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Late liver allograft dysfunction: definition, risk factors and outcomes

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Abstract

Introduction*. Impaired liver transplant function in the long term often leads to graft loss and the recipient death. There are many causes for the development of a late liver allograft dysfunction and different types of its clinical presentation, but there is no generally accepted definition. This hinders its timely diagnosis, analysis of its prevalence, and also makes it difficult to compare the performance of transplantation programs.*

Objective. *To determine the clinical and prognostic value of late liver allograft dysfunction.*

Material and methods. *The study included 103 cases of cadaveric liver transplantation from donors diagnosed with brain death to 100 recipients, of whom 36% were men, aged 48 years old (40;56)(18–68) at the time of transplant, having MELD* score *17 (14;21) (7–41). The follow-up period was 52 months (20;77)(8-180). The cases where the graft loss occurred earlier than 3 months were excluded.*

The late liver allograft dysfunction was defined as a dysfunction of the transplanted liver, which was manifested by at least one of three following signs and occurred at more than 3 months after transplantation: 1) increased aspartate aminotransferase, alanine aminotransferase and/or gamma glutamyl transferase, alkaline phosphatase, bilirubin; 2) impaired synthetic function (increased international normalized ratio, decreased antithrombin III, cholinesterase); 3) liver cirrhosis complications (signs of portal hypertension, ascites, encephalopathy). The following limits were chosen as a diagnostic threshold for laboratory parameter abnormalities: more than 2 upper limits of normal for total bilirubin, more than 1.5 upper limits of normal for the levels of alanine or aspartic aminotransferases, more than 1.5 upper limits of normal for gamma-glutamyltransferase or alkaline phosphatase, more than 1.6 of normal for international normalized ratio.

Results. *Late liver allograft dysfunction was diagnosed at least once in 64% of recipients. Through the postoperative course, the proportion of patients with late dysfunction varied from 22% to 40%. The etiology of late liver allograft dysfunction was viral (38%), unknown (25%), biliary (19%), immune (17%), and vascular (1%). Late liver allograft dysfunction was reversible in 75% of cases, persistent in 17%, progressive in 8% of cases. Progressive late liver allograft dysfunction led to a graft loss in all cases observed.*

Recipients with late liver allograft dysfunction were found to have had a 33% higher incidence of early allograft dysfunction (OR 4.7, 95% CI [1.8–12.3]); the incidence of biliary dysfunction was 3.1 times higher with distant choledochojejunostomy (OR 3.9, 95% CI [1.1–13.9]); in patients with autoimmune and cholestatic disease, the incidence of immune dysfunction was 4.8 times higher (OR 5.8, 95% CI [1.7–20.3]).

Conclusion. *The progressive nature of late liver allograft dysfunction negatively affects the results of transplantation and therefore should be considered as an indication for retransplantation. Reversible and persistent variants of late liver allograft dysfunction have favorable) prognosis. If the etiology of late dysfunction is not established, the regular surveillance with monitoring for fibrosis and repeated attempts to clarify the diagnosis should be continued.*

Keywords: liver transplantation, dysfunction, long term outcomes

Conflict of interests Authors declare no conflict of interest

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ALT, alanine aminotransferase
AST, aspartic aminotransferase
ULN, upper limit of normal
GGT, glutamine transpeptidase
GCS, glucocorticosteroids
CI, confidence interval
BMI, body mass index
CT, computed tomography
INR, international normalized ratio
MRI, magnetic resonance imaging
MRCP, magnetic resonance cholangiopancreatography
CVA, [acute] cerebrovascular accident/stroke
OR, odds ratio
AVT, antiviral therapy
LAD, late [liver] allograft dysfunction
DAAs, direct acting antivirals
PSC, primary sclerosing cholangitis
PCR, polymerase chain reaction
LT, liver transplantation

SVR, sustained virological response
USE, ultrasound examination
ALP, alkaline phosphatase
EAD, early allograft dysfunction
HLA, human leukocyte antigens
MELD, Model for End-stage Liver Disease
NS5A, nonstructural protein 5A

Introduction

Severe or treatment-resistant liver graft dysfunction may require a repeat transplantation, which occurs in 5–10% of cases and reduces the survival of recipients, including that in the long term [1–3]. Preservation of a normally functioning liver graft allows both improving outcomes, and also increasing the availability of organs for other patients in need for transplantation.

Late liver allograft dysfunction (LAD) is detected in the presence of laboratory signs of cytolysis and(or) cholestasis; and most often abnormal test results can be observed without any symptoms; and only in severe cases and at an advanced stage of the dysfunction, it can be accompanied with the signs of hepatic cellular failure, portal hypertension, hepatic encephalopathy, etc. [4, 5].

Late liver allograft dysfunction can be caused by many factors: immunological, viral, vascular, and biliary complications, recurrence of the original disease, drug toxicity, steatohepatitis and others [6–10]. At the same time, there is no generally accepted definition of LAD, diagnostic criteria vary, and the thresholds for abnormal laboratory parameters, above which a further examination is necessary, are determined by local protocols [4, 11]. All this, combined with the variable causes and options of the LAD natural course, makes timely diagnosis difficult and negatively affects the efficacy of treatment measures. Therefore, it is necessary to clarify the definition and diagnostic criteria of LAD, analyze its overall incidence, typical features of

the clinical course and the frequency of its variants, identify risk factors, and assess the impact of LAD on transplantation outcomes.

Material and methods

The study included 103 cases of cadaveric liver transplantation to 100 recipients who were followed-up on an outpatient basis in the Department of Surgery and Liver Transplantation of Moscow Regional Research and Clinical Institute n.a. M.F. Vladimirskiy from March 2016 to March 2020. The study was approved at a Meeting of the Local Ethics Committee.

Inclusion criteria:

- Liver transplantation from a posthumous donor,
- Known laboratory and medical history data of the perioperative period,
- follow-up period being more than 3 months.

Exclusion criteria:

- A graft loss in the early postoperative period (up to 3 months),
- The patient's age under 18 years old at the time of transplantation.

The duration of follow-up ranged from 8 months to 15 years, the median was 52 months (4 years 4 months), the interquartile range was from 20 to 77 months.

