A score model for the continuous grading of early allograft dysfunction severity

Eugenia Pareja[1], Miriam Cortes[1], David Hervás[2], José Mir[1], Andrés Valdivieso[3], José V. Castell [1,4] and Agustín Lahoz[1]*

[1] Unidad de Hepatología Experimental, Instituto de Investigación Sanitaria La Fe, Valencia, Spain

[2] Unidad de Bioestadística, Instituto de Investigación Sanitaria La Fe, Valencia, Spain

[3] Surgery and Liver Transplant Unit. Cruces University Hospital, University of Basque Country, Bilbao, Spain

[4] Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad de Valencia, CIBERehd, Centro de Investigaciones Biomédicas en Red de Enfermedades Hepáticas y Digestivas, FIS, Spain

Short Title: Grading early allograft dysfunction

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1002/lt.23990

List of abbreviations in order of appearance

ALT: alanine aminotransferase; AST: aspartate aminotransferase; AUC: area under the curve; CIT: cold ischemia time; EAD: early allograft dysfunction; ECDF: empirical cumulative distribution function; FFP: fresh frozen plasma; HBcAb: hepatitis B core antibody; INR: international normalized ratio; ICU: intensive care unit; MEAF: model for early allograft function scoring; MELD: model for end-stage liver disease; OLT: orthotopic liver transplant; PNF: primary non function; POD: postoperative days; RBC: red blood cells; RI: relative importance; sPCA: sparse principal component analysis; WIT: warm ischemia time.

Financial support

This work has been supported by the Instituto de Salud Carlos III of the Spanish Ministry of Science and Innovation (PI11/02942). A.L. is grateful for a Miguel Server contract (CP08/00125) from the above Ministry/Instituto de Salud Carlos III.

No author has any conflict of interest to declare

*To whom correspondence should be addressed: Agustín Lahoz.

E-mail: agustin.lahoz@uv.es. Unidad de Hepatología Experimental, Instituto de Investigación Sanitaria-Fundación Hospital La Fe, Av. Campanar, 21, 46009 Valencia, Spain. Tel: +34 96 197 34 85. Fax: +34 96 349 44 1

ABSTRACT

Early allograft dysfunction (EAD) dramatically influences graft and patient outcomes. Lack of consensus in EAD definition hinders comparisons of liver transplant outcomes and recipients management among and within centers. We aimed to develop a model for the quantitative assessment of early allograft function (MEAF) after transplantation.

A retrospective study including 1026 consecutive liver transplantations was performed for MEAF score development. Multivariate data analysis was used to select a small number of postoperative variables that adequately describe EAD. Then, the distribution of these variables was mathematically modeled to assign a score for each actual variable value. A model, based on easily obtainable clinical parameters (i.e., ALT, INR and bilirubin), which scores liver function from 0 to 10, was built. The MEAF score showed a significant association with patient and graft survival for the 3-, 6- and 12-month follow-ups. Hepatic steatosis and age for donors; cold/warm ischemia times, and post-reperfusion syndrome for surgery; ICU and hospital stays, MELD score, Child-Pugh, BMI and fresh frozen plasma transfusion for recipients were factors that associated significantly with EAD. The model was satisfactorily validated by applying it to an independent set of 200 patients who underwent liver transplantation at a different center. In conclusion, a model for the quantitative assessment of EAD severity has been developed and validated for the first time. The MEAF provides a more accurate graft function assessment than current categorical classifications, which may help clinicians to make early enough decisions on retransplantation benefits. Furthermore, the MEAF score is a predictor of recipient and graft survival. The standardization of the criteria used to define EAD may allow the reliable comparison of recipients' treatments and transplants outcomes among and within centers.

Key words: liver, graft, transplantation, dysfunction

INTRODUCTION

Orthotopic liver transplantation (OLT) has become the effective treatment for acute liver failure and end-stage liver diseases. According to the data reported by the National Transplantation Organization in Spain, 2095 patients were on waiting list for OLT in 2013, of whom 1093 were transplanted and 117 patients died awaiting an organ (1). The imbalance between number of donors and patients on waiting list for OLT has led transplant units to use the extended donor criteria liver allografts. This practice has resulted in lower mortality rates among patients on waiting list (2, 3) at the expenses of an increase of post-transplant complications, such as primary graft dysfunction (PDF) and liver failure (4). Most transplanted patients present some type of PDF after the OLT procedure, which ranges from entities with varying degrees of reversible graft dysfunction (known as early allograft dysfunction -EAD-), to an uncommon (2-3%) irreversible dysfunction state called primary non function (PNF), which leads to retransplantation or death during the first postoperative week (5). EAD encompasses a poorly defined clinical entity that represents a condition where the liver graft shows some degree of hepatic injury, but functions sufficiently to support life. Although most authors agree that the graft function finally recovers, EAD is associated, in turn, with increased recipients' susceptibility to: sepsis (6), longer intensive care unit (ICU) and hospital stays (7-9), graft loss (10), higher morbidity and mortality (11-13). Despite its high incidence (15-27%) and its negative impact on OLT success, a consensus about the criteria used to define EAD is still lacking (Table 1). Different diagnostic criteria and benchmarks are employed to establish whether a graft shows any degree of dysfunction (7, 8, 14-20). Graft function after OLT is usually categorized according to arbitrary pre-established clinical variables cut-offs or subjective parameters (e.g., concomitant encephalopathy) in patients with EAD or no-EAD (Table 1). Lack of agreement among

the different criteria is cause of concern; e.g., *Ploeg et al. (18)* and *Gonzalez et al. (14)* agreed only in 34% of EAD cases (21). Such discrepancies make the comparisons of OLT results among and within centers difficult in terms of recipients' clinical evolution and management, which may lead to misleading conclusions; e.g., several authors have reported different EAD incidences (Table 1). These facts have motivated clinicians to make more efforts in improving classifications by incorporating new EAD subcategories (10, 14, 22). However, existing classifications still prove insufficient to accurately grade recipients' liver function after OLT and to maximize the benefits of retransplantation in recipients (23). As pointed out by many authors, a more accurate EAD definition that ranks the liver graft function after OLT is necessary (8, 9, 23, 24).

Here, we describe the development of a model for the continuous grading of early allograft function after transplant (MEAF). Multivariate data analysis was used to select which postoperative biochemical variables adequately described graft function after OLT. Then, the distribution of these clinical variables was mathematically modeled, which allowed us to compute a continuous score for grading EAD severity after OLT. To study the model's capabilities, we investigated its ability to predict graft and patient survival, its relationship with PNF, and also which donor, recipient and surgery factors are associated with EAD.

PATIENTS AND METHODS

Recipient Population

The study cohort included all the consecutive OLTs performed at La Fe Hospital Valencia (Spain) from 1 January 2003 to 31 of December 2012 of patients on waiting lists. This was done in accordance with the model for the end-stage liver disease (MELD) score and Child-Pugh (25, 26), in agreement with our Transplant Committee and after obtaining patients' consent. The patient exclusion criteria were: recipient age under 18, OLT for acute liver failure, receiving a split liver, recipients with early vascular complications and recipients' retransplantation during the first 2 postoperative days (POD). In order to reflect patients' real status, no extra points for hepatocellular carcinoma were added to the MELD score. An independent set of patients was used for MEAF score validation. The validation group included 200 patients who underwent OLT performed at the University Cruces Hospital. The receptor exclusion criteria were equal to those used for model development. Clinical data were collected from the Transplant and Surgery Unit at the Cruces University Hospital.

