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The histological assessment of liver fibrosis in grafts from extended criteria donors predicts the outcome after liver transplantation: A retrospective study

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ABSTRACT

Background: The use of extended criteria donors (ECD) in liver transplantation is increasing due to the organ shortage. Histological evaluation of the liver graft in the context of procurement is an important tool for extending the donor pool without affecting the quality of the transplanted organs. Macrovesicular steatosis is widely accepted as predictor of early allograft dysfunction (EAD), while other features, such as portal fibrosis, are poorly studied.
Aim: To identify morphological features, other than macrovesicular steatosis, that may affect recipients' outcome.
Methods: Between 2014 and 2016, 132 donors with extended criteria underwent pre-transplant liver biopsy during procurement. Histological variables of the graft, donors'/recipients' clinical data, EAD and patient/graft survival were registered.
Results: The recipients who received a graft with histological-proven portal fibrosis had a significant lower patient and graft survival in comparison to patients without fibrosis (P = 0.044 and P = 0.039, respectively). Donors' dyslipidemia was significantly associated with the occurrence of EAD (P = 0.021). When dyslipidemia was combined with histological liver fibrosis a 54.5% incidence of EAD was observed (P = 0.012).
Conclusions: The histological assessment of liver fibrosis in pre-transplant biopsy of ECD grafts, together with donor's clinical data, provides important information on recipients' outcome.

1. Introduction

The extended criteria donors (ECD) have increased the number of liver donors in these last two decades [1]. Donor age is the strongest criteria defining ECD. The literature suggests that the use of aged organs could reduce the number of patients on the waiting list although it can increase the risk of graft dysfunction [2,3]. The age limit for donation is variable and according to the literature ranges from 50 up to 70 years; [4] the original age cut-off for ECD was set at 40 years, and the risk of graft failure progressively increases with age [5]. Our experience has demonstrated similar survival rates in recipients who received grafts from donors >65 years old, 55–65 years old and <55 years old [6]. Besides donor age, other ECD criteria include donation after cardiovascular death (DCD), macrovesicular steatosis (MS), prolonged cold ischemia time (CIT), treatment in intensive care unit for >7 days, high body-mass index (BMI), steatosis, hypernatremia, high levels of alanine/aspartate aminotransferases (AST/ALT), bilirubin or gamma glutamyl-transferase [4,7,8].

The role of pre-transplant liver biopsy in ECD grafts is greatly debated in the different transplant centers worldwide and some

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groups consider it as a time-consuming procedure, without any advantages in the decision making. On the contrary, others consider liver biopsy an important tool to increase the donor pool without affecting the quality of the transplanted organ. Macrovesicular steatosis is recognized as the most important histological prognostic parameter, predicting graft loss [9–11] with acceptable percentages of MS that vary among different centers [11–13]. However, other morphological alterations such as fibrosis, chronic inflammation or arteriolar thickening could be found [14,15].

Despite improvement in pre-allocation clinical imaging early allograft dysfunction (EAD) and primary non-function (PNF) still represent a major concern in LT [16–18]. EAD implies a 10.7-fold risk of dying and a 7.4-fold risk of losing the graft [17], whereas PNF may occur in up to 10% of LT from ECD, even in centers where liver biopsies with MS assessment are performed [19].

The aim of the present study is to identify morphological features of liver graft, other than MS, that may affect short- and long-term recipient's outcome.

2. Materials and methods

2.1. Study design

Liver biopsies were performed during organ procurement in all donors aged more than 65 years, and/or with a history of alcoholic abuse or other causes of hepatic injury, and/or with alterations in liver function tests, according to the more recent European Guidelines [5]. The decision to proceed with the LT was made on the macroscopic appearance of the liver and the histopathological examination of liver biopsy, performed to quantify hepatic steatosis. Digital images of the liver were sent via smartphones in real-time by the junior surgeon to gain a staff surgeon's opinion in the evaluation of the organ during procurement at a remote site. For the purpose of the present study, only ECD after brain death were included, as well as only recipients transplanted in our Institution (S.Orsola-Malpighi Hospital, University of Bologna, Italy) with at least 6 months of available follow-up. The present study conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008).

