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Early liver allograft dysfunction: risk factors, clinical course and outcomes

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Early liver allograft dysfunction (EAD) is associated with a high incidence of graft loss and patient mortality in the first 6 weeks after orthotopic liver transplantation (OLT).

***The aim** of this retrospective single-center study is to identify the risk factors of EAD and to compare the short- and long-term results in EAD and non-EAD groups.*

***Materials and methods.** The results of 213 consecutive deceased-donor liver transplantations performed between December 2004 and February 2015 were included in the analysis. Indications for OLT were non-viral liver cirrhosis in 52% of cases, viral hepatitis C or B in 34 %, hepatocellular carcinoma in 8 %; retransplantations were performed in 6% of cases due to previous liver graft dysfunction. EAD was defined by Olthoff criteria (Olthoff et al., 2010).*

Results. *Overall incidence of EAD was 41.3%, including 5.6% of primary non-function grafts (PNF), i.e. irreversible EAD. No significant differences between EAD and non-EAD groups were seen either among donors in their age, gender, cause of death, bilirubin, plasma sodium level, aminotransferase activities, or among the recipients in their age, gender, body mass index, MELD.*

Retransplantation, donor time on mechanical ventilation in the intensive care unit for more than 2 days, high-risk donor category, transplant surgery duration more than 9.5 hours, and cold ischemia time (CIT) > 8 hours were independent significant risk factors of EAD in a multivariate model.

A 42-day mortality rates were 18.2% in EAD group (mostly due to PNF without urgent retransplantation in 9.1%), and 0% in non-EAD group.

Long-term results in EAD group were also significantly poorer: 1-, 5-, and 10-year graft survival rates were 74%, 68%, and 64%, respectively, versus 96%, 90%, and 83% in non-EAD group, Log-rank p = 0.0001.

Conclusion. *EAD significantly (≈ 20%) decreases the short-term graft and patient survival rates. Meanwhile, a reversible EAD has no impact on long-term results. Despite the increased risk of EAD, the liver grafts from high-risk donors are suitable for transplantation. The most important and modifiable risk factor is CIT (optimal timeframe 6 - 8 h), especially when HTK solution is used. The risk of EAD / PNF dramatically increases in case of combined donor and recipient risk factors.*

Keywords: liver transplantation, early graft dysfunction, survival.

Introduction

The evolution of OLT over the past 25 years has demonstrated outstanding results thanks to advances and inventions in the area of donor selection and management, donor organ preservation, surgical techniques, anesthesia and intensive care, in clinical immunosuppression, and a long-term management of recipients [1, 2]. In order to reduce the gap between the ever-growing Waiting lists of candidates for liver transplantation and the number of available post-mortem donors, the transplant community around the world has been actively resorted to using suboptimal donors, or the so-called expanded criteria donors (ECDs). This liberal use of donors leads to an increased numbers of transplantation and reduced mortality rates of those on the Waiting list; but can also have a negative impact on patient and graft survival rates [3, 4].

An increased risk of the initial poor graft function that develops immediately after transplantation is one of the consequences of the donor pool expansion. In addition, the organ allocation system in the MELD era has markedly changed the practice so that the available grafts are transplanted in the first place to the most severe patients; and this may also adversely affect the patient and graft survival. Unlike kidney donors, the issues of defining the standard criteria for liver donors and the ECDs, of using such donors, extending the boundaries of individual criteria, the post-transplant outcomes have not been developed and studied well enough and still represent the subject of numerous studies with ambiguous results [5-8].

Current practice defines the ECDs basing on donor characteristics that may have potential risks to a recipient. The first among all the risks is a poor or absent initial graft function. The conventional donor-derived risk factors include the following: 1) donation after circulatory arrest, and(or) in

hemodynamic instability requiring a significant vasopressor support; 2) a vascular or anoxic cause of brain death; 3) the old age of the donor; 4) a prolonged cold ischemia time; 5) macrovesicular hepatic steatosis; 6) hypernatremia; 7) the length of the donor stay in the ICU and on mechanical lung ventilation (MLV); 8) split-graft transplantation; 9) increased aminotransferase and(or) bilirubin levels [9, 10].

In addition, the risks may be associated with the transmission of donor's known or potential disease (infection, malignancy), but the latter will not be discussed in the context of this article.

EAD is a relevant clinical and predicting factor of the outcome, and its severity is influenced by the contributing and interacting risk factors derived both from the donor and the recipient [11, 12]. However, a generally accepted clinical definition of specific EAD has not been established until recently, there are numerous definitions using a variety of EAD criteria and characteristics. In literature reports, the EAD incidence varies from 23% to 56.3% depending on the definition and the classification used [11, 13-15].

In 2010, Olthoff et al. [16] revised the EAD definition and linked it up with the graft and recipient prognosis. A cohort study of 300 liver transplants from post-mortem donors was conducted in three major US centers. EAD was identified based on the pre-set value of one or more laboratory parameters reflecting the severity of liver dysfunction and liver injury for the post-transplantation week; EAD was observed with the incidence of 23.2%. For the six month following surgery, 18.8% of patients died in the EAD group, while in the non-EAD group, the rate of deaths for the same period was 1.8% (relative risk [RR]: 10.7 [95% confidence interval (CI): 3.6, 31.9]; $p<0.0001$). The number of recipients with a graft loss was higher in the EAD (26.1%) than in the non-EAD group (3.5%) (RR: 7.4

[95% CI: 3.4, 16.3]; $p<0.0001$). The donor age, and the recipient severity assessed by MELD were significant risk factors for the EAD development in a multivariate analysis.