Donor Population

ABO blood group compatibility and relative size-matching between donor and recipient were required. HBcAb was also matched whenever possible. Donors were accepted after brain death, provided they had no history of liver disease or cancer in the previous 10 years (27). In the selected cases, those donors with extended criteria for advanced age, prolonged ICU stay, high sodium levels in blood (>155 mEq/L), hypotension (systolic blood pressure less than or equal to 60 mmHg) or heart arrest before diagnosing brain death were used (28). Donors with systemic bacterial infection or HBsAg, HCV and HIV antibodies positivity were not accepted. Donor livers with fatty

infiltration (> 40% of macrovesicular steatosis) (29), any degree of fibrosis, and notable atherosclerosis of the hepatic artery were also declined.

Surgical Procedure

The OLT procedure was performed in the recipient following a standard technique (30). In most cases, the recipients' vena cava was preserved. The piggyback technique was adopted with anastomosis to three hepatic veins with neither a veno-venous bypass nor a temporary portal-caval shunt (31, 32). End-to-end anastomosis for the portal vein and the patch of the common hepatic artery with the gastroduodenal artery were used in the recipient for arterial anastomosis. All the patients received similar perioperative intensive care and immunosuppression therapy, consisting in a double therapy with cyclosporine or tacrolimus plus steroids, or a triple therapy with azathioprine for autoimmune cirrhosis. Cold ischemia time (CIT) was defined as the time from *in situ* flushing in the donor until the graft is taken from ice. Warm ischemia time (WIT) was defined as the time that elapses from removing the graft from ice to reperfusion through the portal vein, the hepatic artery, or both, in the recipient (33).

Clinical Data Collection

Data were obtained from the Department of Transplant Surgery Registry, where pre- and post-transplant variables related to donors, recipients and surgery were collected (Tables S1-S3). The clinical and laboratory parameters assessing graft liver function after OLT were obtained by biochemical testing (Table S4). Blood samples were collected 2 h before starting surgery, every 8 h during the first 24 h, and once a day from post- OLT days 2 to 7.

Statistical Analysis and Mathematical Variable Modeling

Data are summarized by their mean and standard deviation, mean (SD) in the case of continuous variables, and relative and absolute frequencies in the case of categorical variables. A sparse principal component analysis (sPCA), a variant of PCA which allows variable selection (34), was applied to select which postoperative clinical variables were the most adequate to model graft function after OLT (Table S4). The distribution of the variables selected to describe early allograft function was explored by their empirical cumulative distribution function (*ecdf*) and a non linear regression model was used to assign a score value to each clinical variable level. Cox proportional-hazards regression was used to evaluate the relationship between the MEAF score and patient/graft survival. All the survival analyses started on the OLT date. Patient and graft survival was followed-up for 3-, 6- and 12-months after surgery. Multivariable regression modeling was used to study the relationship of the donor, surgery and recipient factors (Tables S6-S8) associated potentially with both EAD and the MEAF score. The model-averaged importance of terms using the Bayesian Information Criterion (BIC) was used to examine the relative importance of the different putative factors associated with the MEAF score (i.e., EAD). The relative importance of a variable was computed as the sum of the relative evidence weights of all the models in which that variable appeared. The relative evidence weights were computed as exp (-ΔBIC/2), where ΔBIC is the difference in BIC between each model and the best model. The relative importance (RI) cut-off value for each variable was set at 0.8 to control for the type I risk (35). The relationship between the MEAF score and ICU and Hospital stays was assessed by censored quantile regression. Natural cubic splines were used in the regression equation to account for the non linear trend between the MEAF score and ICU stay. All the statistical analyses were performed with the R software (version

2.15.3) and the glmulti packages (version 1.0.7) for model averaging and mixOmics (version 4.1-4) for the sPCA analyses.

RESULTS

Clinical characteristics of the study cohort and surgery description

The study included 1026 consecutive OLT in 981 recipients, and 152 patients were excluded for the following reasons: 47 pediatric recipients; 45 OLT because of acute liver failure; 10 splits; 16 early hepatic artery thrombosis; 3 hepatic vein thrombosis; 3 patients with early fibrosing cholestatic hepatitis recurrence; 10 retransplants during the first 2 POD and 18 patients with incomplete data. After applying the exclusion criteria, 839 liver transplants in 829 recipients were enrolled in our study. The donor population's baseline characteristics are detailed in Table S1. The mean age was 54.5±17.6 years, and males slightly predominated (60.6%). The main cause of death was cerebral vascular accident (67.6%). The main surgery procedure characteristics are summarized in Table S2. The mean CIT was 311±156 min, while the WIT and arterial anastomosis delay times were 44±15 and 43±19 min, respectively. Of the 829 recipients enrolled in the study, 75.4% were male (mean age of 54±9 years). The commonest primary diagnoses for end-stage liver disease in the cohort study were hepatitis C and alcoholism; the patients' mean MELD score was 16.7± 7.6. Further recipient population details are provided in Table S3.

Selection of postoperative variables to model EAD

sPCA was used to select which postoperative variables were most suitable to model EAD. Table S4 summarizes the clinical parameters examined as potential candidates to describe EAD. Among them, the sPCA selected the AST, ALT, INR, and PT maximum values during the first 3 post-operative days (Max.3DPO) and the bilirubin value on day 3 after LT (3DPO) as the variables which accounted for most data variance (**Fig. S1**). The correlation analysis of the selected variables showed an important positive correlation (r = 0.86) between ALT and AST, and between INR and PT (r = 0.85) (**Fig.**

S2). $ALT_{Max.3DPO}$ was chosen instead of $AST_{Max.3DPO}$ for its specificity on liver parenchymal damage, and $INR_{Max.3DPO}$ was selected instead of $PT_{Max.3DPO}$ because the former is a normalized parameter that is comparable among different laboratories.

Modeling early allograft function

A nonlinear regression model was used to assign a score value to each clinical variable level. Thus, a sigmoid curve was fitted to each variable's *ecdf* (**Fig. 1**). The fit of the different curves was acceptable with residual standard errors of 0.035, 0.034 and 0.032, respectively (**Fig. 1**). To facilitate model interpretation, the final MEAF score range was arbitrarily set at 0-10 points. Therefore, the $ALT_{Max.3DPO}$, $INR_{Max.3DPO}$, and $bilirubin_{3POD}$ scores were set from 0 for the lower *ecdf* value to 3.33 for the highest one. The MEAF score consists in adding the three scores corresponding to the extrapolation of each variable value in its corresponding sigmoid regression fitted function (**Eq. 1**). The MEAF score calculated for our population, by simply substituting the patients' actual $ALT_{Max.3POD}$, $INR_{Max.3POD}$ and $bilirubin_{3POD}$ values in **Eq. 1**, showed a normal shaped distribution, which ranged from 0.06 to 9.73, with a mean value of 5.02 and a standard deviation of 1.99. A nomogram (**Fig. 2**) or an Excel file is provided to easily calculate the MEAF scores (Supplementary Material).

Impact of EAD on patient and graft survival

The association between the MEAF score (i.e., EAD severity) and patient survival was examined by a Cox regression analysis. Of the 829 recipients enrolled in our study, 52 patients died from causes related to OLT during the 3-month follow-up. The survival analysis showed a significant association between the MEAF score and mortality during the first 3 months (*p-value = 0.042*), with an estimation of a hazard ratio increase for each score point of 1.19 and a 95% confidence interval (CI) of [1.01, 1.41] (**Fig. 3A**). The mortality rate rose to 40.6% for the recipients with a MEAF score > 8 (**Fig. 3B**). A significant association was also found for the 6- and 12-month follow-ups (Table S5). Concerning graft survival, 35 recipients were re-transplanted during the first 3 post-operative months. The Cox regression analysis showed a significant association between the MEAF score and graft loss during the 3 postoperative months (*p-value < 0.001*), with an estimation of a hazard ratio increase for each score point of 2.66 with a 95% CI of [2.06, 3.44]. The graft loss rate was 31% for the recipients with a MEAF score > 8 (**Fig. 3D**).