2.2. Setting

Between 2014 and 2016, all patients who met the inclusion criteria, submitted to pre-transplant liver biopsy during organ procurement, were enrolled. Informed consent for organ and tissue donation was signed by the donor's closest relatives or legal representative, according to the Italian law. Donors' clinical data were collected from the Regional Transmission Information System including tobacco use, alcohol abuse, heart, lung or liver disease, hypertension, diabetes, dyslipidemia, nephropathy, drug abuse, body-mass index (BMI). The Regional database does not include details on therapy regimens, and cholesterol/triglycerides serum levels: data on hypercholesterolemia and therapy with statins were retrieved when specifically available.

Recipients' clinical data collected included: model for end-stage liver disease (MELD) score at LT, etiology of liver cirrhosis, BMI. Cold ischemia time (CIT), grafts' and recipients' survivals were recorded as well.

2.3. Histological variables

Liver graft biopsy included both wedge and needle biopsies for frozen-section analysis. Biopsies were performed with a 18 Gauge needle and sent in gauze soaked with saline solution [9]. In order to propose a model as close as possible to the real setting, we considered only the morphological features on frozen section. Permanent sections were evaluated in order to confirm these data.

The organ was considered unacceptable by histological examination if MS was $\geq$30%. Moreover, graft with MS < 30% but with features of patent steatohepatitis —such as hepatocellular necrosis, granulocytic infiltrate and/or evidence of ballooning— were not used for donation. Other histological features were not considered for organ assessment in the present study but retrospectively evaluated: fibrosis of portal tracts (absent or present), portal inflammation (absent, mild, moderate or severe), biliary changes (absent or present), myointimal arteriolar thickening (absent, mild or severe), lobular necrosis (absent or present), lobular inflammation (absent or present), occurrence and percentage of microvesicular steatosis, and cholestasis (absent, mild, moderate or severe). Fibrosis of the portal tract was semi-quantitatively assessed as present when an enlargement of most portal tract was seen, generally with initial short septa (defining Ishak stage 2 fibrosis or more). Severe arteriolar thickening was defined according to the definition used by the Karpinski score system for kidney transplantation [20], i.e. when the wall thickness exceeded the diameter of the lumen and at least one obliterated arteriolar lumen was observed. Biliocyte regression was defined when loss of biliocytes in the duct, nuclear biliocyte pleomorphism, small size and/or irregular shape of the duct were observed.

2.4. Outcome variables

EAD was defined as the presence of at least one of the following clinical parameters on postoperative day 7: bilirubin $\geq$ 10 mg/dL, Prothrombin Time international normalized ratio (PT-INR) $\geq$ 1.6 and/or ALT/AST > 2000 IU/L.

Graft survival (GS) was defined as the time from the day of LT to the patient's death, re-transplant, or last date of follow-up. Patient survival (PS) was calculated from the day of LT to the patient's death or last follow-up.

2.5. Statistical analysis

Data were expressed as mean $\pm$ SD. Differences were explored by the and the Mann–Whitney U test, as appropriate. Categorical variables were compared using the chi-square test. Uni- and multivariate logistic regression analysis (Stepwise Backward Wald logistic regression) was performed to identify independent predictors of EAD. Survival curves were calculated using the Kaplan–Meier method and compared using the log-rank test. All statistical tests were two-tailed, and differences were considered significant at a p-value of $\leq$0.05. Data analysis was performed with SPSS for Windows (ver. 20).

3. Results

During the study period, 258 LT were performed. Among them, 132 (51.2%) had pre-transplant liver biopsy.