In 4 patients, 7 follow-up periods were included: 3 retransplantations took place in the late postoperative period, 1 transplant occurred in the early postoperative period, and in this case, the follow-up after the first transplantation was not included, since the period of graft functioning was less than 3 months.

We have formulated a definition of late liver allograft dysfunction as a dysfunction of the transplanted liver, which is manifested by at least one of the 3 following signs occurring at more than 3 months after transplantation:

- Increased blood levels of alanine aminotransferase (ALT), aspartic aminotransferase (AST) (“cytolysis”) and(or) glutamine transpeptidase (GGT), alkaline phosphatase (ALP), bilirubin (“cholestasis”);
- An impaired synthetic function (increased international normalized ratio (INR), decreased levels of antithrombin III, cholinesterase);
- Liver cirrhosis complications (signs of portal hypertension, ascites, encephalopathy).

The following limits were chosen as the diagnostic thresholds for abnormal laboratory parameters:

- Total bilirubin: more than 2 upper limits of normal (ULN)
- AST or ALT: more than 1.5 ULN
- GGT or ALP: more than 1.5 ULN
- INR: more than 1.6.

Late liver allograft dysfunction was identified based on the clinical examination and laboratory test results: biochemical (determination of total and direct bilirubin, AST, ALT, GGT, ALP, albumin, total protein), and coagulogram (prothrombin, INR). If the results of these tests deviated from the norm, an additional examination was performed: the qualitative measurement of HCV RNA, HBV DNA, and CMV DNA by polymerase chain reaction (PCR), ultrasound examination (USE), magnetic resonance cholangiopancreatography (MRCP), computed tomography (CT) or magnetic resonance imaging (MRI) with contrast, anti-HLA antibodies, and graft biopsy.

The obtained data were subjected to statistical processing using parametric and nonparametric analyses. Differences in variables were considered statistically significant at $p<0.05$. Sets of quantitative variables are presented using median values (Me), lower and upper quartiles (Q1;Q3) and minimum and maximum values (min-max). To compare independent populations in cases where there were no signs of normal data distribution, the Mann–Whitney U test was used. Comparisons of nominal data were made using Pearson's χ^2 test and Fisher's exact test. Odds ratio (OR) was used as a quantitative measure of effect when comparing relative parameters. The patient survival function was assessed using the Kaplan–Meier method.

Results

Parameters of recipients, donors, and surgical interventions are presented in Table 1. All organs were obtained from donors diagnosed as brain dead ones.

Table 1. Recipient and donor characteristics, and surgery features

Recipient characteristics	Value
Number of transplants	103
Of these, retransplantations, %	4 (4%)
Number of recipients	100
Age at the time of LT, years	48 (40;56) (18–68)
Male, %	37 (36%)
BMI at the time of LT, kg/m^2	24 (21;27) (15–37)
MELD, score	17 (14;21) (7–41)
Indications for transplantation	
Liver cirrhosis of viral etiology	33 (32%)
Liver cirrhosis as a result of cholestatic diseases	25 (25%)
Liver cirrhosis as a result of autoimmune hepatitis	8 (8%)
Liver cirrhosis of alcoholic etiology	10 (10%)
Liver cirrhosis of other and unclear etiology	6 (6%)
Hepatocellular cancer	12 (1 2 %)
Other diseases (genetic, congenital anomalies, polycystic disease, alveococcosis)	5 (6%)

Graft cirrhosis	3
Early thrombosis of the graft artery	1
Donor characteristics	
Cause of donor death	
Traumatic brain injury	49 (48%)
CVA	49 (48%)
Others and unknowns	5 (4%)
Donor age, years	41 (33;50) (18–63)
Male	81 (79%)
Surgery characteristics	
Cold ischemia, hours	6.6 (5.5;8.2) (0.9–12.7)
Warm ischemia, minutes	40 (31;50) (15–81)
Surgery duration, hours	8 (7;9.2) (3–14.3)
Biliary reconstruction options	
Choledochocholedochoanastomosis	84 (82%)
Choledochojejunoanastomosis with a Roux intestine loop	14 (14%)
Unknown	5 (5%)

Notes: LT, liver transplantation; BMI, body mass index; CVA, [acute] cerebrovascular accident

Modification of immunosuppressive therapy

Characteristics of immunosuppressive therapy are presented in Table 2 and in Fig. 1. The initial regimen was chosen with regard to the risk factors for complications: immunological or, on the contrary, oncological and infectious.

Table 2. Frequency of using various initial maintenance immunosuppressive therapy regimens

Immunosuppressive therapy	Number of patients (%)
Three-component scheme	41 (41%)
Two-component scheme	17 (17%)
Tacrolimus+methylprednisolone	6 (6%)
Tacrolimus+mycophenolates	9 (9%)
Tacrolimus+everolimus	2 (2%)
Tacrolimus monotherapy	44 (43%)

Table 2 demonstrates that in the presented cases the three-component immunosuppressive therapy or tacrolimus monotherapy prevailed.

All patients who had a disease of autoimmune or cholestatic nature (33 recipients) received multicomponent therapy at the start; of them, at

discharge, 29 recipients (88%) received a 3-component therapy, 2 patients (6%) received tacrolimus and mycophenolates, 2 more patients (6%) received tacrolimus and steroids.