Primary non function and MEAF score

According to the definition of the United Network for Organ Sharing (36), 22 recipients enrolled in our study showed PNF (i.e., 2.1%). 2 of the 22 PNF recipients were re-transplanted during the first 2 POD, thus it was not possible to calculate their score. The mean MEAF score value for the remaining 20 recipients was 8.14 with a standard deviation of 1.06. Logistic regression modeling was used to study the relationship between the MEAF score and PNF, and gave an odds ratio of 3.74 with a 95% CI of [2.53, 6.78]. The likelihood of PNF in accordance with the MEAF score was estimated and showed a sharp increase for the MEAF score values above 7 (**Fig. S3**).

EAD-associated factors

Of all the study factors (Tables S6-S8), the variables selected by model averaging were: age (RI=0.96), hepatic steatosis (RI=0.92) and ICU stay (RI=0.82) for donors (Table S5); CIT (RI=1), post-reperfusion syndrome (RI=0.95) and WIT (RI=0.88) for surgery (Table S6); and MELD score (RI=1), BMI (RI=1), Child-Pugh (RI=1) and FFP transfusion (RI=0.82) for the recipients' examined variables (Table S7). A significant positive association ($p<0.001$) between the MEAF score and recipients' ICU and Hospital stays was also found, showing a non linear increase in days of ICU stay per MEAF score unit (**Fig. 4A**), and 0.92 days for Hospital stay with a 95% CI of [0.47, 1.37] (**Fig. 4B**).

Validation of the MEAF score

The association between the MEAF score calculated for the validation group and patient and graft survival was examined by a Cox regression analysis. The survival analysis showed a significant association between the MEAF score and mortality during the first 3 months (*p-value < 0.01*), with an estimation of hazard ratio increase for each score point of 2.43 and a 95% confidence interval (CI) of [1.58, 3.71]. A significant association was also found for the 6- and 12-month follow-ups. These results are in agreement with those obtained in the analysis performed for the model building group (Table S5). Figure 4 compares the hospitalization and ICU stays between the model building and validation groups. A good agreement was found between the results of both independent groups, showing prolonged hospitalization and ICU stays for those patients with higher MEAF scores. As regards PNF, logistic regression modeling was used to study the relationship between the MEAF score and PNF, which gave an odds ratio of 1.78 with a 95% CI of [1.29, 2.53]. The likelihood of PNF being in accordance

with the MEAF score was estimated, and the same trend as that observed for the model building group was found (Fig. S3).

Comparison between the MEAF score and a current EAD definition

The MEAF score was compared with the EAD categorical definition reported by *Olthoff et al*. (8). Thus following such calcification, the recipients included in our study population were classified as EAD or non EAD. An acceptable agreement was found between the extreme MEAF score values and the categorical classification. By taking a MEAF score > 8, a disagreement of 15% was found between both approaches (i.e., 7 cases). A more in-depth analysis of these results revealed that three obtained INR values ranged from 1.4 to 1.57 (Olthoff's cut-off limit=1.6), one had 9.9 mg/dl of bilirubin (Olthoff's cut-off limit 10mg/dl) and three had higher ALT values, ranging from 1,750 to 1,909 U/l (Olthoff's cut-off limit =2,000 U/l). However when a MEAF score < 2 was considered, disagreement less than 3% was found. In the central MEAF score values, different degrees of discrepancy were observed in accordance with the MEAF score intervals set.

DISCUSSION

The difficulty to assess EAD, since a clear definition is lacking, may not only compromise recipients' management but also affect liver transplant outcome. Most current classifications based on arbitrarily chosen laboratory cut-off values or subjective parameters simply categorize recipients as showing a dysfunction or not (Table 1), and do not grade recipients' graft dysfunction. EAD has been demonstrated to be an independent risk factor for graft loss and patient death (9, 10, 12, 37). EAD incidence has increased to 23.7% in recent years, mainly because of the use of extended criteria donors (2, 8, 37). In the clinical arena, prompt discrimination between patients with dysfunction who quickly recover from those who do not would be of much importance. Thus, there is a need to objectively grade and standardize EAD severity. This study describes the development of a model for the continuous assessment of early allograft function (MEAF).

The MEAF score uses the $INR_{Max.3DPO}$, $ALT_{Max.3DPO}$ and $Bilirrubin_{3POD}$ as the adequate postoperative variables to model graft function after OLT. The chosen variables are objective and have a proven influence on transplant outcome (7, 8). Indeed two of them are also used by the MELD score (25), which accurately evaluates the preoperative liver function. These facts reinforce their appropriateness for modeling the post-transplant liver graft function. For easy use, the model's range was arbitrarily set from 0 to 10, thus EAD severity increases proportionally to the MEAF score. Recipients' MEAF score can be easy calculated at bedside using a nomogram (**Fig. 2**) or in laboratories by with **Eq.1** or an Excel file (Supplementary Material).

The key advantage of the MEAF score is its continuous character, which allows patients to be ranked according to EAD severity, instead of merely classifying them all into two or three groups. This feature also enables the more efficient use of the Cox regression

model for patient and graft survival analysis. Interestingly, we found a significant association between the MEAF score and patient survival for the 3-, 6- and 12-month follow-up. We focused on the 3-month results, as we believed that severe EAD would lead to death or retransplant during this time, and that later graft failure could be attributed more to other related complications, such as rejection or disease recurrence. However, we also found a significant association for the 6- and 12-month follow-ups. Significant association between graft loss and MEAF score was also found for 3-month follow-up. The regression analysis allowed us to build a function to estimate the likelihood of each graft's and recipient's survival according to the score obtained. Our results indicate that the higher the MEAF score is, the lower the recipients' and grafts' survival rates, which demonstrates its relationship with transplant outcome. Although PNF is uncommon after liver transplantation (with an incidence of around 2%), it is the most severe form of graft dysfunction and needs urgent re-transplantation during the first postoperative week (5). Therefore, a method that can help with the early assessment of potential PNF may be of much relevance to maximize the benefits of retransplantation. The MEAF score is significantly associated with PNF occurrence, which indicates that those recipients with a MEAF score > 8.5 have a PNF likelihood of 40% in the building cohort, or an even higher one in the validation cohort (Fig. S3). Obviously, the MEAF score is not the only factor to delineate PNF, but it can help make quick decisions in due time.

The cumulative incidence of several risks factors during OLT may lead to increased EAD severity, which ultimately has an impact on patient and graft survival (38). Therefore, multiple donor, surgery and recipient variables were examined as potential factors associated with EAD occurrence. Multivariable regression modeling shows that age, hepatic steatosis and ICU are still factors that predispose to EAD, which highlights

the impact of surgery on transplant outcome. These results reinforce the assumption stated by *Busuttil et al.* (39), who suggested preventing the combination of donor steatosis (30-60%) and prolonged CIT. The MELD and Child Pugh scores were found to be graft dysfunction-related parameters that indicate the relevance of recipients' disease severity for EAD occurrence (8, 10). Prolonged hospital stays have an impact on hospital bed and resources utilization (9). Interestingly, the MEAF score showed a significant positive association with longer ICU and hospital stays for patients with EAD, which reinforces its clinical usefulness. Some of the described factors can be used to prevent EAD and to maximize the use of available resources. Model performance was successfully evaluated by applying it to an independent group of patients who underwent OLT at a different hospital, with higher rates for patient mortality and graft loss, as well as prolonged hospitalization and ICU stays for the high MEAF score values (**Fig. 4 and Table S5**). These results are in good agreement with those obtained for the assessment of the model building group, which reinforces the clinical utility of the MEAF score.

