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THE "MICROBIOME" OF POST-LIVER TRANSPLANT COMPLICATIONS

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This paper reviews modern literature and presents a brief analysis of our own data on one of the most pressing issues in modern transplantology and, in particular, transplant hepatology – the role and place of gut-liver axis (GLA) in the early post-transplant period. **Objective:** to compare the correlation between gut microbiome palette and incidence of certain early postoperative complications in liver transplantation. **Materials and methods.** The study design is presented as a pilot, prospective, observational, double-blind study based on investigation of the composition of the microbiome residing in the large intestinal in patients that underwent orthotopic liver transplantation (OLTx). The primary cohort of patients consisted of 12 patients who underwent OLTx from a postmortem donor. To assess the gut microbiome palette, biomaterial was collected from all patients in the pre- and post-transplant period followed by next-generation sequencing. The study was conducted as primary study results registered under number NCT04281797. **Results.** In the preoperative period, differences close to statistically reliable in relation to *Actinobacteria* were observed in patients included in the liver transplant waiting list for cirrhosis (LC) and hepatocellular carcinoma (HCC) in cirrhosis. However, due to the pilot nature of the study, this study cohort was limited to an extremely small sample. In turn, in the post-transplant period, there was a statistically significant difference in the taxonomic range of *Actinobacteria* ($p < 0.05$) between the above groups, indicating a possible effect of liver transplantation on the gut microbiome. In addition, in the early post-transplant period, there was a marked difference in the microbiome palette between patients with and without acute cellular rejection. **Conclusion.** GLA and the gut microbiome play a critical role in many liver diseases, and may also have a significant impact on the post-transplant period. In this regard, further research in this direction will not only characterize the predictors and risk factors of bacterial infection and rejection episodes, but will also allow us to form a completely new approach to the treatment tactics for certain complications, including through formation of a microbiota-oriented pharmacotherapy.

Keywords: liver transplantation, bacterial complications, gut-liver axis, hepatocellular carcinoma, acute cellular rejection, sequencing, gut microbiota.

INTRODUCTION

The term gut-liver axis (GLA) in its present lexicon was introduced for the first time in 1978 by Volta et al. [1] of the University of Bologna, Italy, to denote a special relationship between the liver and intestines in relation to the production of IgA antibodies directed against intestinal microorganisms in liver cirrhosis [1]. Subsequently, GLA became referred to as an independent "virtual human organ" [2]. In 2010–20s, at numerous sessions of the European Association for the Study of the Liver (EASL), American Association for the Study of Liver Diseases (AASLD), Asian Pacific Association for the Study of the Liver (APASL), etc. the key role of GLA in the development and progression of non-alcoholic fatty liver disease (NAFLD) was clearly defined. Later this concept was applied to the recently formed and largely unstudied acute-on-chronic liver failure (ACLF), as well as the variability of its course depending on various factors associated with GLA [3–6]. Over time, the traditional concept of the physiological principles of GLA functioning, under the influence of new discoveries, began to undergo significant changes. So, in the spectrum of the concepts of immunobiological interaction regulation, GLA is now considered rather from the position of symbiotic two-vector dualism rather than the previously familiar monism theory, in which both organs work independently of each other.

In turn, GLA cannot exist without the gut microbiome palette. This fact was clearly demonstrated in a paper entitled 'Our "other" genome' published in Nature in 2010. It was then, in the context of international research, an active review of the etiological links and pathogenetic mechanisms of a number of infectious and noninfectious diseases began, taking into account

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new data on the human microbiome [7]. At the same time, the role of GLA has often been overestimated in a particular pathological process. For instance, from time to time, this concept has stimulated an extremely large number of expected and unexpected scientific findings and conclusions. Over time, increasing importance has been given to the intestinal microbiota, the functioning of the intestinal barrier, the innate immune response of the intestinal mucosa, the transfer of antigens from the liver to the intestine, the involvement of the liver itself in infectious patterns and, ultimately, metabolic damage [1, 8].

Objective: to compare the correlation between gut microbiome palette and incidence of certain early postoperative complications of liver transplantation.

MATERIALS AND METHODS

The study design is presented as a pilot, prospective, observational, double-blind study based on investigation of the microbiome composition of the large intestine in patients that underwent OLTx.

The study was conducted as primary study results registered under number NCT04281797.

The sample consisted of 12 patients who underwent OLTx for cirrhosis of various etiologies. All patients were hospitalized with cirrhosis and HCC in cirrhosis. One patient was hospitalized with autosomal dominant polycystic kidney disease (ADPKD) and autosomal dominant polycystic liver disease (ADPLD), which resulted in liver failure.

Meanwhile, 2 patients were excluded from the analysis because of concomitant enterocolitis. Patients with previous gastrointestinal surgical interventions and inflammatory bowel diseases were not included in the analysis due to proven changes in gut microbiome composition in this category of patients. The main characteristics of the patients are presented in Table.