The EAD definition made by Olthoff has been recognized and is widely used today as a universal endpoint in native and foreign clinical trials aimed at identifying the contributing factors, and finding possible therapeutic options to enhance the primary function of the liver graft. Modified and extended methods of EAD assessment have been already proposed for a more accurate and earlier prediction of the transplant immediate outcome and a timely decision making concerning the treatment tactics and the need for retransplantation. [13, 17].

EAD is a sign of a severe ischemia-reperfusion injury (IRI) of the liver graft. However, the interactions of different non-modifiable IRI-contributing factors derived both from donor and recipient have been still poorly studied. Minimizing the time of cold ischemia is often the only clinically implementable possibility of EAD prevention while using ECDs [18].

This paper presents a retrospective analysis of the liver graft initial function depending on the donor category, the donor and recipient individual characteristics, and the specific features of the actual surgery. The EAD effects on the immediate and long-term outcomes of liver transplantation have been studied.

Material and Methods

In the period from December 2004 to February 2015, 220 consecutive OLTs from post-mortem donors were performed in a single center: the Liver and Kidney Transplantation Unit (headed by Prof. Ya.G. Moysyuk) at

V.I.Shumakov Federal Research Center of Transplantology and Artificial Organs (Director: Prof. S.V.Gautier, Academician of the Russian Academy of Sciences [RAS]) within the RF Healthcare Ministry. Seven transplantations were excluded from the study on the following causes: recipient age under 10 years old (n=3); death within the first 24 hours post-transplantation due to cardiovascular complications (n=2); lack of necessary data for analysis (n=2). So, the study included total 213 OLTs in 206 patients, 7 of whom underwent retransplantation at various dates during the study period.

The indications for surgery in the majority of cases included liver cirrhosis resulted from different diseases: hepatitis C (in 24%), primary biliary cirrhosis (in 14%), hepatitis B (in 11%), autoimmune hepatitis (in 11%), alcoholic cirrhosis (in 10%), hepatocellular carcinoma in the presence of viral cirrhosis (in 8%), primary sclerosing cholangitis (in 4%), allograft cirrhosis after live related transplantation (in 3%), and other diseases (15%). Recipient age ranged from 10 to 67 years (median 44 years); 43% of patients were male, 57% were female; the patient severity was assessed from 8 to 40 by MELD (median 17).

Effective donors were the individuals who died as a result of traumatic brain injury (TBI) (56%), acute cerebrovascular accident (CVA) (42%), brain malignancy (1.5%), anoxic brain injury (0.5%). Donor age ranged from 18 to 71 years (median 40); 83% were male, 17% were female. All the donors were pronounced brain-dead.

The donor liver was obtained s a result of multiple organ retrieval in conditions of maintained circulation. Cold perfusion was performed via the aorta with HTK solution in the amount of 10-15 L; the organ preservation was made in the same solution. Organ-specific assessment of the donor liver

and the decision on its appropriateness to be used for transplantation was made at following stages: before retrieval at a primary donor evaluation, and further in dynamics based on the donor management results; then during the explantation, and after the perfusion guided by the established algorithm for liver allograft assessment [19]. Liver biopsy to evaluate the steatosis severity before the start of cold perfusion was performed in 103 cases (48%).

In all cases, the transplantation was made without veno-venous bypass. The cava reconstruction was performed using a traditional technique of the inferior vena cava resection (60%) or using various options of a Piggy-back technique with preservation of inferior vena cava (40%). The choice of the technique was based on the intraoperative situation. In 6 cases, a split transplantation of the expanded right lobe of the liver was performed.

Immunosuppression was performed with cyclosporine in the initial 11 clinical cases, further on, as a tacrolimus monotherapy or in combination with mycophenolates and(or) glucocorticosteroids using protocols established by the center. Basiliximab was used for the induction of immunosuppression in most of the patients. The effect of immunosuppression regime on the primary graft function was beyond the scope of this study.

By studying the case history, donor medical records, and dynamic observation records of recipients, we created an electronic database that contained the following data:

- *For a donor:* gender, age, cause of death, length of stay in the ICU and on mechanical ventilation, transaminases, bilirubin, blood plasma sodium, stable/unstable hemodynamics, asystole episodes, the need for inotropic and vasopressor support, the severity of macrovesicular globular steatosis assessed by histology, cold ischemia time;

- *For a recipient:* gender, age, diagnosis, preoperative evaluation of patient severity by MELD, BMI, the maximum post-surgery levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), bilirubin level, and the international normalized ratio (INR) on the 7th postoperative day, the surgical revision (relaparotomy), complications, length of stay in the ICU, the use of extracorporeal methods, outcome by 03.01.2015;

- *For surgery parameters:* surgery duration, anhepatic period, warm ischemia time for the graft, the amounts of packed red blood cells (RBCs) and fresh frozen plasma (FFP) transfused, amount of autologous blood reinfusion, retransplantation.