To assess MEAF score capabilities in comparison to current classifications, we compared the new model with the EAD definition recently reported by *Olthoff et al*. (8). The extreme score values showed acceptable agreement (i.e., immediate graft function, severe dysfunction, respectively). The discrepancies observed are attributed mainly to the circumstance that the recipients with INR, ALT or bilirubin values that came close to the established cut-offs are classified as non EAD when, in fact, their overall variable values are more indicative of poor graft function. The observed misclassification may be attributable to the use of subjective cut-off values to classify patients. This approach assumes a flat relationship between the predictor and the response within intervals,

which leads to inaccurate classifications, especially in those values that come close to the pre-established cut-offs (40).

In conclusion, a model for the continuous scoring of EAD severity based on well-established biochemical parameters has been developed for the first time. All the advantages of this model derive from its ability to assess graft function continuously, which implies that the individual scoring of each recipient clinical outcome can provide more precise information than current categorical classifications. Although model refinement and improvement is anticipated by the authors, the MEAF score may help to standardize the EAD definition and can enable a comparison of recipients' clinical outcome, treatments and clinical results both among and within medical centers. Furthermore, the model shows a significant association with both patient and graft outcome, and allows survival to be estimated according to each recipient's score. High score values may warn clinicians that a patient is at risk of PNF and can help them make decisions on potential retransplantation. The MEAF score may also help in translational studies that require a precise and objective graft function endpoint.

References

1. ONT. Dossier de la Organización Nacional de Trasplante. 2012.
2. Schaubel DE, Sima CS, Goodrich NP, Feng S, Merion RM. The survival benefit of deceased donor liver transplantation as a function of candidate disease severity and donor quality. Am J Transplant. 2008;8(2):419-25.
3. Barshes NR, Horwitz IB, Franzini L, Vierling JM, Goss JA. Waitlist mortality decreases with increased use of extended criteria donor liver grafts at adult liver transplant centers. Am J Transplant. 2007;7(5):1265-70.
4. Maluf DG, Edwards EB, Kauffman HM. Utilization of extended donor criteria liver allograft: Is the elevated risk of failure independent of the model for end-stage liver disease score of the recipient? Transplantation. 2006;82(12):1653-7.
5. Uemura T, Randall HB, Sanchez EQ, Ikegami T, Narasimhan G, McKenna GJ, et al. Liver retransplantation for primary nonfunction: analysis of a 20-year single-center experience. Liver transpl. 2007;13(2):227-33.
6. Howard TK, Klintmalm GB, Cofer JB, Husberg BS, Goldstein RM, Gonwa TA. The influence of preservation injury on rejection in the hepatic transplant recipient. Transplantation. 1990;49(1):103-7.
7. Deschenes M, Belle SH, Krom RA, Zetterman RK, Lake JR. Early allograft dysfunction after liver transplantation: a definition and predictors of outcome. National Institute of Diabetes and Digestive and Kidney Diseases Liver Transplantation Database. Transplantation. 1998;66(3):302-10.
8. Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. Liver transpl. 2010;16(8):943-9.
9. Croome KP, Hernandez-Alejandro R, Chandok N. Early Allograft Dysfunction Is Associated With Excess Resource Utilization After Liver Transplantation. Transplantation Proceedings. 2013;45(1):259-64.
10. Salvalaggio P, Afonso RC, Felga G, Ferraz-Neto BH. A proposal to grade the severity of early allograft dysfunction after liver transplantation. Einstein. 2013;11(1):23-31.
11. Croome KP, Wall W, Quan D, Vangala S, McAlister V, Marotta P, et al. Evaluation of the updated definition of early allograft dysfunction in donation after brain death and donation after cardiac death liver allografts. Hepatobiliary & pancreatic diseases international : HBPD INT. 2012;11(4):372-6.
12. Pokorny H, Gruenberger T, Soliman T, Rockenschaub S, Langle F, Steininger R. Organ survival after primary dysfunction of liver grafts in clinical orthotopic liver transplantation. Transplant international : official journal of the European Society for Organ Transplantation. 2000;13 Suppl 1:S154-7.
13. Briceno J, Ciria R. Early graft dysfunction after liver transplantation. Transplant Proc. 2010;42(2):631-3.
14. Gonzalez FX, Rimola A, Grande L, Antolin M, Garcia-Valdecasas JC, Fuster J, et al. Predictive factors of early postoperative graft function in human liver transplantation. Hepatology. 1994;20(3):565-73.
15. Greig PD, Forster J, Superina RA, Strasberg SM, Mohamed M, Blendis LM, et al. Donor-specific factors predict graft function following liver transplantation. Transplant Proc. 1990;22(4):2072-3.
16. Makowka L, Gordon RD, Todo S, Ohkohchi N, Marsh JW, Tzakis AG, et al. Analysis of donor criteria for the prediction of outcome in clinical liver transplantation. Transplant Proc. 1987;19(1 Pt 3):2378-82.
17. Mor E, Klintmalm GB, Gibbs JF, Watemberg I, Goldstein RM, Husberg BS. The use of marginal donors for liver transplantation, a retrospective study of 365 liver dnors. Transplantation. 1992;53(2):383-6.

18. Ploeg RJ, D'Alessandro AM, Knechtle SJ, Stegall MD, Pirsch JD, Hoffmann RM, et al. Risk factors for primary dysfunction after liver transplantation--a multivariate analysis. Transplantation. 1993;55(4):807-13.
19. Strasberg SM, Howard TK, Molmenti EP, Hertl M. Selecting the donor liver: risk factors for poor function after orthotopic liver transplantation. Hepatology. 1994;20(4 Pt 1):829-38.
20. Nanashima A, Pillay P, Verran DJ, Painter D, Nakasuji M, Crawford M, et al. Analysis of initial poor graft function after orthotopic liver transplantation: experience of an australian single liver transplantation center. Transplant Proc. 2002;34(4):1231-5.
21. Maring J, Klompmaker I, Zwaveling J, Kranenburg K, Ten Vergert E, Sloof M. Poor initial graft function after orthotopic liver transplantation:can it be predicted and does it affect outcome?An analysis of 125 adult primary transplantations. Clin Transplant. 1997;11:373-9.
22. Silberhumer GR, Pokorny H, Hetz H, Herkner H, Rasoul-Rockenschaub S, Soliman T, et al. Combination of extended donor criteria and changes in the Model for End-Stage Liver Disease score predict patient survival and primary dysfunction in liver transplantation: a retrospective analysis. Transplantation. 2007;83(5):588-92.
23. Wagener G, Raffel B, Young AT, Minhaz M, Emond J. Predicting early allograft failure and mortality after liver transplantation: the role of the postoperative model for end-stage liver disease score. Liver transpl. 2013;19(5):534-42.
24. Lock JF, Schwabauer E, Martus P, Videv N, Pratschke J, Malinowski M, et al. Early diagnosis of primary nonfunction and indication for reoperation after liver transplantation. Liver transpl. 2010;16(2):172-80.
25. Wiesner R, Edwards E, Freeman R, Harper A, Kim R, Kamath P, et al. Model for end-stage liver disease (MELD) and allocation of donor livers. Gastroenterology. 2003;124(1):91-6.
26. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg. 1973;60(8):646-9.
27. Buell J, Alloway R, Woodle E. How can donors with a previous malignancy be evaluated? Journal of Hepatology. 2006;45:483–513.
28. Levesque E, Hoti E, Khalfallah M, Salloum C, Ricca L, Vibert E, et al. Impact of Reversible Cardiac Arrest in the Brain-Dead Organ Donor on the Outcome of Adult Liver Transplantation. Liver Transplantation. 2011;17:1159-66.
29. Burt A, Mutton A, Day C. Diagnosis and interpretation of steatosis ans steatohepatitis. Semin Diagn Pathol. 1998;15:246-58.
30. Starzl T, Iwatsuki S, Esquivel C, Todo S, Kam I, Lynch S, et al. Refinements in the Surgical Technique of Liver Transplantation. Semin Liver Dis. 1985;4:349-56.
31. Tzakis A, Todo S, Starzl TE. Orthotopic liver transplantation with preservation of the inferior vena cava. Annals of surgery. 1989;210(5):649-52.
32. Aggarwal S, Kang Y, Freeman J, Fortunato F, Pinsky M. Postreperfusion syndrome: cardiovascular collapse following hepatic reperfusion during liver transplantation. . Transplant Proc. 1987;19:54-5.
33. IJtsma A, Van der Hilst C, de Boer M, de Jong K, Peeters P, Porte R, et al. The Clinical Relevance of the Anhepatic Phase During Liver Transplantation. Liver Transplantation. 2009;15:1050-5.
34. Lee S, Epstein MP, Duncan R, Lin X. Sparse principal component analysis for identifying ancestry-informative markers in genome-wide association studies. Genet Epidemiol. 2012;36(4):293-302.
35. Burnham K, Anderson D. Model selection: understanding AIC and multimodel inference, with contrasts to BIC. . Methods in Sociological Research. 2004 33: 261–304.
36. United Network for Organ Sharing Liver Disease Severity Score C. www.UNOS.org.
37. Briceño J, Ciria R, de la Mata M, Rufián S, López-Cillero P. Prediction of Graft Dysfunction Based on Extended Criteria Donors in the Model for End-Stage Liver Disease Score Era. Transplantation. 2010;90:530-9.

