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# Risk factors, surgical complications and graft survival in liver transplant recipients with early allograft dysfunction

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ABSTRACT

*Background:* Early allograft dysfunction (EAD) is a severe complication after liver transplantation. The associated risk factors and complications have re-gained recent interest. This study investigated risk factors, survival and complications associated with EAD in a large liver transplant center in Latin America.

*Methods:* Retrospective, unicenter, cohort, based on data from adult patients undergoing first deceased-donor liver transplant from January 2009 to December 2013. EAD was defined by one or more of the following: (i) bilirubin ≥10 mg/dL on postoperative day 7; (ii) international normalized ratio ≥1.6 on postoperative day 7, and (iii) alanine aminotransferase or aspartate aminotransferase >2000 IU/L within the first seven days after transplant.

*Results:* A total of 602 patients were included; of these 34.2% developed EAD. Donor risk factors were male ($P=0.007$), age between 50 and 59 years ($P=0.034$), overweight ($P=0.028$) or grade I obesity ($P=0.012$), sodium >157 mmol/L ($P=0.002$) and grade IV ischemia/reperfusion injury ($P=0.002$). Cold ischemia time ≥10 h ($P=0.008$) and warm ischemia time ≥40 min ($P=0.013$) were the surgical factors. Male ($P<0.001$) was the only recipient protective factor. Compared with the non-EAD group, patients with EAD were submitted to more reoperations (24.3% vs. 13.4%, $P=0.001$) and had higher graft loss rates (37.9% vs. 21.2%, $P<0.001$), with similar patient survival rates ($P=0.238$).

*Conclusions:* EAD risk factors are related to donor, surgical procedure and recipient. Donor risk factors for EAD were male, age between 50 and 59 years, donor overweight or grade I obesity, sodium >157 mmol/L and grade IV ischemia/reperfusion injury. Cold ischemia time ≥10 h and warm ischemia time ≥40 min were the surgical risk factors. Male was the only recipient protective factor. Patients with EAD had higher reoperations and graft loss rates.

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## Introduction

Liver transplantation (LT) is the treatment of choice for patients with end-stage liver disease. Early allograft dysfunction (EAD) is a severe complication after LT, affecting 5.2%–38.7% of the recipients [1–4]. EAD has been associated with post-transplant complications and compromises patient and graft survival [2,5,6]. EAD is related to ischemia/reperfusion (I/R) injury to the transplanted organ [6–8]. Primary non-function (PNF) is the most severe manifestation [2,7].

Different parameters and cutoffs were proposed to define and diagnose EAD [4,5,9–11]. In 2010, Olthoff and a group of experts examined an updated definition of EAD to validate previously used criteria, and correlated this definition with graft and patient outcome [5]. In that study, the EAD definition included one or more of the following variables: (i) bilirubin levels ≥10 mg/dL on postoperative day 7; (ii) international normalized ratio (INR) ≥1.6 on postoperative day 7 and (iii) alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels >2000 IU/L within the first seven days after transplant. There is no clear accepted definition of PNF. However, early retransplantation or patient death are universally accepted as a common PNF outcome [3–5,7,12–14].

EAD is associated with the transplant procedure, but also with donor and recipient clinical and epidemiological characteristics and respective local surgical practice [2,7]. Despite the large body of literature on EAD risk factors, analysis are mainly based on data from single small center reports with low statistical power and short-term follow-up in most studies, precluding accurate and consensual definition of these characteristics. Moreover, significant

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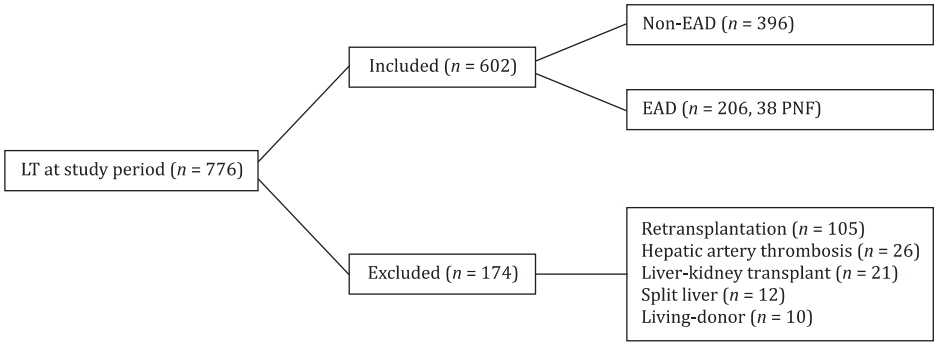


**Fig. 1.** Liver transplantation (LT) patients during period of the study.

knowledge regarding EAD is originated in the early LT years prior to the current model for end-stage liver disease (MELD) era. Finally, EAD data from Latin America are scarce. Thus, one could hypothesize the incidence of EAD and the donor, recipient and transplant risk factors might have changed over the past decades. This study was to identify potential risk factors for EAD and to assess current morbidity and mortality associated with this entity in patients who underwent LT in a high-volume Latin American transplant center.

