia. *Liver Int* 2012; **32**: 467–75.
66. Vlachogiannakos J, Viazis N, Vasianopoulou P, *et al.* Long-term administration of rifaximin improves the prognosis of patients with decompensated alcoholic cirrhosis. *J Gastroenterol Hepatol* 2013; **28**: 450–5.
67. Stadlbauer V, Mookerjee RP, Hodges S, *et al.* Effect of probiotic treatment on deranged neutrophil function and cytokine responses in patients with compensated alcoholic cirrhosis. *J Hepatol* 2008; **48**: 945–51.
68. Lata J, Novotný I, Príbramská V, *et al.* The effect of probiotics on gut flora, level of endotoxin and Child-Pugh score in cirrhotic patients: results of a double-blind randomized study. *Eur J Gastroenterol Hepatol* 2007; **19**: 1111–3.
69. Besselink MG, van Santvoort HC, Buskens E, *et al.* Probiotic prophylaxis in patients with predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. *Ned Tijdschr Geneeskd* 2008; **152**: 685–96.
70. Muñoz P, Bouza E, Cuenca-Estrella M, *et al.* Saccharomyces cerevisiae fungemia: an emerging infectious disease. *Clin Infect Dis* 2005; **40**: 1625–34.
71. Watanabe A, Sakai T, Sato S, *et al.* Clinical efficacy of lactulose in cirrhotic patients with and without subclinical hepatic encephalopathy. *Hepatology* 1997; **26**: 1410–4.
72. Tang Y, Forsyth CB, Banan A, Fields JZ, Keshavarzian A. Oats supplementation prevents alcohol-induced gut leakiness in rats by preventing alcohol-induced oxidative tissue damage. *J Pharmacol Exp Ther* 2009; **329**: 952–8.
73. Marotta F, Barreto R, Wu CC, *et al.* Experimental acute alcohol pancreatitis-related liver damage and endotoxemia: synbiotics but not metronidazole have a protective effect. *Chin J Dig Dis* 2005; **6**: 193–7.
74. Riordan SM, Skinner NA, McIver CJ, *et al.* Synbiotic-associated improvement in liver function in cirrhotic patients: relation to changes in circulating cytokine messenger RNA and protein levels. *Microb Ecol Health Dis* 2007; **19**: 7–16.