**Degree of Philosophy**

# Prediction Model for Survival after Liver Transplantation In Acute-on-Chronic Liver Failure : Novel Risk Score Integrating Cardiac Biomarkers

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# Prediction Model for Survival after Liver Transplantation In Acute-on-Chronic Liver Failure : Novel Risk Score Integrating Cardiac Biomarkers

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# Prediction Model for Survival after Liver Transplantation In Acute-on-Chronic Liver Failure : Novel Risk Score Integrating Cardiac Biomarkers

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**Abstract**

**Background & Aims**: Acute-on-Chronic Liver Failure (ACLF) is a syndrome associated with high mortality, especially among patients with three or more failed organ systems, yet with acceptable survival benefit of liver transplantation (LT). Of well-known prognostic scores of ACLF, such as Chronic Liver Failure Consortium (CLIF-C) Organ Failure score (CLIF-C OFs) and ACLF score (CLIF-C ACLFs), category "Circulatory Failure" is evaluated only by mean blood pressure or use of vasopressor, thus may not adequately reflect cardiac function. Given that cardiovascular mortality is the leading cause of death after LT, we aimed to develop and validate a new prognostic score (CLIF-C CARDIACs) incorporating cardiac biomarkers to improve outcome prediction after LT.

**Methods:** Data from the Asan LT Registry between January 2008 and February 2019 were prospectively collected. CLIF-C ACLFs, Model for End-Liver Disease score (MELDs), and Child-Pugh score (CPs) were calculated, and survival data were collected. Troponin I (TnI) and B-type natriuretic peptide (BNP) were independently selected from random survival forest analysis and were subsequently combined to develop CLIF-C CARDIACs. Area under the curve, Concordance index, Net Reclassification Index (NRI) and Integrated Discrimination Index (IDI) were performed to compare the discrimination abilities across models.

**Results:** Of 2845 patients, 685 (24%) showed ACLF. During a median 3.4 year of follow-up, 28, 90, 180 and 365-day mortality after LT was 35 (5.1%), 66 (9.6%), 93 (13.6%), and 115 (16.8%), respectively in patients with ACLF. CLIF-C CARDIACs had superior predictive accuracy of predicting mortality than CLIF-C ACLFs, MELDs, and CPs at 28, 90, 180, and 365 days after LT. NRI showed that the CLIF-C CARDIACs improved the post-LT mortality predictions by 18% to 42% as compared to the CLIF-C ACLFs, MELDs and CPs. Calibration of CLIF-C CARDIACs

by Hosmer-Lemeshow test showed good fit (28-day, p=0.601; 90-day, p=0.351; 180-day, p=0.504; 365-day, p=0.552).

**Conclusion**: The CLIF-C CARDIACs, integrating objective cardiac biomarkers, was superior to CLIF-C ACLFs in predicting short- and long-term mortality after LT. The CLIF-C CARDIACs therefore may be used to identify a high-risk cohort in need of perioperative intensive care management.

**Keywords**: acute-on-chronic liver failure; liver transplantation; cardiac evaluation; risk score

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## Introduction

Acute-on-Chronic Liver Failure (ACLF), characterized by acute decompensation of cirrhosis, organ failure(s), is associated with high short-term mortality.[1)] Specifically, in patients with multiple organ failures, ACLF grade 3, 28-day mortality is reported to be as high as 80% without liver transplantation, indicating very poor prognosis.[2, 3)] Given the higher mortality associated with higher grade of ACLF, liver transplantation (LT) is potentially important intervention for these patients. In addition, studies regarding LT in patients with ACLF grade 3 have demonstrated a substantial survival benefit of LT, with 1-year posttransplant survival greater than 80%.[4, 5)]

However, type of organ failure has significant effect on the post-LT outcome in patient with ACLF. It is consistently reported that post-LT survival rates in patients with circulatory and respiratory failure are worse compared to those with liver, coagulation, kidney, or cerebral failure.[6, 7)] Moreover, the main causes of death after LT at present are associated with early cardiovascular complication.[8, 9)]

Considering the escalating risk profiles of LT candidates with ACLF, pretransplant assessment of cardiac risk is essential, yet clinically challenging.[10)] Typical symptoms or signs of cardiac disorders are often non-specific and may be overlapped with the clinical manifestation of liver cirrhosis, raising the risk of under- or mis-diagnosis of underlying cardiac disorder. Meanwhile, quantitative cardiac biomarkers, such as troponin I (TnI) for myocardial injury and B-type natriuretic peptide (BNP) for the presence and severity of hemodynamic cardiac stress and heart failure, are considered to have powerful prognostic value in cardiovascular assessment. [11-14)] However, prognostic value of TnI and BNP on survival after LT in patients with ACLF needs the be researched.

Current prognostic score (Chronic Liver Failure Consortium [CLIF-C] ACLF

score)[15], was designed to evaluate each organ failure, however, it lacks cardiovascular risk assessment. Category "Circulatory Failure" is evaluated by mean blood pressure or use of vasopressor, thus may not adequately reflect cardiac function.

Therefore, main objective of current study was to develop a new score (CLIF-C CARDIACs) reflecting cardiac function by integrating BNP and TnI, aiming at higher prognostic accuracy to predict outcomes after LT, compared to current scoring systems such as CLIF-C ACLFs, Model for End-stage Liver Disease score (MELDs), and Child-Pugh score (CPs). The study had three main aims. First aim was to assess the prognostic value and clinical applicability of BNP and TnI levels in patients with ACLF to prognosticate the post-LT mortality. Second aim was to develop a new scoring system integrating cardiac functions which has higher prognostic value compared to current scoring method. Third aim was to evaluate whether the prognostic accuracy of CLIF-C CARDIACs is higher than other currently widely-used scores in predicting 28-, 90-, 180-, and 365-day mortality after LT.

## Method

*Patients and data collection*

The Asan LT Registry included a total of 4836 consecutive, prospectively registered patients who underwent LT from January 2008 to February 2019. Of these patients, 1518 were excluded, including 204 patients with < 18 years old, 197 patients who underwent re-transplantation, 84 with concomitant end-stage renal disease, 256 with toxic or fulminant hepatitis, 777 without preoperative TnI or BNP measurement. Finally, this observational cohort study was a retrospective review of data from 2845 end-stage liver disease who underwent liver transplantation.

Parameters, including baseline demographic data, MELDs, and perioperative variables were collected using our institution's database extraction software, a fully computerized Asan Medical Center research information system (ABLE, Asan BiomedicaL rEsearch) after approval from the local research ethics committee, which waived the requirement for the written informed consent. The study protocol conforms to the ethical guidelines of the 2013 Declaration of Helsinki.

The diagnosis of ACLF was based on the CLIF-C Organ Failure (CLIF-C OF) criteria[15], simplified version of CLIF-C Sepsis Organ Failure Assessment score (SOFAs) which is as follows; a) presence of at least renal failure, b) any other single organ failure if associated with renal dysfunction (serum creatinine 1.5–1.9 mg/dl) and/or grade I–II hepatic encephalopathy (ACLF grade 1). Patients with two organ failures were graded as ACLF grade 2 and those with three or more organ failures as ACLF grade 3. The CLIF-C ACLFs was calculated using data from 685 patients who had ACLF at the time of admit or during hospitalization before LT.

*Assessment of TnI and BNP*

High-sensitivity TnI (ADVIA Centaur XP TnI-Ultra, Siemens Healthcare Diagnostics, Tarrytown, NY; the 99th percentile reference limit = 0.040 ng/mL) and BNP (ADVIA Centaur; Bayer Diagnostics, Tarrytown, NY, USA) concentration tests were conducted at the clinical laboratories of Asan Medical Center, which is certified by the College of American Pathologists and Korean Society for Laboratory Medicine. was estimated using a chemiluminescence immunoassay. They were measured at least once preoperatively in all recipients according to the institution's routine protocol. Among patients with >1 pretransplant laboratory results, the BNP and TnI most proximate to the date of transplant was used.

*Study outcomes*

The main study outcomes included all-cause mortality at 28, 90, 180, and 365 days after LT.

All the data required to compute CLIF-C CARDIACs (as well as those used to compute CLIF-C ACLFs, MELDs and CPs) were measured at the time of LT (either at admit or within the hospitalization for LT)

*Statistical analysis*

In univariate statistical comparisons, the chi-square test or Fisher's exact test was used for categorical variables, Student's t-test and Mann-Whitney test for continuous variables, as appropriate.

The functions of "rms" and "survival" in R packages were employed to construct estimated hazard ratio curves with splines for all-cause mortality. Restricted cubic spline analysis with four knots was utilized to graphically display and evaluate nonlinear associations of mortality with TnI and BNP on a continuous scale. The importance of TnI and BNP on outcome after LT were assessed four different methods.

First, we performed Receiver operating characteristic (ROC) analyses and calculated areas under ROC curves (AUC).

Second, we developed a random survival forest model to compute a variable importance (VIMP) score using "randomForestSRC" and "ggRandomForests" packages in R software.[16] Briefly, the central elements of the random survival forest algorithm are growing the survival tree and constructing the ensemble cumulative hazard function. By assuming randomness for variable selection and resampling, the random survival forest method improves predictive ability and are widely used for variable selection because they produce a variable importance score.[17] The main advantage of the random survival forest algorithm is that it does not use restrictive assumptions such as proportional hazards and parametric or linear effects of the variables. Therefore, it can be used flexibly for researching the highly associated variable with outcome without regards to number of entered variables and automatically takes care of the possible interaction between variables and reflects them in the importance scores.[18, 19] The importance score is a metric of how much the prediction error rate of a model is improved by addition of each variable (more influential factors have higher scores). Specifically, the importance score of a covariate x is the prediction error for the original ensemble trees subtracted from the prediction error for the new ensemble trees obtained using randomizing x assignments. Here, we computed the prediction error using Harrell's c-index, and the splitting rule we employed is the log-rank rule that splits tree nodes maximizing the log-rank statistics.

Thirdly, a Cox proportional hazard regression model was used to assess factors that independently contributed to 28-day all-cause mortality after LT. Factors analyzed included preoperative demographic characteristics including CLIF-C ACLFs, TnI, BNP, parameters derived from echocardiography and other clinically

relevant variables which has high VIMP score or has a P value <0.1 on univariate analysis to predict post-LT mortality. Selected variables with were included in the multivariate analysis with stepwise backward elimination. Finally, we performed 1000 bootstraps of automated multivariable cox proportional regression analysis with resamples and computed the relative selection frequency. The relative selection frequency was calculated for TnI and BNP to evaluate the association with 28-day mortality after LT.

After identifying the independent association of TnI and BNP with mortality, we developed CLIF-C CARDIACs based on the results obtained from the multivariable Cox-proportional hazards model with BNP, TnI and CLIF-C ACLFs and 28-day all-cause mortality after LT as outcome. The calibration of the modified scoring system, CLIF-C CARDIACs, was assessed by comparing the actual observed risk and the average probability of dying at different time points predicted by the score. The Hosmer-Lemeshow test was used to assess the corresponding goodness-of-fit.

To evaluate the discrimination ability, the Harrell's concordance index (C-index) was compared between scores at all time points. Percentage improvement was obtained with CLIF-C CARDIACs by calculating prediction error rate with respect to the other scores (computed as percentage reduction in discordance rate of CLIF-C CARDIACs vs. Reference score, i.e. 100 [$\text{C-index}_{\text{CLIF-C CARDIACs}}$ - $\text{C-index}_{\text{Reference}}$]/[1 - $\text{C-index}_{\text{Reference}}$]). Furthermore, time-dependent ROC curves were performed using the R software package "timeROC" to assess and compare the discriminative ability of different scoring methods at 28, 90, 180, 365-day all-cause mortality. Time-dependent ROCs were estimated non-parametrically using the inverse probability of a censored weighting approach.[20, 21] Finally, Net Reclassification Index (NRI) and Integrated Discrimination Index (IDI) were performed to investigate the improved performance of CLIF-C CARDIACs using

the R package of "survIDINRI".[22] The NRI (theoretical range -2 to +2) is computed by assessing the change (movement "up" or "down" within categories) in the classification of the risk/probability of patients with respect to the end point (in this case 28-day all-cause mortality) by the addition of the new marker in question (TnI and BNP in the current study), that is, NRI = *P*(up|event) – *P*(down|event) + *P*(down|nonevent) - *P*(up|nonevent). In the absence of understandable and well-verified risk categories, a category-free version is available NRI (>0)[23], which may be more robust as the NRI has been demonstrated to be computationally sensitive to the number of risk categories used.[24] The NRI (>0) ". . .captures the marginal strength of the new predictor after accounting for correlations with variables included in the baseline model".[25] The IDI, a complement to the AUC, is defined as: IDI = (ISnew – ISold) – (IPnew – IPold), where IS is the integral of sensitivity over all possible cutoff values and IP is the corresponding integral of "1-specificity".[26] The IDI magnitude indicates the increase in the separation of mean predicted risks/probabilities for events and nonevents that has occurred by the incorporation of the new parameters.[25] The NRI and IDI represent new metrics for the formal assessment of model performance, to supplement the improvement in the AUC.

Finally, we internally validated the performance of the CLIF-C CARDIAC score by bootstrap method with 10,000 resamples with replacement, which accounts for the generalizability error and to cope with overfitting issue.[27] Simulation studies have shown that this approach provides the least biased and most stable estimates of optimism-corrected performance among the various proposed methods for internal validation[28, 29]; with "optimism" referring to the inherent bias toward an overestimated performance in the derivation dataset.[27, 30] In brief, optimism in a performance measure, such as c-index, with this method is estimated by the average of ($\text{C-index}_{\text{bootstrap sample}}$ – $\text{C-index}_{\text{original dataset}}$) for a large number of models derived from respective bootstrap samples: the C-index of

each of the bootstrap sample-derived models is evaluated on the bootstrap sample ("training" dataset) and back to the original dataset ("validation" dataset). The average of ($C\text{-index}_{\text{bootstrap sample}} - C\text{-index}_{\text{original dataset}}$) is the optimism, which is subtracted from the original model's C-index to provide a more realistic estimate. This approach moderates our expectations from the model and sets an upper limit for performance in future external validation.

All tests were 2-tailed, with a P value<0.05 considered statistically significant. All statistical and graphical analyses were performed using R 3.4.1 (http://www.rproject.org).

## Result

*Patients' baseline characteristics*

Of 2845 LT recipients, 685 (24.1%) patients presented with ACLF. Table 1 lists the baseline characteristics of 685 patients with ACLF. Of all ACLF patients, 71.2% were male with a median age of 53 (45-58) years-old and a median MELD score of 32 (26-40). Main etiologies of liver cirrhosis were viral hepatitis (48.0%) and alcoholic liver disease (35.9%), along with biliary disease (6.6%) and others (0.4%). Median BNP were 144 (67-348) pg/ml and median TnI were 0.014 (0.006-0.050) ng/ml. During the follow-up period, 28, 90, 180, and 365-day mortality rate were 35 (5.1%), 66 (9.6%), 93 (13.6%), and 15 (16.8%), respectively.