## Methods

This study was conducted in compliance with the *Declaration of Helsinki* and with local and national regulations. The study was exempted from informed consent and was approved by the Internal Review Board (CAAE No. 40520214.3.0000.0071).

This was a retrospective cohort study evaluating patients who underwent LT at the same organ transplant center, from January 1st, 2009 to December 31st, 2013. Follow-up database ended on May 31st, 2018.

Patients underwent LT with piggyback technique and side-to-side inferior vena cava reconstruction. Immunosupression was tacrolimus based without antibody induction with the use of a 3-month steroid taper. After 3-month patients remained on tacrolimus monotherapy. During the period of the study, anti-HCV treatment consisted of a Peg-interferon based regimen for selected patients.

Patients with new changes in aminotransferases have initially a new check on potential vascular complications by ultrasound followed by a 30% raise in tacrolimus levels. Those who persist with altered liver function tests will have an ultrasound-guided percutaneous liver biopsy that was checked by an experienced and fully dedicated liver transplant pathologist. Acute cellular rejection was defined and graded according to Banff criteria report [15,16]. At our center, only recipients with viral hepatitis will have annual liver protocol biopsies.

Adult patients aged over 18 years who had their first deceased-donor LT were included. Recipients submitted to combined liver-kidney transplant or retransplantation, those receiving split grafts, or diagnosed with vascular thrombosis within the first week after transplant were excluded (Fig. 1).

Patients met criteria for EAD when at least one of the following variables were present: (i) bilirubin levels $\geq$10 mg/dL on postoperative day 7, (ii) INR $\geq$1.6 on postoperative day 7, or (iii) ALT or AST levels >2000 IU/L within the first seven days post-transplant [5]. The study population was divided into two groups (EAD and non-EAD) according to the EAD criteria of Olthoff et al. [5]. Patients who had PNF (most severe degree of EAD) were included in the EAD group. PNF was defined according Organ Procurement and Transplantation Network (OPTN) and United Network for Organ Sharing (UNOS) criteria [17].

The following donor data were included: sex, age, body mass index (BMI), cause of death, length of intensive care unit (ICU) stay, arterial blood pH, sodium level, I/R injury and macrovesicular steatosis in time-zero biopsy. Surgical procedure data included cold ischemia time (CIT) and warm ischemia time (WIT), use of blood products. Recipient data included sex, age, race, body mass index, blood type, cause of LT, origin at LT, biological MELD score at LT, length of follow-up (day) and clinical outcomes and complications (length of transplant-related hospital stay, rejection, infection, opportunistic infections, transplant related reoperations, biliary and vascular complications, graft and patient survival).

Age was stratified according to the work of Feng et al. [18]. Donors and recipients were considered overweight or obese according to World Health Organization (WHO) definition and classification [19,20].

Transplant-related reoperations were defined as those performed within 90 days of the first surgery, including delayed abdominal wall closure, investigation of intra-abdominal bleeding, surgical management of intra-abdominal fluid collection, treatment of biliary complications and resuture of the abdominal wall. Incisional hernia repair within the first year after transplant was also defined as reoperation.

Length of hospital stay related to transplant was defined as the time from LT to hospital discharge. Biliary fistula and/or stenosis were the biliary complications considered. Vascular complications were hepatic artery thrombosis or stenosis, portal thrombosis or stenosis and supra-hepatic vein thrombosis, occurring after the first week of LT. Rejection was defined according to the Banff criteria [15,16]. Infections were defined according to Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) criteria and the following conditions considered: pneumonia, urinary tract infection, surgical site infection, and venous catheter related blood stream infection [21]. Patients positive for cytomegalovirus, herpesvirus or clostridium, among other agents, were included in the opportunistic infection category.

Data concerning I/R injury severity based on histological assessment of time-zero biopsies were also included. I/R injuries were grade zero (grade I), mild (grade II), moderate (grade III) or severe (grade IV), according to the presence of neutrophilic infiltrates, apoptotic bodies and changes in hepatocyte cell architecture [22].

### *Statistical analysis*

Categorical variables were described with absolute and relative frequencies. Numerical variables were described as mean and standard deviations (SD) or median and ranges.

Factors associated with EAD were investigated using univariate and multivariate logistic regression analysis. Variables with $P \leq 0.2$ in the univariate analysis were included in the multiple regression

model. For the multivariate analysis, *P* values under 0.05 were considered statistically significant.

Results were expressed as estimated odds ratio (OR), 95% confidence interval (CI) and *P* value. Donor arterial blood pH, donor sodium level, donor length of ICU stay, CIT and WIT cutoffs were determined using ROC curve analysis. Respective areas under the receiver operating characteristic curve and 95% CI were calculated. CIT and WIT cutoffs used in literature were also included in the univariate model [2,23]. Large amount of missing data of the donor biopsies precluded inclusion of steatosis in the logistic regression model.

Comparative analysis of clinical outcomes of patients in the EAD and non-EAD group was based on the Chi-square and the Mann–Whitney *U* tests.

Patient and graft survival were evaluated at different time points: 30 days, 90 days, 1 year and 5 years. All information outside the periods of interest was censored. Patient and graft survival curves were prepared using the Kaplan–Meier method; the log-rank test was employed for comparative analysis of EAD and non-EAD groups.