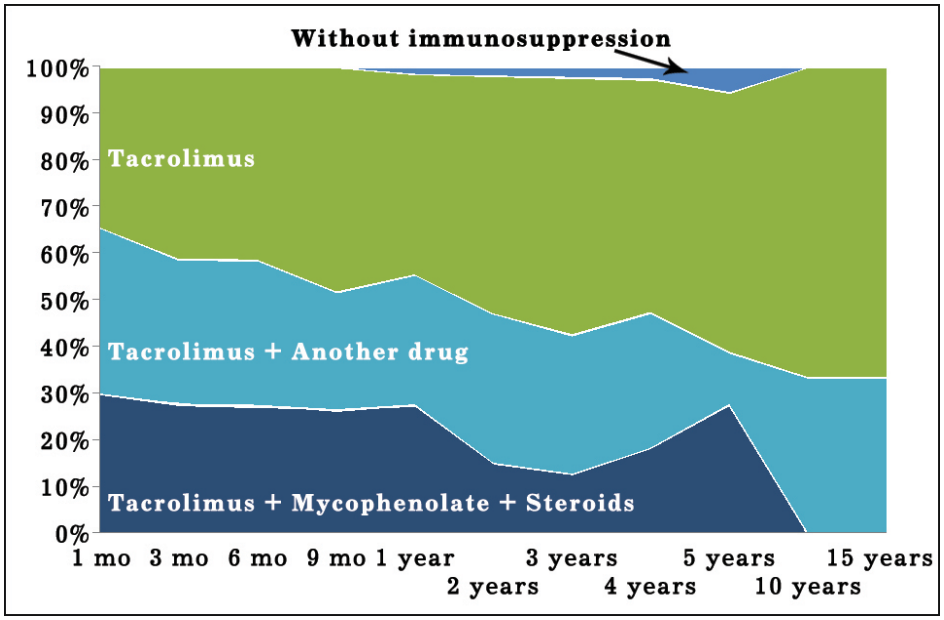

Fig. 1. Dynamics of adjusting the immunosuppressive therapy regimens in liver recipients from the first day of its administration

Immunosuppressive therapy regimens were adjusted over time (Fig. 1). Of the 60 recipients receiving a multicomponent regimen, 40 (67%) had at least 1 component discontinued. Drugs were added to the initial regimen less frequently: in 19 cases (18%).

In addition to the data presented in Fig. 1, it should be noted that, mycophenolate drugs were most often discontinued for reasons of infection (mainly hepatitis B and C), pancytopenia, or as planned in low risks of rejection: in 27 (53%) of 51 cases of their prescriptions. Mycophenolates were returned for intake to six patients: after diagnosis of acute rejection in 2 cases, for LAD of unknown etiology in 2 more cases, for satisfactory graft

function after coping with adverse events in the others. In 2 cases, mycophenolates were replaced with azathioprine for the treatment of autoimmune hepatitis.

Methylprednisolone was discontinued in 16 cases (33%) of 48. In one of these cases, the three-drug regimen was reintroduced after the treatment of acute rejection. In the rest, GCSs were no longer prescribed.

In 13 (13% of all cases) of 44 patients who initially received tacrolimus monotherapy, everolimus was added for a safer tacrolimus dose reduction in the presence of worsened renal function or cancer development. Of these, 3 patients receiving everolimus had the drug discontinued due to pancytopenia, the rest continued to receive everolimus in combination with tacrolimus.

One patient independently discontinued immunosuppressive therapy (tacrolimus with everolimus) after being diagnosed with recurrent hepatocellular carcinoma.

By 5 years after transplantation, the proportion of patients on triple therapy had decreased; the proportion of patients receiving tacrolimus in combination with another immunosuppressant remained the same, and the proportion of recipients on monotherapy had increased. However, despite the general trend towards a decrease in the amount of immunosuppressive therapy over time, not all patients could undergo such a maneuver. In some cases of LAD (rejection, viral hepatitis, recurrence of an autoimmune disease), n individual modification of immunosuppression was required as a part of therapeutic measures to normalize the graft function.

Incidence and etiological variants of late graft dysfunction

When analyzing 103 cases of the long-term period after liver transplantation, 66 patients (64%, 95% confidence interval (CI) [54–73]),

were diagnosed with 76 episodes of LAD, 8 patients being identified as having 2 LAD episodes of different etiology. In one of the patients, LAD was diagnosed three times: hepatitis B, bile duct stricture, and LAD of immune etiology.

The distribution of LAD etiology is shown in Fig. 2. As can be seen in Fig. 2, LADs of viral and biliary etiology predominate. The vascular complication was represented by a case of portal vein thrombosis. No recurrent primary biliary cirrhosis, drug-induced hepatitis, alcoholic illness or steatohepatitis were diagnosed in our case series.

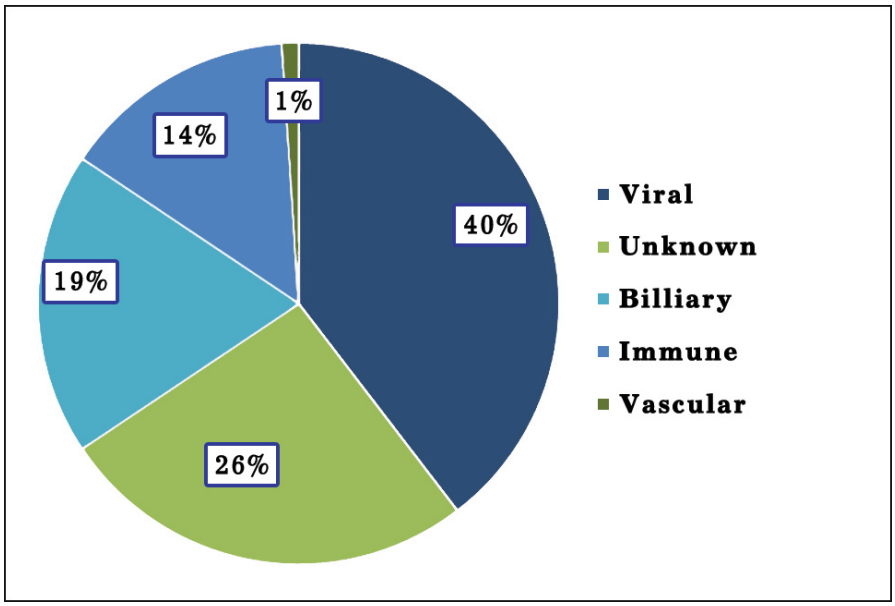

Fig. 2. Etiology of the late liver allograft dysfunction

It should be noted that some patients were operated on before the implementation of direct acting antivirals (DAAs) into clinical practice for the treatment of hepatitis C, therefore, LAD of viral etiology took a leading place due to 15 cases of hepatitis C.

Depending on the follow-up period after LT, the proportion of patients with LAD varied from 22 to 40% without a tendency to decrease. The maximum LAD incidence was observed at 1–2 years after transplantation,

mainly due to LAD of viral etiology, which accounted for 71% of all LAD at that period. The incidence of biliary complications turned out to be maximum at 3–6 months, the incidence of LAD of immune etiology fluctuated at approximately the same level, the incidence of LAD of unknown etiology increased with increasing the period after surgery (Fig. 3, Table 3).