**Table 1. Demographics according to acute on chronic liver failure grade in liver transplant recipients**

| | ACLF grade 1 (N=285) | ACLF grade 2 (N=215) | ACLF grade 3 (N=185) | Total (N=685) | P value |
|---|---|---|---|---|---|
| **Demographics** | | | | | |
| Age (yr) | 52 (45-58) | 52 (45-58) | 54 (46-59) | 53 (45-58) | 0.063 |
| Sex (male) | 192 (67.4) | 158 (73.5) | 138 (74.6) | 488 (71.2) | 0.163 |
| Body mass index (kg/m$^2$) | 23.8 (20.8-26.7) | 24.1 (21.5-26.9) | 24.1 (21.6-26.6) | 24.0 (21.3-26.7) | 0.178 |
| MELD score | 26 (23-30) | 34 (30-40) | 40 (37-44) | 32 (26-40) | <0.001 |
| CP score | 10 (9-12) | 11 (11-12) | 12 (11-13) | 11 (10-12) | <0.001 |
| Cardiovascular disease | 22 (7.7) | 23 (10.7) | 12 (6.5) | 57 (8.3) | 0.280 |
| Diabetes mellitus | 73 (25.6) | 42 (19.5) | 42 (22.7) | 157 (22.9) | 0.277 |
| Hypertension | 57 (20.0) | 29 (13.5) | 23 (12.4) | 109 (15.9) | 0.046 |
| Beta blocker | 79 (27.7) | 54 (25.1) | 48 (25.9) | 181 (26.4) | 0.796 |
| Donor type (living /deceased) | 202 (72.9)/83 (29.1) | 101 (47.0)/114 (53.0) | 62 (33.5)/123 (66.5) | 365 (53.3)/320 (46.7) | <0.001 |
| **Cardiac related parameter** | | | | | |
| B-type natriuretic peptide (pg/ml) | 116 (49-264) | 150 (74-318) | 209 (91-600) | 144 (67-348) | <0.001 |
| Troponin I (ng/ml) | 0.006 (0.006-0.021) | 0.015 (0.006-0.047) | 0.044 (0.015-0.188) | 0.014 (0.006-0.050) | <0.001 |

Table 1. continued

| | ACLF grade 1 (N=285) | ACLF grade 2 (N=215) | ACLF grade 3 (N=185) | Total (N=685) | P value |
|---|---|---|---|---|---|
| LVEF (%) | 65 (62-68) | 64 (62-68) | 66 (62-69) | 65 (62-68) | 0.110 |
| E/A ratio (n=2769) | 1.10 (0.86-1.39) | 1.11 (0.84-1.37) | 1.11 (0.85-1.34) | 1.11 (0.85-1.38) | 0.889 |
| **Etiology of liver cirrhosis** | | | | | |
| Viral cirrhosis | 113 (39.6) | 112 (52.1) | 104 (56.2) | 329 (48.0) | 0.001 |
| Alcoholic cirrhosis | 110 (38.6) | 77 (35.8) | 59 (31.9) | 246 (35.9) | 0.334 |
| Biliary disease | 30 (10.5) | 8 (3.7) | 7 (3.8) | 45 (6.6) | 0.002 |
| Other disease | 3 (1.1) | 0 (0.0) | 0 (0.0) | 3 (0.4) | 0.121 |
| **Data used to compute CLIF-C ACLF score** | | | | | |
| Serum bilirubin (mg/dl) | 15.3 (5.9-25.8) | 28.4 (16.4-35.0) | 29.5 (18.8-36.7) | 22.5 (12.8-32.7) | <0.001 |
| Serum creatinine (mg/dl) | 0.95 (0.63-1.51) | 1.18 (0.73-2.10) | 2.00 (1.04-2.76) | 1.18 (0.74-2.11) | <0.001 |
| Renal replacement therapy | 34 (11.9) | 51 (23.7) | 119 (64.3) | 204 (29.8) | <0.001 |
| HE grade I-II | 46 (16.1) | 82 (38.1) | 72 (38.9) | 200 (29.2) | <0.001 |
| HE grade III-IV | 8 (2.8) | 13 (6.0) | 74 (40.0) | 95 (13.9) | <0.001 |
| PT, INR | 1.95 (1.55-2.30) | 2.51 (2.00-2.95) | 2.57 (2.09-3.04) | 2.23 (1.8-2.71) | <0.001 |

Table 1. continued

| | ACLF grade 1 (N=285) | ACLF grade 2 (N=215) | ACLF grade 3 (N=185) | Total (N=685) | P value |
|---|---|---|---|---|---|
| Mean blood pressure (mmHg) | 81 (75-88) | 81 (75-89) | 78 (73-86) | 80 (74-88) | 0.026 |
| Use of vasopressors | 15 (5.3) | 24 (11.2) | 108 (58.4) | 147 (21.5) | <0.001 |
| Mechanical ventilation | 11 (3.9) | 35 (16.3) | 133 (71.9) | 179 (26.1) | <0.001 |
| White-cell count (x$10^9$ cells/L) | 4.9 (3.3-7.4) | 6.0 (4.3-9.6) | 9.2 (5.6-13.9) | 6.1 (4.1-10.3) | <0.001 |
| Serum sodium (mEq/L) | 136 (132-139) | 135 (131-138) | 136 (133-140) | 136 (132-139) | 0.019 |
| **Mortality rates** | | | | | |
| 28-Day mortality | 6 (2.1) | 11 (5.1) | 18 (9.7) | 35 (5.1) | 0.001 |
| 90-Day mortality | 12 (4.2) | 16 (7.4) | 38 (20.5) | 66 (9.6) | <0.001 |
| 6-Month mortality | 20 (7.0) | 21 (9.8) | 52 (28.1) | 93 (13.6) | <0.001 |
| 1-Year mortality | 30 (10.5) | 27 (12.6) | 58 (31.4) | 115 (16.8) | <0.001 |

Continuous variables are expressed as mean ± standard deviation or as median (interquartile range) and categorical variables as n (%). MELD, model for end-stage liver disease; CP score. Child-Pugh score; LVEF, left ventricular ejection fraction; E/A, transmitral early and late diastolic velocity ratio; CLIF-C ACLF score, Chronic Liver Failure Consortium Acute on Chronic Liver Failure score; HE, hepatic encephalopathy; PT, prothrombin time; INR, international normalized ratio.

*Part1: BNP and TnI levels and outcome*

Increased BNP and TnI levels were associated with higher organ failure states, as presented by higher grade of ACLF (Fig. 1). Interestingly, within the group of patients with same ACLF grade, higher BNP or TnI levels were associated with higher risk of 28, 90, 180 and 365-day mortality (Fig. 2, 3).

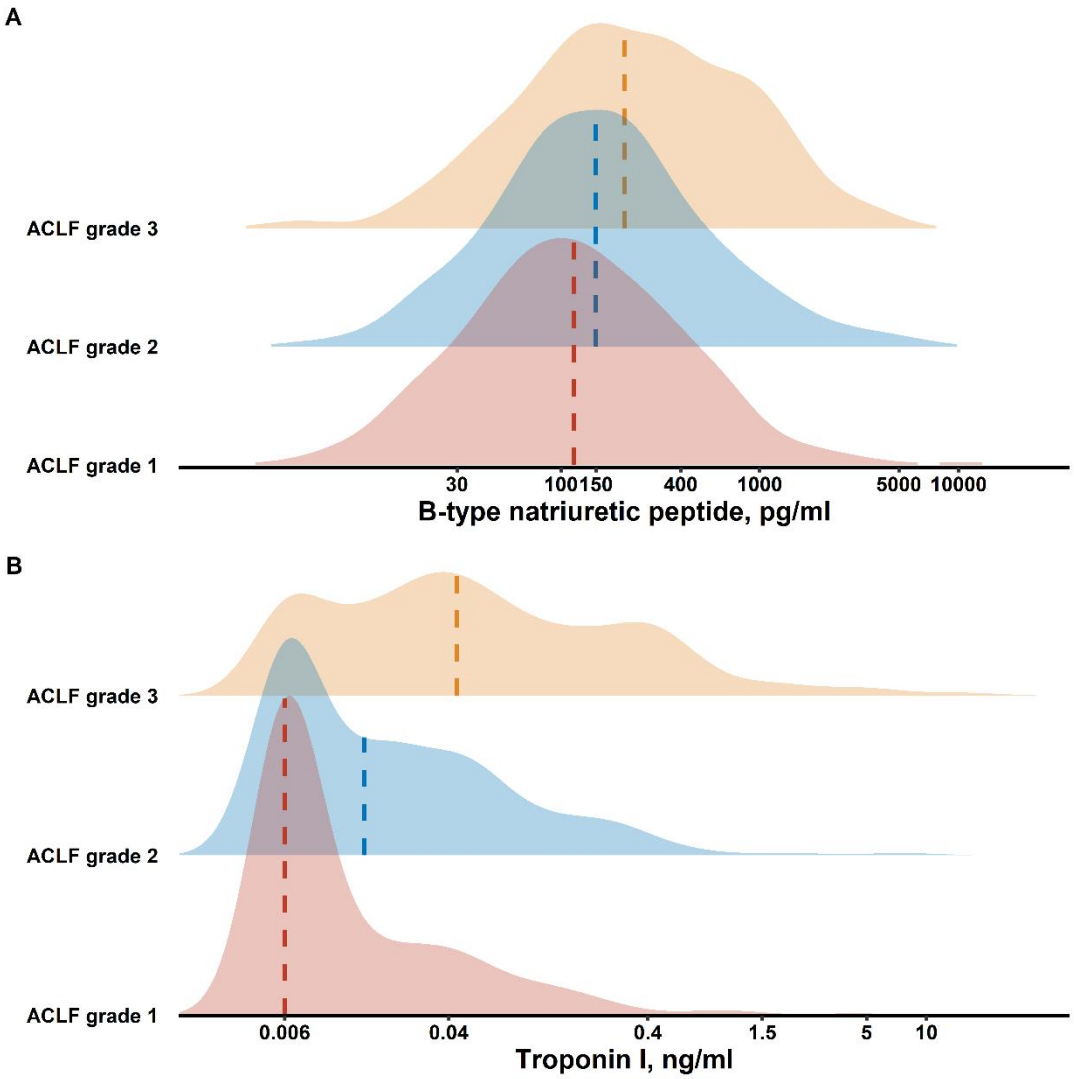


**Fig. 1. Distribution of B-type natriuretic peptide (BNP, A) and troponin I (TnI B) according to Acute-on-Chronic Liver Failure (ACLF) grade.** The median BNP and TnI is shifting to the rightward in proportion to ACLF grade. Vertical dashed lines indicate median value.

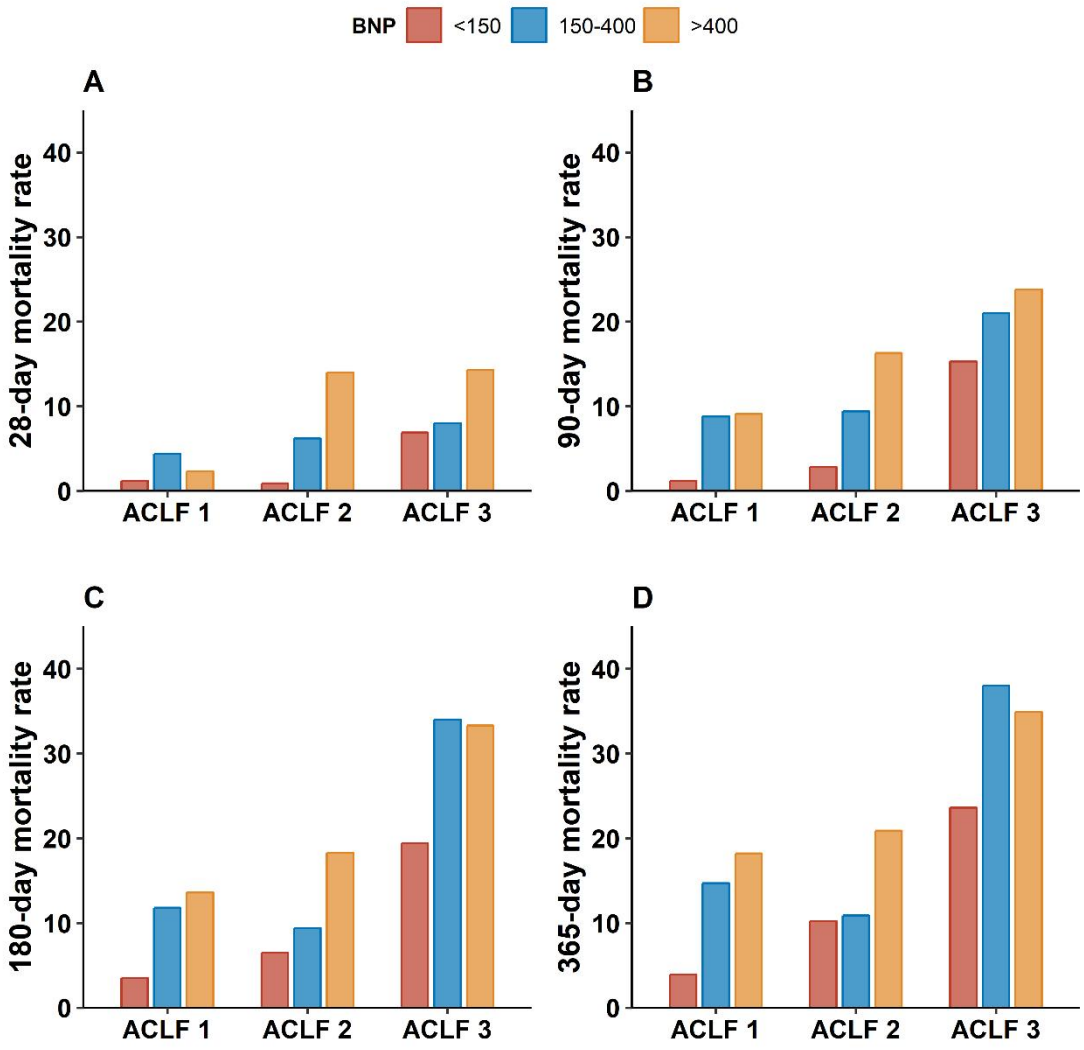


**Fig. 2. Within each grade of Acute-on-Chronic Liver Disease, higher B-type natriuretic peptide level was associated with higher level of mortality at 28, 90, 180, and 365 days after liver transplantation.**

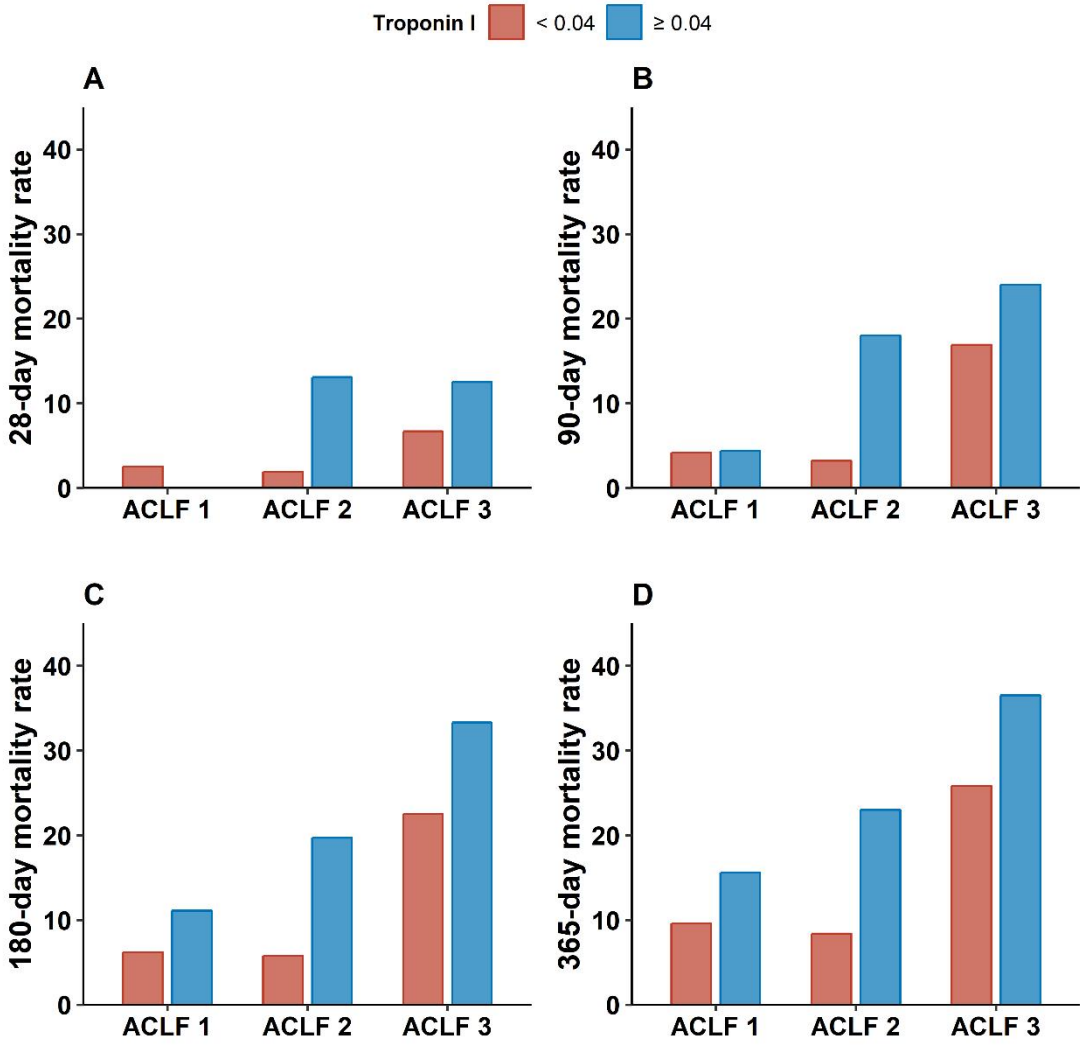


**Fig. 3. Within each grade of Acute-on-Chronic Liver Disease, higher Troponin I level was associated with higher level of mortality at 28, 90, 180, and 365 days after liver transplantation.**