For descriptive analysis and survival curves, $P < 0.05$ was considered statistically significant. Statistical analyses were performed using *R* statistical software (Team RC, R Foundation for Statistical Computing, Vienna, Austria) [24,25] and the cmprsk package [26].

## Results

There were 776 liver recipients within the period of the study, 602 of them met the inclusion criteria. A total of 206 (34.2%) patients developed EAD, of which 38 (6.3% of all included) had PNF. Data concerning donor characteristics, surgical procedure and recipient demographics are displayed on Table 1.

Mean patient follow-up was 1011.4 ± 669.7 days, with 17.6% (106/602) mortality rate. Median transplant related hospital stay was 10 days. Acute cellular rejection rate at year 1 of follow-up was 17.8% (107 patients), of which 75.7% (81 patients) were steroid treated. Graft loss rates was higher in the EAD patients than in the non-EAD patients (37.9% vs. 21.2%, $P < 0.001$). LT related reoperations were required in 24.3% of patients in the EAD and in 13.4% of patients in the non-EAD groups ($P = 0.001$). Re-transplant rates was 18.9% in the non-EAD compared to 34.4% in the EAD group. Clinical outcomes and complications are displayed on Table 2.

EAD risk factor analysis is shown in Table 3. Male ($P = 0.013$), age group 40 to 49 or 50 to 59 years ($P = 0.115$ and $P = 0.010$, respectively), overweight, grade I or grade II obesity subgroups ($P = 0.004$, $P = 0.001$ and $P = 0.152$, respectively), ICU stay >5 days ($P = 0.162$), arterial blood pH >7.35 ($P = 0.056$), serum sodium level >157 mmol/L ($P = 0.006$) and grade III or IV I/R injury in time-zero biopsy ($P = 0.054$ and $P = 0.003$, respectively) were the donor variables included in the multiple regression model. CIT ≥10 h ($P = 0.001$) and WIT ≥40 min ($P = 0.002$) were the variables associated with surgical procedure with the highest levels of significance in the simple regression analysis, and were therefore included in the multiple regression model. As regards recipient factors, grade I obesity ($P = 0.101$), male ($P = 0.004$), age groups 40–49, 50–59 or 60–69 years ($P = 0.097$, $P = 0.141$ and $P = 0.065$, respectively) and patients transferred from the hospital ward ($P = 0.148$) or ICU ($P = 0.139$) were associated with higher EAD risks. All of these factors were included in the multiple regression model. Male (OR = 1.73, 95% CI: 1.16–2.60, $P = 0.007$), age group 50 to 59 years (OR = 1.73, 95% CI: 1.04–2.87, $P = 0.034$), overweight (OR = 1.59, 95% CI: 1.05–2.41, $P = 0.028$) and grade I obesity (OR = 2.28, 95% CI: 1.19–4.35, $P = 0.012$), sodium levels above 157 mmol/L (OR = 1.84, 95% CI: 1.26–2.68, $P = 0.002$) and grade IV I/R injury (OR = 4.18, 95% CI: 1.70–10.60, $P = 0.002$) were significant donor risk factors in the multivariate analysis. CIT ≥ 10 h (OR = 1.78, 95%