3.1. Donors and recipients' characteristics

Mean age of donors was 65 years (range 19–93). It was reported a history of tobacco use in 41 donors (31.3%), alcohol abuse in 4 (3.0%), heart disease in 39 (29.5%), lung disease in 9 (6.8%), hypertension in 78 (59.1%), diabetes in 18 (13.6%), nephropathy in 10 (7.6%), and drug abuse in 2 (1.5%). A history of liver disease was reported in 10 (7.6%) donors: in particular, HCV infection in 5, previous HBV infection in 1, combined HCV-HBV infection in 1, previous HAV hepatitis in 1, and previous resection for benign lesion in 2 (one biliary cyst and one hemangioma). Dyslipidemia was present in 31 (23.5%) donors, among which a hypercholesterolemia

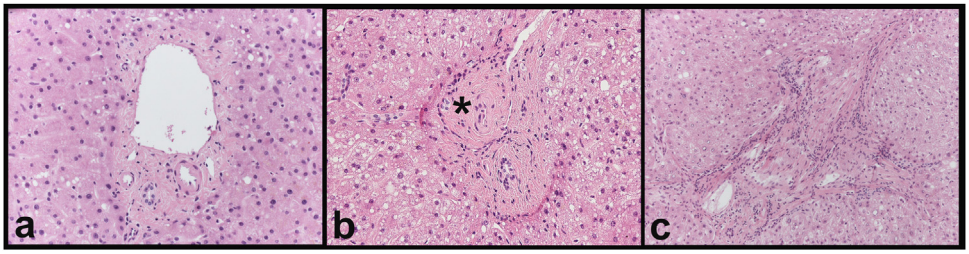

Fig. 1. Histology of liver grafts. (a) An example of regular portal tract. (b) Mild portal fibrosis with myointimal thickening of a portal artery (asterisk). (c) A fibrotic portal tract with septa.
Haematoxylin-Eosin stain, 20× (a,b) and 4× (c) magnification.

Table 1
Histological features normally assessed in the check-list for the intraoperative pathology report of our Institution. Frequencies (n) and percentages (%) in our series.

Histological features		n (%)
Portal fibrosis	Absent	36 (27.3)
	Present	96 (72.7)
Portal inflammation	Absent	13 (9.8)
	Mild	113 (85.6)
	Moderate	6 (4.5)
	Severe	0 (0.0)
Biliary changes	Absent	35 (34.1)
	Present	87 (65.9)
Myointimal arteriolar thickening	Absent	17 (12.9)
	Mild	51 (38.6)
	Severe	64 (48.5)
Lobular necrosis	Absent	115 (87.1)
	Present	17 (12.9)
Lobular inflammation	Absent	106 (80.3)
	Present	26 (19.7)
Macrovesicular steatosis	<10%	104 (78.7)
	10–30%	28 (21.3)
	>30%	0 (0.0)
Microvesicular steatosis	<10%	75 (56.9)
	10–30%	53 (40.1)
	>30%	4 (3.0)
Cholestasis	Absent	106 (80.3)
	Mild	21 (15.9)
	Moderate	3 (2.3)
	Severe	2 (1.5)

in statins therapy was reported in 20 (15.2%). Mean donors' BMI was 16.2 ± 40.4 (range 26.4–4.2).

Recipients' group included 98 (74.2%) males and 34 (25.8%) females, mean age was 53.82 ± 8.65 years (range 24–69), mean recipients' BMI was 25.4 ± 3.9 (range 15.4–37.7). The main indication for LT was represented by chronic viral hepatitis (87/132 patients, 66%) with hepatocellular carcinoma representing the most common complication (n = 58, 44%). Preoperative mean MELD score was 18.5 ± 6.9 (range 7–37), mean cold ischemia time was 369.3 ± 52.2 min (range 245–500 min), life support was needed in 4 of 132 (3%) patients before LT.