Accepted definitions

Donor categories

As a mandatory standard (liver) donor (SD) criteria we have taken the following:

• the age not exceeding 50 years old;

• the length of stay in the ICU and on mechanical ventilation no more than 5 days;

• no episodes of asystole and(or) hypotension below 80/60 mm Hg for over 2 hours;

• dopamine inotropic support not exceeding 10 mcg/kg/min;

• vasopressor support with noradrenaline not exceeding 500 ng/kg/ min;

• normal levels of total bilirubin (up to 20 mmol/L), AST and ALT (lower than 40 U/L);

• plasma sodium concentration lower than 155 mmol/L;

• imaging technique (ultrasound) assessment and visual evaluation of steatosis to be no more than 30%;

• graft cold ischemia time not exceeding 6 hours.

Donors not satisfying at least one of the above SD criteria were classified as ECDs.

In order to determine the acceptable limits of expanding the donor criteria among the ECDs, we were first to identify a subgroup of high-risk donors (HRDs). That included donors who met at least one of the following criteria:

• the age of 60 years and older;

• length of stay in the ICU and on mechanical ventilation of 7 days or more;

• asystole episodes before or at the time of explantation;

• AST and(or) ALT activities more than 3 times exceeding the laboratory normal values (i.e. being over 120 U/L);

• total bilirubin level twice exceeding the laboratory normal value (i.e. being over 40 mmol/L);

• blood plasma levels of sodium being 170 mg/dL or higher;

• graft cold ischemia time of 9 hours or longer.

Donor category distribution was as follows: there were 17 (8.0%) SDs, 122 (57.3%) ECDs, and 74 (34.7%) HRDs.

The graft function

EAD was identified using Olthoff criteria [16]:

• AST and(or) ALT activities of more than 2000 U/L during 7 days immediately following OLT;

• INR$\geq$ 1.6 on the 7^{th} day following OLT;

• total bilirubin ≥ 10 mg/dL (≥ 171 mmol/L) on the 7th day following OLT.

A reversible EAD (READ) and an irreversible EAD (IEAD) were distinguished retrospectively, with regard to the outcome. Grafts with severe (irreversible) EAD, lost grafts (requiring retransplantation or resulted in a patient death) without function restoration in the early postoperative period (up to 21 days in our series) for causes not related to surgical complications (thrombosis, biliary complications, bleeding, primary infection etc.), or acute rejections were considered as PNF.

All PNF graft assessments were consistent with known UNOS laboratory criteria [20], assessed in the period from 24 hours to 7 days after OLT:

• AST > 3000 U/L and at least one of the following:

- INR≥ 2.5;

- acidosis (arterial pH <7.30, and(or) venous pH ≤7.25);

- lactate≥ 4 mmol/L.

Statistical methods

Quantitative variables are presented as median (min-max); the incidence per cent was stated for qualitative variables. A non-parametric two-sided Mann-Whitney test was used to determine the differences in quantitative characteristics between two independent samples. A two-sided Fisher's exact test was used to identify the differences in the incidence of qualitative characteristics in two independent samples. In a univariate analysis, the difference of $p<0.2$ was considered significant for the inclusion in the logistic regression model. In order to determine the threshold values of quantitative variables that displayed the significant differences in the

univariate analysis, we made ROC analysis. The obtained data were further used in the logistic regression model. The significance level in multivariate analysis was set as $p<0.05$.

The survival rate was calculated using the Kaplan-Meier method. Differences in survival between two independent groups were evaluated using a Log-rank test. The differences were considered statistically significant at $p<0.05$. Differences in survival in three independent groups were assessed using a Chi-square test. The differences were considered statistically significant at $p<0.05$.

Results and Discussion

Primary graft function, case allocation between groups, and their characteristics

EAD, as defined by Olthoff, was diagnosed in 88 cases (41.3%) in our series. In turn, the EAD group was divided into two subgroups: "READ" with 76 cases (35.7% of total number of cases) and "PNF" with 12 cases (5.6% of total number of cases).

The incidence of the individual EAD signs and their combinations are given in Table 1.

Table 1. The incidence of individual EAD signs

AST and(or) ALT> 2000 U/L in the first 7 days following OLT	INR≥ 1.6 on the 7th post-OLT day	Total bilirubin≥ 10 mg/dL (≥171 mmol/L) on the 7th post-OLT day	N,% of total EAD cases
+	-	-	26 (29.5%)
-	+	-	27 (30.7%)

-	-	+	4 (4.5%)
+	+	-	16 (18.2%)
-	+	+	7 (8.0%)
+	-	+	0 (0.0%)
+	+	+	8 (9.1%)

In most cases (50 of 88, or 56.8%), EAD was diagnosed within the first 24-48 hours post surgery basing on aminotransferase levels increased over 2000 U/L. They exceeded 3000 U/L in 29 cases (58%), and 5000 U/L in 18 cases (36%). However, the EAD appeared reversible in 26 patients (89.7%) with maximum enzyme levels over 3000 U/L, and in 9 patients (50%) with maximum enzyme levels over 5000 U/L. Thus, there is reason to believe that the hyperenzymemia sign may be the earliest marker of EAD, but solely this is not a sufficient sign for the diagnosis of PNF.

Alone or in combination with other signs, the INR$\geq$ 1.6 on the 7th postoperative day was observed in 58 patients (65.9%), however severe, clinically significant coagulopathy occurred much rarer. Total bilirubin over 171 mmol/L on the 7^{th} day after OLT was observed with the lowest incidence in 19 patients (21.6%) with EAD.