**Table 1**
Donor, transplant and recipient characteristics by EAD status.

| Variables | Non-EAD ($n = 396$) | EAD ($n = 206$) |
|---|---|---|
| Donor characteristics | | |
| Age (yr) | 42.7 ± 18.1 | 46.1 ± 14.3 |
| Male | 210 (53.0%) | 131 (63.6%) |
| BMI (kg/m$^2$) | 25.0 ± 4.4 | 26.8 ± 4.3 |
| Cause of death | | |
| Stroke | 235 (59.3%) | 126 (61.2%) |
| Trauma | 139 (35.1%) | 70 (34.0%) |
| Anoxia | 8 (2.0%) | 5 (2.4%) |
| Other | 14 (3.5%) | 5 (2.4%) |
| ICU stay (d) | 4.7 ± 3.8 | 6.0 ± 7.0 |
| Arterial blood pH | 7.33 ± 0.17 | 7.29 ± 0.22 |
| Serum sodium (mmol/L) | 155.1 ± 14.0 | 159.0 ± 13.8 |
| Donor risk index | | |
| <1.5 | 61 (15.4%) | 31 (15.0%) |
| 1.5–2.0 | 187 (47.2%) | 106 (51.5%) |
| >2.0 | 148 (37.4%) | 69 (33.5%) |
| Ischemia/reperfusion injury[a] | | |
| I | 62 (16.0%) | 23 (11.2%) |
| II | 184 (47.5%) | 82 (40.0%) |
| III | 125 (32.3%) | 80 (39.0%) |
| IV | 16 (4.1%) | 20 (9.8%) |
| Macrovesicular steatosis[a] | | |
| 0 | 124 (76.1%) | 46 (68.7%) |
| <30% | 23 (14.1%) | 16 (23.9%) |
| 30%–59% | 10 (6.1%) | 3 (4.5%) |
| ≥60% | 6 (3.7%) | 2 (3.0%) |
| Surgical procedure characteristics | | |
| Cold ischemia time (h) | 8.4 ± 1.9 | 8.6 ± 2.1 |
| Warm ischemia time (min) | 42.4 ± 9.7 | 45.0 ± 10.1 |
| Blood units transfusion | | |
| 0 | 192 (48.5%) | 99 (48.1%) |
| 1–2 | 113 (28.5%) | 61 (29.6%) |
| 3–5 | 71 (17.9%) | 35 (17.0%) |
| >5 | 20 (5.1%) | 11 (5.3%) |
| Others blood products | 118 (29.8%) | 65 (31.6%) |
| Recipient characteristics | | |
| Age (yr) | 52.6 ± 11.9 | 50.9 ± 12.0 |
| Male | 296 (74.7%) | 131 (63.6%) |
| Race | | |
| White | 299 (75.5%) | 149 (72.3%) |
| Afro | 15 (3.8%) | 13 (6.3%) |
| Other | 82 (20.7%) | 44 (21.4%) |
| BMI (kg/m$^2$) | 26.6 ± 5.2 | 27.2 ± 5.1 |
| Blood type | | |
| A | 164 (41.4%) | 78 (37.9%) |
| AB | 20 (5.1%) | 9 (4.4%) |
| B | 60 (15.2%) | 29 (14.1%) |
| O | 152 (38.4%) | 90 (43.7%) |
| Etiology of liver disease | | |
| HCV | 145 (36.6%) | 74 (35.9%) |
| HBV | 35 (8.8%) | 14 (6.8%) |
| Alcoholic cirrhosis | 74 (18.7%) | 32 (15.5%) |
| Cryptogenic cirrhosis | 42 (10.6%) | 24 (11.7%) |
| Acute liver failure | 15 (3.8%) | 15 (7.3%) |
| NASH | 18 (4.5%) | 10 (4.9%) |
| Primary biliary cirrhosis | 21 (5.3%) | 11 (5.3%) |
| Others | 46 (11.6%) | 26 (12.6%) |
| Location of patient at LT | | |
| Home | 246 (62.1%) | 144 (69.9%) |
| Hospital | 81 (20.5%) | 34 (16.5%) |
| ICU | 69 (17.4%) | 28 (13.6%) |
| Biological MELD score at LT | 22.3 ± 10.0 | 21.0 ± 10.3 |
| MELD scores | | |
| <15 | 106 (26.8%) | 63 (30.6%) |
| 15–19 | 64 (16.2%) | 31 (15.0%) |
| 20–30 | 144 (36.4%) | 73 (35.4%) |
| ≥30 | 82 (20.7%) | 39 (18.9%) |
| Follow-up (d) | 1057.8 ± 637.9 | 922.1 ± 720.3 |

[a] Only available data. BMI: body mass index; EAD: early allograft dysfunction; LT: liver transplantation; NASH: non-alcoholic steatohepatitis; HBV: hepatitis B virus; HCV: hepatitis C virus; ICU: intensive care unit; MELD: model for end-stage liver disease.

**Table 2**
Clinical outcomes and complications after LT by EAD status.

| Variables | Non-EAD ($n=396$) | EAD ($n=206$) | $P$ value |
|---|---|---|---|
| Transplant-related hospital stay (d) | 10 (7–16) | 11 (7–19) | 0.272 |
| Graft rejection | 71 (17.9%) | 36 (17.5%) | 0.980 |
| Infections | 71 (17.9%) | 39 (18.9%) | 0.849 |
| Opportunistic infections | 35 (8.8%) | 26 (12.6%) | 0.188 |
| Transplant-related reoperations | 53 (13.4%) | 50 (24.3%) | **0.001** |
| Hernia repair (within 1-year) | 21 (5.3%) | 18 (8.7%) | |
| Delayed abdominal wall closure | 13 (3.3%) | 14 (6.8%) | |
| Intra-abdominal bleeding or fluid collection | 10 (2.5%) | 11 (5.3%) | |
| Others | 9 (2.3%) | 7 (3.4%) | |
| Biliary complications | 19 (4.8%) | 11 (5.3%) | 0.926 |
| Vascular complications | 8 (2.0%) | 9 (4.4%) | 0.164 |
| Graft loss | 84 (21.2%) | 78 (37.9%) | **<0.001** |
| Recipient death | 64 (16.2%) | 42 (20.4%) | 0.238 |

LT: liver transplantation. EAD: early allograft dysfunction.