Table

Main patient characteristics

Criteria	Number	Mean	Interval
Age		52.3	29–64
Gender			
– female	3		
– male	9		
Etiology			
– HCV	2		
– HBV			
– HCV + HBV	1		
– Cryptogenic	2		
– AIH	1		
– PBC	1		
– Wilson–Konovalov disease	1		
– Toxic	1		
– HCC + LC	2		
– ADPLD	1		
Child–Turcotte–Pugh			
– A	4	6	(5–7)
– B	5	8	(7–9)
– C	*3*	*10*	*(9–11)*
MELD		15	(6–30)
Ascites			
– absent	2		
– minimal	8		
– average	1		
– pronounced	1		
TIPS in pre-transplant period	2		
Immunosuppressive regimen			
TACROLIMUS + MMF + GKS	11		
TACROLIMUS + MMF + GKS + Azathioprine	1		
Advagraf + MMF + GKS + Sertikan	1		

In our opinion, the study showed several interesting results. In particular, on the ratio of microbiome between patients with cirrhosis and patients with HCC in cirrhosis. It should be noted that there was no statistically significant difference between patients of our own groups in the pre- and postoperative periods in terms of taxonomic typology. However, the difference in microbiome palette among patients with cirrhosis and patients with HCC in cirrhosis seems interesting. Although no significant differences in microbiome composition in the pre- and postoperative periods in each of these cohorts were detected, which in our opinion is directly related to the small sample of patients, values close to significant were achieved for a number of indicators (Fig. 1).

Moreover, the significance of differences in the gut microbiome composition in patients with HCC has been pointed out by several recent studies. Thus, according to Wang and Chen [9], "It is undoubted that gut microbiota play a critical role in the pathogenesis of HCC; this fact can be used not only as an early diagnosis of HCC, but also as a tool for improvement.

In addition, a seminal study by Ren et al. also points to the association between HCC and gut microbiota. In particular, the authors identified differences between patients with cirrhosis and HCC according to actinobacteria taxon [10]. This fact was confirmed by our study, which showed a statistically significant difference in this type between patients with cirrhosis and patients with HCC in cirrhosis (Fig. 2).

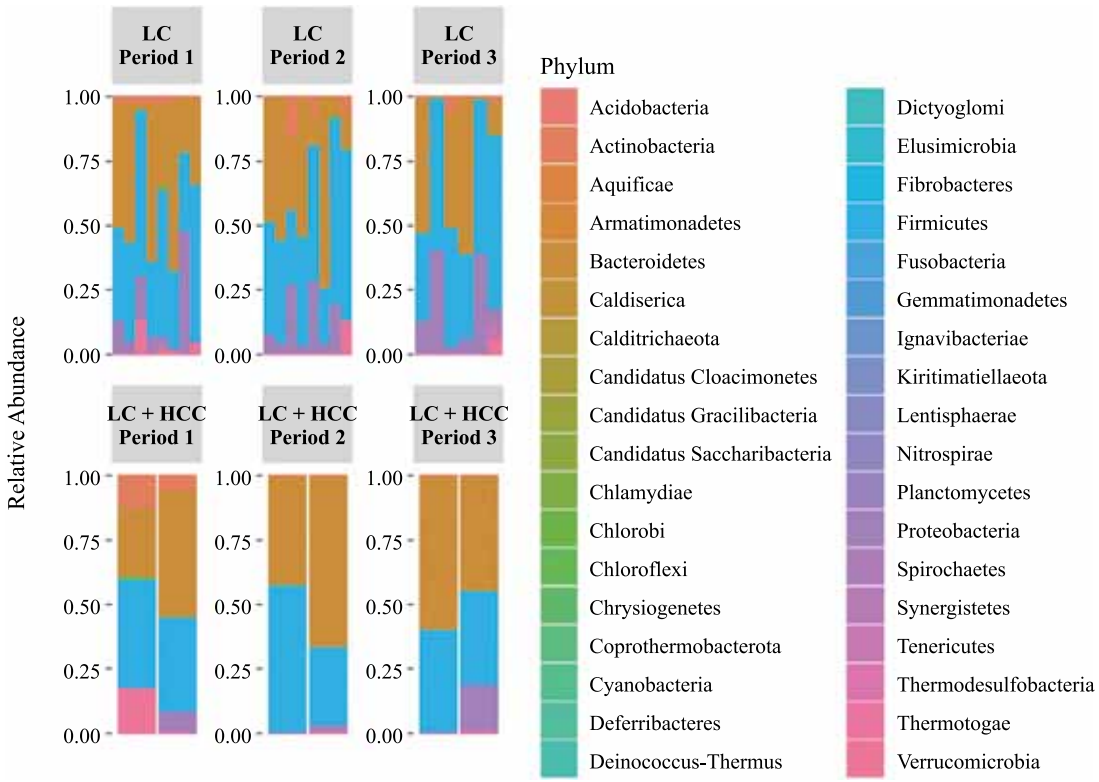
Furthermore, our pilot study identified significant differences in gut microbiota in patients with acute cellular rejection. For instance, we observed a pronounced change in the gut microbiome pattern in the postoperative period compared to the samples obtained before

transplantation. It should be noted that the mentioned taxonomic difference was not observed in patients who had no acute cellular rejection (ACR) (Fig. 3).