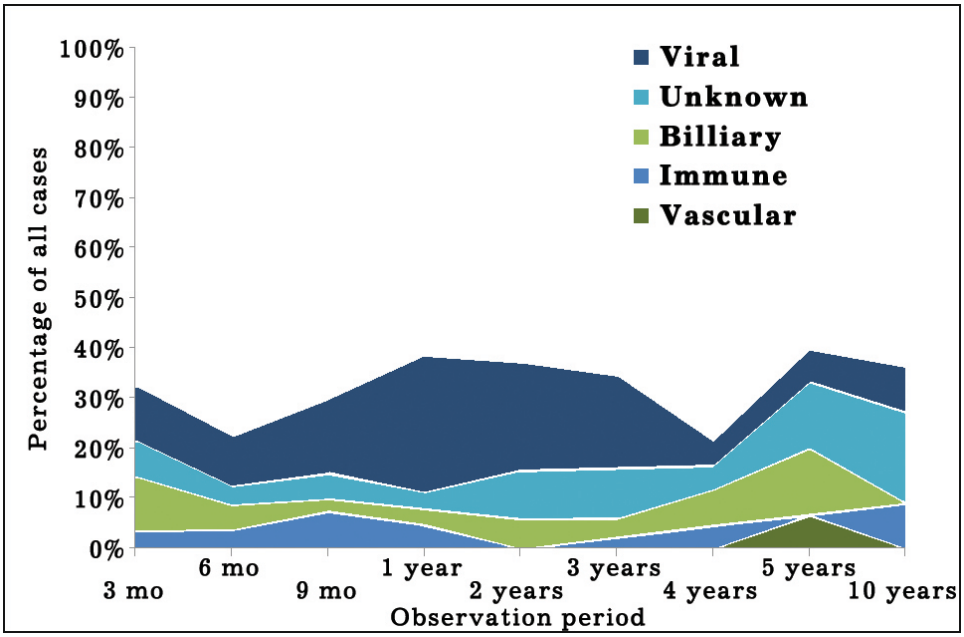

Fig. 3. Incidence of a late liver allograft dysfunction and its variants with regard to the time period elapsed after liver transplantation

Table 3. Late allograft dysfunction incidence at different time periods after liver transplantation

Term after LT	3-6 months	6-9 months	9-12 months	1-2 years	2-3 years	3-4 years	4-5 years	5-10 years	10-15 years
Number of cases in analysis, n	83	81	80	62	51	49	41	15	11
LAD rate, %	33%	22 %	30 %	39%	37%	35%	22 %	40 %	36%
(95% CI)	[23–44]	[13–32]	[20–41]	[27–52]	[24–52]	[22–50]	11–38]	[16–68]	[11–69]

Late allograft dysfunction of viral etiology

Late graft dysfunction of viral etiology occurred in 29 cases (38%) of 76. The causes were hepatitis C and B.

Hepatitis C virus

Late dysfunction of HCV etiology was diagnosed in 15 cases; in 2 more cases, hepatitis C proceeded without cytolysis signs. Despite 97% of relapses of HCV infection in the graft (spontaneous clearance of the virus occurred in one patient after transplantation), no cases of advanced fibrosis, severe dysfunction with hepatic cell failure or fibrosing cholestatic hepatitis C were observed. No grafts were lost due to hepatitis C in the study group.

Antiviral therapy was administered to 17 patients (Table 4).

Table 4. Characteristics of patients with hepatitis C after transplantation who received antiviral therapy

Parameter	n, (%)
Number of patients	17
"Naive" patients	10 (59)%
Fibrosis F3-4 according to METAVIR	0%
1st genotype	14 (82)%
Pegylated interferon with ribavirin	3 (18)%
Ombitasvir + dasabuvir + paritaprevir with ritonavir	7 (41)%
Sofosbuvir+ NS5A inhibitors	7 (41)%
Adding ribavirin	9 (53%)
Treatment duration	
12 weeks	10 (59%)
24 weeks	6 (35%)
48 weeks	16%)
SVR	16 (94%)
MELD score at treatment initiation	10 (9;12) (9–15)
MELD score at 24 weeks after treatment completion	9 (9;10) (8–15)

Notes: AVT, antiviral therapy, SVR, sustained virological response, MELD, Model for End-stage Liver Disease

In addition to the data presented in Table 4, it should be noted that three patients were successfully treated with interferons, the rest were followed-up and regularly monitored for the stage of fibrosis, while the appearance of DAAs was awaiting, and received AVT as soon as they became available. Only one patient did not respond to treatment (12-week course of sofosbuvir with daclatasvir). In all recipients who achieved aviremia, a biochemical response and normalization of graft function were observed.

Hepatitis B virus

In the study group, 19 patients were transplanted while being positive for HBsAg, of whom 10 had co-infection with HDV and 3 with HCV. Prevention of hepatitis B relapse of was ensured by using analogs of nucleos(t)ides, without anti-B immunoglobulin.

Late dysfunction of HBV etiology was diagnosed in 14 cases, of whom 10 patients got HBV infected de novo after transplantation, the rest patients had a relapse of hepatitis B. All patients with relapse had co-infection: three had HDV, one had HCV, meanwhile the reactivation of HBV infection occurred during the course of antiviral therapy for hepatitis C, despite the use of entecavir. All patients received treatment with nucleos(t)ide analogues (tenofovir, entecavir, or their combination) with the effect of achieving aviremia of HBV and HDV and normalization of transaminase activities. There were no cases of graft loss due to hepatitis B or D.

Thus, all cases of LAD of viral etiology are classified as reversible due to an effective antiviral therapy.

Late liver allograft dysfunction due to biliary complications

Biliary complications were diagnosed in 14 cases of LAD of 76 at the postoperative outpatient follow-up, making 19%. Among them, 4 cases of choledochocholedochoanastomosis strictures, 3 cases of biloma, one case each of kinking, biliary sludge, papillostenosis and cholangitis with abscesses. These conditions were resolved by puncture, endoscopy or surgery.