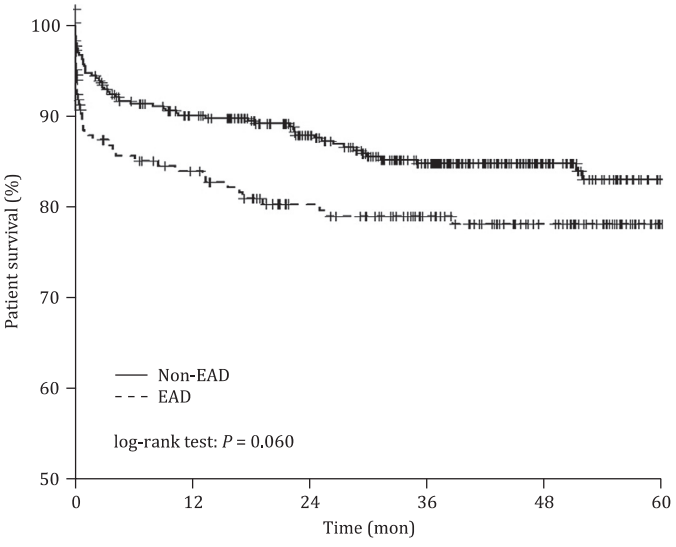


**Fig. 2.** Patient survival after LT by EAD status. LT: liver transplantation; EAD: early allograft dysfunction.

CI: 1.16–2.72, $P=0.008$) and WIT ≥40 min (OR = 1.67, 95% CI: 1.12–2.50, $P=0.013$) were independent risk factors. Male (OR = 0.45, 95% CI: 0.29–0.69, $P < 0.001$) was the only recipient protective factor in this study. Recipient age from 60 to 69 years (OR = 0.52, 95% CI: 0.28–0.95, $P=0.033$) and transfer from ICU for LT (OR = 0.55, 95% CI: 0.32–0.94, $P=0.032$) were associated with lower EAD risks compared to recipients aged under 40 years or those admitted from home.

The EAD and non-EAD groups were evaluated for patients and grafts survival at different time points (30-day, 90-day, 1-year and 5-year) (Table 4). For the EAD group, patient survival rates at 30-day, 90-day, 1-year and 5-year were 86.7%, 85.6%, 82.2% and 76.4%, respectively. For the non-EAD group, patient survival rates were 93.9%, 91.4%, 88.3% and 81.3%, respectively. For the EAD group, graft survival rates at 30-day, 90-day, 1-year and 5-year were 75.7%, 74.7%, 70.3% and 60.5%, respectively. For the non-EAD group, graft survival rates were 93.9%, 90.9%, 85.2% and 74.3%, respectively.

In addition, the overall patient and graft survival were analyzed by log-rank tests. Patient survival did not differ significantly between EAD and non-EAD groups ($P=0.060$; Fig. 2). Graft survival was significantly higher in the non-EAD group ($P<0.0001$; Fig. 3).

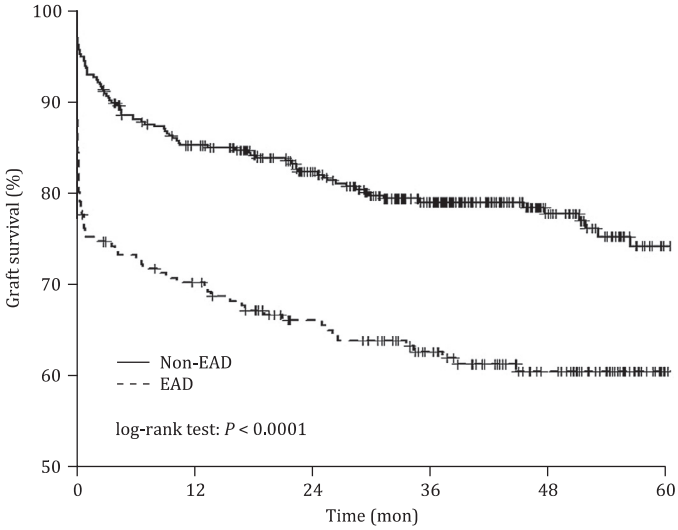


**Fig. 3.** Graft survival after LT by EAD status. LT: liver transplantation; EAD: early allograft dysfunction.

## Discussion

The study aimed to identify current risk factors and post-transplant survival and complications associated with EAD in a high-volume Latin American transplant center. Included were 206 (34.2%) patients with EAD, 38 (6.3%) of whom progressed to PNF. EAD affects up to 38.7% of LT recipients and up of 7.2% LT recipients suffer from PNF [1,2,5,27]. The percentage of patients with EAD and PNF in this study is consistent with data in literature [2,5,28]. Differences in the incidence rates are potentially related to differences in the EAD diagnostic criteria in different papers [2,3,5,12].

Male donor was an independent risk factor for EAD in our study. However, previous EAD studies did not report donor sex as a relevant EAD factor [6,18,28]. Male is thought to be a "marginally significant" donor factor for EAD by other researchers [1,2]. Donor sex may be indirectly associated with other variables, such as the cause of death and BMI, explaining therefore why this variable is not always included in all EAD studies.

The investigation of the association of donor age as an EAD risk factor has been inconclusive. The present analysis revealed that donors aged from 50 to 59 years had a higher chance of developing EAD. Donor age over 60 years also fall into the expanded criteria donor category and some transplant centers tend to select recipients in less severe conditions in these cases which also can introduce potential selection biases in analyzing donor age as a risk factor for EAD. Probably the adoption of more strict selection criteria in grafts from older donors justify lower EAD rates in patients that received grafts from donor age over 60 years. Universally accepted age cutoffs remain to be determined. However, 50 years is thought to be the approximate age limit for risk of EAD [1,4,5,18,29].