However, taking into account that ours is a pilot study that was based on a preliminarily small cohort of patients, we can assume that in the study of a large cohort of patients, the results will provide answers to many questions related to etiology and pathogenesis of ACR, thereby defining the "points of application of efforts" on the path to correcting this severe complication.

Meanwhile, we cannot consider our data to be sufficiently comparable with the above-mentioned studies

a



b

Phylum	.y.	group 1	group 2	p	p.adj	p.format	p.signif	method
Firmicutes	Abundance	LC	LC + HCC	0.8889	1	0.889	ns	Wilcoxon
Bacteroidetes	Abundance	LC	LC + HCC	0.7111	1	0.711	ns	Wilcoxon
Proteobacteria	Abundance	LC	LC + HCC	0.5333	1	0.533	ns	Wilcoxon
Verrucomicrobia	Abundance	LC	LC + HCC	0.8889	1	0.889	ns	Wilcoxon
Actinobacteria	Abundance	LC	LC + HCC	0.08889	0.44	0.089	ns	Wilcoxon

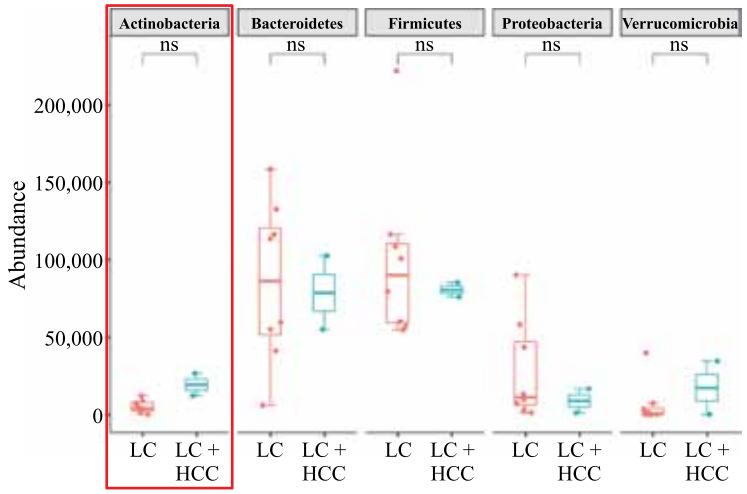


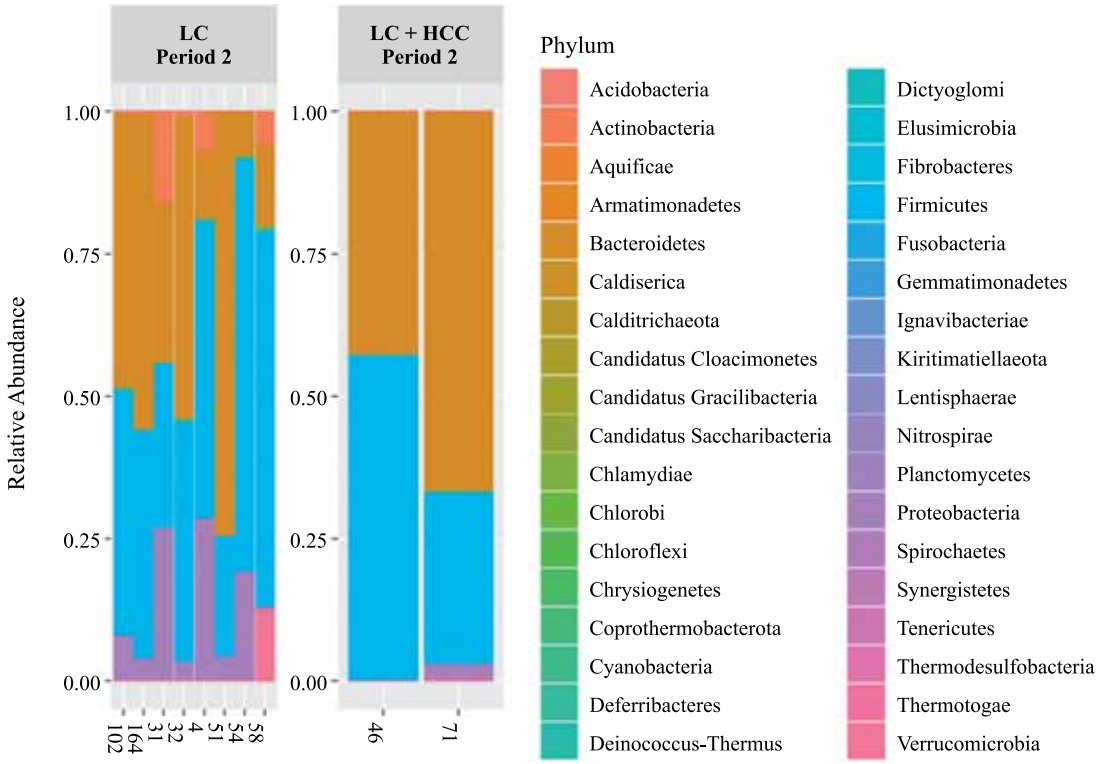
Fig. 1. Microbiome composition of different patient groups: a, total distribution of taxonomic types in patients with liver cirrhosis and with cirrhosis and HCC; b, total distribution of taxonomic types in patients with liver cirrhosis and with cirrhosis and HCC in pretransplant period. "Period 1", material collection before liver transplantation; "Period 2", material collection on the 3rd day after liver transplantation; "Period 3", material collection on the 10th day after liver transplantation)