For further analysis, all cases were allocated in three groups depending on the initial graft function, and the comparisons of donor and recipient characteristics were made between the groups (Table 2, 3).

Table 2. Demographic characteristics of liver donors in non-EAD, READ, and PNF groups

Parameter	Study groups		
	Non-EAD n = 125 (58.7%)	**READ n = 76 (35.7%)**	**PNF n = 12 (5.6%)**
Donor age, years *median (min - max)*	38 (18-69)	40 (18-71)	41 (21-56)
Males, n (%)	103 (82.4)	64 (84.2)	10 (83.3)
Cause of death TBI, n (%) CVA, n (%) Other, n (%)	 73 (58.4) 50 (40.0) 2 (1.6)	 40 (52.6) 33 (43.4) 3 (4.0)	 7 (58.4) 4 (33.3) 1 (8.3)
Length of stay in ICU and on mechanical ventilation, days *median (min - max)*	2 (1-15)	3 (1-14)	3 (2-8)
AST/ALT, U/mL *median (min - max)*	40 (11-308)	45 (15-492)	57 (33-511)
Bilirubin, mcmol/L *median (min - max)*	11 (3-55)	13 (2-39)	13 (4-25)
Na, mmol/L *median (min - max)*	150 (130-190)	151 (132-172)	153 (135-200)
Cold ischemia time, min *median (min – max)*	400 (205-690)	420 (220-763)	420 (315-736)
Donor category SD, n (%) ECD, n (%) HRD, n (%)	 13 (10.4) 78 (62.4) 34 (27.2)	 4 (5.3) 37 (48.7) 35 (46.0)	 0 (0) 7 (58.4) 5 (41.6)

Table 3. Demographic characteristics of liver transplant recipients in non-EAD, READ, and PNF groups

Parameter	Study groups		
	Non-EAD n = 125 (58.7%)	**READ n = 76 (35.7%)**	**PNF n = 12 (5.6%)**
Recipient age, years *median (min - max)*	46 (10-66)	43 (12-63)	39 (18-76)
Males, n (%)	65 (52.0)	24 (31.6)	3 (25.0)
BMI, kg/m^2 *median (min - max)*	24 (16-38)	22 (15-32)	22 (16-36)
MELD, score *median (min - max)*	17 (8-39)	16 (8-40)	18 (10-34)

No statistically significant differences were established between the groups in any of the above donor or recipient characteristics. Meanwhile, there were significant differences between the groups in surgery parameters (Table. 4).

Table 4. OLT surgery parameters for non-EAD, READ, and PNF groups

Parameter	Study groups		
	Non-EAD n = 125 (58.7%)	**READ n = 76 (35.7%)**	**PNF n = 12 (5.6%)**
Surgery duration, min *median (min - max)*	514 (247-852)	**580 (320-930) ***	**592 (360-1040) ***
Anhepatic period, min *median (min - max)*	53 (26-110)	57 (26-130)	57 (40-150)
Warm ischemia time, min	36	40	**40**

median (min - max)	(19-81)	(20-80)	**(35-60) ***
Packed RBC transfusion, ml *median (min – max)*	610 (0-5350)	**948** **(0-4962) ***	**2460** **(210-5928) * †**
FFP transfusion, ml *median (min - max)*	2915 (0-16350)	3735 (0-18410)	**7350** **(1500-14770) * †**
Blood reinfusion, ml *median (min - max)*	352 (0-4426)	351 (0-10811)	**1400** **(0-5861) * †**
Re-transplantation, n (%)	3 (2.4)	6 (7.9)	**4 (33,3) * †**

* Statistically significant differences ($p < 0.05$) when compared to non-EAD group.
† Statistically significant differences ($p < 0.05$) when compared to READ group.

Table 4 clearly demonstrates the maximum surgery duration in READ and especially PNF groups; they were characterized by the largest amounts of transfused blood and blood components, including autologous blood reinfusion. This causal relationship is dual by nature, in our opinion. We may give arguments that the complex technical aspects of major surgery and anesthesia accompanied by massive blood loss at hepatectomy stage (retransplantation in the long-term period, prior laparotomy procedures, history of peritonitis, severe baseline portal hypertension and coagulopathy, portal vein thrombosis) are associated with EAD and PNF. During such extraordinary complicated surgical procedures, the reperfusion often takes place in situation of unstable hemodynamics (hypotension), anemia, and pronounced metabolic disorders than aggravate the graft IRI. On the other hand, even in situation of a standard hepatectomy performed, a missing function of the originally suboptimal graft (coagulation factor deficiencies and fibrinolysis) immediately after reperfusion could have aggravated the coagulopathy intensifying bleeding that would require massive blood transfusions and prolong the surgery duration.

We should emphasize the fact that in the third of cases in the PNF group, retransplantations were performed in initially extremely severe patients.

Risk factors of early allograft dysfunction

All donor- and recipient-derived characteristics, as well as OLT surgery parameters listed in Tables 2-4 were considered for evaluation as potential risk factors. For that part of the study, READ and PNF groups were united into EAD group that was compared with no-EAD group. Univariate analysis results are shown in Table 5.