Donor serum sodium levels is another controversial risk factor for EAD [2,11,30–34]. It has been hypothesized that changes in the extracellular osmolarity in liver grafts could lead to water accumulation within the hepatocytes, and ultimately result in cell injury following reperfusion [11]. Serum sodium level >157 mmol/L was an independent risk factor for EAD in our multivariate analysis. High serum sodium levels may also indirectly reflect poor maintenance of potential donors after brain death and must be further investigated as potential avoidable and reversible risk factor for EAD.

Donor variables such as BMI, body weight and height were often investigated in EAD and PNF studies [5,7,18,28,32]. Body mass index >30 kg/m$^2$ was associated with EAD [31] and BMI

**Table 3**
Univariate and multivariate analysis for EAD risk factors.

| Variables | Non-EAD ($n=396$) | EAD ($n=206$) | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|---|---|
| | | | OR (95% CI) | *P* value | OR (95% CI) | *P* value |
| Donor sex | | | | | | |
| Female | 186 (47.0%) | 75 (36.4%) | 1.00 | | 1.00 | |
| Male | 210 (53.0%) | 131 (63.6%) | 1.55 (1.10–2.19) | **0.013** | 1.73 (1.16–2.60) | **0.007** |
| Donor age (yr) | | | | | | |
| <40 | 159 (40.2%) | 66 (32.0%) | 1.00 | | 1.00 | |
| 40–49 | 80 (20.2%) | 48 (23.3%) | 1.45 (0.91–2.29) | **0.115** | 1.35 (0.80–2.26) | 0.256 |
| 50–59 | 78 (19.7%) | 58 (28.2%) | 1.79 (1.15–2.80) | **0.010** | 1.73 (1.04–2.87) | **0.034** |
| 60–69 | 56 (14.1%) | 25 (12.1%) | 1.08 (0.61–1.85) | 0.796 | 0.94 (0.50–1.73) | 0.838 |
| ≥70 | 23 (5.8%) | 9 (4.4%) | 0.94 (0.40–2.08) | 0.888 | 0.81 (0.31–1.93) | 0.641 |
| Donor BMI (kg/m$^2$) | | | | | | |
| <25 | 209 (52.8%) | 77 (37.4%) | 1.00 | | 1.00 | |
| 25–29.9 | 148 (37.4%) | 93 (45.1%) | 1.71 (1.18–2.47) | **0.004** | 1.59 (1.05–2.41) | **0.028** |
| 30–34.9 | 30 (7.6%) | 29 (14.1%) | 2.62 (1.48–4.67) | **0.001** | 2.28 (1.19–4.35) | **0.012** |
| ≥35 | 9 (2.3%) | 7 (3.4%) | 2.11 (0.73–5.86) | **0.152** | 1.98 (0.60–6.27) | 0.247 |
| Cause of death | | | | | | |
| Stroke | 235 (59.3%) | 126 (61.2%) | 1.00 | | | |
| Trauma | 139 (35.1%) | 70 (34.0%) | 0.94 (0.65–1.34) | 0.733 | | |
| Anoxia | 8 (2.0%) | 5 (2.4%) | 1.17 (0.35–3.57) | 0.792 | | |
| Other | 14 (3.5%) | 5 (2.4%) | 0.67 (0.21–1.79) | 0.445 | | |
| Donor ICU stay (d) | | | | | | |
| ≤5 | 276 (69.7%) | 132 (64.1%) | 1.00 | | 1.00 | |
| >5 | 120 (30.3%) | 74 (35.9%) | 1.29 (0.90–1.84) | **0.162** | 1.42 (0.95–2.11) | 0.085 |
| Donor arterial blood pH | | | | | | |
| ≤7.35 | 206 (52.0%) | 124 (60.2%) | 1.00 | | 1.00 | |
| >7.35 | 190 (48.0%) | 82 (39.8%) | 0.72 (0.51–1.01) | **0.056** | 0.72 (0.49–1.05) | 0.090 |
| Donor sodium (mmol/L) | | | | | | |
| ≤157 | 237 (59.8%) | 99 (48.1%) | 1.00 | | 1.00 | |
| >157 | 159 (40.2%) | 107 (51.9%) | 1.61 (1.15–2.26) | **0.006** | 1.84 (1.26 - 2.68) | **0.002** |
| Ischemia/reperfusion injury | | | | | | |
| I | 62 (16.0%) | 23 (11.2%) | 1.00 | | 1.00 | |
| II | 184 (47.5%) | 82 (40.0%) | 1.20 (0.70–2.10) | 0.509 | 1.45 (0.81–2.67) | 0.217 |
| III | 125 (32.3%) | 80 (39.0%) | 1.73 (1.00–3.05) | **0.054** | 1.58 (0.86–2.95) | 0.144 |
| IV | 16 (4.1%) | 20 (9.8%) | 3.37 (1.51–7.71) | **0.003** | 4.18 (1.70–10.60) | **0.002** |
| Cold ischemia time (h) | | | | | | |
| <10 | 316 (79.8%) | 140 (68.0%) | 1.00 | | 1.00 | |
| ≥10 | 80 (20.2%) | 66 (32.0%) | 1.86 (1.27–2.73) | **0.001** | 1.78 (1.16–2.72) | **0.008** |
| Warm ischemia time (min) | | | | | | |
| <40 | 157 (39.6%) | 55 (26.7%) | 1.00 | | 1.00 | |
| ≥40 | 239 (60.4%) | 151 (73.3%) | 1.80 (1.25–2.62) | **0.002** | 1.67 (1.12–2.50) | **0.013** |
| Blood transfusion | | | | | | |
| No | 192 (48.5%) | 99 (48.1%) | 1.00 | | | |
| Yes | 204 (51.5%) | 107 (51.9%) | 1.02 (0.73–1.43) | 0.921 | | |
| Other blood product | | | | | | |
| No | 278 (70.2%) | 141 (68.4%) | 1.00 | | | |
| Yes | 118 (29.8%) | 65 (31.6%) | 1.09 (0.75–1.56) | 0.657 | | |
| Recipient sex | | | | | | |
| Female | 100 (25.3%) | 75 (36.4%) | 1.00 | | 1.00 | |
| Male | 296 (74.7%) | 131 (63.6%) | 0.59 (0.41–0.85) | **0.004** | 0.45 (0.29–0.69) | **<0.001** |
| Recipient age (yr) | | | | | | |
| <40 | 56 (14.1%) | 42 (20.4%) | 1.00 | | 1.00 | |
| 40–49 | 83 (21.0%) | 39 (18.9%) | 0.63 (0.36–1.09) | **0.097** | 0.64 (0.34–1.20) | 0.163 |
| 50–59 | 137 (34.6%) | 71 (34.5%) | 0.69 (0.42–1.13) | **0.141** | 0.67 (0.38–1.17) | 0.156 |
| 60–69 | 107 (27.0%) | 49 (23.8%) | 0.61 (0.36–1.03) | **0.065** | 0.52 (0.28 −0.95) | **0.033** |
| ≥70 | 13 (3.3%) | 5 (2.4%) | 0.51 (0.15–1.48) | 0.237 | 0.40 (0.11–1.28) | 0.140 |
| Recipient BMI (kg/m$^2$) | | | | | | |
| <25 | 170 (42.9%) | 79 (38.3%) | 1.00 | | 1.00 | |
| 25–29.9 | 133 (33.6%) | 66 (32.0%) | 1.07 (0.72–1.59) | 0.746 | 1.10 (0.70–1.73) | 0.677 |
| 30–34.9 | 69 (17.4%) | 47 (22.8%) | 1.47 (0.93–2.31) | **0.101** | 1.37 (0.81–2.31) | 0.235 |
| ≥35 | 24 (6.1%) | 14 (6.8%) | 1.26 (0.60–2.53) | 0.531 | 1.25 (0.56–2.73) | 0.576 |
| MELD score at LT | | | | | | |
| <30 | 296 (74.7%) | 163 (79.1%) | 1.00 | | | |
| ≥30 | 100 (25.3%) | 43 (20.9%) | 0.78 (0.52–1.16) | 0.232 | | |
| Location of patient at LT | | | | | | |
| Home | 246 (62.1%) | 144 (69.9%) | 1.00 | | 1.00 | |
| Hospital | 81 (20.5%) | 34 (16.5%) | 0.72 (0.45–1.12) | **0.148** | 0.65 (0.39–1.06) | 0.089 |
| ICU | 69 (17.4%) | 28 (13.6%) | 0.69 (0.42–1.12) | **0.139** | 0.55 (0.32–0.94) | **0.032** |