due to the small sample size, absence of NAFLD cirrhosis and/or cirrhosis complicated by ACLF in the patient cohort in which the influence of microbiome and GLA has been studied and proven. However, the results we obtained can be considered promising and point to the extremely high importance of further research in this area both academically and practically.

DISCUSSION

The role of GLA in infectious complications

Infectious complications have been shown to be the leading cause of mortality after liver transplantation (LTx). Intra-abdominal infection, primary bacteremia and post-transplant pneumonia are the most common complications. The most frequently detected



Phylum	.y.	group 1	group 2	p	p.adj	p.format	p.signif	method
Firmicutes	Abundance	LC	LC + HCC	0.4	1	0.400	ns	Wilcoxon
Bacteroidetes	Abundance	LC	LC + HCC	0.5333	1	0.533	ns	Wilcoxon
Proteobacteria	Abundance	LC	LC + HCC	0.08889	0.36	0.089	ns	Wilcoxon
Verrucomicrobia	Abundance	LC	LC + HCC	0.3578	1	0.358	ns	Wilcoxon
Actinobacteria	Abundance	LC	LC + HCC	0.04444	0.22	0.044	*	Wilcoxon

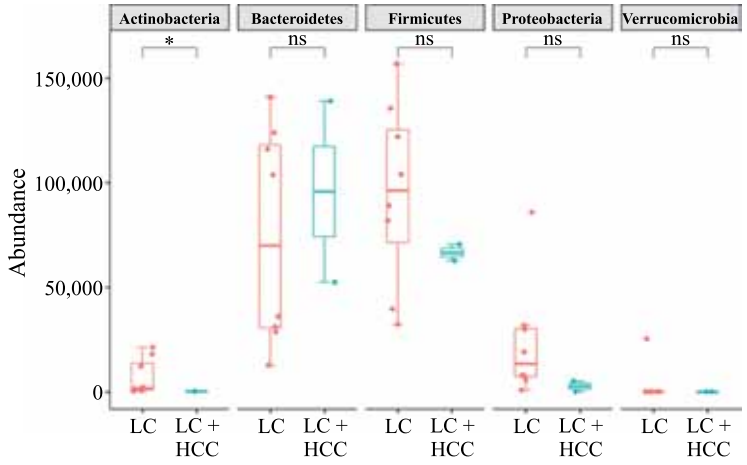


Fig. 2. Comparison of microbiota composition in patients with liver cirrhosis and liver cirrhosis. Statistically significant difference in actinobacteria is observed in patients who underwent liver transplantation due to liver cirrhosis a and liver cirrhosis + HCC. *, a statistically significant difference. “Period 2”, material collection after liver transplantation

microorganisms are staphylococci, enterococci and E. coli. In turn, high MELD, biliodigestive anastomosis, and pre-transplant infections are also known prognostic factors of this type of post-LTx complications [11–15]. In addition, colonization by multidrug-resistant bacteria and the severity of immunosuppressive therapy after LTx significantly aggravate the prognosis of overall survival and graft survival [13, 16–20]. Since the liver is constantly exposed to bacterial products of intestinal microbiome origin by means of anatomical and physiological connection between the intestine and liver, conditioned by portal blood inflow on one side and biliary tract on the other, which in their totality make up the GLA concept, it becomes clear that that GLA seems to play a significant role in these complications and risk factors [11, 12, 21]. This is increasingly supported by recent studies pointing to bacterial commensals and products of bacterial commensalism, such as pathogen-associated molecular patterns (PAMPs), which can move freely from the intestinal lumen into the liver against the background of a body compromised by pathological process, thereby triggering a cascade of immune and proinflammatory reactions [22, 23]. The altered balance of immune response regulation, known as cirrhosis-associated immune dysfunction, is well studied today in patients suffering from chronic diffuse liver disease. This alteration of adaptive immune processes decreases the body's ability to remove cytokines, bacteria and lipopolysaccharides from the general bloodstream, thereby negatively affecting the reparative characteristics of the body [12, 22, 24–26]. In the meantime, monocyte migration, chemotaxis and bacterial phagocytosis are significantly reduced in patients with cirrhosis compared to a healthy population; patients with ACLF have lower expression of antigen-presenting HLA-DR molecules on monocytes, which may lead to decreased monocyte activation and cytokine secretion. In experimental models, microbial translocation in mice induced type I interferon production, which led to interleukin-10 production by myeloid cells and subsequent loss of control over the infectious agent and higher mortality in the experimental animals [27–29]. At the same time, the number of works devoted to the problem of changes in immune response in a GLA context in patients and liver transplant recipients is very small, but the results of these studies will significantly increase the understanding of the role of intestinal microbiota in post-transplant complications. So, Wu et al, observed high levels of endotoxin and IL-6 expression in plasma among patients with liver cirrhosis, and the results of the study correlated with specific phenotypes of the gut microbiota in many parameters. In this study, LTx was used to restore gut microbiota,