In 3 cases, bile duct strictures were multiple, being manifestations of secondary (bacterial) cholangitis in two, and a PSC recurrence in the third case. There were no signs of hepatic artery thrombosis in any of the cases. All 3 cases led to the occurrence of secondary biliary cirrhosis and retransplantations, which were performed at periods of 1.7 years, 5.5 years and 7 years. Thus, the incidence of graft loss in multiple strictures was 100% (lower limit of CI: 30).

Cold ischemia time, its excess of more than 8 hours, and warm ischemia time had no statistically significant effect on the likelihood of developing biliary complications ($p>0.05$ for each parameter).

In patients with PSC performance of choledochojejunoanastomosis was statistically significantly higher than choledocheal anastomosis: 66% versus 11% ($p<0.001$; OR 17.4; 95% CI [2.8–107]). With choledochojejuno anastomosis, the incidence of biliary dysfunction was statistically significantly higher making 28% (95% CI [10–54]) versus 9% (95% CI [4–17]) with biliary reconstruction ($p=0.04$, OR 3, 9 95% CI [1.1–13.9]).

Late dysfunction of immune etiology

Rejection and autoimmune hepatitis after transplantation were combined into one category of LAD of immune etiology, given the difficulty of differentiating these conditions in liver recipients. The diagnosis of LAD

of immune etiology was established in the presence of pathomorphological signs of autoimmune hepatitis or rejection in combination with a laboratory and clinical response to increased immunosuppressive therapy, and(or) pulse therapy with glucocorticosteroid hormones, or the prescription of therapy for autoimmune hepatitis.

Late dysfunction of immune etiology was seen in 13 cases (17%) of 76. A typical acute rejection with an increase in transaminase activities to 10–20 norms was diagnosed and confirmed morphologically in 5 cases. In 4 cases, the course of rejection was characterized by a mild clinical presentation in the form of intermittent increases in transaminases to 2-3 norms; the morphological examination revealed signs of chronic rejection or low-level activity hepatitis. In 4 cases, the diagnosis of autoimmune hepatitis or plasma cell hepatitis of the transplanted liver was made.

Among the cases of LAD of immune etiology, episodes of severe dysfunction with severe cholestasis and hepatic cellular failure were seen: in 2 patients with acute rejection and in 1 patient with autoimmune hepatitis. In case of acute rejection, pulse therapy with methylprednisolone was performed, which made it possible to achieve positive clinical and laboratory dynamics, but the subsequent chronic rejection with ductopenia and progressive fibrosis became an indication for including this patient on the waiting list for a second transplant. Azathioprine was administered to a patient with autoimmune hepatitis after ineffective pulse therapy; the remission was achieved. In 3 of 13 patients, graft fibrosis F3-4 as detected by elastometry and F2-3, according to METAVIR, as assessed with biopsy.

In patients with autoimmune and cholestatic liver diseases, the incidence of LAD of immune etiology was 24% (95% CI [12–41]), which is statistically significantly higher than the incidence of this complication

making 5% (95% CI [1–13]) in others recipients, p=0.003. The probability of developing LAD of immune etiology in this cohort was 4.8 times higher, OR 5.8 (95% CI [1.7–20.3]).

At discharge from hospital, 88% of recipients with autoimmune and cholestatic diseases were receiving tacrolimus with mycophenolates and steroids, and the remainder received tacrolimus in combination with one of these immunosuppressants. More than half of them (19 of 33, 57%) were withdrawn from mycophenolates, but that did not statistically significantly affect the incidence of LAD of immune etiology in this subgroup, nor did the development of rejection in the early postoperative period or episodes of decrease in tacrolimus concentration less than 5 ng/ml (p>0.05).

Late allograft dysfunction of unknown etiology

Patients, in whom the cause of dysfunction remained unclear, despite the undertaken examination, were diagnosed with LAD of unknown etiology, which made 19 cases (25% of LAD). In 9 cases (47%), the dysfunction resolved on its own, in 1 it led to retransplantation, and in 9 cases it persisted. 3 jo 9 patients had cardiometabolic factors of metabolic-associated fatty liver disease, but refused a biopsy, which did not allow confirmation of this diagnosis. In the remaining 6 cases of persistent unclear LAD, morphological examination of the biopsy specimen did not reveal specific signs of certain pathology; these patients required monitoring for the stage of fibrosis and repeated attempts to establish a diagnosis.

In one case, a graft dysfunction was suspected based on clinical manifestations in the form of hepatic encephalopathy with satisfactory protein synthetic function and no increase in transaminases. The examination revealed increased liver stiffness and numerous portacaval shunts in the abdominal

cavity. The cause of the cirrhosis development could not be established. The patient was included in the waiting list, and retransplantation was performed at a follow-up period of 12 years. Thus, the incidence of the graft loss in LAD of unclear etiology was 5% (95% CI [0.1–26]).

The autoimmune and cholestatic etiology of the underlying disease, the presence of EAD, the initial immunosuppression regimen and the withdrawal of its components did not affect the incidence of LAD of unknown etiology ($p>0.05$).

Late allograft dysfunction as a result of vascular complications

A vascular complication late in the follow-up period was registered in 1 case; portal vein thrombosis was detected. The clinical course was characterized by severe ascites and esophageal varices, which required ligation; but as a result of angiocoagulant therapy, the thrombosis was resolved conservatively.

Diverse late allograft dysfunction course

When classifying LAD based on reversibility, we revealed that LAD was reversible in 75% (95% CI [64–84]) (57 of 76 cases). These include all cases of viral and most cases of biliary and immune LAD (Fig. 4). An increase in the MELD score of more than 15, reflecting the severity of the acute condition, was noted in 8 cases of reversible LAD; after an effective therapy, the graft function recovered and the MELD decreased to score 7–8.