OR: odds ratio; EAD: early allograft dysfunction; BMI: body mass index; ICU: intensive care unit; MELD: model for end-stage liver disease; LT: liver transplantation.

**Table 4**
Patient and graft survival after LT.

| Time after LT | Patient survival rate (%) | | *P* value | Graft survival rate (%) | | *P* value |
|---|---|---|---|---|---|---|
| | Non-EAD | EAD | | Non-EAD | EAD | |
| 30-day | 93.9 (91.1–95.9) | 86.7 (81.0–90.8) | **0.016** | 93.9 (91.1–95.9) | 75.7 (69.3–81.0) | **<0.0001** |
| 90-day | 91.4 (88.2–93.8) | 85.6 (79.7–89.9) | 0.124 | 90.9 (87.6–93.4) | 74.7 (68.2–80.1) | **<0.0001** |
| 1-year | 88.3 (84.6–91.1) | 82.2 (75.8–87.0) | 0.161 | 85.2 (81.3–88.4) | 70.3 (63.5–76.0) | **<0.0001** |
| 5-year | 81.3 (76.3–85.3) | 76.4 (69.3–82.0) | 0.241 | 74.3 (68.3–79.3) | 60.5 (53.2–67.0) | **<0.0001** |

EAD: early allograft dysfunction; LT: liver transplantation.