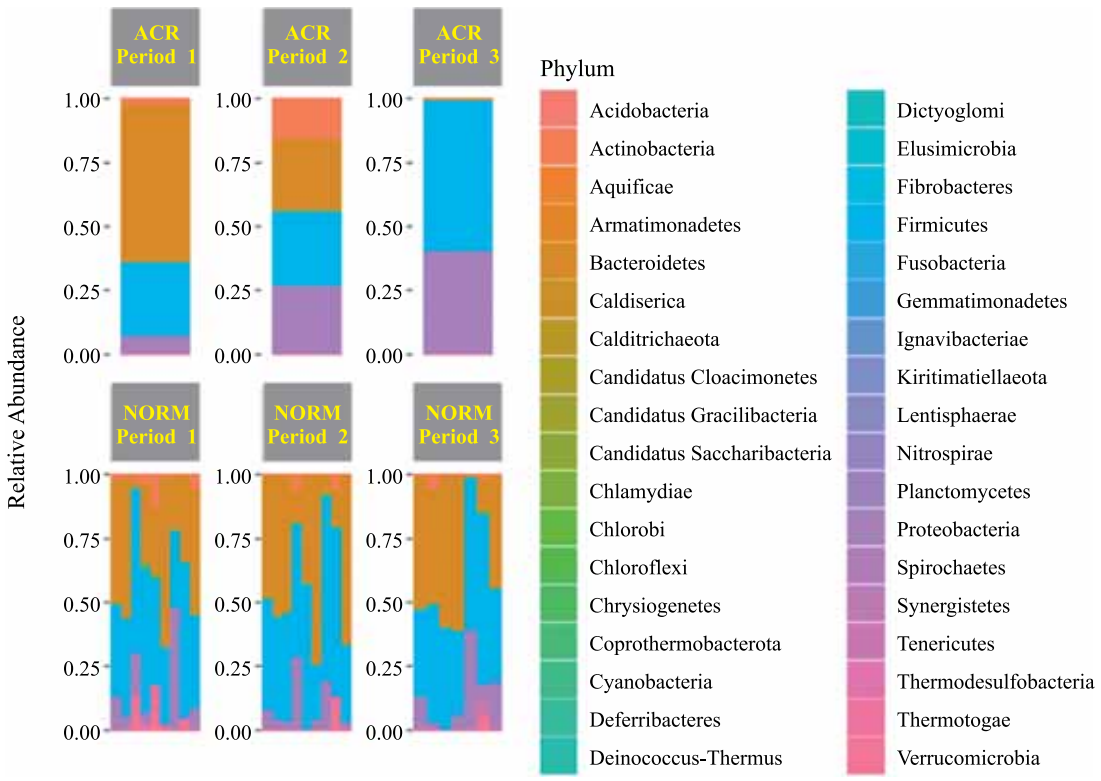


Fig. 3. Microbiota composition in patients with acute cellular rejection episode and a successful posttransplant period. "ACR", acute cellular rejection; "Period 1", material collection before liver transplantation; "Period 2", material collection on the 3rd day after liver transplantation; "Period 3", material collection on the 10th day after liver transplantation. There is a marked difference in the microbiome palette in patients before and after liver transplantation

as well as reduce plasma endotoxin levels in IL-6, which were directly associated with the incidence of post-LTx infectious complications [30]. In addition, in one of the most significant recent studies conducted at Kyoto University Hospital [31], the authors, in their prospective study, found a statistically significant difference in the microbiome map in patients who developed bacterial infection after undergoing LTx compared with the control group. Patients with resurgent bloodstream infections (BSI) had a significantly lower Shannon's diversity index (SDI) at the onset of bloodstream infection than in the pre-transplant period ($P = 0.026$). In the posttransplant period, SDI was also lower in BSI patients than in the non-BSI patients ($P = 0.040$).

Moreover, in the same study, the authors found a statistically significant difference with regard to SDI in ACR patients.

So, it can be assumed that microbiome restoration in the post-transplant period can significantly reduce the risks of infections by reducing microbial translocation and subsequent inflammation. In addition, given the works devoted to the immune response and immune regulation of processes occurring within the GLA, it is possible that a deeper understanding of the functioning of the "virtual organ" will also shed light on many unresolved issues associated with both the frequency of infectious complications and the frequency of liver transplant rejection.