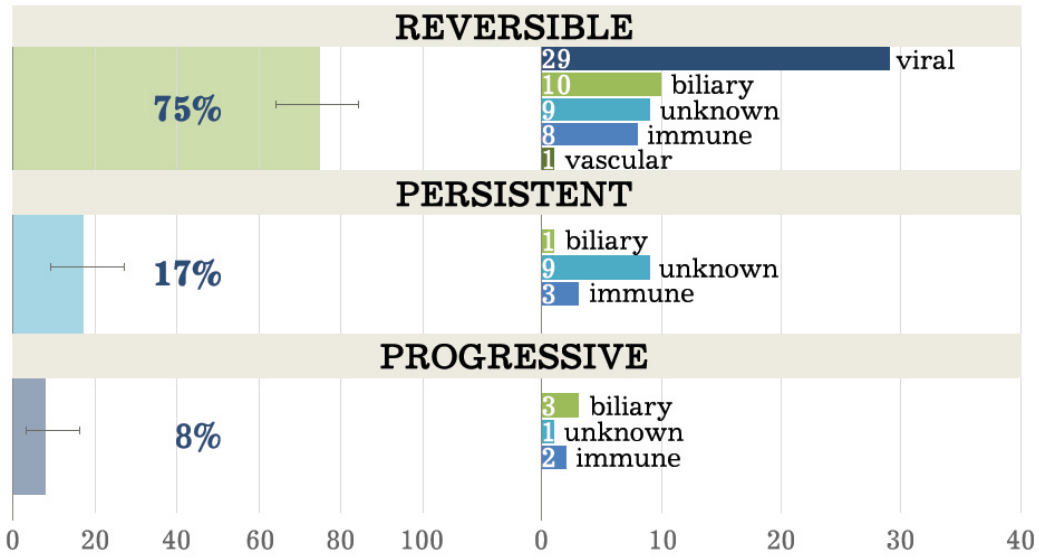

Fig. 4. Variants of the late allograft dysfunction clinical course and their structure

In 13 cases (17%, 95% CI [9–27]), LAD had a persistent course with constant or occasionally occurring abnormalities in test results. Of these, in case of immune dysfunction, an increased immunosuppressive therapy did not lead to normalization of transaminase activities; in case of biliary dysfunction, recurrent cholangitis persisted. In other cases, LAD of unknown etiology was observed. These patients should be monitored for the stage of fibrosis and require repeated attempts of making diagnosis.

A progressive course was observed in 6 (8%, 95% CI [3–16]) LAD cases with the formation of severe graft fibrosis. In all cases, except for the case of unclear LAD, there was an increase in the MELD score to more than 15. Of those, in 3 cases, an indication for retransplantation was biliary cirrhosis with the present multiple strictures of bile ducts; 3 retransplantations were successfully performed. In one case, LAD was diagnosed clinically at the stage of transplanted liver cirrhosis with the

development of hepatic encephalopathy, for which retransplantation was performed. Two female patients with the progressive LAD of immune etiology leading to cirrhosis, continue to be followed-up, being on the waiting list at the moment of the study completion.

Thus, indications for retransplantation for LAD were established in 6 of 103 cases, all of them related to LAD of a progressive course. In 4 cases, successful retransplantations were performed at the end of the study. The incidence of graft loss due to LAD was 6% (95% CI [2–13]).

Death occurred in 2 cases due to the causes unrelated to the transplanted liver function. The death rate was 2% (95% CI [0.2–6.8]).

Stage of fibrosis and degree of steatosis

When performing a protocol elastometry in recipients, the measurements of liver stiffness yielded the following results: more than 15 kPa in 2 cases (2%), from 7 to 15 kPa in 24 cases (23%), lower than 7 kPa in 66 cases (64%); in 4 cases no valid measurements were obtained, and in 10 cases the measurement was not performed.

The liver parenchyma stiffness was 6.5 kPa (6.0;7.2) (3.2–10.8) in patients with satisfactory function, 7.6 kPa (6.0;9.2) (4.0–15.8) in the recipients with current LAD, 6.2 kPa (5.6;6.4) (4.0–22) in those in whom LAD was cured before the current study. We found that in patients with current LAD, the liver stiffness was statistically significantly higher than in patients without LAD and those who had a history of LAD ($p=0.03$). The liver stiffness between the patients without LAD and with the LAD controlled before elastometry did not differ statistically significantly ($p=0.05$) (Fig. 5).

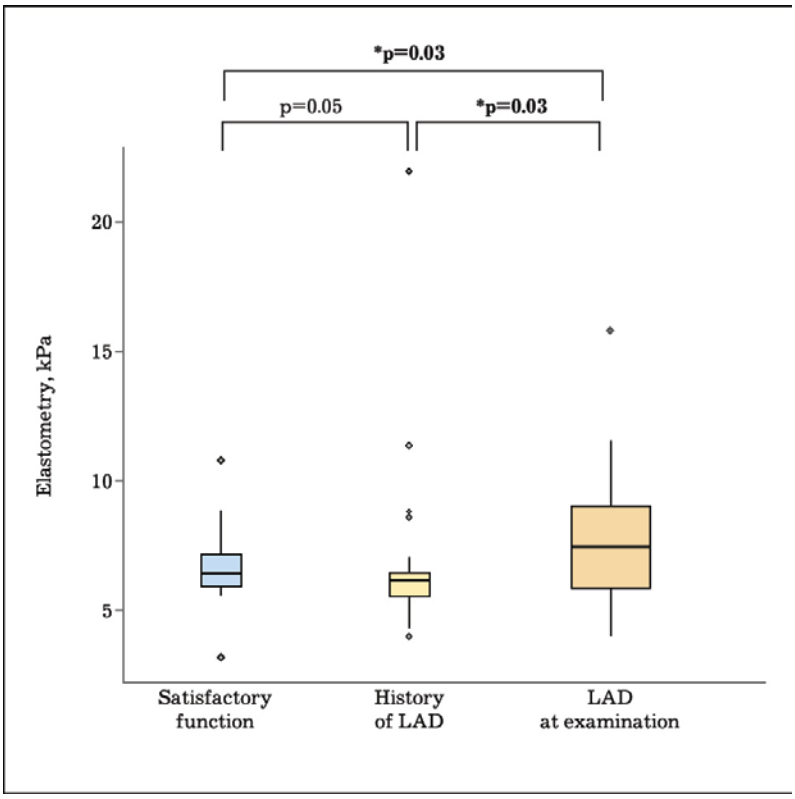

Fig. 5. Comparison of elastometry results

In one of the cases of increased liver stiffness (15.8 kPa), the elastometry result corresponded to biopsy F3 as assessed by METAVIR. In another case, the patient refused a biopsy.