38. Pokorny H, Langer F, Herkner H, Schernberger R, Plochl W, Soliman T, et al. Influence of cumulative number of marginal donor criteria on primary organ dysfunction in liver recipients. Clin Transplant. 2005;19(4):532-6.
39. Busuttil RW, Tanaka K. The utility of marginal donors in liver transplantation. Liver Transplantation. 2003;9(7):651-63.
40. Altman DG, Lausen B, Sauerbrei W, Schumacher M. Dangers of using "optimal" cutpoints in the evaluation of prognostic factors. Journal of the National Cancer Institute. 1994;86(11):829-35.

Figure legends

Figure 1. Modeling the MEAF postoperative variables. To approach each variable empirical cumulative distribution function (shaded lines), a non linear sigmoid regression was performed (dashed lines). Scores were computed using each fitted regression function for the $ALT_{Max.\ 3POD}$ **(A)**, $INR_{Max.\ 3POD}$ **(B)** and $bilirubin_{3POD}$ **(C)** score ranges, set from 0 to 3.33. The final MEAF score consisted in adding the three individual scores and ranges from 0 to 10. $ALT_{Max.\ 3POD}$ and $INR_{Max.\ 3POD}$ represent the maximum variable value during the first 3POD, while $bilirubin_{3POD}$ represent variable value on day 3 after transplantation.

Figure 2. Nomogram to calculate the MEAF score. To use the nomogram, connect the patient actual values of $ALT_{Max.\ 3POD}$ (U/L), $INR_{Max.\ 3POD}$ and $bilirubin_{3POD}$ (mg/dL) with the score scale by an orthogonal line and add the three values.

Figure 3. Estimation of 3-month recipient and graft survival according to the MEAF score. **(A)** The Cox regression model showed a significant association between the MEAF score and patient survival during the first 3 months (*p-value = 0.042*). The hazard ratio increase estimation per score point was 1.19 with a 95% CI of 1.01; 1.41. **(B)** Mortality rates according to the MEAF score intervals **(C)** Graft loss rates according to the MEAF score intervals.

Figure 4. **Relationship between the MEAF score, ICU and Hospital stays**. **(A)** A statistically significant positive relationship was found between increasing MEAF score values and longer median ICU stays ($p<0.001$). **(B)** A statistically significant positive relationship was observed between increasing MEAF score values and longer median hospital stays ($p<0.001$). Black dots represent the median values and 95% confidence interval bars for each MEAF score interval. The dashed line represents the regression line calculated by censored quantile regression.

Equation 1.

MEAF = (Score ALT $_{\text{Max. 3POD}}$ + Score INR $_{\text{Max. 3POD}}$ + Score Bilirubin $_{\text{3POD}}$)

$$Score\ ALT_{Max.3POD} = \frac{3.29}{1 + e^{-1.9132(\ln(ALT_{Max.3POD}) - 6.1723)}}$$

$$Score\ INR_{Max.3POD} = \frac{3.29}{1 + e^{-6.8204(\ln(INR_{Max.3POD}) - 0.6658)}}$$

$$Score\ Bilirubin_{3POD} = \frac{3.4}{1 + e^{-1.8005(\ln(Bilirubin_{3DPO}) - 1.0607)}}$$

Tables

Author	n	Parameters to define EAD	Diagnosis Time Frame (days)	Incidence (%)
Makowka et al.1987	219	AST>3500 ALT>2500 PT>25	1	15
Greig et al. 1990	83	AST>2500	2-7	--
Mor et al. 1992	365	AST/ALT>2000	1	13.2
Ploeg et al. 1993	331	AST>2000 PT>16 NH_4>50	2-7	22
Strasberg et al.1 994	--	AST>1500 PT>20	1-7	
Gonzalez et al. 1994	168	ALT>2500 PT(%)<60 Bile output<40	3	27
Maring et al. 1997	125	AST>2500 PT>16 NH_4>50	2-7	13
Deschênes et al. 1998	710	Bb>10 PT>7 Encephalopathy	1-7	23
Nanashima et al. 2002	93	AST/ALT>1500	3	18
Pokorny et al. 2005	734	AST>2500 Clotting support>2d	5	13.1
Olthoff et al. 2010	300	AST>2000 Bb ≥ 10	1-7	23.2

INR>1.6

Table 1. Summary of the different criteria used to define EAD. ALT: alanine aminotransferase (U/L); AST: aspartate aminotransferase (U/L); Bb: bilirubin (mg/dl); Bile output (ml/day); INR: International Normalized Ratio; NH_4: ammonia (μmol/L); PT: prothrombin time (seconds). This brief overview is representative of the classifications used by the researchers, and it is not intended to be a comprehensive review.