VIMP obtained after random survival forest analysis, showed that BNP on a logarithmic scale and TnI on a logarithmic scale had high relative importance (One of the highest top 5 of all covariates) with mortality at all main time points. (Fig. 4-7)

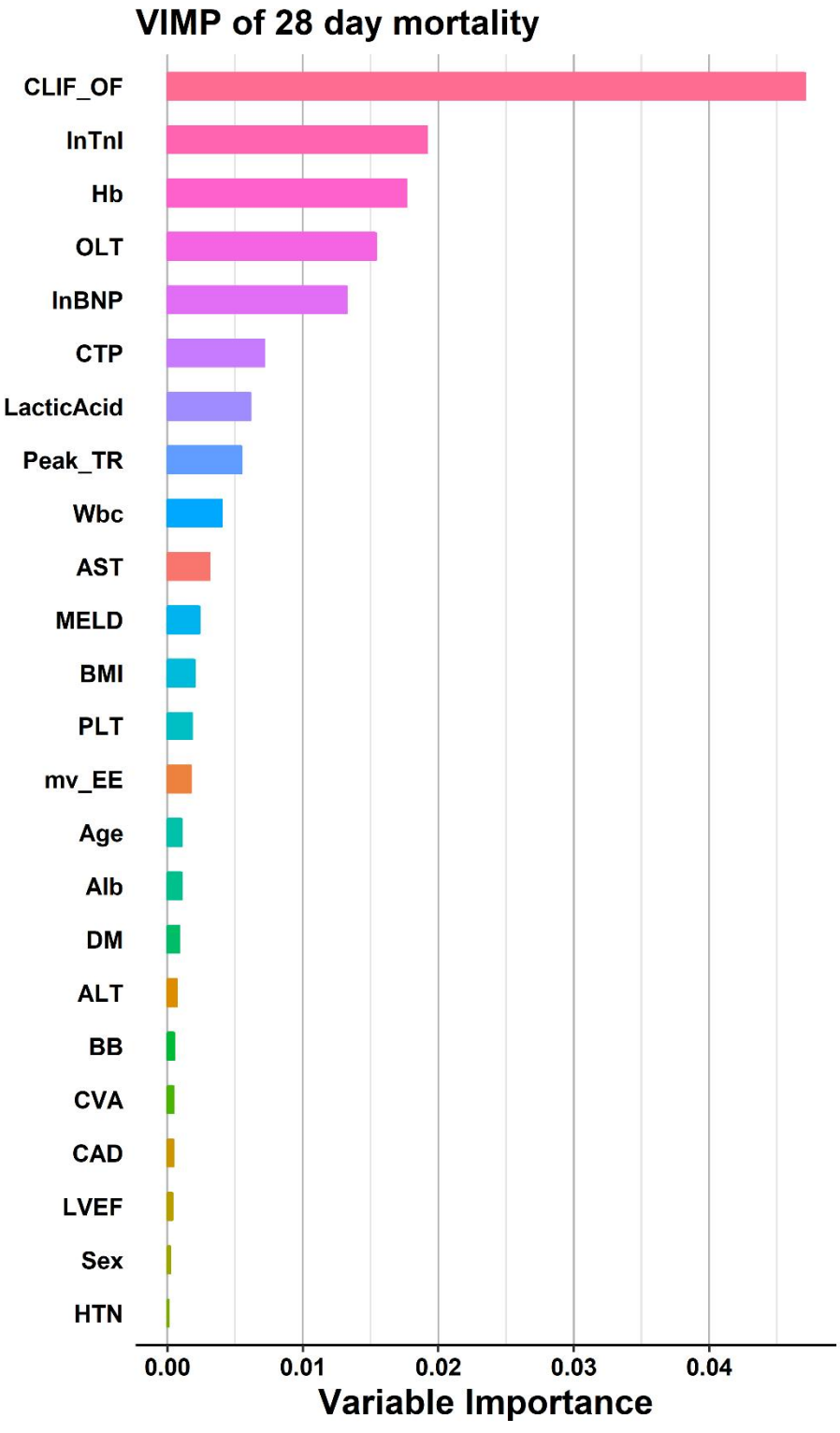


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**Fig. 4. Variable importance of predicting 28-day mortality.**

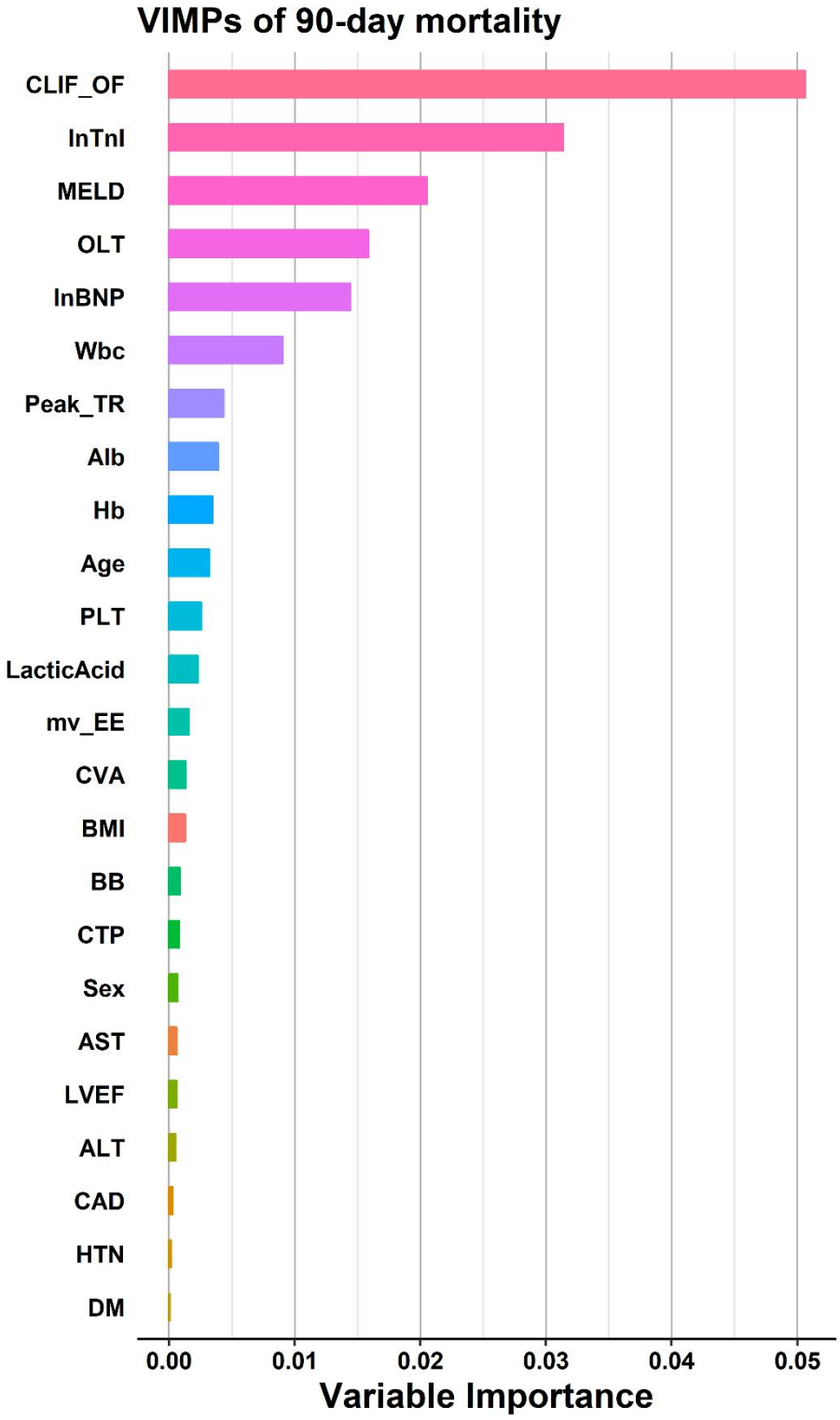



**Fig. 5. Variable importance of predicting 90-day mortality.**

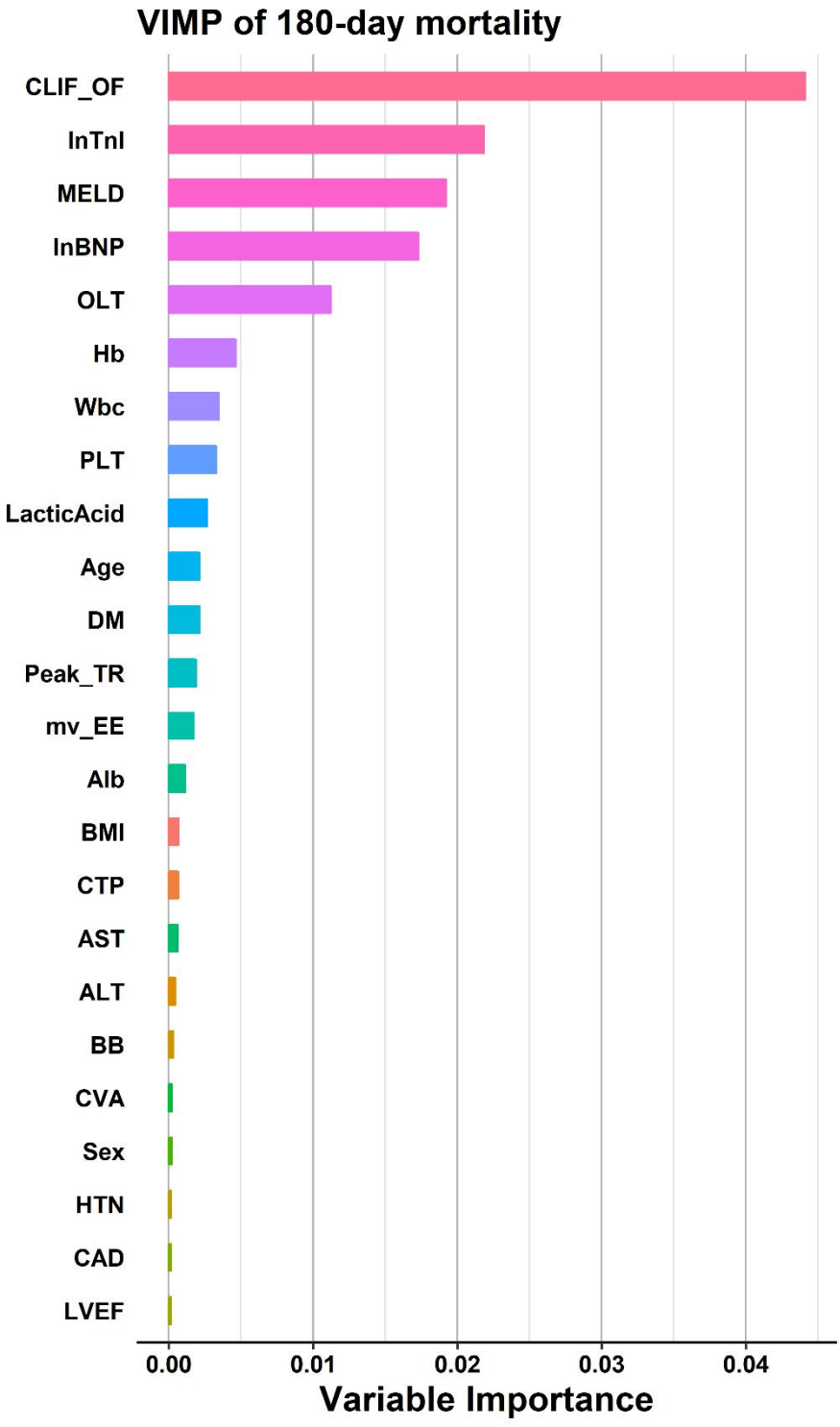



**Fig. 6. Variable importance of predicting 180-day mortality.**

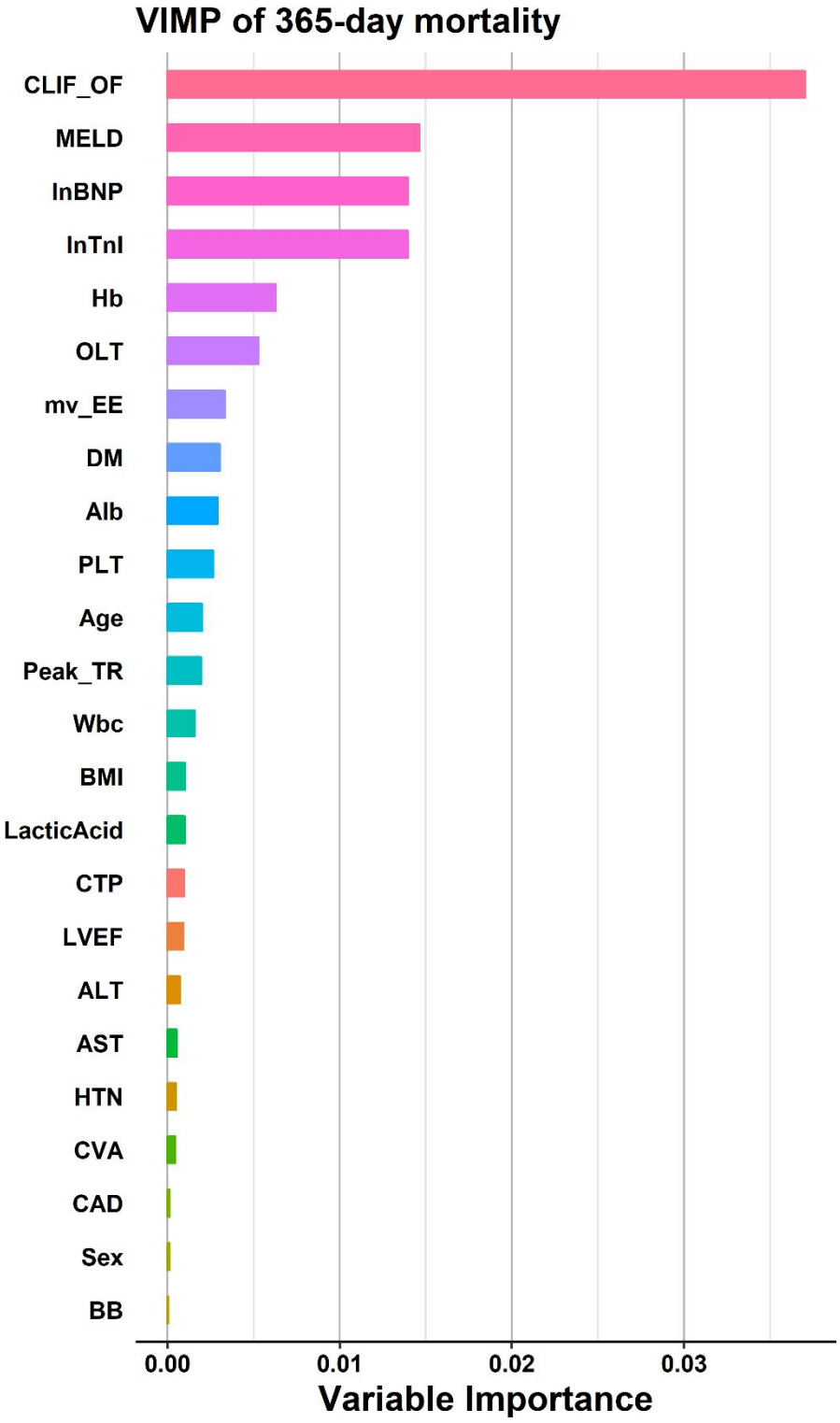



**Fig. 7. Variable importance of predicting 365-day mortality.**

In patients with ACLF, preoperative BNP and TnI were independently associated with 28, 90, and 180-day mortality, and higher 365-day mortality, after adjusting for significant variables in univariate analysis, which were age, sex, CLIF-C ACLF score, hypertension, left ventricular ejection fraction and deceased-donor liver transplantation in patients with ACLF (Table 2). To obtain robustness for the predictive value of BNP and TnI, we performed automated backward variable selection with respect to the Cox-proportional hazards model and computed the relative selection frequency for 1000 bootstrap resamples. Relative frequency of BNP and TnI were > 500 for 28, 90, 180 and 365-day mortality, which clearly shows the strong relationship between BNP and TnI with post-LT mortality (Table 3).