Overall, although this is a promising field, there is currently little data on the modulation of immune response by the microbiome. The stratification of trigger mechanisms and systematization of risk factors is an urgent task facing modern transplantology [30, 32].

Pathogen-associated molecular patterns and immune response

Activation of toll-like receptors (TLRs), which are analogues of recognition receptors for various antigenic patterns in mammals, is important evidence of the close relationship between immune response and translocation of the intestinal microbiome within GLA [12, 30, 33–39]. According to Albilos et al., changes in the functionality of the gut microbiome appear to be more relevant to immune response activation than changes in its composition [12]. In turn, the infectious patterns of the intestinal microbiome are the so-called "PAMPs" which are products of microbial metabolism specifically produced only by pathogens, in this case bacteria and viruses; this term implies a large number of molecules such as lipopolysaccharides, lipids and nucleic acids [34, 37, 38]. In turn, 13 types of mammalian TLRs are currently known. In humans, there are 10 TLRs ranging from TLR1 to TLR10. TLR2, 4, 5, 9 play the greatest role in terms of GLA and microbiome influence on liver tissue [40]. TLRs are expressed in cells of the immune system, as well as in epithelial cells and fibroblasts. However, with respect to pathogen-associated molecular pattern recognition or TLR-PAMP recognition by TLRs, not all TLRs play the same role. For example, a significantly lower number of TLR2 was found in patients suffering from chronic progressive liver diseases compared to the healthy group, while the number of expressed TLR2 was significantly higher in patients suffering from chronic viral hepatitis and nonalcoholic steatohepatitis (NASH) [41]. As for TLR3, many authors point out their protective and anti-inflammatory role [40].

TLR4 selectively recognizes lipopolysaccharide (LPS), heat shock proteins, fibronectin or specific viral envelope proteins [42, 43]. This group of receptors is the most studied in terms of GLA. They have been noted to be significantly elevated in those patients suffering from chronic liver disease (CLD), whose portal blood had high levels of circulating LPS [40, 44–46]. In addition, the association between TLR4 and liver fibrosis has been demonstrated in a number of experimental models. For example, in experimental mice, TLR4-mediated MyD88-NF-κB activation enhances proinflammatory cytokine production, α-SMA, TIMP1 and TGF-β expression, and is associated with disturbances in the extracellular matrix architecture [40, 47].

In turn, TLR5 are less studied, but they are known to play a projective role in NASH pathogenesis. Meanwhile, peritoneal infiltration by flagellin, which is a ligand for TLR5, stimulates massive expression of interleukins, neutrophil and macrophage infiltration of the liver [48]. A large number of experimental studies have been devoted to TLR7, but the full range of their functions is still the subject of debate and discussion. However, their role in both NASH and other chronic progressive liver diseases is known. The presence of these diseases is always associated with a large number of expressed TLR7, as well as a large amount of production of SMA and type 1 collagen [49].

TLR9 appears to be of great importance in patients suffering from alcohol-related CLD, which has been proved by numerous experimental models. Their role in NASH development and progression has also been demonstrated [40].

PAMPs recognition by TLR usually leads to activation of the proinflammatory pathway signaling cascade, which initiates the activation of genes encoding the release of inflammatory cytokines and acute phase inflammation proteins [23, 34, 50–53]. This response mechanism is physiological and necessary for protection against pathogens, but its excessive or prolonged activation can cause functional and morphological changes, leading to a compensatory decrease in immune system activity during chronic pathogenic stimulation. Thus, chronic susceptibility to some infectious agents is formed [34]. For example, prolonged exposure to gram-negative bacteria presented by LPS can induce tolerance to this endotoxin, which is subsequently characterized

by impaired antigen presentation, decreased expression of proinflammatory mediators and overexpression of anti-inflammatory signal molecules [51, 53].

Apart from PAMPs, TLRs can recognize the so-called danger-associated molecular patterns (DAMPs), which originate from apoptotic destruction cells and also play an important role in immune-inflammatory response [43].

Thus, PAMPs translocation, including in the form of LPS and lipoteichoic acid as bacterial cell walls and DAMPs in the form of dead bacterial fragments, lead to initiation of the interaction of various cells of the immune system and production of inflammatory cytokines, followed a related response to their release into systemic circulation [12, 33, 34, 37, 54, 55].

Besides, the balance of proinflammatory and anti-inflammatory cytokines may shift the course of the underlying disease toward progression or regeneration in patients with chronic progressive liver disease [12, 33, 34, 55, 56–59].