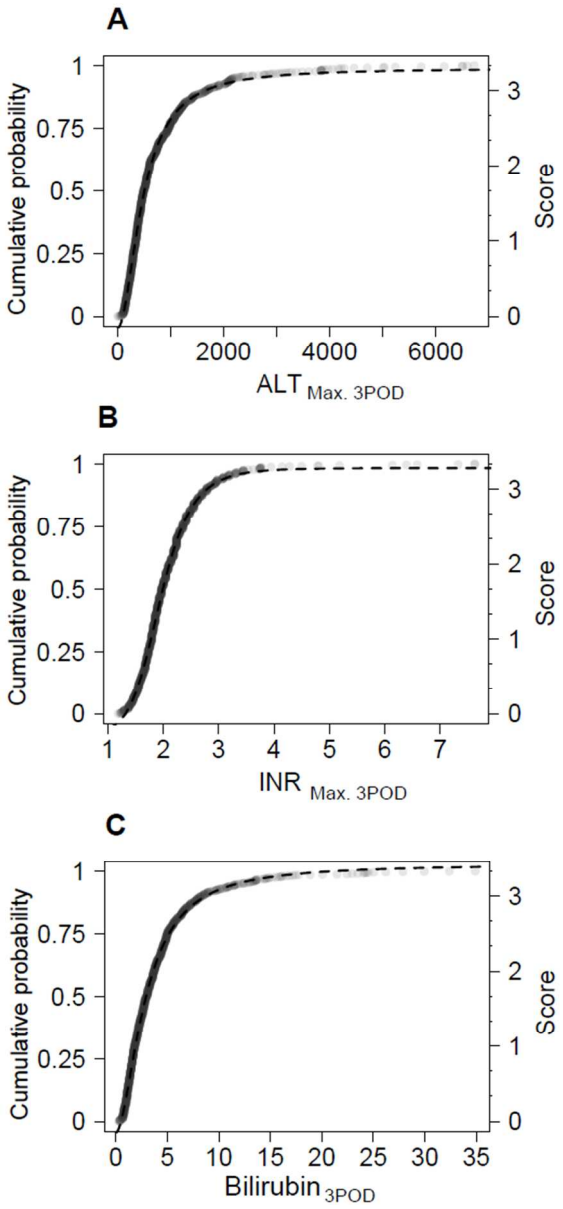


Figure 1. Modeling the MEAF postoperative variables. To approach each variable empirical cumulative distribution function (shaded lines), a non linear sigmoid regression was performed (dashed lines). Scores were computed using each fitted regression function for the ALTMax. 3POD (A), INR Max. 3POD (B) and bilirubin3POD (C) score ranges, set from 0 to 3.33. The final MEAF score consisted in adding the three individual scores and ranges from 0 to 10. ALTMax. 3POD and INR Max. 3POD represent the maximum variable value during the first 3POD, while bilirubin3POD represent variable value on day 3 after transplantation.
684x1468mm (120 x 120 DPI)

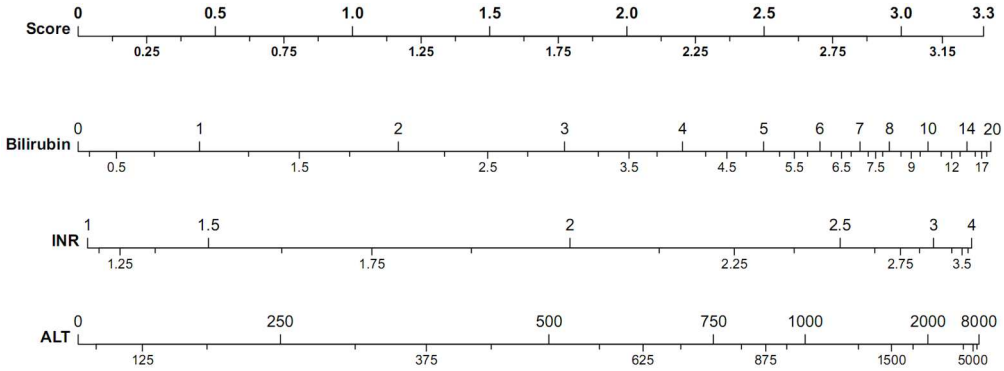


Figure 2. Nomogram to calculate the MEAF score. To use the nomogram, connect the patient actual values of ALT Max. 3POD (U/L), INR Max. 3POD and bilirubin3POD (mg/dL) with the score scale by an orthogonal line and add the three values.
355x131mm (120 x 120 DPI)

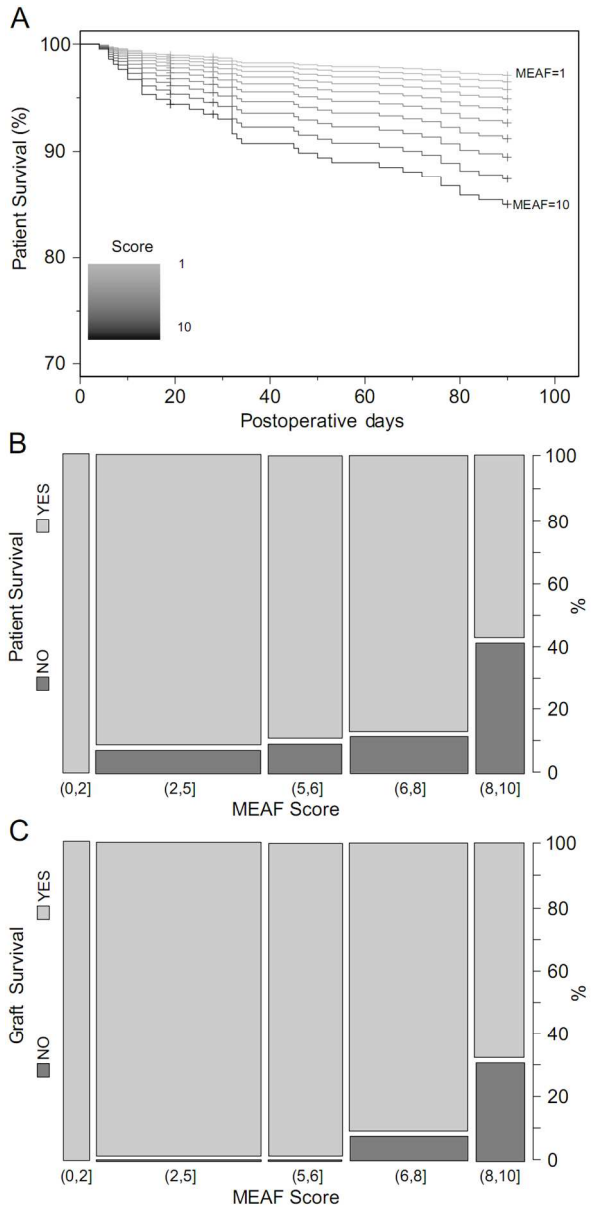
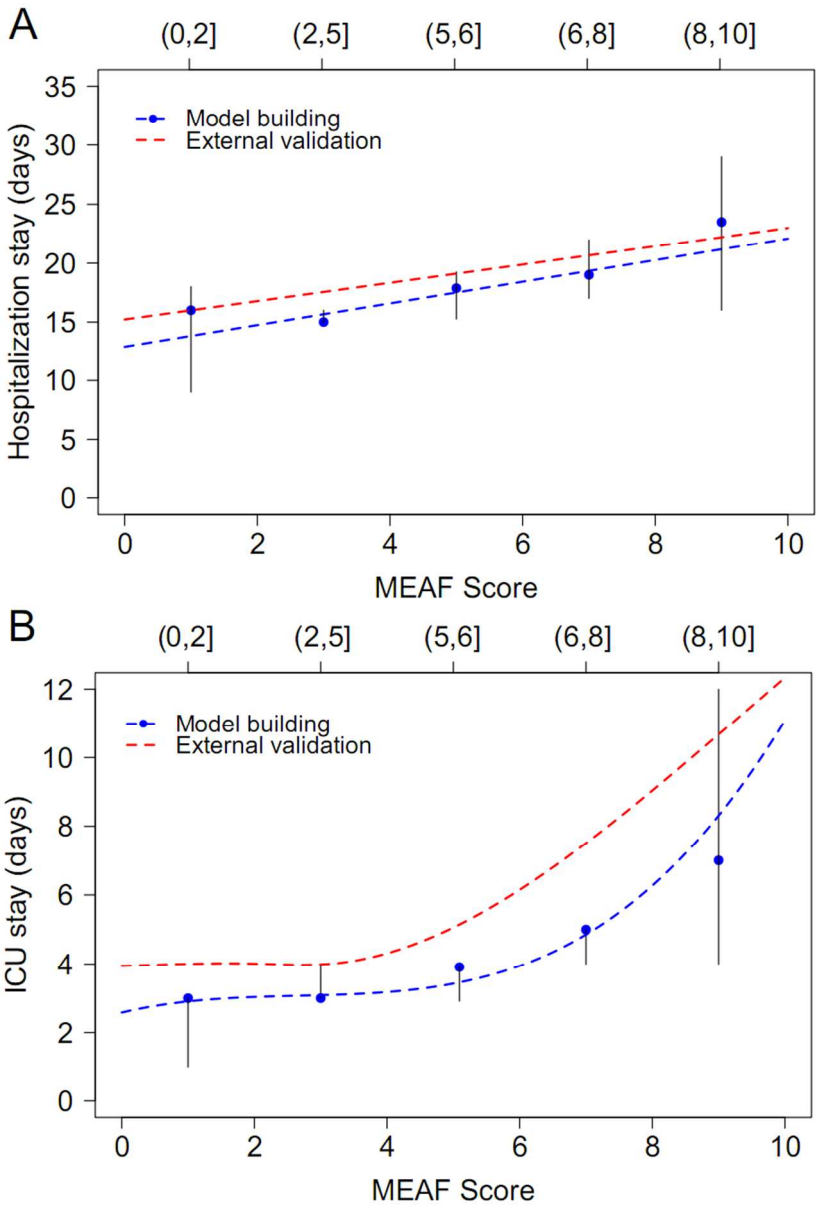