Liver biopsies were performed on indications - to clarify the etiology of LAD. Based on the results of 32 liver biopsies performed during the period of outpatient follow-up, except for the above-mentioned case of stage 3 fibrosis, no patient had advanced fibrosis. In 6 patients, the transplanted liver steatosis was detected; steatosis was identified in less than 33% of hepatocytes in 2 cases, in more than 33% of cells, but less than 66% in one case, and steatosis was present in more than 66% of liver cells in 3 cases. Of those 6 patients, 4 were diagnosed with LAD of viral etiology (HCV, HBV), one patient had no signs of dysfunction, and another patient had signs of LAD, but in presence of severe steatosis (more than 66%), there were no signs of steatohepatitis,

which, however, did not encouraged us to reject steatohepatitis as a cause of LAD, since liver biopsy has its diagnostic limitations.

Graft survival

The calculation of graft survival does not include the cases of graft loss that occurred in the early postoperative period, given the inclusion and exclusion criteria. A cumulative endpoint was used: a graft loss was defined as retransplantation, placing the recipient on the waiting list, or recipient death. The incidence of graft loss was 3% (95% CI [0.07–14]). In the LAD group, 7 grafts were lost, 11% (95% CI [4–21]): in 2 cases, the patients were included in the waiting list; in 4 cases, retransplantations were performed, and in 1 case, the death of a patient with a functioning graft occurred. In the satisfactory function group, there was one death of a recipient with a functioning graft. There was no statistically significant difference in survival between the groups with and without LAD, calculated by the log-rank test, $p>0.05$ (Fig. 6, Table 5).

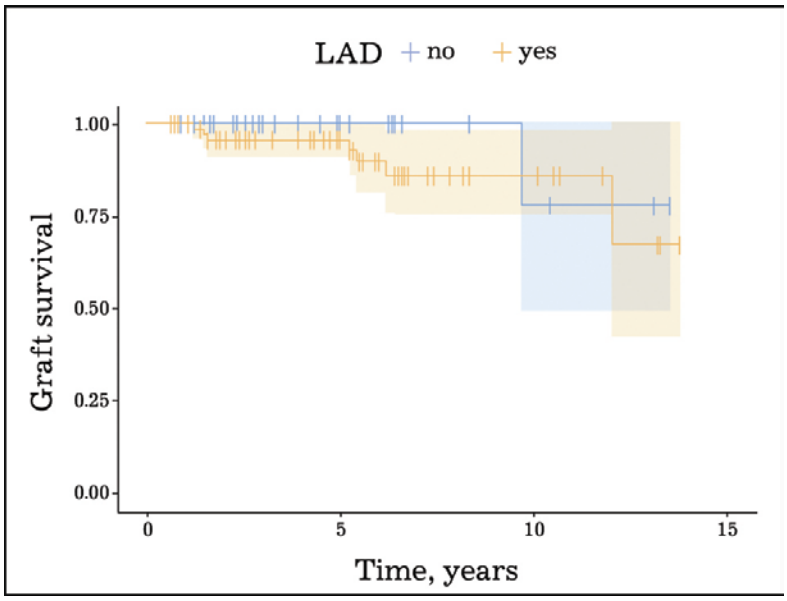

Fig. 6. Liver graft survival in patients with late allograft dysfunction and his satisfactory function

Table 5. Graft survival (cumulative time point was used: inclusion on the waiting list, retransplantation, recipient death)

Graft survival	1 year	5 years	10 years
Overall survival	100%	97%	81%
LAD	100%	95%	84%
Satisfactory function	100%	100%	75%

Risk factors for late liver allograft dysfunction

It was revealed that the occurrence of EAD in recipients with LAD is statistically significantly higher (by 2.7 times) p=0.001 (OR 4.7; 95% CI [1.8–12.3]), than in recipients with a satisfactory graft function (Table 6).

Table 6. Risk factors for late graft dysfunction

Characteristic	All patients, n=103	LAD, n=66	Without LAD, n=37	p
Cold ischemia time, hours	6.6 (5.5;8.2) [0.9–12.7]	6.8 (5.5;8.3) [0.9–12.3]	6.3 (5.4;7.9) [2.0–12.7]	0.4
EAD	41	34 (52%)	7 (19%)	**0.001**
Choledochojejunostomy	14	9 (14%)	5 (14%)	1.0
Autoimmune and cholestatic liver diseases	33	23 (70%)	10 (27%)	0.5
Rejection in the early postoperative period	6	5 (8%)	1 (3%)	0.7
Monotherapy	44	26 (39%)	18 (49%)	0.4
Withdrawal of immunosuppressive components	40	11 (%)	29 (%)	0.2
Addition of immunosuppressive components	19	14 (21 %)	5 (14 %)	0.4
Reducing the concentration to less than 5 ng/mL	68	47 (71 %)	21 (57 %)	0.1
Ratio of actual visits to scheduled ones	0.79 (0.69;0.91) (0.12–1.93]	0.84 (0.73;0.94) [0.32–1.93]	0.75 (0.61;0.96) [0.12–1.17]	0.3

Notes: LAD, late allograft dysfunction; EAD, early allograft dysfunction

As can be seen from Table 6, only the development of EAD had a statistically significant effect on the incidence of LAD. Our data have demonstrated that other risk factors known from the literature and cited there produced no statistically significant effect on the incidence of all-cause LAD cases.

Reducing tacrolimus trough level to lower than 5 ng/ml registered in 68 cases (66% of cases), but did not have a statistically significant effect on the LAD incidence. Fifteen (22%) of these were the recipients taking everolimus in combination with tacrolimus, which trough level was maintained in the range of 3 to 5 ng/ml (92% of cases using combination with everolimus. In the remaining 53 cases, an unintentional decrease in tacrolimus trough level was recorded to a level lower than 5 ng/ml. It should

be noted that such a decrease in trough level was observed occasionally, most often once, and when this abnormal value was detected, the attending physician immediately adjusted the dose of tacrolimus.

When assessing patient compliance, the proportion of visits was calculated as the ratio of actually performed visits to the scheduled number of visits. As it was established, the majority of recipients adhered to the given schedule, and the number of visits did not differ between the patients with and without LAD.