Table 5. Comparisons of recipient- and donor-derived characteristics and surgery parameters between non-EAD and EAD groups. Univariate analysis results

Parameter	Study group		P
	Non-EAD (N = 125)	EAD (N = 88)	
Recipient parameters			
Age, years	46 (10-66)	42 (12-67)	0.3407
Female	**49%**	**69%**	**0.0030**
BMI, kg/m^2	**24 (16-38)**	**22 (15-36)**	**0.0223**
MELD, score	17 (8-39)	17 (8-40)	0.7347
Donor parameters			
Age, years	38 (18-69)	40 (18-71)	0.5726
Female	18%	16%	0.8530
Cause of death			
TBI	59%	53%	0.4861
CVA	39%	42%	
Other	2%	5%	

Length of stay in the ICU and on mechanical ventilation, days	**2 (1-15)**	**3 (1-14)**	**0.0026**
AST/ALT, U/mL	**40 (11-308)**	**48 (15-511)**	**0.1075**
Bilirubin, mcmol/L	11 (3-55)	13 (2-39)	0.3232
Na, mmol/L	**150 (130-190)**	**151 (132-200)**	**0.1567**
Cold ischemia time, min	**400 (205-690)**	**420 (220-763)**	**0.0613**
Donor category SD ECD **HRD**	 10% 63% **27%**	 5% 50% **45%**	 **0.0056**
Surgery parameters			
Duration, min	**514 (214-852)**	**580 (320-1040)**	**0.0079**
Anhepatic period, min	**53 (26-110)**	**57 (26-150)**	**0.1347**
Warm ischemia time, min	**36 (19-81)**	**40 (20-80)**	**0.0261**
Packed RBC transfusion, mL	**610 (0-5350)**	**960 (0-5928)**	**0.0054**
FFP transfusion, mL	**2915 (0-16350)**	**3750 (0-18410)**	**0.1202**
Blood reinfusion, mL	352 (0-4426)	360 (0-10811)	0.6722
Retransplantation	**2%**	**11%**	**0.0091**

Univariate analysis demonstrated that there were more women in the cumulative EAD group, and the patients had lower BMI. A recipient severity as assessed by MELD did not differ between no-EAD and EAD groups.

Having analyzed the donor and recipient characteristics, we found that EAD group donors had longer ICU stays and time on mechanical ventilation, and often belonged to HRD category.

It is important to note that significant differences were established between the two groups in interrelated actual OLT surgery parameters: the surgery duration, warm ischemia time, amount of blood transfusion, and liver retransplantation.

Further, the variables that displayed differences between no-EAD and EAD groups in a univariate analysis at a significance level of $p<0.2$ were included in a multinomial logistic regression model. Those variables include: a recipient female gender, a recipient BMI, the donor length of stay in the ICU and on mechanical ventilation, donor aminotransferase levels, donor plasma sodium, cold ischemia time, HRD, surgery duration, anhepatic period of warm ischemia, packed RBC transfusion, FFP transfusion, re-transplantation.

All continuous variables that had displayed significance in the univariate analysis were subjected to ROC analysis for establishing the thresholds; the analysis results are shown in Table 6.

Table 6. Threshold values of continuous parameters. ROC-analysis results

Parameter	Threshold	Sensitivity% / specificity,%
Recipient BMI, kg/m^2	<23	56/64
Donor length of stay in the ICU, days	> 2	67/56
Donor AST/ALT, U/mL	> 40	69/45
Donor Na level, mmol/L	> 145	85/29
Cold ischemia time, h	> 8	74/40
Surgery duration, h	> 9.5	57/67
Anhepatic period, min	> 55	60/55

Warm ischemia time, min	> 40	57/60
Packed RBC transfusion, ml	> 740	61/61
FFP transfusion, ml	> 3000	62/51

The thresholds obtained were characterized by the best sensitivity and specificity ratio. Parameters that appeared statistically significant in the multivariate analysis, i.e. the parameters that represent the risk factors of EAD development, the odds ratio (OR), 95% confidence intervals, and p values are presented in Table 7.

Table 7. Risk factors for EAD. The results of multivariate analysis

Factor	RR	95% CI,	P
Re-transplantation	6.3	2.4-16.6	0.0030
Donor length of stay in the ICU > 2 days	4.4	1.7-11.4	0.0020
HRD	2.4	1.1-6.4	0.0421
Surgery duration > 9.5 h	3.6	1.4-9.4	0.0102
Cold ischemia time > 8 hours	2.5	1.1-6.4	0.0490

Risk factors for primary nonfunctioning graft

Potential risk factors for the liver graft non-function were identified by studying two groups: READ group (n=76), and PNF group (n=12). The groups were compared on donor- and recipient-derived characteristics, and surgery parameters; the univariate analysis results are shown in Table. 8.

Table 8. Comparisons of recipient- and donor-derived characteristics and surgery parameters between READ and PNF groups. Univariate analysis results

Parameter	Study groups		P
	READ (N = 76)	PNF (N = 12)	
Recipient parameters			
Age, years	43 (12-63)	39 (18-67)	0.5123
Female gender	67%	75%	0.7449
BMI, kg/m^2	22 (15-32)	22 (16-36)	0.3631
MELD, score	16 (8-40)	18 (10-34)	0.6012
Donor parameters			
Age, years	40 (18-71)	41 (21-56)	0.6762
Female gender	16%	17%	1.0000
Cause of death TBI CVA Other	 53% 43% 4%	 59% 33% 8%	0.7649
ICU length of stay, days	3 (1-14)	3 (2-8)	0.8846
AST/ALT, U/mL	45 (15-492)	57 (33-511)	0.3809
Bilirubin, mcmol/L	12 (2-39)	13 (4-25)	0.6927
Na, mmol/L	151 (132-175)	153 (135-200)	0.8652
Cold ischemia time, min	420 (220-763)	420 (315-736)	0.7962
Donor category SD	5% 49%	0% 58%	1.0000