Figure 3. Estimation of 3-month recipient and graft survival according to the MEAF score. (A) The Cox regression model showed a significant association between the MEAF score and patient survival during the first 3 months (p-value = 0.042). The hazard ratio increase estimation per score point was 1.19 with a 95% CI of 1.01; 1.41. (B) Mortality rates according to the MEAF score intervals (C) Graft loss rates according to the MEAF score intervals.
799x1638mm (120 x 120 DPI)



748x1092mm (120 x 120 DPI)

Supplementary Material

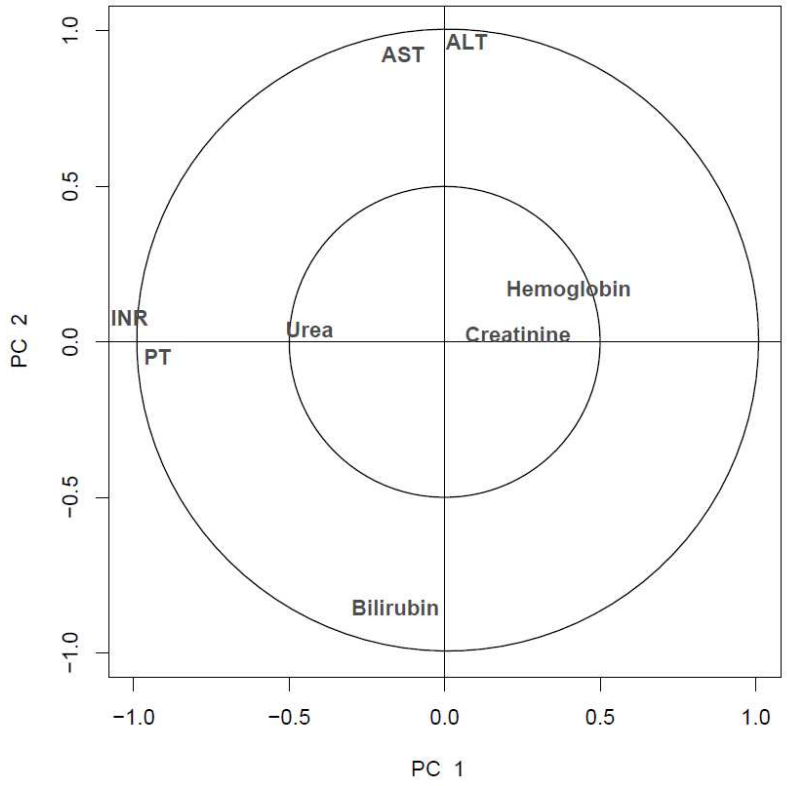


Figure S1. Sparse Principal Component Analysis (sPCA) correlation circle plot. The plot shows which variables among the recipients' variables summarized in Table S4 are selected by the sPCA. In this plot variables are represented according to their correlation with the principal components generated by sPCA. The two plotted circumferences correspond to correlations of 0.5 and 1. Variables with a strong correlation are projected in the same direction from the origin.

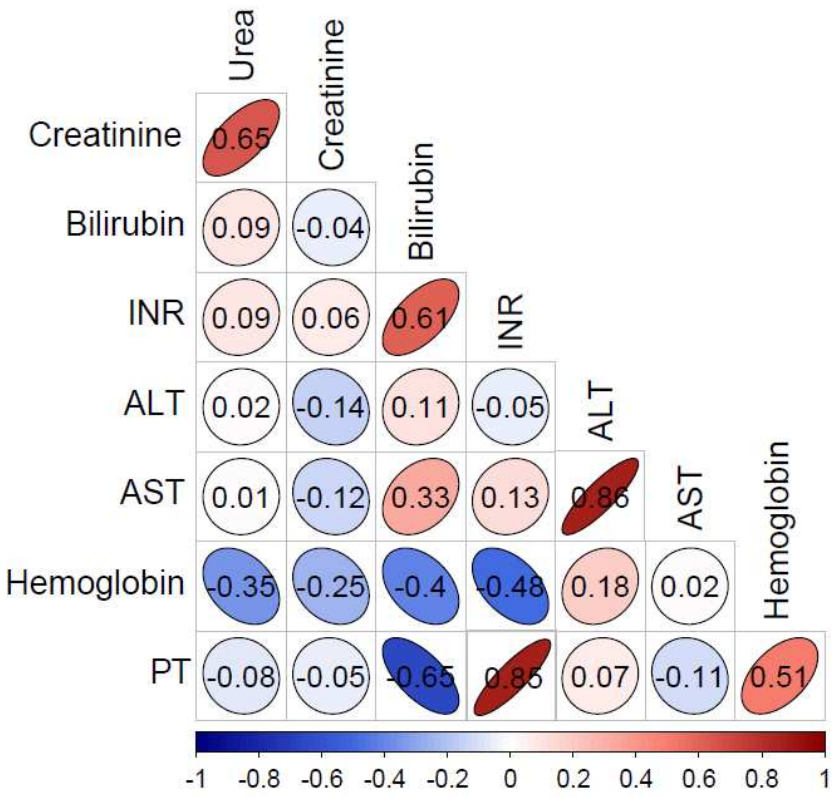


Figure S2. Correlation analysis between the recipient's clinical variables selected by sPCA.

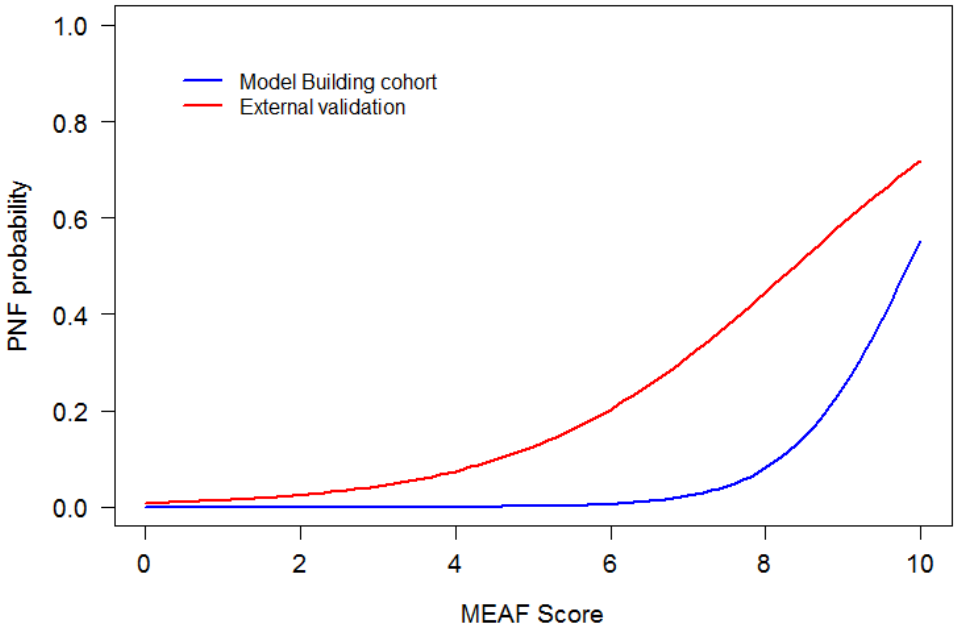


Figure S3. Estimated probabilities of PNF derived from the logistic regression analysis in both the model building and the external validation cohorts. PNF: primary non-function.