**Table 2.** Hazard ratios of mortality according to b-type natriuretic peptide and troponin I levels in patients with acute on chronic liver failure

| | Crude | | Multivariable adjusted | |
|---|---|---|---|---|
| | **HR [95% CI]** | **P value** | **HR [95% CI]** | **P value** |
| **B-type natriuretic peptide** | | | | |
| 28-day | 1.52 [1.18–1.96] | 0.001 | 1.34 [1.03-1.74] | 0.027 |
| 90-day | 1.44 [1.19-1.73] | <0.001 | 1.26 [1.04-1.53] | 0.018 |
| 180-day | 1.39 [1.19-1.63] | <0.001 | 1.23 [1.05-1.45] | 0.010 |
| 365-day | 1.29 [1.12-1.48] | <0.001 | 1.14 [0.98 -1.32] | 0.088 |
| **Troponin I** | | | | |
| 28-day | 1.42 [1.20-1.68] | <0.001 | 1.24 [1.02-1.49] | 0.028 |
| 90-day | 1.40 [1.24-1.58] | <0.001 | 1.18 [1.01-1.38] | 0.033 |
| 180-day | 1.37 [1.23-1.53] | <0.001 | 1.17 [1.03-1.33] | 0.018 |
| 365-day | 1.30 [1.17-1.43] | <0.001 | 1.12 [0.99-1.26] | 0.064 |

*Multivariate analysis adjusted for age, sex, Chronic Liver Failure Consortium Acute-on-Chronic liver failure score, hypertension, left ventricular ejection fraction and deceased-donor liver transplantation.

HR, hazard ratio; CI, confidence interval

**Table 3**. Relative Selection Frequency Based on 1000 Bootstrap resampling of multivariable cox-proportional regression analysis

| **Variable** | **Troponin I** | **Age** | **Sex** | **CLIF-C ACLF score** | **Hypertension** | **LVEF** | **DDLT** |
|---|---|---|---|---|---|---|---|
| 28-day mortality | 663 | 491 | 185 | 824 | 989 | 524 | 544 |
| 90-day mortality | 665 | 621 | 383 | 991 | 665 | 762 | 575 |
| 180-day mortality | 743 | 493 | 304 | 993 | 512 | 710 | 552 |
| 365-day mortality | 656 | 617 | 194 | 977 | 667 | 627 | 653 |
| **Variable** | **B-type natriuretic peptide** | **Age** | **Sex** | **CLIF-C ACLF score** | **Hypertension** | **LVEF** | **DDLT** |
| 28-day mortality | 720 | 462 | 192 | 924 | 970 | 481 | 521 |
| 90-day mortality | 799 | 656 | 404 | 998 | 357 | 723 | 563 |
| 180-day mortality | 839 | 477 | 261 | 1000 | 438 | 686 | 561 |
| 365-day mortality | 675 | 602 | 178 | 991 | 566 | 650 | 672 |

CLIF-C ACLF, Chronic Liver Failure Consortium Acute-on-Chronic liver failure score; LVEF, left ventricular ejection fraction; DDLT, deceased-donor liver transplantation

Restricted cubic spline analysis demonstrating the relationship between mortality risk with BNP and TnI on a continuous scale are shown in Fig. 8 (for BNP) and 9 (for TnI), which demonstrates the dose-dependent relationship between the cardiac markers and 28, 90, 180 and 365-day mortality. Furthermore, impact of combination of BNP and TnI on mortality is illustrated in a Kaplan-Meier plot (Fig. 10), which shows that increase of both BNP and TnI has higher association with mortality.

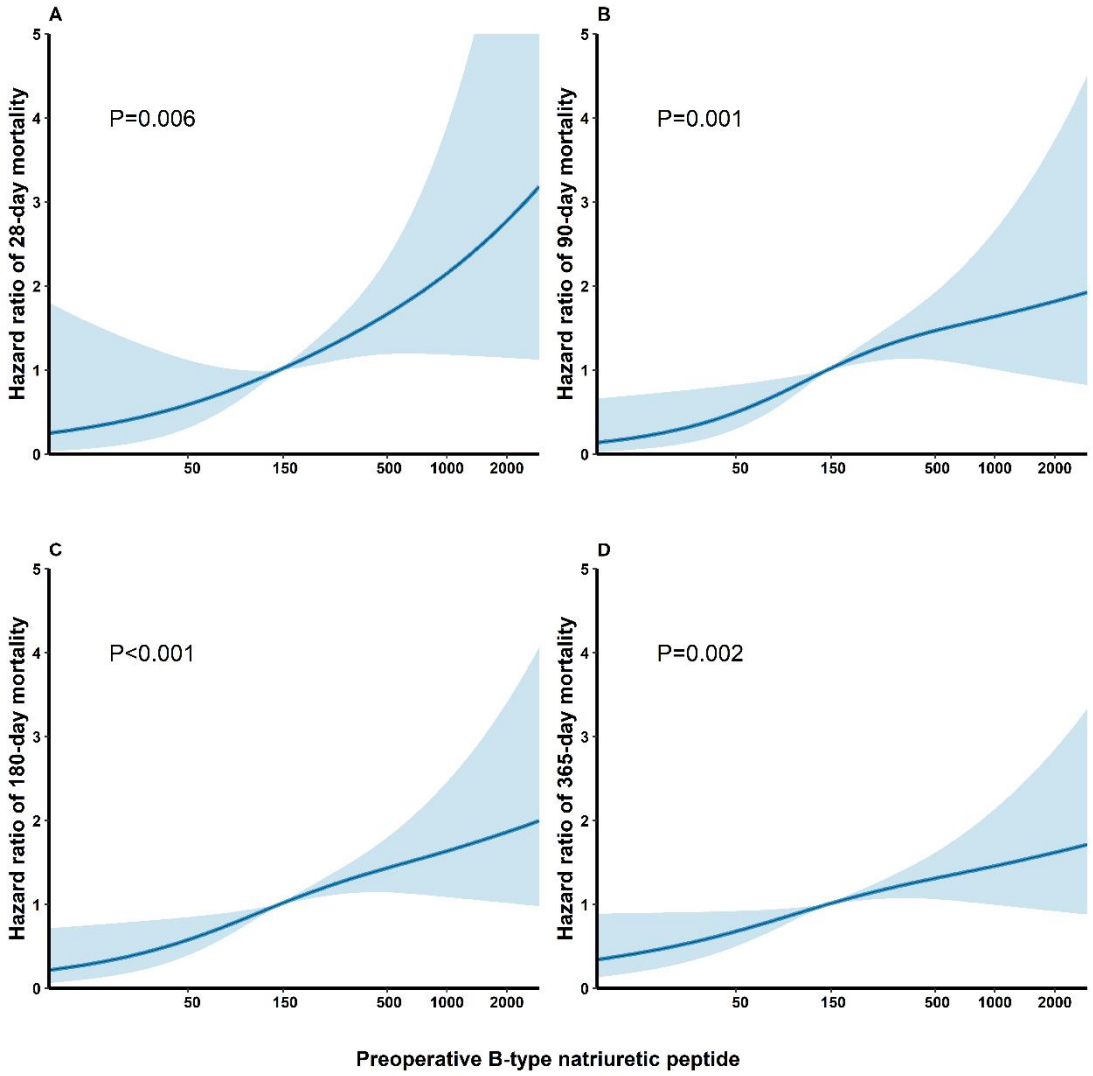



**Fig. 8. Restricted cubic spline showing dose-dependent relationship with b-type natriuretic peptide with (A) 28, (B) 90, (C), 180, (D) 365-day mortality.**

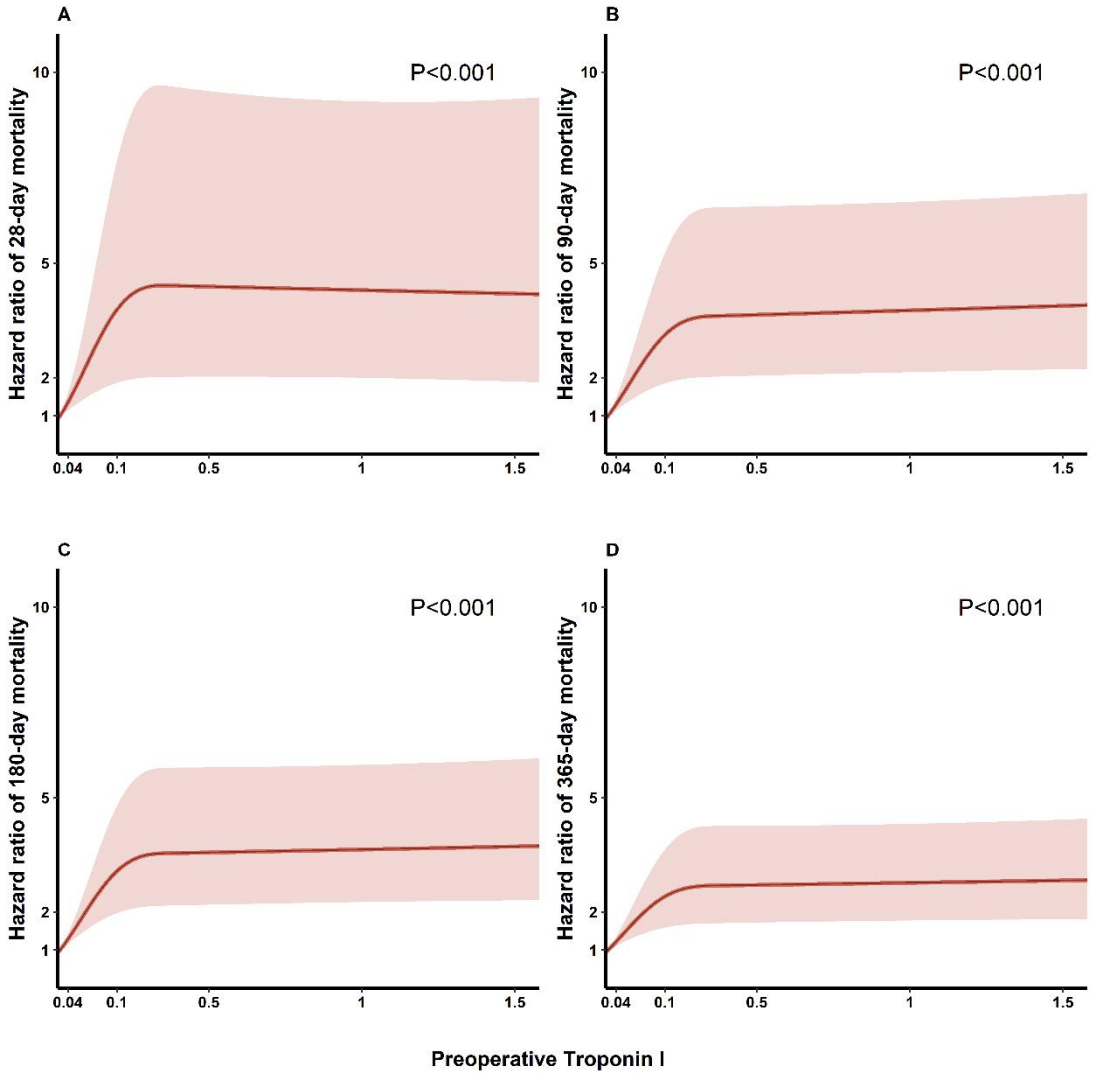



**Fig. 9. Restricted cubic spline showing dose-dependent relationship with b-type natriuretic peptide with (A) 28, (B) 90, (C), 180, (D) 36- day mortality**

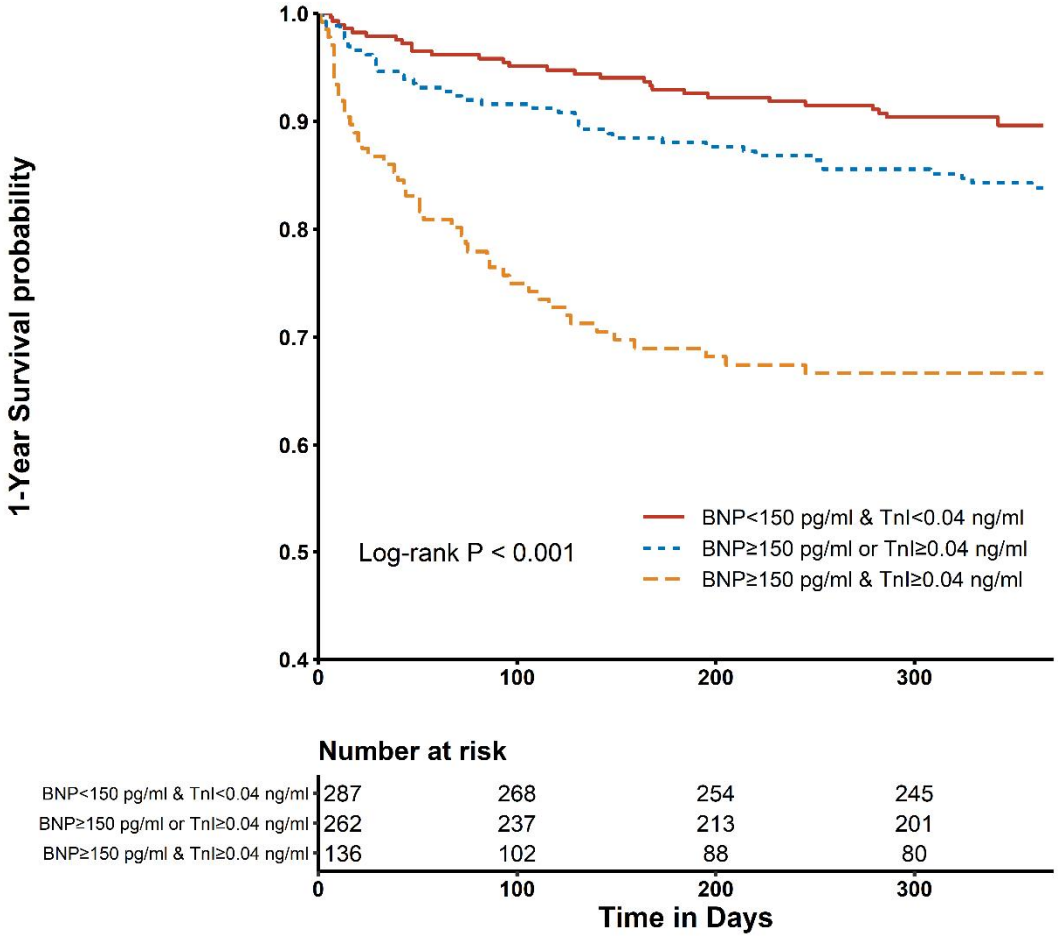



**Fig. 10. Kaplan-Meier plot showing higher cumulative mortality with higher level of troponin I and B-type natriuretic peptide**

*Part2: Development of CLIF-C CARDIAC score*

Since BNP and TnI levels were independently associated with post-LT mortality, we aimed to assess whether integrating BNP and TnI improves accuracy of the CLIF-C ACLF score in evaluation of prognosis after LT. We incorporated BNP and TnI in the calculation of CLIF-C ACLF score, based on the results obtained from the multivariable proportional hazards model with 28-day mortality as outcome. The modified CLIF-C ACLF score (CLIF-C CARDIAC score) was developed by following formula;

$$\textit{CLIF-C CARDIAC score} = 10*[0.03*\textit{CLIF-C OFs} + 0.04*\textit{Age} + 0.63*\ln(\textit{WBC count}) - 2] + 5.14*\ln(\textit{BNP}) + 3.05*\ln(\textit{TnI})$$

$$= \textit{CLIF-C ACLFs} + 5.14*\ln(\textit{BNP}) + 3.05*\ln(\textit{TnI})$$

Range of CLIF-C CARDIAC score (20.9-110.0; median 59.7) was slightly broader compared to CLIF-C ACLF scores (21.1-81.3; median 46.3).