ECD HRD	46%	42%	
Surgery parameters			
Duration, min	580 (320-930)	592 (360-1040)	0.5626
Anhepatic period, min	57 (26-130)	57 (40-150)	0.4663
Warm ischemia time, min	40 (20-80)	40 (35-60)	0.2728
Packed RBC transfusion, ml	948 (0-4962)	2460 (210-5928)	0.0277
FFP transfusion, ml.	3735 (0-18410)	7350 (1500-14733)	0.0796
Blood reinfusion, ml	352 (0-10811)	1399 (0-5861)	0.0025
Re-transplantation	8%	33%	0.0275

No statistically significant differences were found between the groups in donor and recipients characteristics. The variables that displayed significant difference of $p<0.2$ in a univariate analysis (namely, packed RBC transfusion, FFP transfusion, blood reinfusion, re-transplantation) were included in the multivariate logistic regression model.

All continuous variables that showed significance in the univariate analysis were subjected to ROC analysis aimed at establishing the threshold values (Table. 9).

Table 9. Threshold values of continuous parameters. ROC-analysis results.

Parameter	Threshold	Sensitivity% / Specificity,%
Packed RBC transfusion, ml	> 2200	64/91
FFP transfusion, ml	> 7200	55/94
Blood reinfusion, ml	> 900	73/85

None of the parameters included in the model displayed a statistical significance in predicting a PNF. Nevertheless, re-transplantation, and large amounts of packed RBC transfusion (over 2200 ml), FFP transfusion (over 7,200 ml), and the amount of blood reinfusion exceeding 900 ml may be considered the factors predisposing to PNF.

The impact of EAD and PNF on transplantation outcome

In the early postoperative period (up to 42 days after transplantation), 16 recipients died (7.8%), 11 (68.8%) of whom because of PNF and a subsequent development of multiple organ dysfunction syndrome (MODS) (Table 10). Re-transplantations for PNF were performed in 3 cases, but all of the patients died due to a repeated PNF (n=1), or MODS progression (n=2). The rest 5 patients who died from other causes also developed EAD. There were no deaths or retransplantations in the non-EAD group in the studied period.

Table 10. Causes of deaths during the first 42 days following OLT

Cause of death	N
PNF	11
EAD, hepatic artery thrombosis	1

EAD, bleeding from esophageal varices	1
EAD, neurological complications (CVA, CPM)	2
EAD, fulminant sepsis	1

Note: CPM: central pontine myelinolysis.

The main signs of PNF in the first 24-48 hours post-surgery, as a rule, were the following: a rapid elevation in aminotransferase activities and bilirubin levels, a severe hepatic deficit of coagulation factors, continued coagulopathic bleeding, hypoglycemia, high levels of lactate in venous blood (more than 4.0 mmol/L with a tendency to increase), unstable hemodynamics, failed weaning from mechanical ventilation, or the need in re-intubation, encephalopathy progression, and coma, an acute renal failure. The condition was diagnosed by the dynamic integrated assessment of all clinical and laboratory parameters, as well as the trends in their development. Definitive diagnosis of PNF can be difficult and temporarily delayed in certain cases because of "masking" effect of undertaken life support measures.

All recipients with PNF needed the mechanical ventilation to be continued (until the death or retransplantation), required a renal replacement therapy (continuous veno-venous hemofiltration, albumin dialysis), massive plasma and blood transfusions, the infusion of coagulation factors, and also the administration of vasopressors (noradrenaline) in high and(or) increased doses. Relaparotomy for ongoing bleeding was performed in 4 patients on the 1^{st}-3^{rd} day post-surgery.

Despite the intensive care measures undertaken, 8 patients with PNF graft died consecutively on the 1^{st}, 3^{rd}, 4^{th}, 5, 7^{th}, 12^{th}, 19^{th}, and 21^{st} day after transplantation. The first two of those died could not have been subjected to

retransplantation because of extremely unstable hemodynamics and deep coma. The other 6 recipients had the indications for urgent retransplantation, but that could not be performed because of lacking a donor; and their deaths occurred in condition of the MODS progression and associated systemic infection.

We must emphasize that transplantations were technically standard only in 3 of these 8 patients, and in the other 5 cases the surgery was performed with great technical difficulties (3 cases were retransplantations in the long-term period, 2 cases were primary transplantations after previous major abdominal surgery), associated with an unusually massive blood loss, unstable hemodynamics, a prolonged ischemia time and transplant surgery duration. Moreover, the recipients were characterized as being high-risk as per MELD score. These data suggest that PNF after a strict donor selection is greatly contributed by the recipient-derived factors and transplant surgery parameters.