Variable	Mean	SD	Min. Val.	Max. Val.
Age (years)	54.5	17.6	12	86
Gender (male/female)	509/330			
Gender mismatch				
No	499			
Male(D) → Female (R)	116			
Female(D) → Male (R)	224			
BMI(kg/m^2)	26.5	4	13.8	49.4
Cause of death				
CVA	567			
Head trauma	205			
Anoxia	43			
Brain tumour	8			
Others	16			
ICU Stay (days)	3.48	3	1	45
Hypotension (no/yes)	487 /352			
Heart arrest (no/yes)	710/129			
Inotropic drugs				
No	541			
<2	218			
≥2	80			
Na (mEq/L)	149	10.6	120	188
Steatosis				
No	678			
Mild (<30%)	143			
Moderate (30-59%)	18			

Table S1. Donor Information Summary. Continuous variables are described as the mean, categorical variables are expressed as an absolute number. BMI: body mass index; CVA: cerebral vascular accident; D: donor; ICU: Intensive Care Unit; Min. Val: minimum value; Max. Val: maximum value; Na: sodium; R: recipient, SD: standard deviation minimum value.

Variables	Mean	SD	Min. Val.	Max. Val.
Surgery length (min)	254	48	100	545
CIT (min)	311	156	22	850
WIT (min)	44	15	15	135
Arterial anastomosis delay (min)	43	19	10	180
Cell saver (ml)	710	698	0	10000
RBC (units)	3	2.8	0	33
FFP (units)	2.5	1.7	0	14
Graft wash				
Saline	83			
Blood	289			
Saline + Blood	467			
Post-reperfusion syndrome (no/yes)	717/122			
T-tube (no/yes)	323 /516			
Relaparotomy for bleeding (no/yes)	767/72			

Table S2. Surgery Information Summary. Continuous variables are described as the mean, categorical variables are expressed as an absolute number. CIT: cold ischemia time; FFP: fresh frozen plasma; Min. Val: minimum value; Max. Val: maximum value; RBC: red blood cells; SD: standard deviation; WIT: warm ischemia time.

Variable	Mean	SD	Min. Val.	Max. Val.
Age (years)	54.14	9	17	70
Gender (male/female)	633/206			
BMI (kg/m^2)	27	4.4	15.6	45.4
Primary Diagnosis				
Viral	195			
Alcohol	184			
Cholestatic	25			
Tumor	19			
Viral + tumour	147			
Alcohol + viral	87			
Alcohol + tumour	56			
Alcohol + viral + tumour	55			
Others	71			
Tumour (HCC/Neuroendocrine)	267/10			
MELD score	16.7	7.6	6	57
Child-Pugh store				
A	152			
B	284			
C	403			
ICU stay (days)	6	5	1	115
Hospital stay (days)	23	21	1	240
Post-Tx RBC Transfusion (yes/not)	681/158			
Post-Tx FFP Transfusion (yes/not)	662/177			
CMV (positive/negative)	698/141			
CMV mismatch				
No	590			
D (-)→R(+)	156			
D(+)→R(-)	93			
HBcAb (+/-)	403/436			

Table S3. Recipient Information Summary in 839 OLTs. Continuous variables are described as the mean, categorical variables are expressed as an absolute number. Continuous variables are described as mean, standard deviation (SD), minimum value (Min. Val) and maximum value (Max. Val); categorical variables are expressed as an absolute number. From the 23 patients who underwent a retransplantation, 13 had the first transplant before the studied period. D: donor; BMI: body mass index; CMV: cytomegalovirus; FFP: fresh frozen plasma; HBcAb: hepatitis B core antibodies; HCC: hepatocellular carcinoma; ICU: intensive care unit; MELD: model for end-stage liver disease; Min. Val: minimum value; Max. Val: maximum value; Post-OLT: post-orthotopic liver transplant; R: recipient; RBC: red blood cells; SD: standard deviation minimum value.

Biochemical test	Full blood cell count	Coagulation parameters
Albumin (g/dl)	Haematocrit (%)	APTT (s)
ALT/GPT (U/L) *, **	Haemoglobin (g/dl)	Fibrinogen (mg/dl)
AST/GOT (U/L) *	Leukocytes (10^3/µl)	INR *, **
Bilirubin (mg/dl) *, **	Platelets (10^3/µl)	Prothrombin time (s)*
Creatinine (mg/dl)		
Glucose (mg/dl)		
Ions		
Total proteins (g/dl)		
Urea (mg/dl)		

Table S4. Summary of recipients' clinical variables candidates to model EAD. *, parameter frequently used to define EAD, see Table 1; **, parameters used in our score. ALT: aspartate alanine transferase; AST: aspartate alanine transferase; APTT: activated partial thromboplastin time; INR: international normalised ratio, LDH: lactate dehydrogenase.

Model building cohort			
Follow-up (months)	**Hazard ratio**	**Confidence Interval (95%)**	***p-value***
3	1.19	1.01; 1.41	0.042
6	1.23	1.05; 1.44	0.009
12	1.21	1.05; 1.41	0.009
External validation cohort			
Follow-up (months)	**Hazard ratio**	**Confidence Interval (95%)**	***p-value***
3	2.43	1.58; 3.73	<0.001
6	2.24	1.52; 3.31	<0.001
12	1.95	1.38; 2.75	<0.001

Table S5. Survival analysis by Cox regression: for 3-, 6- and 12-month follow-ups using MEAF score as continuous predictor in both the model building and the external validation cohort.

Variable	Model averaged importance
Age	0.96
Hepatic steatosis	0.92
ICU stay	0.83
Vasoactive drugs	0.49
Sodium levels	0.35
Heart arrest	0.05
Rh	0.05
BMI	0.04
Hypotension	0.03
Cause of death	0.01

Table S6. Importance of Donor's parameters estimated by model averaging. BMI: body mass index; ICU: Intensive Care Unit.

Variable	Model averaged importance
Cold ischemia time	1.00
Postreperfusion syndrome	0.95
Warm ischemia time	0.88
RBC units	0.69
FFP units	0.58
Relaparatomy	0.29
Operation duration	0.18
T-tube	0.14
Cell saver	0.09

Table S7. Importance of Surgery's parameters estimated by model averaging. FFP: fresh frozen plasma; RBC: red blood cells. Post-reperfusion syndrome is defined as hypotension (mean arterial blood pressure 30% lower than the value immediately at the end of a hepatic stage, lasting for more than 1minute within 5 minutes) or asystole after unclamping.

Variable	Model averaged importance
MELD	1.00
BMI	1.00
Child Pugh	1.00
FFP	0.82
ICU stay	0.82
Hospital stay	0.81
Age	0.30
Gender	0.13
RBC	0.12
Retransplantation	0.03
Renal function	0.03
SBP	0.03
UGIB	0.03
Encephalopathy	0.02
Ascites	0.01
HCC	0.01
CMV	0.00

Table S8. Importance of Recipient's parameters estimated by model averaging. BMI: body mass index; CMV: citomegalovirus; FFP: fresh frozen plasma; HCC: heaptocellular carcinoma; ICU: intensive care unit; MELD: model for end stage liver disease; RBC: red blood cells; SBP: spontaneous bacterial peritonitis; UGIB: upper gastrointestinal bleeding.