Probability of death (*P*) at a given time "t" can be estimated by the formula below;

$$P = 1 - e^{[-CI(t) * \exp(\beta(t) * \text{CLIF-C Cardiac})]}$$

CI (t) refers to the cumulated baseline hazard and β(t) is the beta coefficient estimated by the modified model fitted for time "t". At each main time points, CI(28) = 0.0055, β(28) = 0.0504; CI(90) = 0.0108, β(90) = 0.0502; CI(180) = 0.0159, β(180) = 0.0471; CI(365) = 0.0204, β(365) = 0.0374, respectively. Predicted and observed probabilities of death at 28, 90, 180, 365-days after LT across the quintiles of the CLIF-C CARDIAC scores were similar, which showed good agreement (Fig. 11). Calibration of the CLIF-C CARDIACs assessed by Hosmer-Lemeshow Goodness-of-fit test for each study time period showed that proposed score was adequately fitted, which are as follows, 28-day: $\chi^2 = 2.749$, p=0.601; 90-day: $\chi^2 = 4.429$, p=0.351; 180-day: $\chi^2 = 3.328$, p=0.504; 365-day: $\chi^2 = 3.033$, p=0.552. (Fig. 12)

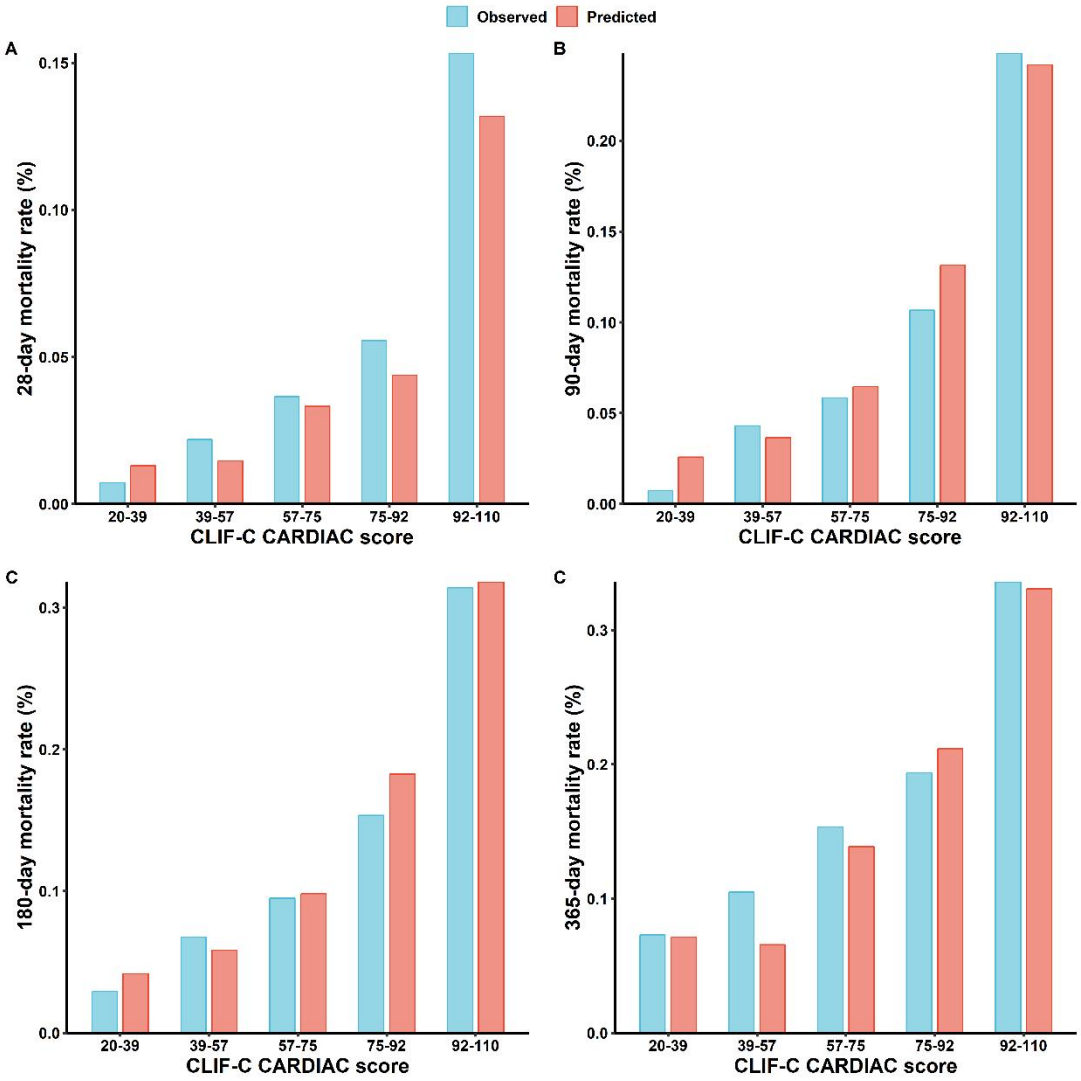



**Fig. 11. Observed (blue) vs. predicted (red) mortality at (A) 28-, (B) 90-, (C) 180-, (D) 365-days after liver transplantation according to the quintiles of the CLIF-C CARDIAC score in patients with ACLF.** The mortality probability predicted using CLIF-C CARDIAC score were similar to those observed, indicating a good performance of the score throughout the whole range of CLIF-C CARDIAC scores across all main time points.

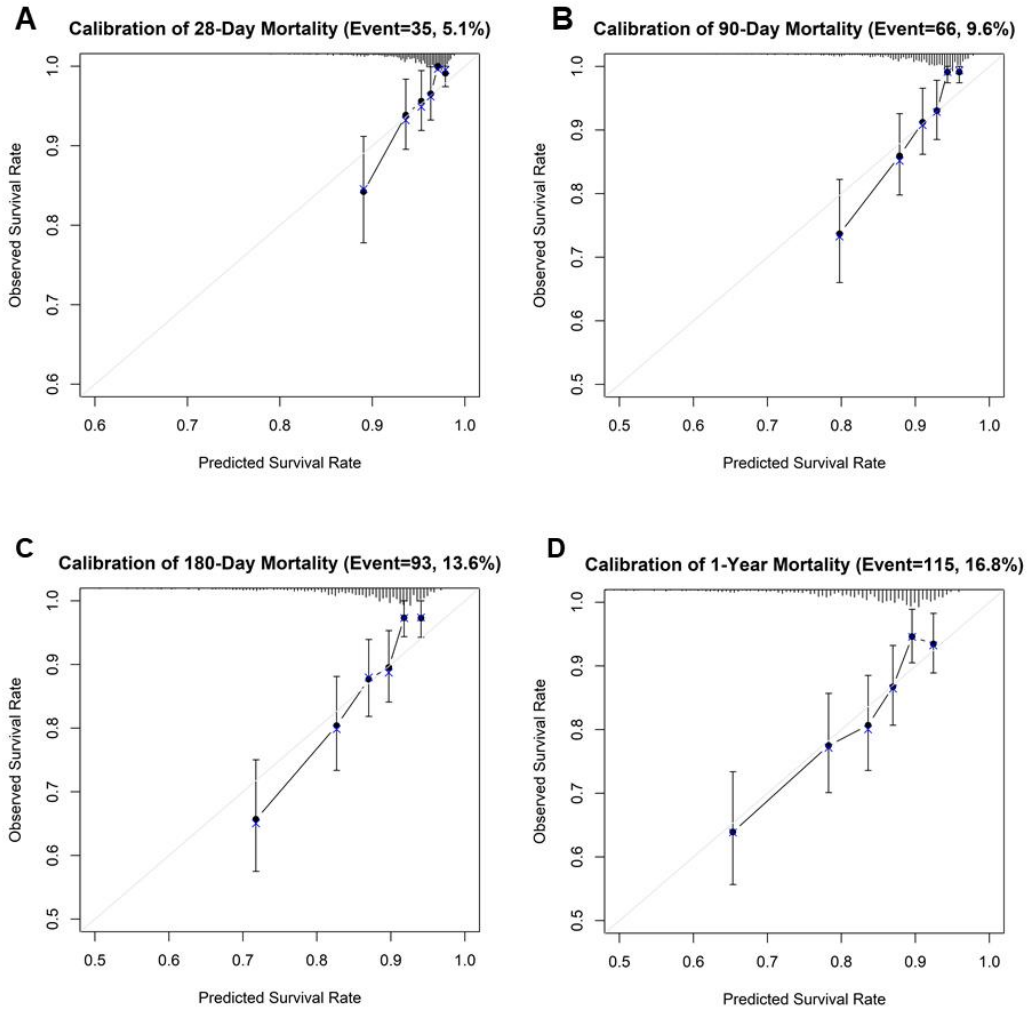



**Fig. 12. Calibration plot of CLIF-C CARDIAC score for predicting mortality at (A) 28-, (B) 90-, (C) 180-, (D) 365-days after liver transplantation**

C-index was evaluated to compare the discrimination ability of CLIF-C CARDIACs (0.76, 0.758, 0.737, and 0.696) with CLIF-C ACLFs (0.728 [p<0.001], 0.736 [p=0.033], 0.716 [p=0.033], and 0.677 [p<0.001]), MELDs (0.600 [p=0.04],0.614 [p<0.001],0.618 [p<0.001] and 0.574 [p=0.007] ) and CPs (0.593 [p<0.001], 0.578 [p<0.001], 0.553 [p<0.001], and 0.534 [p<0.001]) for 28, 90, 180, 365-day post-LT mortality, respectively, which showed that discriminability of CLIF-C CARDIACs was significantly higher compared to other prognostic scores at 28, 90, 180, and 365-days (Table 4). CLIF-C CARDIACs improved mortality prediction by up to 12.0 %, 40.1%, and 42.6%, as compared to CLIF-C ACLFs, MELDs, and CPs, respectively. (Fig. 13).

**Table 4. Predictive discrimination ability of the CLIF-C CARDIAC score as compared with the CLIF-C ACLF, MELD and Child-Pugh score in patients with Acute-on-Chronic Liver Failure**

| | CLIF-C CARDIAC C-index (95% CI) | CLIF-C ACLF score C-index (95% CI) | MELD score C-index (95% CI) | Child-Pugh score C-index (95% CI) |
|---|---|---|---|---|
| 28-day mortality | 0.760 (0.664-0.856) | 0.728 (0.632-0.824) | 0.600 (0.504-0.696) | 0.593 (0.499-0.688) |
| *p* value* | | <0.001 | 0.040 | <0.001 |
| 90-day mortality | 0.758 (0.688-0.828) | 0.736 (0.667-0.806) | 0.614 (0.545-0.684) | 0.578 (0.509-0.647) |
| *p* value* | | 0.033 | <0.001 | <0.001 |
| 180-day mortality | 0.737 (0.678-0.796) | 0.716 (0.658-0.775) | 0.618 (0.559-0.676) | 0.553 (0.495-0.611) |
| *p* value* | | 0.033 | <0.001 | <0.001 |
| 365-day mortality | 0.696 (0.643-0.749) | 0.677 (0.624-0.730) | 0.574 (0.541-0.647) | 0.534 (0.482-0.587) |
| *p* value* | | <0.001 | 0.007 | <0.001 |

*p values *vs.* CLIF-C CARDIAC from the Integrated Discrimination Improvement statistics test.

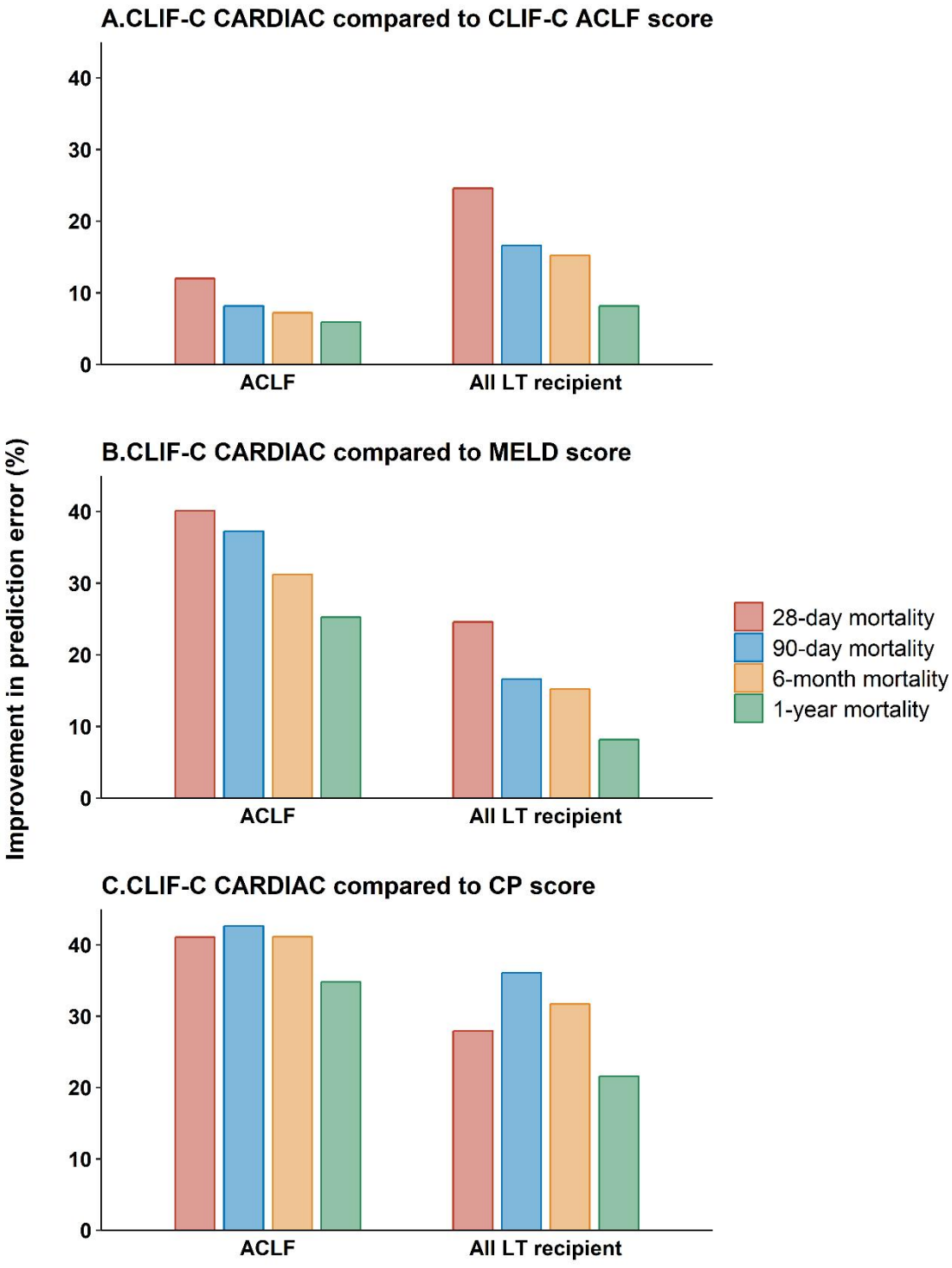
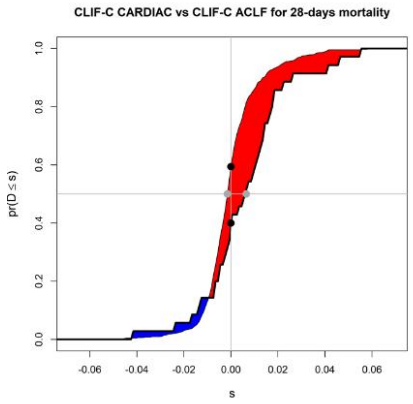
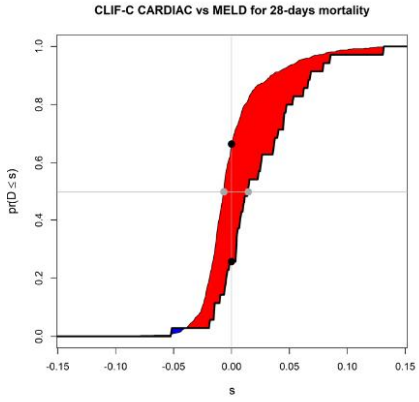