Three patients with PNF underwent retransplantation on the 2^{nd}, 20^{th}, and 21^{st} day, and died 2, 21, and 18 days later, respectively, as a result of the pre-existing MOF progression and sepsis, and a repeated PNF in one case. Retrospectively revising the unfavourable experience, we may talk about over-expanded indications to retransplantation in the first case (extremely unstable hemodynamics and excessive metabolic disorders), and about the improperly delayed retransplantation in the other two cases

In this regard, a great interest arises to the recently published study of the Essen Group [17]. The aim of the study was to evaluate and identify the factors contributing to the patient and graft survival in cases of EAD and transaminase activities over 5000 U/L at 24 hours after transplantation. Such transaminase level elevations were recorded in 64 (7.0%) of 917 adult

patients after OLT. Of those, a 30-day and a 1-year survival rates made 21.4% in the patients with post-transplant (at 24 hours) MELD scores of 31 or more, and 80% and 71.8 %, respectively (p <0.001), in the patients with post-transplant MELD scores under 31. The authors suggested that using MELD score at 24 hours after OLT allows a transplant surgeon to make an objective decision on retransplantation without further waiting for the graft function recovery.

Thus, EAD, and particularly PNF, are the most dangerous and life-threatening complications of OLT, and also may serve as reliable independent predictors of the transplantation adverse outcome in the early postoperative period. Therefore, the differential diagnosis of EAD and PNF, and timely decision-making on retransplantation acquire an essential importance.

Long-term results

The long-term outcomes in the EAD group were significantly worse, and exclusively due the early graft loss and death (Fig. 1).

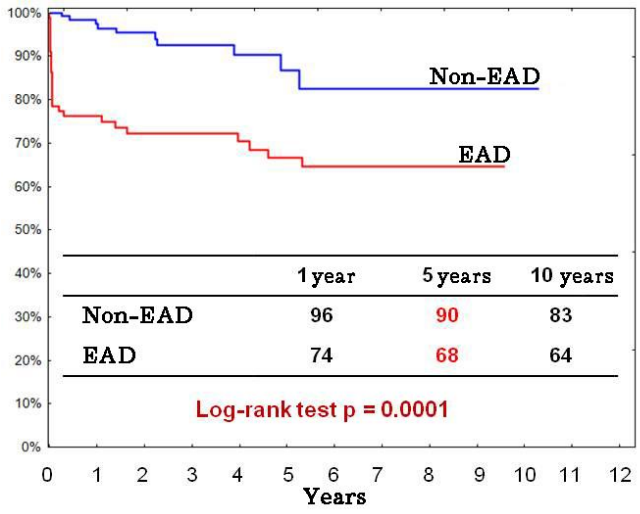

Fig. 1. Graft survival in the non-EAD and EAD groups. Two endpoints: recipient death and retransplantation were considered

However, the dysfunction in the majority of EAD patients (86%) was reversible and did not affect the survival rate (Fig. 2).

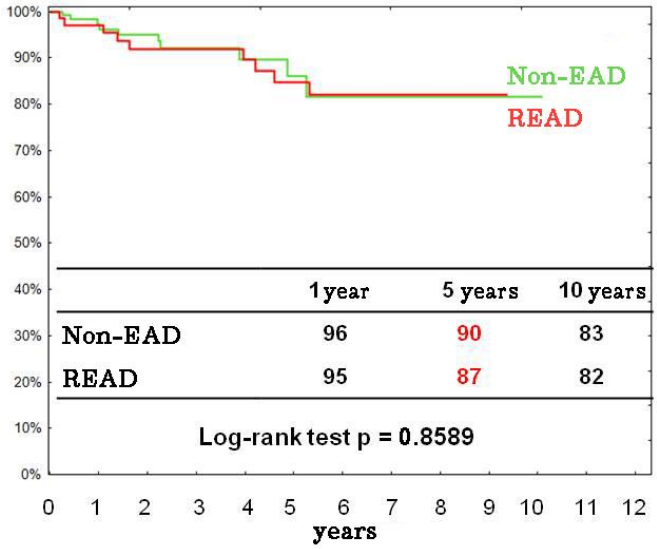

Fig. 2. Graft survival in the non-EAD and READ groups. Two endpoints: recipient death and retransplantation were considered

Despite the fact that the development of EAD and PNF represents a multifactorial process, the liver donor category has a significant impact on the transplantation outcome (Fig. 3).

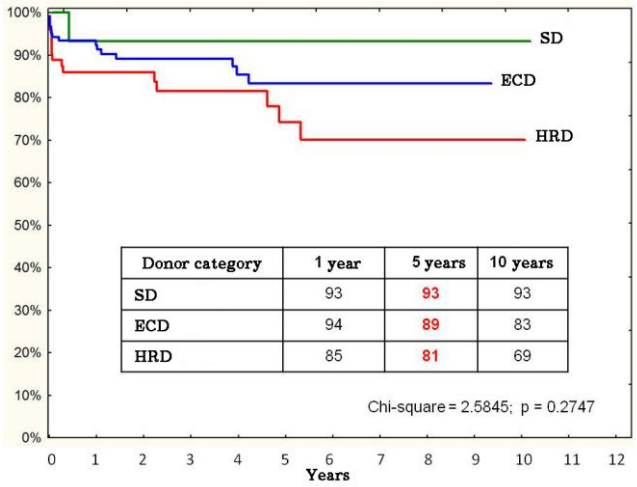

Fig. 3. Graft survival with regard to donor category. Two endpoints: recipient death and retransplantation were considered.

Conclusion

Our data, being completely consistent with literature, have demonstrated that EAD, including READ and PNF, were associated with high mortality rates and a high incidence of early graft loss, and also constitute the main indication to retransplantation in the early post-OLT period. Risk factors for EAD are well-known and were confirmed in our study. The most significant of them include:

- Retransplantation;
- HRD;
- The surgery duration exceeding 9.5 hours;
- Graft cold ischemia time more than 8 hours.