3.2. Histological features

The mean number of portal tracts evaluated was 16.9 ± 3.2 (range 12–23). Histological analysis showed portal fibrosis in 96 patients (72.2%). Portal inflammation was absent in 13 cases (9.8%), mild in 113 (85.6%) and moderate in 6 (4.5%). Biliocyte regression was observed in 87 cases (65.9%). Lobular necrosis was confirmed in 17 cases (12.9%), whereas lobular inflammation in 26 (19.7%). Cholestasis was absent in 106 cases (80.3%), mild in 21 (15.9%), moderate in 3 (2.3%), and severe in 2 (1.5%). Mean MS was 5.13 ± 6.26% (range 0–30) and microvesicular steatosis 9.67 ± 11.75% (range 0–65). Sixty-four cases (48.5%) showed severe arteriolar thickening (Fig. 1; Table 1).

3.3. Outcome following LT

Major complications (Dindo-Clavien ≥ grade 3A) occurred in 35 out of 132 patients (26.5%), whereas biliary complications (leak and/or stenosis) in 19 (14.4%). EAD was recorded in 38 (29.7%) recipients, including 4 cases of PNF. Ten out of 38 (26.3%) patients experienced graft loss and 5 of them were successfully re-transplanted. Ninety-day mortality was 3%. Donors of patients with EAD showed more frequently dyslipidemia (P = 0.021) and nephropathies (P = 0.024). At multivariate analysis, recipients from dyslipidemic donors (p = 0.016, OR = 2.89, 95% CI = 1.22–6.85) and recipient's MELD (p = 0.023, OR = 1.07 95% CI = 1.01–1.13) confirmed to have a significant higher risk of EAD.

Even though there were no significant differences between patients with and without EAD in terms of histopathological features, combining liver fibrosis with the only independent predictor of EAD among all donor factors examined (i.e. donors' dyslipidemia), the association with EAD became even stronger: we observed 12 EAD in 22 (54.5%) recipients with dyslipidemic grafts and portal fibrosis, versus 5 EAD cases in 27 (18.5%) recipients from non-dyslipidemic donors without portal fibrosis (P = 0.012). The presence of only one out of two risk factors had 29.8% of EAD incidence.

3.4. Survival analysis

Mean follow-up was 28.7 ± 14.3 months. At the end of follow-up 17 (12.9%) patients died, 1 for graft failure, 9 for sepsis/infective episodes, 4 for hepatocellular carcinoma recurrence, and 3 for other causes. One- and 5-year patient survival (PS) was 90% and 83.1%, respectively. One- and 5-year graft survival (GS) was 86.3% and 79.1%, respectively.

Taking singularly main histological features (Table 2), we observed that recipients who received a graft with histological-proven portal fibrosis had a significant lower GS (Fig. 2a) and PS (Fig. 2b) in comparison to patients without fibrosis (P = 0.039 and P = 0.044, respectively). No significant difference in survival was seen when other histological features were compared.

3.5. Role of arteriolar thickening

Thirty out of 132 (22.7%) recipients needed liver biopsy within 6 months post-OLT due to rises in transaminases and/or cholestasis markers. Biopsies were performed at 7.0 ± 6.5 weeks after transplantation (range 1.2–19.6).

Briefly, histopathological analysis of graft biopsies showed recurrent hepatitis C in 12 cases and acute T-cell-mediated rejection in 7 cases. The other 11 cases showed other biliary changes and lobular necrosis (mild in 8 cases, moderate in 3 and severe in 1). Cholestasis was present in 8 biopsies (mild in 4, moderate in 1, severe in 3). We did not observe any case of preservation/reperfusion injury.