**Fig. 13. Improvement in prediction error by CLIF-C CARDIAC score compared to CLIF-C ACLF, MELD, Child-Pugh score at the main study points**

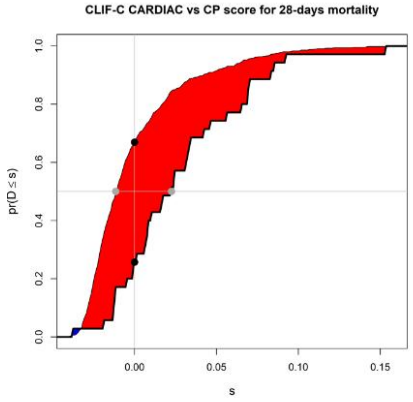
We further explored the added value of TnI and BNP to CLIF-C ACLF score by comparing the predictive value with CLIF-C ACLF, MELD, and CP scores using NRI and IDI.[23] Regarding 28-day mortality as outcome, estimates of IDI of CLIF-C CARDICs (model integrating CLIF-C ACLF score, logarithm of TnI and logarithm of BNP) were significantly higher compared to CLIF-C ACLFs, MELDs, and CPs (all $P < 0.05$). NRI (>0) were 0.194 (95% CI 0.069–0.419; $P = 0.007$), 0.407 (0.219–0.567; $P < 0.001$) and (0.219–0.567; $P < 0.001$) for CLIF-C ACLFs, MELDs, and CPs, respectively, suggesting substantial increased predictability of CLIF-C CARDIAC score. These are shown graphically in Fig. 14, in which the shaded area and the span of NRI (>0) demonstrates the clear added value (paired difference between risk scores) of integrating TnI and BNP to develop CLIF-C CARDIACs compared to CLIF-C ACLFs (Fig. 14A), MELD score (Fig. 14B), and CP score (Fig. 14C). The predictive ability for CLIF-C CARDIACs were significantly higher than for the CLIF-C ACLF, MELD and CP score at each main time points. NRI showed that the CLIF-C CARDIAC score improved the 90-, 180-, and 365-day post-LT mortality predictions by about 18% to 42% as compared to the CLIF-C ACLF, MELD and CP score (Fig. 15-17).




| | Improvement | P value |
|---|---|---|
| IDI | 0.025 (0.009-0.049) | <0.001 |
| NRI (>0) | 0.407 (0.219-0.567) | <0.001 |
| Median risk score | 0.021 (0.009-0.051) | <0.001 |




| | Improvement | P value |
|---|---|---|
| IDI | 0.007 (0.001-0.022) | 0.040 |
| NRI (>0) | 0.194 (0.048-0.403) | 0.013 |
| Median risk score | 0.008 (0.000-0.023) | 0.047 |



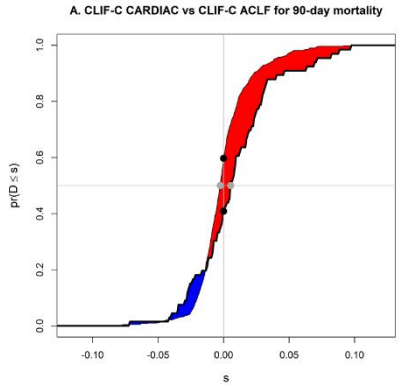

| | Improvement | P value |
|---|---|---|
| IDI | 0.030 (0.013-0.059) | <0.001 |
| NRI (>0) | 0.412 (0.211-0.550) | <0.001 |
| Median risk score | 0.033 (0.014-0.063) | <0.001 |

**Fig. 14. The additional value of integrating troponin I and b-type natriuretic peptide to CLIF-C ACLF score as assessed by the paired difference of risk scores.**

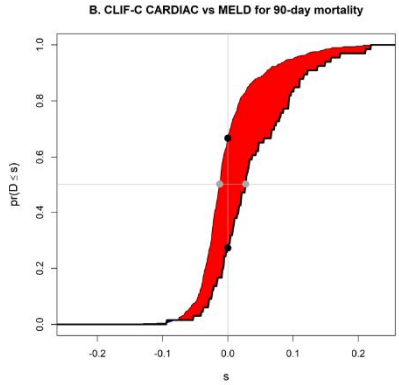
Figure shows the empirical distribution function of the paired difference (^D) between the risk scores (on the probability scale) estimated at Time = 28 days

between CLIF-C CARDIAC score and (A) CLIF-C ACLF score, (B) Model for End-stage Liver Disease score, and (C) Child-Pugh score. The empirical distribution function of the change in estimated risk scores for non-survivors (thick solid line) and survivors (thin solid line) was assessed. Additional discrimination value of CLIF-C CARDIAC score is proportional to the area of the red-color shaded graphic while blue-color shaded graphics indicates negative discrimination value. The distances between the two black dots and between the two grey dots represent the continuous NRI and median improvement, respectively.

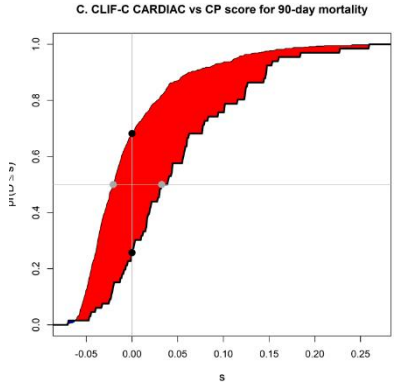
y-axis, cumulative probability; x-axis, s; difference between two model risk scores.




| | Improvement | P value |
|---|---|---|
| IDI | 0.009 (0.001-0.033) | 0.033 |
| NRI (>0) | 0.188 (0.051-0.336) | 0.020 |
| Median risk score | 0.008 (0.001-0.026) | 0.007 |



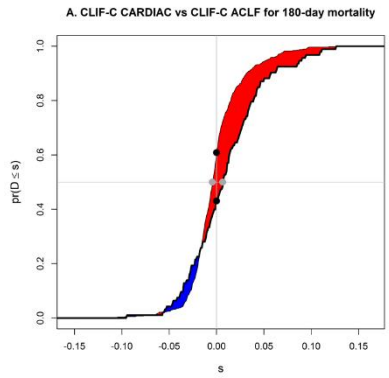

| | Improvement | P value |
|---|---|---|
| IDI | 0.044 (0.023-0.080) | <0.001 |
| NRI (>0) | 0.395 (0.255-0.529) | <0.001 |
| Median risk score | 0.041 (0.023-0.061) | <0.001 |



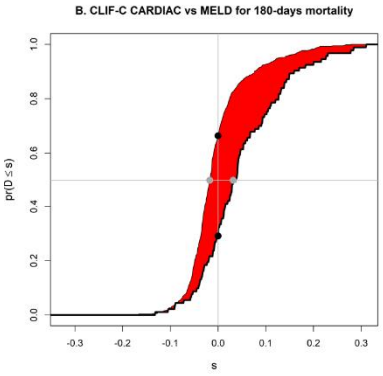

| | Improvement | P value |
|---|---|---|
| IDI | 0.055 (0.028-0.093) | <0.001 |
| NRI (>0) | 0.424 (0.273-0.532) | <0.001 |
| Median risk score | 0.053 (0.030-0.098) | <0.001 |

**Fig. 15. The additional value of integrating troponin I and b-type natriuretic peptide to CLIF-C ACLF score as assessed by the paired difference of risk scores.**

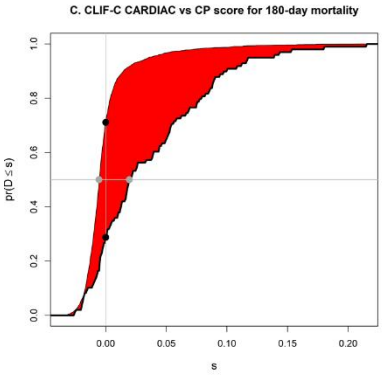
Figure shows the empirical distribution function of the paired difference (^D) between the risk scores (on the probability scale) estimated at Time = 90 days between CLIF-C CARDIAC score and (A) CLIF-C ACLF score, (B) Model for End-stage Liver Disease score, and (C) Child-Pugh score.




| | Improvement | P value |
|---|---|---|
| IDI | 0.011 (0.001-0.039) | 0.033 |
| NRI (>0) | 0.178 (0.047-0.284) | 0.020 |
| Median risk score | 0.011 (0.001-0.033) | 0.007 |



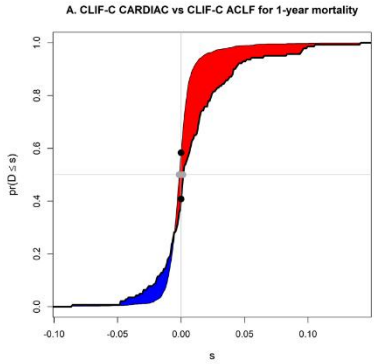

| | Improvement | P value |
|---|---|---|
| IDI | 0.055 (0.027-0.093) | <0.001 |
| NRI (>0) | 0.373 (0.222-0.480) | <0.001 |
| Median risk score | 0.055 (0.029-0.46) | <0.001 |



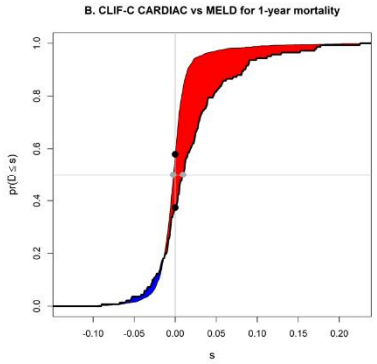

| | Improvement | P value |
|---|---|---|
| IDI | 0.038 (0.023-0.067) | <0.001 |
| NRI (>0) | 0.424 (0.261-0.553) | <0.001 |
| Median risk score | 0.024 (0.013-0.055) | <0.001 |

**Fig. 16. The additional value of integrating troponin I and b-type natriuretic peptide to CLIF-C ACLF score as assessed by the paired difference of risk scores.**

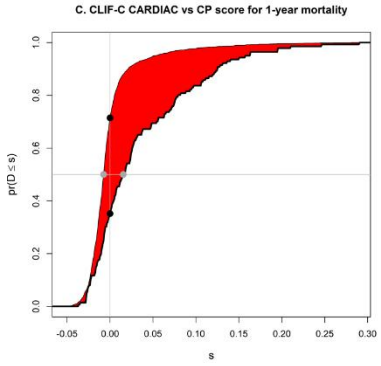
Figure shows the empirical distribution function of the paired difference (^D) between the risk scores (on the probability scale) estimated at Time = 180 days between CLIF-C CARDIAC score and (A) CLIF-C ACLF score, (B) Model for End-stage Liver Disease score, and (C) Child-Pugh score.




| | Improvement | P value |
|---|---|---|
| IDI | 0.009 (0.001-0.027) | <0.001 |
| NRI (>0) | 0.176 (0.068-0.266) | <0.001 |
| Median risk score | 0.003 (0.001-0.012) | <0.001 |




| | Improvement | P value |
|---|---|---|
| IDI | 0.020 (0.006-0.044) | 0.007 |
| NRI (>0) | 0.206 (0.049-0.325) | 0.020 |
| Median risk score | 0.012 (0.004-0.025) | 0.007 |




| | Improvement | P value |
|---|---|---|
| IDI | 0.038 (0.022-0.065) | <0.001 |
| NRI (>0) | 0.363 (0.194-0.456) | <0.001 |
| Median risk score | 0.025 (0.009-0.039) | <0.001 |

**Fig. 17. The additional value of integrating troponin I and b-type natriuretic peptide to CLIF-C ACLF score as assessed by the paired difference of risk scores.**

Figure shows the empirical distribution function of the paired difference (^D) between the risk scores (on the probability scale) estimated at Time = 365 days between CLIF-C CARDIAC score and (A) CLIF-C ACLF score, (B) Model for End-stage Liver Disease score, and (C) Child-Pugh score.

Fig. 18 shows the time-dependent AUC between the scores, which clearly shows that predictive ability of CLIF-C CARDIAC score is statistical significantly higher than MELD score ($P < 0.001$ for all time period) and CP score ($P < 0.001$ for all time period) and showed higher AUROC compared to CLIF-C ACLF score, although statistical significance was not reached in this difference ($P = 0.159$, 0.193, 0.163 and 0.123 for 28, 90, 180, and 365-day).

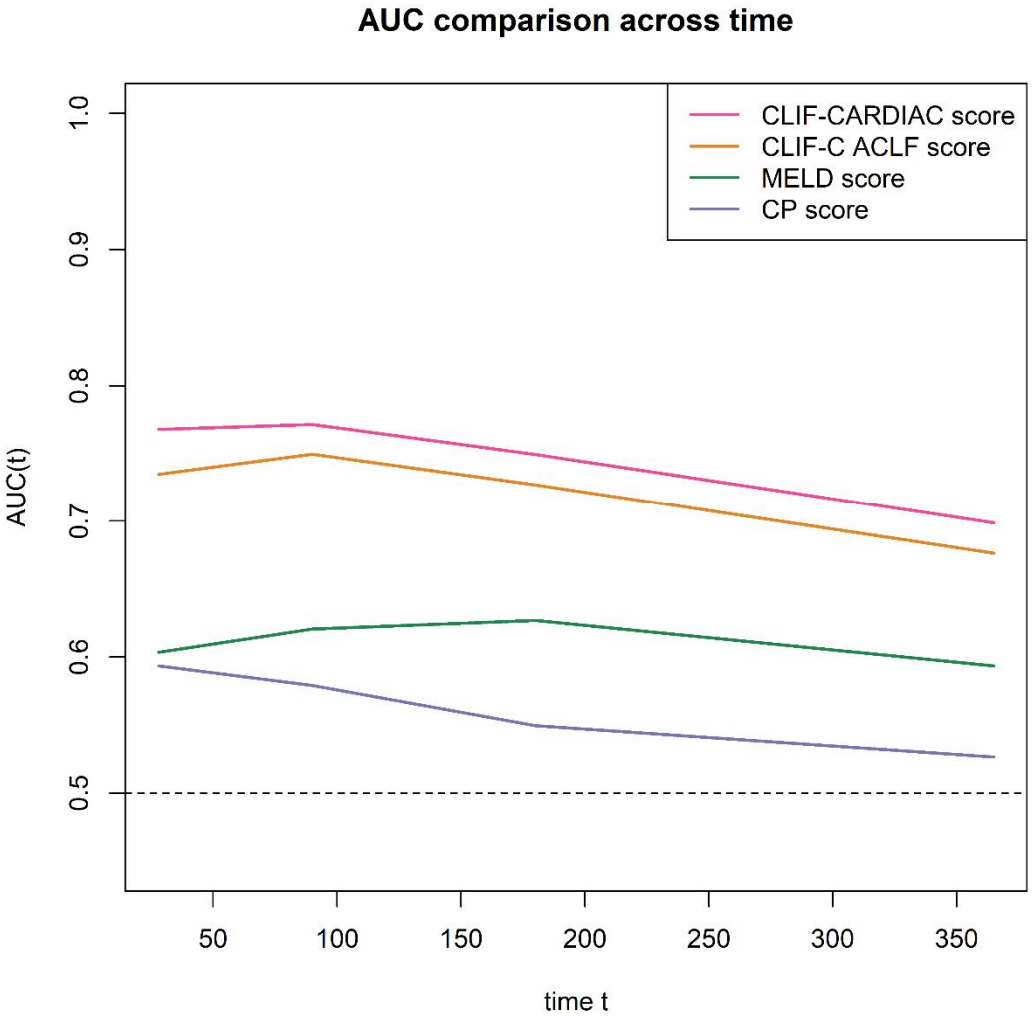



**Fig. 18. Time-dependent area under the curve of CLIF-C CARDIAC, CLIF-C ACLF, MELD and CP score to predict post-LT mortality in patients with ACLF.**

*Part3. Applications of CLIF-C CARDIAC score to overall LT recipients*

Since cardiovascular complication is the leading cause of early mortality after LT, we further evaluated if CLIF-C CARDIACs is better prognostic score not only in ACLF but also in overall LT recipients, compared to other conventional scores. Basic demographics of overall LT recipients are shown in Table 5. Median age was 54 (49-59) years and 74.8% were male. The median MELD score was 14 (9-22). The number of non-survivors at 28, 90, 180, and 365 days after LT was 43 (1.5%), 89 (3.1%), 131 (4.6%) and 189 (6.6%), respectively.