>25.3 kg/m$^2$ was described by Hoyer et al. as an isolated risk factor for EAD [1]. We opted to stratify BMI for better construction of the multiple regression model. Results from donor BMI data analysis in this study clearly support a positive association between BMI and EAD. Similar associations have been reported in recent studies [1,2,27,31]. We introduce the use of the WHO classification for BMI analysis. This classification certainly allows a reproducible assessment method for BMI stratification in future EAD studies.

Some authors have also studied the association of graft steatosis with EAD [27,35,36]. Recently, an extensive retrospective from the Mayo clinic found that graft steatosis (>30% on a wedge biopsy 1-h after reperfusion) was an important risk factor for EAD [37]. However, these authors did not discuss about I/R injury or obesity, only about steatosis. The occurrence of EAD is related to the I/R injury in the graft [1,2,5,7]. Ali et al. [22] reported on an extensive review of liver biopsies after reperfusion. They found that grade IV I/R injury was associated with lower graft survival within the first year after transplant. In their publication most severe I/R injuries were related to donation of cardiac death donors and at least moderate graft steatosis. We did not include donation of cardiac death donors in our study nor found a direct relation with graft steatosis, but with obesity. The association between grade IV I/R injury, independently of graft steatosis, and EAD in this study emphasizes the relevance of graft histopathological data. Reperfusion biopsies can be easily incorporated into a transplant center routine. This finding stands out as the major contribution of our study. Early biopsy findings of severe I/R injury can lead to potential early therapeutic targets in future studies.

Ischemia time is a risk factor with high impact on solid organ transplant outcomes [6,18,28,37,38]. CIT ≥10 h was a significant risk factor for EAD in the multiple regression model in this study. CIT is directly related to logistic aspects of transplant and is therefore predictable and potentially controllable once the organ has been accepted [1]. Other factors, such as intraoperative coagulopathy leading to extended time to hemostasis, use of organs imported from other centers or split grafts may also contribute to prolonged CIT [2]. As previously reported [23,29,33], WIT ≥40 min was a significant factor in this study. As with CIT, studies describing prolonged WIT as risk factor for EAD date from the pre-MELD era [3,29]. New surgical standards and increased surgical expertise may explain the lower CIT and WIT cutoffs reported in more recent studies and highlight the importance of these variables in the early and late post-transplant outcomes.

Recipient sex is weakly associated with transplant outcomes. No studies describing recipient sex as a risk factor for EAD were found in the literature. In this analysis, male recipients had 55% less chance of developing EAD. Therefore, female was thought to be an independent protective factor for EAD. Sex may be indirectly related to graft size and liver mass, explaining the association of female recipient and EAD. Future studies might consider to better match donor and recipient liver mass to minimize EAD.

Surprisingly, recipients from 60 to 69 years and who were at ICU at the transplant date were associated with lower EAD risks. These findings may reflect the application of more strict criteria in allocation of organs to less debilitated recipients.

The relation between EAD and some LT outcomes was also investigated in this study. Transplant-related reoperations and graft loss rates differed significantly between EAD and non-EAD groups, despite similar patient survival between groups. Initial studies reported that EAD associated was higher susceptibility of recipients to infection [39], prolonged ICU and hospital stay [5,6,40], graft loss [41,42] and higher patient morbidity and mortality [2,31,32]. These factors may indirectly translate into higher EAD patient costs, suggesting that there might be clinical and financial implications of interventions aimed to overcome EAD, and justifying specific studies on this topic.

This study has several limitations. Firstly, retrospective nature and unicenter which may not be universally applicable. Secondly, despite well-established associations between macrovesicular steatosis and EAD, biopsies are not routinely performed at donor surgery at our center and this investigation was limited to only part of the study population. However, we have opted to maintain the biopsy report because those who had grade IV I/R in the EAD group had worse patient survival. This is an important finding and as far we understand this has not yet been addressed in the literature and is the most important and novel finding of our work. Thirdly, the time lapsed between laboratory test sample collection from potential donors, diagnosis of brain death and organ extraction surgery varied widely and was very difficult to control. Such wide variations may be a limiting factor in several studies investigating correlations between laboratory test findings and EAD. Finally, the utilization of different preservation solutions is another potentially limiting factor [43].

In summary, EAD is associated with donor factors, surgical factors and recipient factors. EAD group had higher transplant related reoperation and graft loss rates compared to patients in the non-EAD group, despite similar patient survival between two groups. This study is the first to emphasize the significance of time-zero biopsy as a routine low-cost test for prediction of EAD. Lastly, BMI stratification based on WHO classification introduced a universally accepted criteria with good reproducibility potential in future studies involving different organ transplant centers.

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## Contributors

BND designed the study, collected and analyzed the data, and wrote the first draft. OSPR designed the study, analyzed the data, and reviewed the manuscript. AMD designed the study and reviewed the manuscript. All authors contributed to further drafts. BND is the guarantor.

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## Ethical approval

This study was conducted in compliance with the *Declaration of Helsinki* and with local and national regulations. The study was exempted from informed consent and was approved by the Internal Review Board (CAAE No. 40520214.3.0000.0071).

## Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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