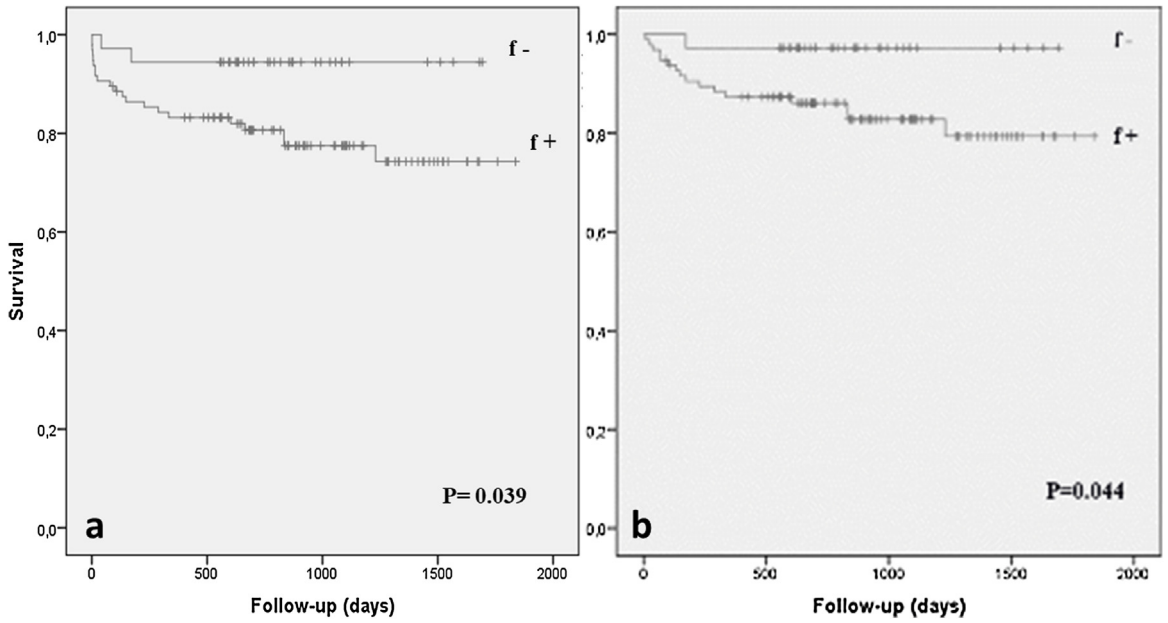

Fig. 2. Survival curves (graft –a- and recipient –b-) of transplantations with (f +) and without (f –) portal fibrosis at donors' liver biopsy (P = 0.039 and P = 0.044, respectively).

Table 2
Correlation coefficients of the donors' histological and clinical variables towards the main follow-up endpoints of the present study.

Graft histology	Recipient survival (Log rank)	Graft survival (Log rank)	EAD (chi-square)
Arteriolar thickening	P = 0.439	P = 0.317	P = 0.243
Portal fibrosis	**P = 0.044**	**P = 0.096**	P = 0.207
Portal infiltrate	P = 0.962	P = 0.435	P = 0.108
Bile duct regression	P = 0.944	P = 0.781	P = 0.468
Lobular necrosis	P = 0.367	P = 0.834	P = 0.470
Lobular infiltrate	P = 0.941	P = 0.700	P = 0.364
Macrovesicular steatosis	P = 0.491	P = 0.814	P = 0.329
Microvesicular steatosis	P = 0.454	P = 0.670	P = 0.724
Cholestasis	P = 0.380	P = 0.156	P = 0.801
Donor clinical data			
Tobacco use	P = 0.782	P = 0.113	P = 0.418
Alcohol abuse	P = 0.550	**P = 0.017**	P = 0.184
Cardiopathy	P = 0.910	P = 0.863	P = 0.772
Pneumopathy	P = 0.901	P = 0.946	P = 0.235
Epatopathy	P = 0.855	P = 0.941	P = 0.481
Hypertension	P = 0.686	P = 0.822	P = 0.399
Diabetes	**P = 0.065**	P = 0.359	**P = 0.054**
Dyslipidemia	P = 0.527	P = 0.956	**P = 0.021**
Nephropathy	P = 0.646	P = 0.263	**P = 0.024**
Drug abuse	P = 0.644	P = 0.724	P = 0.110

Arteriolar myointimal thickening was the only donor variable that statistically correlated with the need for liver biopsy within the early 6 months post-OLT. In particular, 22 out of 30 (73.3%) recipients who received grafts with arteriolar thickening needed post-OLT biopsies, versus 29 out of 81 (35.8%) recipients without arteriolar thickening (P < 0.001, chi-square test).