Discussion

The lack of a generally accepted definition of liver graft dysfunction in the long-term postoperative period and undefined limits in laboratory parameters, beyond which an additional examination is required, worsen diagnosis and complicate scientific studies of this condition. Therefore, the term “late graft dysfunction” was proposed and its definition was formulated. The cutoff of 3 months after LT was chosen to exclude the interference with early complications: EAD, hepatic artery thrombosis, which outcome had most often been known by that time.

LAD is often asymptomatic and is detected during routine laboratory monitoring, thanks to which, in the vast majority of cases, it is possible to detect it before the development of consequences, diagnose the cause, and perform treatment, as evidenced by literature data and research [4, 5].

The limits in laboratory parameters for diagnosing LAD were selected based on a review of literature publications [4, 11]. Clinical signs of liver disease are also included in the definition of LAD, since the signs of portal hypertension may persist at ultrasound after LT; and when compensated

cirrhosis is formed in the absence of ongoing inflammation, there may be no biochemical abnormalities, as was observed in one case included in the study.

Identifying the signs of graft dysfunction is an indication for a systematic search for its causes, including studying the medical history of the recipient, donor, surgery, analysis of surgical complications, the treatment received, immunosuppressive therapy, as well as the patient's adherence to treatment and recommendations. Laboratory and instrumental examination for LAD includes a hematology and and biochemistry blood test, coagulogram, trough level of calcineurin inhibitors, virological examination (identification of hepatitis B, C, A, E viruses, cytomegalovirus, Epstein-Barr virus), ultrasound with Doppler measurements of blood flow through the hepatic artery and veins, and if this is not enough, then CT, MRCP, and biopsy [4, 5, 7, 12, 13]. The use of these methods made it possible to diagnose the cause of LAD in 75% of cases.

Despite ongoing screening for dysfunction, it seems prudent to consider risk factors for LAD and time since transplant when managing recipients. Vulnerable groups of patients with regard to the development of LAD are the patients with autoimmune and cholestatic diseases, jejunostomy, HCV viremia at the time of transplantation, co-infection with HBV+HDV or HBV+HCV, as well as the patients who do not have a protective anti-HBs titer and have had EAD. Regarding the timing, in the first 6 months after transplantation, the development of biliary complications and viral hepatitis should be monitored, and after 2 years, steatohepatitis can be considered among the causes of LAD. Warning regarding the immune type of LAD should remain at any time and especially in relation to patients with autoimmune and cholestatic liver diseases.

Despite the fact that the assessment of patient compliance calculating the proportion of patient actual visits in relation to the scheduled ones did not reveal differences between the groups with and without LAD; in clinical practice we observe a pattern of worsening the results in patients who miss a large number of doctor's visits, which requires further study.

Cases where the LAD etiology could not be determined should be a subject of particular interest. In the study group, about half of them (47%) resolved independently without intervention. Causes could include drug toxicity, alcohol injury, an alloimmune reaction, given the liver's unique multiple mechanisms of combating immunological aggression, or other factors. In 3 cases of persistent unclear LAD, there were reasons to suspect steatohepatitis, but, given the refusal of a biopsy, this diagnosis could not be confirmed. In all cases of persistent LAD of unknown etiology, it is necessary to make repeated attempts to establish a diagnosis and monitor the stage of fibrosis.

The most threatening variant of the LAD course is progressive. This included cases of multiple bile duct strictures and chronic rejection. In these cases, the prognosis was unfavorable, since there is no effective treatment for these complications: in the study group, all cases of progressive course led to cirrhosis and graft loss. Based on this, we have concluded that the progressive course of graft dysfunction and the lack of prospects for treatment are an indication for including the patient on the retransplantation waiting list. Considering the high risk of repeated surgery and the uncertain waiting period, the progressive LAD should be considered as a sufficient indication for retransplantation, which should be performed before the development of severe hepatocellular failure, since the decompensation of

graft function and a high MELD score negatively affect the patient survival during the waiting period and after retransplantation [3, 14].

Conclusion

In the long-term postoperative period, regular monitoring of liver graft function is required using laboratory and instrumental investigation techniques, especially in the presence of autoimmune and cholestatic diseases, choledochojejunostomy, viremia associated with hepatitis C at the time of surgery and early allograft dysfunction. When abnormalities and clinical signs of dysfunction are identified, an examination is necessary to determine its cause. The prognosis for the recipient life and the graft preservation is favorable if the diagnosis and treatment of dysfunction is successful and is clarified during dynamic observation if it is persistent; and the prognosis is unfavorable with the progressive course of late dysfunction.

We can summarize the study results, making the following conclusions

- Late allograft dysfunction is disrupted work of the transplanted liver, which is manifested by the syndrome of cytolysis and(or) cholestasis; and(or) impaired synthetic function; and(or) complications of liver cirrhosis and occurs at more than 3 months after transplantation. Late liver allograft dysfunction should be diagnosed in case the blood level of bilirubin exceeding 2 upper limits of normal, alanine aminotransferase or aspartic aminotransferase more than 1.5 upper limits of normal, gamma-glutamyltransferase or alkaline phosphatase being over 1.5 upper limits of normal, and international normalized ratio is raised by more than 1.6.
- Late liver allograft dysfunction is diagnosed in more than half of recipients during the post-transplant period.

- The most common is a late liver allograft dysfunction of viral or unknown etiology, next by incidence comes the late liver allograft dysfunction as a result of biliary complications, and then a late liver allograft dysfunction of immune nature. Late liver allograft dysfunction due to vascular complications is rare. In 75% of cases, the late liver transplant dysfunction is reversible, in 17% it is persistent, and in 8% of cases it is progressive.

- The incidence of late liver allograft dysfunction is 2.7 times higher in the patients who have developed an early liver allograft dysfunction than in the recipients with satisfactory initial liver graft function (OR 4.7, 95% CI [1.8–12.3]), 3.1 times higher in those with choledochojejunoanastomosis in (OR 3.9, 95% CI [1.1–13.9]); in patients with autoimmune and cholestatic diseases, the incidence of the biliary type of late liver allograft dysfunction increases significantly, by 4.8 times (OR 5.8, 95% CI [1.7–20.3]), higher is the incidence of the immune type late liver allograft dysfunction.

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