**Table 5. Demographics of enrolled liver transplant recipients**

| | Total (N=2845) |
|---|---|
| **Demographics** | |
| Age (yr) | 54 (49-59) |
| Sex (male) | 2127 (74.8) |
| Body mass index (kg/m$^2$) | 24.2 (22.0-26.6) |
| MELD score | 14 (9-22) |
| CP score | 8 (6-10) |
| Cardiovascular disease | 299 (10.5) |
| Diabetes mellitus | 690 (24.3) |
| Hypertension | 507 (17.8) |
| Beta blocker | 945 (33.2) |
| Donor type (living /deceased) | 406 (14.3)/2439 (85.7) |
| **Cardiac related parameter** | |
| B-type natriuretic peptide (pg/ml) | 51 (23-121) |
| Troponin I (ng/ml) | 0.006 (0.006-0.010) |
| LVEF (%) | 64 (62-67) |
| E/A ratio (n=2769) | 1.12 (0.88-1.36) |

Table 5. Continued

| | **Total (N=2845)** |
|---|---|
| **Etiology of liver cirrhosis** | |
| Viral cirrhosis | 1850 (65.0) |
| Alcoholic cirrhosis | 671 (23.6) |
| Biliary disease | 109 (3.8) |
| Other disease | 5 (0.2) |
| **Data used to compute CLIF-C ACLF score** | |
| Serum bilirubin (mg/dl) | 2.00 (1.00-6.60) |
| Serum creatinine (mg/dl) | 0.79 (0.64-1.00) |
| Renal replacement therapy | 219 (7.7) |
| HE grade I-II | 250 (8.8) |
| HE grade III-IV | 95 (3.3) |
| PT, INR | 1.41 (1.20-1.81) |
| Mean blood pressure (mmHg) | 81 (75-88) |
| Use of vasopressors | 147 (5.2) |
| Mechanical ventilation | 179 (6.3) |
| White-cell count ($x10^9$ cells/L) | 3.5 (2.4-5.1) |
| Serum sodium (mEq/L) | 139 (135-141) |

Table 5. Continued

| | **Total (N=2845)** |
|---|---|
| **Mortality rates** | |
| 28-Day mortality | 43 (1.5) |
| 90-Day mortality | 89 (3.1) |
| 6-Month mortality | 131 (4.6) |
| 1-Year mortality | 189 (6.6) |

Continuous variables are expressed as mean ± standard deviation or as median (interquartile range) and categorical variables as n (%).

MELD, model for end-stage liver disease; CP score. Child-Pugh score; LVEF, left ventricular ejection fraction; E/A, transmitral early and late diastolic velocity ratio; CLIF-C ACLF score, Chronic Liver Failure Consortium Acute on Chronic Liver Failure score; HE, hepatic encephalopathy; PT, prothrombin time; INR, international normalized ratio.

Calibration of the CLIF-C CARDIAC score in overall LT recipients by Hosmer-Lemeshow test showed good agreement between observed and predicted outcome, which are as follows, 28-day: $\chi^2$ = 1.9, p=0.7; 90-day: $\chi^2$ = 5.2, p=0.3; 180-day: $\chi^2$ = 5.7, p=0.2; 365-day: $\chi^2$ = 4.4, p=0.4.

Of note, CLIF-C CARDIACs showed a significantly better discrimination ability than those corresponding scores at all study time period in overall LT recipients. C-index of CLIF-C CARDIAC score was significantly higher compared to CLIF-C ACLF score, MELD score, and CP score at predicting 28-day and rest of the study time periods (Table 6, all P < 0.05). CLIF-C CARDIAC score consistently improved prediction error rates observed for the CLIF-C ACLF score, MELD score, CP score by 8.2 % - 36.2% at all time points (Fig. 13). After reclassifying the mortality risk according by CLIF-C CARDIAC score by NRI method, the ability to predict mortality was significantly improved by 16.6%-54.8% at all observed time period, compared with other scores (Supplementary Fig. 19-22). AUROC of CLIF-C CARDIAC score to predict 90, 180, 365-day mortality were significantly higher compared to CLIF-C ACLF score, MELD score and CP score, indicating higher predictive ability. (Table 7, Fig. 23)

**Table 6. Predictive discrimination ability of the CLIF-C CARDIAC score as compared with the CLIF-C ACLF, MELD and Child-Pugh score in overall LT recipients**

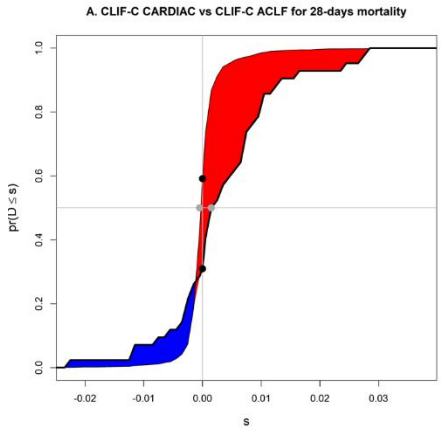
| | CLIF-C CARDIAC C-index (95% CI) | CLIF-C ACLF score C-index (95% CI) | MELD score C-index (95% CI) | Child-Pugh score C-index (95% CI) |
|---|---|---|---|---|
| 28-day mortality | 0.872 (0.785-0.958) | 0.83 (0.744-0.916) | 0.845 (0.759-0.931) | 0.822 (0.736-0.908) |
| *p* value* | | 0.013 | <0.001 | <0.001 |
| 90-day mortality | 0.854 (0.794-0.914) | 0.825 (0.765-0.885) | 0.816 (0.756-0.876) | 0.772 (0.712-0.831) |
| *p* value* | | 0.013 | <0.001 | <0.001 |
| 180-day mortality | 0.831 (0.782-0.881) | 0.801 (0.751-0.85) | 0.795 (0.746-0.844) | 0.753 (0.704-0.802) |
| *p* value* | | 0.007 | <0.001 | <0.001 |
| 365-day mortality | 0.756 (0.715-0.797) | 0.734 (0.693-0.775) | 0.722 (0.681-0.763) | 0.689 (0.648-0.73) |
| *p* value* | | 0.013 | <0.001 | <0.001 |

*p* value*, compared with CLIF-C CARDIAC score with the Integrated Discrimination Improvement statistics test.

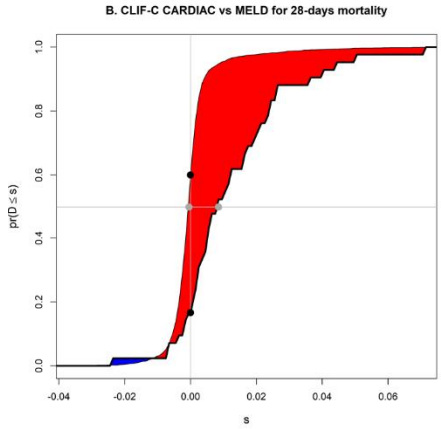
**Table 7. Time dependent Area under the Receiver Operating Characteristic curve of the CLIF-C CARDIAC score as compared with the CLIF-C ACLF, MELD and Child-Pugh score in overall LT recipients**

| | CLIF-C CARDIAC C-index (95% CI) | CLIF-C ACLF score C-index (95% CI) | MELD score C-index (95% CI) | Child-Pugh score C-index (95% CI) |
|---|---|---|---|---|
| 28-day mortality | 0.881 (0.825-0.938) | 0.850 (0.780-0.920) | 0.846 (0.801-0.890) | 0.823 (0.778-0.868) |
| *p* value* | | 0.0042 | 0.1577 | 0.0552 |
| 90-day mortality | 0.859 (0.817-0.902) | 0.830 (0.780-0.881) | 0.821 (0.776-0.865) | 0.775 (0.725-0.824) |
| *p* value* | | 0.0187 | 0.0076 | <0.001 |
| 180-day mortality | 0.837 (0.798-0.875) | 0.806 (0.761-0.851) | 0.801 (0.760-0.841) | 0.757 (0.716-0.797) |
| *p* value* | | 0.0104 | 0.0180 | <0.001 |
| 365-day mortality | 0.754 (0.713-0.795) | 0.733 (0.761-0.851) | 0.720 (0.679-0.762) | 0.686 (0.646-0.726) |
| *p* value* | | 0.0398 | 0.0213 | <0.001 |

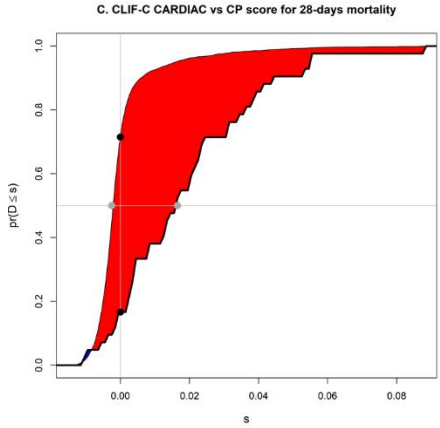
*p* value*, compared with CLIF-C CARDIAC.




| | Improvement | P value |
|---|---|---|
| IDI | 0.003 (0.000-0.012) | 0.013 |
| NRI (>0) | 0.282 (0.056-0.394) | 0.013 |
| Median risk score | 0.002 (0.000-0.009) | 0.033 |

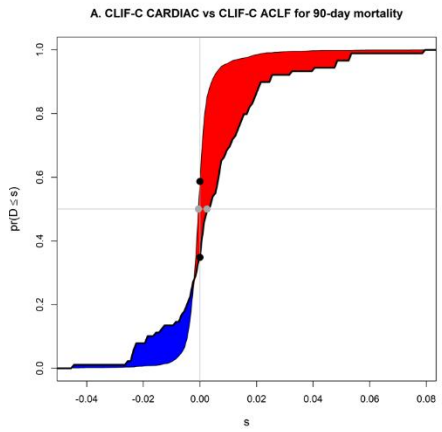



| | Improvement | P value |
|---|---|---|
| IDI | 0.013 (0.006-0.026) | <0.001 |
| NRI (>0) | 0.434 (0.207-0.546) | <0.001 |
| Median risk score | 0.009 (0.004-0.021) | <0.001 |

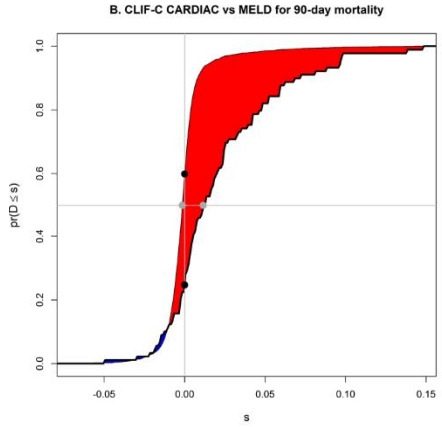



| | Improvement | P value |
|---|---|---|
| IDI | 0.019 (0.011-0.036) | <0.001 |
| NRI (>0) | 0.548 (0.373-0.678) | <0.001 |
| Median risk score | 0.018 (0.008-0.032) | <0.001 |

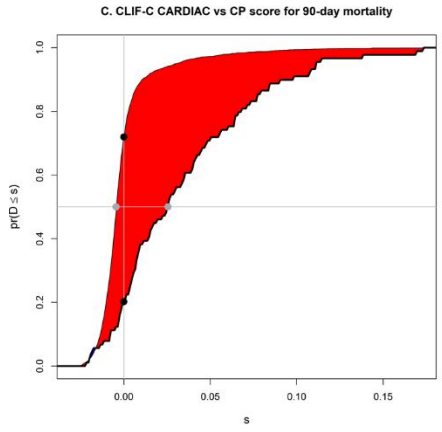
**Fig. 19. The additional value of integrating troponin I and b-type natriuretic peptide to CLIF-C CARDIAC score to predict 28-day mortality as assessed by the paired difference of risk scores, compared to CLIF-C ACLF, MELD and CP score in overall LT recipients.**




| | Improvement | P value |
|---|---|---|
| IDI | 0.006 (0.001-0.019) | 0.013 |
| NRI (>0) | 0.239 (0.075-0.340) | 0.013 |
| Median risk score | 0.004 (0.001-0.011) | 0.013 |

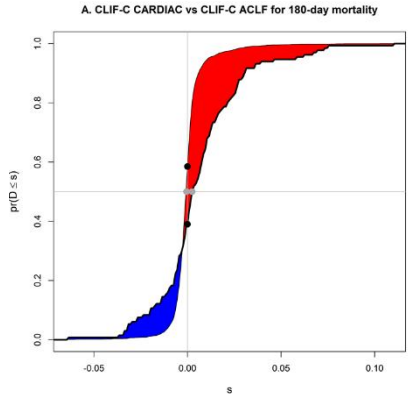



| | Improvement | P value |
|---|---|---|
| IDI | 0.023 (0.011-0.040) | <0.001 |
| NRI (>0) | 0.352 (0.187-0.484) | <0.001 |
| Median risk score | 0.014 (0.006-0.029) | <0.001 |

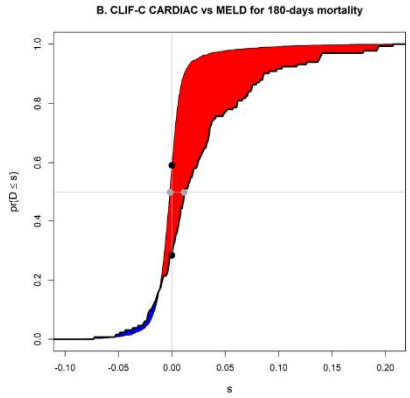



| | Improvement | P value |
|---|---|---|
| IDI | 0.036 (0.022-0.057) | <0.001 |
| NRI (>0) | 0.517 (0.373-0.611) | <0.001 |
| Median risk score | 0.029 (0.014-0.047) | <0.001 |

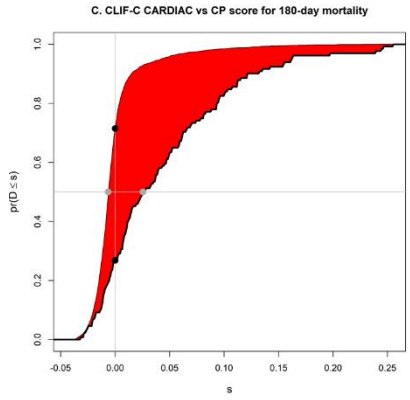
**Fig. 20. The additional value of integrating troponin I and b-type natriuretic peptide to CLIF-C CARDIAC score to predict 90-day mortality as assessed by the paired difference of risk scores, compared to CLIF-C ACLF, MELD and CP score in overall LT recipients.**




| | Improvement | P value |
|---|---|---|
| IDI | 0.007 (0.001-0.019) | 0.007 |
| NRI (>0) | 0.195 (0.016-0.286) | 0.033 |
| Median risk score | 0.003 (0.000-0.013) | 0.027 |