Systemic inflammation in patients with CLD compared with healthy individuals is thought to be caused by translocation of PAMP and DAMP into the portal and systemic circulation through the compromised intestinal barrier [12, 23, 30, 34]. In this case, the physiologically slow blood flow in the liver sinusoids provides a close and complete interaction of intestinal molecules with parenchymal and nonparenchymal liver cells and, importantly, with immune cells [60]. Thus, induction of inflammatory response mediators formed due to active cytokine expression, plays an important role in activation of profibrotic and proinflammatory signals cascade, promoting further deterioration of CLDs [12, 17, 26, 33, 34, 54]. In response to the triggered immune cascade, T cells and additional macrophages originating from monocytes are recruited to the liver. Further, through TLR4 presented on the surface of macrophages, bacterial LPS is recognized leading to activation of tumor necrosis factor (TNF)-α synthesis [43, 51, 55]. Ultimately, PAMPs and/or DAMPs create a proinflammatory environment leading to hepatocyte injury, Ito cell activation, and liver fibrosis. It is this pathway that is of great importance today in the development of complications after LTx, particularly infectious complications, acute and chronic graft rejection, and post-transplant liver fibrosis [11, 19, 23, 54]. Furthermore, the importance of GLA and the intestinal microbiome in terms of immune response is further emphasized by studies demonstrating a link between HCC and chronic liver inflammation caused by microbial translocation, in particular by development of liver carcinoma in NASH [23, 61].

Hepatic regulation of gut microbiota

Intestinal microbiota and bacterial products influence liver function by influencing immune reactions occurring in the liver. The GLA concept also implies a reverse pathway, a pathway that regulates the microbiome colonizing the gut. This regulation fully reflects the bidirectionality of the GLA concept [12, 55, 57, 62]. Thus, the liver "delineates" the gut microbiota through IgA and bile release.

The latter is known to contain bile acids synthesized from cholesterol in the liver. These acids have a direct effect on gut microbes, causing membrane damage and disrupting the function of proteins, DNA, and bacteria. In turn, bile acids are metabolized in the intestine by the microbiota to form secondary bile acids that activate specific receptors, particularly the nuclear farnesoid X receptor (FXR) and the G protein-coupled bile acid receptor (GPBAR1), also called TGR5. These receptors regulate numerous immunological and metabolic pathways in the host, which may also indirectly influence the intestinal microbiota [8, 11, 12, 57].

In turn, bile acid composition can be indirectly regulated by the microbiota through the Myd88 signaling pathway, which changes the profile of bile acids [40, 63]. As a result of direct or indirect mechanisms of influence on the composition of bile acids, the intestinal microbiota composition may also change. So, for example, the number of Bacterioids may decrease and the number of Firmicutes may increase [7, 64]. As another example, the growth of Clostridium difficile can be suppressed by re-regulating secondary bile acid production. The liver is an important source of IgA production, which is transported to the intestine via the biliary tract. In turn, IgA is important for controlling the gut microbiota quantitatively, as well as protecting the intestinal mucosal layer [65, 66]. Impaired IgA production has been shown to result in a significant increase in the biomass of anaerobic microbes in the small intestine. In addition, it seems interesting that the transition to adult microbiota is also controlled by IgA, which has been proved by relevant studies [64, 67]. In particular, IgA-lacking mice show persistent colonization by gammaproteobacteria, which are normally present in neonates but are lost in adults [67]. Prolonged presence of these bacteria can induce pro-inflammatory cytokines in the colon and increase intestinal inflammation [68].

Since bile acids and the microbiome mutually influence each other, it is obvious that decreased secretion of bile acids into the intestine as observed, for example, in liver cirrhosis, promotes severe dysbiosis with the formation of multiple pathobionts [12, 65]. As liver cirrhosis progresses, changes in the microbiota lead to inflammatory intestinal phenomena, damage to the intestinal barrier and, as a consequence, initiation of inflammatory phenomena of the liver, which, in turn, further suppresses its secretion of bile acids. Moreover, decreased intestinal FXR signaling impairs the function of the intestinal barrier by reducing the thickness of the mucosa and antibac-

terial protein synthesis, thereby damaging the intestinal vascular barrier [8, 12].

Microbiome and graft rejection

To date, it is known that the immune system is a kind of "bridge" to maintain the symbiotic relationship between the microbiome and the host. As described above, the gut microbiota modulates the host immune system to a certain extent, and the immune system has an inverse effect on the gut microbiota composition [63, 69]. In turn, gut lymphoid tissue represented by T and B cells, antigen-presenting cells and many others play an important role in systemic and local immune responses [23, 41, 57]. The microbiota is also known to actively shape the host's systemic immune response [2, 57, 70, 71]. Dendritic cells migrate to mesenteric lymph nodes, where they present antigens to stimulate the production of effector T cells [23, 63]. These mechanisms play an important role after LTx, especially in hepatic ischemia-reperfusion injury (IRI) [23, 69]. At the same time, one should take into account the fact that hepatic IRI is always present to some extent after LTx [73, 74].

Thus, IRI leads to parenchymal metabolic disorders and hepatocyte death by releasing DAMPs, which signal through TLRs to activate innate immune cells (including Kupffer cells). Subsequent reperfusion enhances this pro-inflammatory innate immune response, which, if further preserved, can indirectly influence the adaptive immune response [74].