Due to the objectively questionable end-point, these findings will not be discussed further, albeit representing interesting incentives for further studies on graft arteriolar thickening.

4. Discussion

In our study, we demonstrated that portal fibrosis affects patient and graft survival after LT and that graft fibrosis in association with donor's dyslipidemia may contribute to increase the risk of EAD. Other histopathological features significantly impacted neither on EAD nor on patient survival.

The histological evaluation of donor liver biopsy can provide useful information in the prediction of the outcome of recipients, and in particular it can assess the degree of MS, which still represents an independent risk factor for graft dysfunction [9,11,19]. However, besides MS, other histological features may play a role in the recipient outcome. In fact, ECD pushed the limit of organ transplantation, and the evaluation of donor biopsy is likely to be essential to define other morphological features that could correlate with aging or concurrent systemic diseases [14,15].

Our data revealed that recipients who received a graft with histological-proven portal fibrosis had a significant lower survival in comparison to patients without fibrosis. Although liver fibrosis can be assessed by histological examination to help in the decision-making for organ viability, the impact of fibrosis in the recipient's outcome has still to be cleared. A recent paper by Wadhera et al. studied the role of graft fibrosis on recipients' outcome, finding no impact on long-term survival [21]. Nevertheless, further studies are needed in order to understand the exact role of graft fibrosis in LT outcome, and whether fibrosis from ECD organs implies a different risk than fibrosis from a standard donor graft. Moreover, in our study we have assessed the fibrosis only with Haematoxylin-Eosin on frozen section. The usefulness of Reticulin or other stains on frozen section to better define portal or lobular fibrosis is under evaluation [22].

According to our results, portal fibrosis was not directly associated to EAD. The etiopathogenesis of EAD is multifactorial and previous studies failed to identify a correlation between histological features —different from MS— and EAD. However, in our study, when combining this feature with the presence of donor dyslipidemia, EAD occurred in more than 50% of recipients. The term "dyslipidemia" is used in our setting to indicate the presence of cholesterolemia and/or hypertriglyceridemia. In the current setting it is not possible to assess cholesterol and triglycerides blood levels in the donor population, and with this definition, dyslipidemia may reflect a more general metabolic disorder not necessary in the context of a non-alcoholic fatty liver disease (NAFLD). The prevalence of NAFLD is increasing on a global scale and non-alcoholic steatohepatitis (NASH) is expected to be the leading cause of LT in the next years. Likewise, an increased incidence of metabolic disorders in donors has to be expected too. In the present study, according to

the current guidelines, donors with NAFLD, defined as with more than 5% of MS but without necrosis or lobular alterations [23] were used for donation after histological analysis.

A hypothetical mechanism linking metabolism and liver fibrosis can be represented by the gut-liver axis, described to alter lipid metabolism, before the occurrence of histological MS [24]. Part of bacterial products from gut microbiota reaches the liver trough the blood portal flow, and these alterations might be transferred also to the recipient, thus increasing the risk after LT also in standard recipients, as also confirmed by experimental animal models [25,26].

This observation surely deserves further analysis, since portal fibrosis is a common observation among elderly ECD, and dyslipidemia is an increasing health problem in the general population.

In conclusion, in the ECD era transplant setting, the donor liver biopsy provides important information regarding the outcome of the recipient. Our results indicate that portal tract fibrosis and donor dyslipidemia could represent useful predictors of recipients' outcome. In particular, portal fibrosis is an independent predictor of recipients' survival. Further studies in this direction are required to improve the algorithm of graft selection.

Conflict of interest statement

None declared.

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