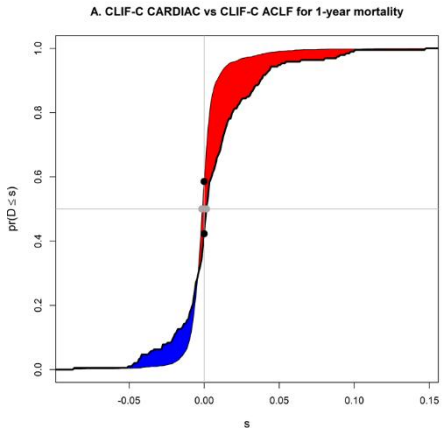



| | Improvement | P value |
|---|---|---|
| IDI | 0.028 (0.014-0.051) | <0.001 |
| NRI (>0) | 0.308 (0.127-0.414) | <0.001 |
| Median risk score | 0.014 (0.007-0.029) | <0.001 |

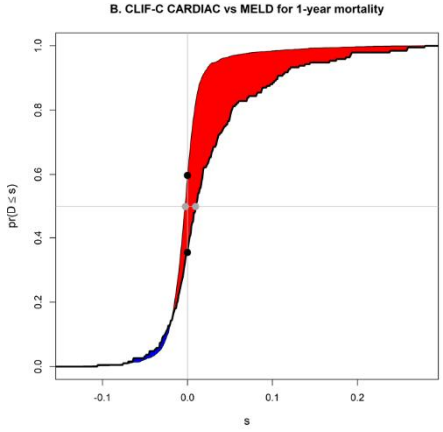



| | Improvement | P value |
|---|---|---|
| IDI | 0.047 (0.029-0.073) | <0.001 |
| NRI (>0) | 0.447 (0.331-0.549) | <0.001 |
| Median risk score | 0.032 (0.017-0.055) | <0.001 |

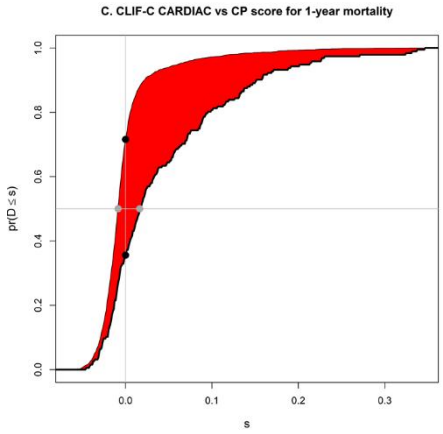
**Fig. 21. The additional value of integrating troponin I and b-type natriuretic peptide to CLIF-C CARDIAC score to predict 180-day mortality as assessed by the paired difference of risk scores, compared to CLIF-C ACLF, MELD and CP score in overall LT recipients.**




| | Improvement | P value |
|---|---|---|
| IDI | 0.006 (0.001-0.018) | 0.013 |
| NRI (>0) | 0.162 (0.022-0.235) | 0.027 |
| Median risk score | 0.002 (0.000-0.009) | 0.007 |




| | Improvement | P value |
|---|---|---|
| IDI | 0.028 (0.013-0.050) | <0.001 |
| NRI (>0) | 0.242 (0.084-0.331) | <0.001 |
| Median risk score | 0.013 (0.005-0.023) | <0.001 |




| | Improvement | P value |
|---|---|---|
| IDI | 0.048 (0.030-0.074) | <0.001 |
| NRI (>0) | 0.360 (0.250-0.446) | <0.001 |
| Median risk score | 0.026 (0.015-0.040) | <0.001 |

**Fig. 22. The additional value of integrating troponin I and b-type natriuretic peptide to CLIF-C CARDIAC score to predict 365-day mortality as assessed by the paired difference of risk scores, compared to CLIF-C ACLF, MELD and CP score in overall LT recipients.**

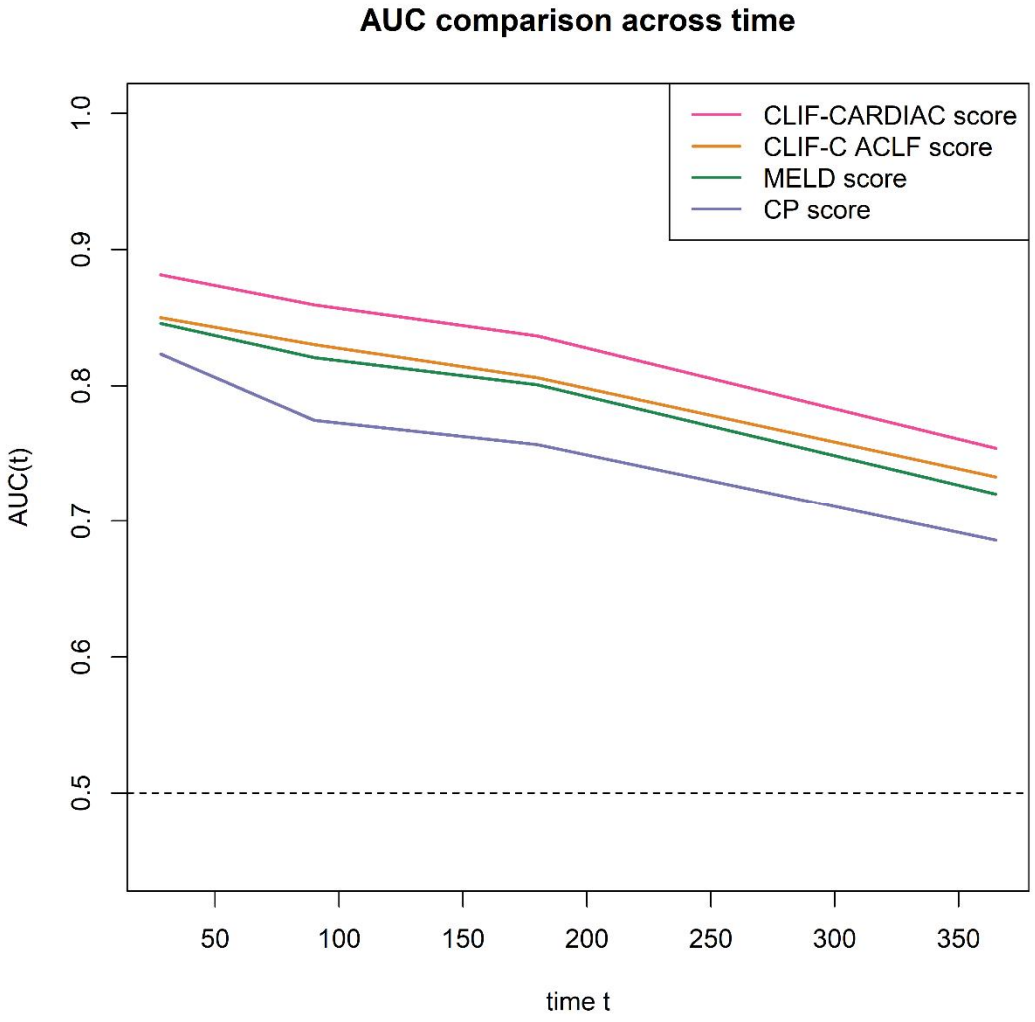



**Fig. 23. Time-dependent area under the curve of CLIF-C CARDIAC, CLIF-C ACLF, MELD and CP score to predict post-LT mortality in overall LT recipients.**

*Part4. Validation of CLIF-C CARDIAC score*

We validated our results using the bootstrap method with 10,000 resamples, which yield optimism-corrected c-index 95% confidence interval for the CLIF-C CARDIAC score. The optimism-corrected c-index was 0.76 (0.671-0.822) for 28-day, 0.758 (0.701-0.804) for 90-day, 0.7369 (0.687-0.780) for 180-day, and 0.696 (0.647-0.740) for 365-day, which supports the validity of the CLIF-C CARDIAC score.

## Discussion

Currently ACLF is recognized as fatal syndrome with high mortality and many prognostic score has been developed.[6, 15] As it is reported that LT gives a substantial benefit of survival, early LT may be the only life-saving option for patients in ACLF. However, prognostic scoring aiming at predicting outcome after LT in patient with ACLF is lacking. In the current study, after identifying the independent association of BNP and TnI with short- and long-term mortality after LT, a strategy used was to analyze patients with incorporating cardiac biomarkers to subsequently fit a final survival model with the CLIF-C ACLFs. Our novel prognostic scoring system, CLIF-C CARDIACs, is superior in predicting short- and long-term mortality than their predecessor CLIF-C ACLFs and MELDs, and CPs, shown by C-index, NRI and IDI. Moreover, it is more accurate at predicting short and long-term mortality not only in patient with ACLF but in overall LT recipients.

ACLF is an increasingly diagnosed syndrome characterized by acute decompensation of a patient with compensated cirrhosis or relatively stable decompensated cirrhosis. Accurate evaluation and timely investigation of its prognosis is of great interest since ACLF is associated with high short-term mortality. The conventional scoring systems, MELDs and CPs is reported to have limited accuracy to predict prognosis in ACLF patients.[31, 32] From the CANONIC study, CLIF-SOFA score and its simplified version, CLIF-C Organ Failure (CLIF-C OF) score were developed to determine the presence of ACLF and its prognosis.[33] In line with the study, Jalan *et* al.[15] developed and independently validated a new scoring system (CLIF-C ACLF score) with higher prognostic accuracy than CLIF-

SOFA and other predecessor scores. However, these scores were developed to predict the prognosis of ACLF, therefore may have limitation when assessing the post-LT risk in LT recipient.

Cardiovascular function is highly associated with mortality after LT.[6-8] The patients with preoperative 'Circulatory Failure' by CLIF-C OF score are reported have higher mortality[6, 7], and occurrence of cardiovascular complication is the leading cause of short-term mortality.[8] Therefore, objective parameter of evaluating cardiac function may be beneficial in accurate evaluation of patient in preoperative period. The use of TnI, an indicator of myocardial injury, has been frequently to diagnosis patient myocardial infarction. However, currently it is recommended to be more wide range of usage, such as monitoring of cardiac function in asymptomatic patients, suggest as "perioperative Troponin screening". [34] BNP is a cardiac hormone that is secreted from the ventricle in response to pressure or volume overload.[35] In LT recipients, higher BNP was reported to predict cirrhotic cardiomyopathy in perioperative period.[14] In the current study, we rigorously identified the association with BNP and TnI with post-LT mortality. Classical multivariate analysis showed the independent association in current cohort and higher importance score after random forest analysis compared to other cardiac variables such as echocardiography-derived parameters may indicate that cardiac biomarker may be more influential factor. Furthermore, the usefulness of biomarkers is that it is objective and could be evaluated in every patient, therefore may be useful to be incorporated in to current prognostic score.

The LT in severely ill patients may be beneficial but may be life-threatening at the same time. Physiologic changes[36, 37] such as compromised ventricular response to stress and decreased beta-agonist transduction, combined with severe intraoperative hemodynamic disturbance[38-40], such as post-reperfusion

syndrome, and postoperatively altered hemodynamic stress, such as sudden increase in preload after reperfusion, all contribute to the potential for cardiovascular complications, eventually increasing mortality after LT.[8, 41] In this regard, VanWagner et al. developed a score specifically to evaluate post-LT cardiovascular morbidity, which identified presence of preoperative heart failure as influential preoperative risk factor.[28] Likewise, considering a high prevalence of pretransplant underlying cirrhotic cardiomyopathy, it may be due to these reasons that out new prognostic score, more accurately reflecting cardiac functions, has higher prognostic value compared to previous score.

Our study has limitation. Although large cohort was used to develop current score, population from one hospital were used. Therefore, studies in different populations, including multicenter studies that included patients of different ethnicities and races, are therefore needed. Thus, our results are needed to be validated externally in the future multicenter studies.

## Conclusion

We developed a novel predicting score of CLIF-C CARDIACs, incorporating cardiac biomarkers, which provides a significant improvement of the discrimination ability as compared to CLIF-C ACLFs, MELDs and CPs as indicated by reduction in percent prediction errors observed. It may provide clinicians an additional are risk-determination information in pre-LT patients evaluation.

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**국문요약**

**연구제목**: 만성 간질환의 급성 악화에 따른 간부전 (Acute-on-chronic liver failure)에서 간 이식 후 생존율 예측 모델: 심기능 지표를 포함한 새로운 위험 예측 점수

**연구배경 및 목적**: 만성 간질환의 급성 악화에 따른 간부전 (acute-on-chronic liver failure, ACLF)은 높은 사망률을 보이는 임상적 질환으로 특히 다장기 부전을 동반하는 경우 더욱 높은 조기사망률을 보이나, 간이식 후 예후는 타 원인으로 인한 간이식과 비슷한 정도로 보고된다. 현재 널리 사용되는 예측점수로는 Chronic Liver Failure Consortium (CLIF-C) Organ Failure score (CLIF-C OFs)과 ACLF score (CLIF-C ACLFs)이 있다. 이러한 점수에서 "순환부전"은 혈압과 승압제의 사용으로 평가하고 있으나 이러한 지표만으로는 심기능을 충분히 반영하지 못할 수 있다. 현재 심혈관 합병증으로 인한 사망률이 간이식 후 사망의 가장 중요한 원인임은 고려하여 본 연구에서는 객관적인 심장관련 지표 (biomarker)를 포함하여, 이전보다 개선된 새로운 예후점수 (CLIF-C CARDIAC)을 개발하고 검증하고자 한다.

**연구대상 및 방법**: 본 연구는 2008 년 1 월부터 2019 년 2 월까지 간이식을 시행 받은 환자의 자료를 후향적으로 분석하였다. CLIF-C ACLF 점수, Model for end-liver disease (MELD), and Child-Pugh (CP) 점수를 계산하였으며 Random survival forest 분석을 통해 선별된 Troponin I (TnI) 와 B-type natriuretic peptide (BNP)을 포함하여 새로운 예후점수 (CLIF-C CARDIAC)을 개발하였다. 각 점수의 예측력을 비교하기 위해 곡선 하 영역 (Area under the curve), Concordance index, Net Reclassification Index (NRI) 그리고 Integrated Discrimination Index (IDI)을 시행하였다.

**결과**: 간이식 받는 환자 2848 명 중 685 (24%)명의 ACLF 환자가 관찰되었다. ACLF 환자에서 전체 3.4 년의 관찰기간 중 간이식 후 28 일, 90 일, 180 일, 그리고 365 일 사망률은 35 (5.1%), 66 (9.6%), 93 (13.6%), 그리고 115 (16.8%) 명으로 관찰되었다. CLIF-C CARDIAC 점수는 기존의 CLIF-C ACLF, MELD, 그리고 CP 점수에 비해 간 이식 후 28, 90, 180, 그리고 365 일 사망 예측력이 더욱 높았다. NRI 를 통해 비교해본 결과 CLIF-C CARDIAC 점수의 간이식 후 28, 90, 180, 그리고 365 일 사망 예측력은 18%에서 42%까지 향상되었다. CLIF-C CARDIAC 점수는 Hosmer-Lemeshow 적합성 검정에서 모두 적합한 것으로 판단되었다 (28-day, p=0.601; 90-day, p=0.351; 180-day, p=0.504; 365-day, p=0.552).

**결론**: 객관적인 심혈관기능 지표를 통합하는 CLIF-C CARDIAC 점수는 기존 예측 점수에 비해서 간이식 후 조기 및 후기 사망률의 예측 정확도가 더욱 우수하였다. CLIF-C CARDIAC 점수는 수술 전 집중 치료 관리가 필요한 고위험 환자군을 조기 식별하는 데 이용될 수 있을 것으로 기대된다.