It is known that the severity of post-LTx IRI predicts early allograft dysfunction, the probability of complications, and long-term graft survival [75–80]. At the same time, the severity of IRI, according to a number of scientists, is of particular importance for studying the impact of the gut microbiome on innate immunity in the early post-transplant period [78, 81, 82]. For example, one study has shown that administration of probiotics, particularly bifidobacterium and lactobacillus, reduces the severity of IRI by reducing plasma endotoxin levels and restoring intestinal barrier function [82]. In addition, in rat experiments, preliminary ischemic preconditioning of the liver (short IRI periods to condition the tissue against prolonged periods of IRI) restores the intestinal microbial composition and reduces IRI, in particular increasing the number of lactobacillus, bifidobacterium and clostridiales, with a decrease in proteobacteria [82]. In addition, short-chain fatty acids (SCFAs) are powerful immunomodulators and can inhibit the activation of macrophages, a critical IRI mediator, with intravenous butyrate administration reducing the severity of IRI [73].

It has also been shown that FXR-mediated bile acid signaling affects the severity of IRI by restoring the bacterial composition involved in the synthesis of secondary bile acids. This fact can be considered as a potential idea of influencing the severity of IRI by means of obeticholic acid and lithocholic acid [83]. However, to date, we have not found any published evidence for this, nor have we found any large study devoted to this issue.

It has also been proven that the microbiome can influence adaptive immunity and changes in the gut microbiota are associated with ACR [10, 84]. Interestingly, early scientific works devoted to this issue did not confirm the link between the microbiota and ACR incidence. In our opinion, this is due to the limitation of these studies to the use of drugs directly affecting the microbiome characteristics. In particular, these studies focused on the relationship between gut decontamination, the use of prebiotics and probiotics, and their association with ACR [81]. At the same time, the relationship between microbiome and ACR was not considered from the perspective of GLA.

In turn, many modern experimental studies have been able to establish a significant association between the intestinal microbiome composition and ACR incidence [85–87]. For example, Ren et al. demonstrated a dramatic change in gut microbiome composition in rats that developed liver ACR compared to the group without ACR. This was assessed on days 3 and 7 after transplantation (the days that are most critical for ACR) [10]. Other original studies have shown an association between dysbiosis and ACR in LTx patients. Thus, changes in the following bacterial families were observed in patients with advanced ACR: Bacteroides, Enterobacteriaceae, Streptococcaceae and Bifidobacteriaceae, with a decrease in Enterococcus, Lactobacillus, Clostridium difficile, Ruminococcus and Peptostreptococcus.

In our own observation, two patients developed acute graft rejection in the immediate postoperative period. Of course, because of the small number of observations, it becomes impossible to perform a qualitative comparative statistical analysis. At the same time, as indicated today, an increasing number of theoretical and experimental studies point to the potential importance of the intestinal microbiome composition in liver ACR pathogenesis of a liver transplant [10, 83, 84]. In this regard, the obtained results of microbiome palette mapping in patients with ACR in a transplant seem promising to us.

Thus, the listed studies, including our own observations, indicate the possibility of changing treatment approaches in the management of LTx recipients. Prebiotic treatment options for ACR are considered promising, and their effect on ACR was evaluated in a meta-analysis of 3 randomized controlled trials. All of these included a study of the use of lactobacilli as probiotics in LTx patients [88–90]. Although there was some difference in the incidence of ACR, no statistical significance was noted by the authors. At the same time, it is known that there are presently a lot of works in this direction.

CONCLUSION

Considering all of the above, it becomes obvious that GLA plays a critical role in the course and progression of many liver diseases, and in some cases may act as the initial mechanism of etiological determinacy for certain diseases. In turn, it is also known that the intestinal microbiome is a key link in the functioning of this "virtual organ". So, the role of GLA in NAFLD and NASH has been proven. The contribution of GLA in fighting the condition of patients with ACLF is beyond doubt. Multi-author works point to the confirmed role of GLA in infectious complications in patients who underwent LTx [91–96]. In addition, the influence of GLA on some immune-inflammatory processes has been demonstrated. At the same time, the influence of the liver itself on the formation of the "architecture of the intestinal microbiome" is not in doubt today [97, 98]. In this regard, numerous scientific works of recent years have been devoted specifically to the study of the influence of GLA and the intestinal microbiota on certain processes in the body, including complications associated with the immune response and bacterial infection after LTx. These studies have become possible due to application of 16S rRNA profiling of the microbiome by means of next-generation sequencing (NGS), which is a group of methods for determining the nucleotide sequence of DNA and RNA to obtain a formal description of its primary structure [95, 96, 99, 100]. NGS methods make it possible to "read" several genome sites at once, in this case, the intestinal microbiome [101–105]. The resulting nucleotide pattern allows to determine the relationship between a particular microbiome and the frequency of certain postoperative complications. The pattern also allows for deepening the understanding of the pathophysiology of these complications. In turn, the results will facilitate not only the characterization of predictors and risk factors of bacterial infection and rejection episodes, but also the formation of a completely new approach to the treatment tactics for certain complications, including through formation of a microbiota-oriented pharmacotherapy.

The authors declare no conflict of interest.

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