In actual clinical practice, given that most donors are ECDs (57.3%), and even belong to the high-risk donor category (34.7%), a special attention should be given to modifiable factors: the prevention and correction of donor hypernatremia during the donor management; the minimization of the graft cold ischemia time, blood loss and OLT surgery duration; the exclusion, as far as possible, of combined risks related to the donor, recipient, and surgical procedure.

The donor stratification as belonging to a high risk category should not be considered as an absolute contraindication to the use of his liver graft, but requires a special attention at recipient selection that would allow the minimization of the cold ischemia time and ensuring the most possible blood-saving surgery. For example, such donors should not be used either for split-transplantation, or for retransplantation in the long-term period. Indications for such retransplantation should be established electively and planned in advance, rather than in urgent order, to allow the optimal graft selection.

Factors responsible for a PNF development as the most severe and irreversible EAD, have not been established with statistical significance in our study, perhaps due to the small sample size. However, we may suggest that the listed EAD prevention tools should reduce the risk of PNF.

Donor and recipient characteristics remained actually unchanged over the 11 years of the OLT program. Obviously, the EAD/PNF development might be considerably contributed by surgical procedure peculiarities (the surgeon experience, recipient's history of previous surgery, the selected implantation technique, transfusion tactics, anesthesia, and intensive care). These factors have been subjected to substantial modifications since the start of the program to date. By 31.12.2015 (the time of writing the article) 270 OLT were performed (67 in the period of 2004-2009; 70 in 2010-2012; 133 in 2013-2015). The annual increase in the number of operations was accompanied by a two-fold decrease in the amount of transfused blood components and blood products (Fig. 4), and by a significant reduction in the incidence of READ, PNF, and in mortality rates (Fig. 5).

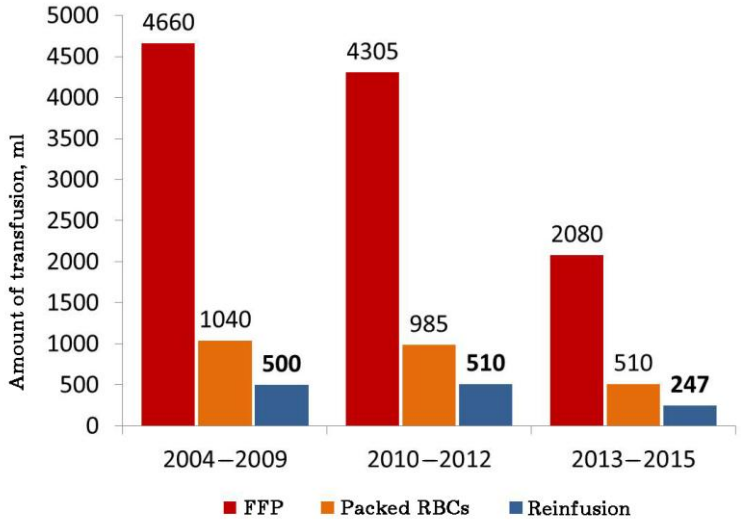

Fig. 4. Changes in intraoperative use of blood components and reinfusion in different periods of OLT program

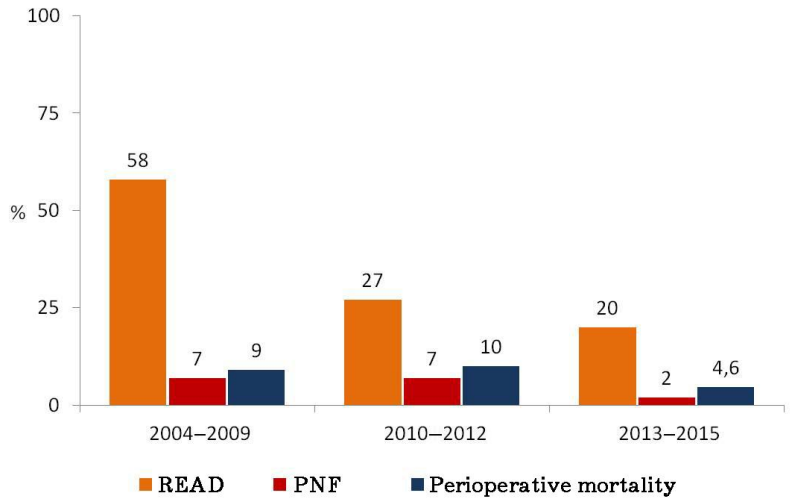

Fig. 5. Changes in the incidence of READ, PNF, and mortality rates in different periods of OLT program

In conclusion, we should emphasize that in case of evident severe (extreme) EAD requiring a whole complex of the described intensive care measures, the main question that must be answered as soon as possible is whether this condition is reversible and how urgently a retransplantation required. The use of UNOS PNF criteria alone for this purpose may not be enough in situation when metabolic disorders and blood coagulation impairments are corrected by means of sophisticated intensive care techniques, including exstracorporeal organ replacement therapies. It is interesting to note that in all those patients with PNF in our series, in whom MELD score was assessed retrospectively, it exceeded 31. Further prospective studies are needed to develop and optimize the objective criteria of correct tactics selection.

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