Discovery and Validation of a Biomarker Model (PRESERVE) Predictive of Renal Outcomes After Liver Transplantation

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Abstract

BACKGROUND AND AIMS: A high proportion of patients develop chronic kidney disease (CKD) after liver transplantation (LT). We aimed to develop clinical/protein models to predict future glomerular filtration rate (GFR) deterioration in this population.

APPROACH AND RESULTS: In independent multicenter discovery (CTOT14) and single-center validation (BUMC) cohorts, we analyzed kidney injury proteins in serum/plasma samples at month 3 after LT in recipients with preserved GFR who demonstrated subsequent GFR deterioration versus preservation by year 1 and year 5 in the BUMC cohort. In CTOT14, we also examined correlations between serial protein levels and GFR over the first year. A month 3 predictive model was constructed from clinical and protein level variables using the CTOT14 cohort (n = 60). Levels of β−2 microglobulin and CD40 antigen and presence of hepatitis C virus (HCV) infection predicted early (year 1) GFR deterioration (area under the curve [AUC], 0.814).

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We observed excellent validation of this model (AUC, 0.801) in the BUMC cohort (n = 50) who had both early and late (year 5) GFR deterioration. At an optimal threshold, the model had the following performance characteristics in CTOT14 and BUMC, respectively: accuracy (0.75, 0.8), sensitivity (0.71, 0.67), specificity (0.78, 0.88), positive predictive value (0.74, 0.75), and negative predictive value (0.76, 0.82). In the serial CTOT14 analysis, several proteins, including β−2 microglobulin and CD40, correlated with GFR changes over the first year.

CONCLUSIONS: We have validated a clinical/protein model (PRESERVE) that early after LT can predict future renal deterioration versus preservation with high accuracy. This model may help select recipients at higher risk for subsequent CKD for early, proactive renal sparing strategies.

Chronic kidney disease (CKD) is a significant clinical problem following liver transplantation (LT). A majority of LT recipients have stage 3 CKD by 2 years after LT and ~20% progress to   stage 4 within 5 years.[1–4] Moreover, recipients with renal dysfunction after LT have reduced survival, particularly if renal replacement therapy is required.[1,3] The etiology of CKD after LT is multifactorial and includes recipient factors, perioperative kidney injury, and calcineurin-inhibitor (CNI)-induced nephrotoxicity.[3,5,6]

Serum creatinine and estimated (eGFR) glomerular filtration rate (GFR) lack sensitivity and specificity and represent late manifestations of significant kidney injury, yet these remain the primary measures currently used to monitor renal function. Measured GFR (mGFR) is more precise than eGFR measures, but is not commonly performed or used to serially monitor renal function. Several studies have suggested that individual biomarkers may be useful in predicting intraoperative renal injury or in improving eGFR accuracy after LT.[7–12] For instance, cystatin C–based GFR equations are somewhat superior in estimating mGFR compared to creatinine-based estimations.[4,13–15]

Even with advances in estimating GFR, most previous investigations have focused on biomarkers that correlate with the presence of already established CKD. We previously performed such an initial exploratory study comparing a large unbiased multianalyte panel (Rules Based Medicine [RBM] DiscoveryMAP; n = 189 proteins; discovery cohort) and a renal injury panel (RBM KidneyMAP; n = 16 proteins; validation cohort) between LT recipients with and without late CKD.[10] Of these large sets of plasma proteins, only 10 had levels that were significantly higher in LT recipients with CKD, most being established renal injury proteins such as cystatin C, α−1 microglobulin, β−2 microglobulin, trefoil factor-3, and CD40. However, this profile merely demonstrates proteins present at CKD diagnosis. What would have more tangible clinical impact would be to determine whether these renal injury proteins were present before the occurrence of GFR deterioration and irreversible CKD. This could allow preclinical identification of renal injury such that early interventions (e.g., CNI withdrawal) could be instituted to avoid future CKD. As such, we performed an interim exploratory study comparing the same large unbiased discovery proteins in LT recipients with normal GFR early post-transplant who either had future renal deterioration versus continued preservation by year 5.[16] Of the large panel spanning multiple pathways, only six proteins (cystatin C, α−1 microglobulin, β−2 microglobulin, macrophage colony stimulating factor 1, CD40 antigen, and erythropoietin) were higher in patients subsequently

developing CKD and all were renal injury proteins that were remarkably similar to those observed in our late CKD study.[10]

With this preliminary discovery work identifying the key proteins involved in early preclinical and late CKD, we turned our attention toward combining these proteins and clinical risk factors to construct an early predictive model to detect patients *at risk for* (e.g., before) deterioration of GFR. The goal was to validate a model that could be implemented in clinical practice early after LT to identify patients at need for preemptive nephroprotective strategies.

Materials and Methods

OVERVIEW

The study was designed to test the central hypothesis that a model combining preidentified blood renal injury proteins and clinical factors can detect early subclinical renal injury to predict future progression to CKD after LT. Our first aim was to analyze samples from the multicenter 1-year prospective study, NIAID CTOT14 (National Institute of Allergy and Infectious Diseases Clinical Trials in Organ Transplantation 14; NCT01672164), and a single-center 5-year biorepository cohort at Baylor University Medical Center (BUMC), to respectively discover and validate this clinical/protein model. Our second aim was to analyze serial samples collected from the CTOT14 cohort to study changes in peripheral blood protein levels during the course of GFR changes in LT recipients. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the institutional review committee at each center. No donor organs were obtained from executed prisoners or other institutionalized persons. Informed consent was obtained from all enrolled patients under IRB approval.

BIOMARKER PLATFORM

The multianalyte panels (Myriad RBM, Inc., Austin, TX) are comprehensive, quantitative, validated immunoassay products measuring human proteins in both serum and plasma using Luminex Bead technology. For both cohorts, we used a targeted 16 renal injury protein panel (KidneyMAP v.1.0) given that all of the proteins of interest from our previous unbiased discovery work were within this panel.[10,16] The following proteins were included: β−2 microglobulin, cystatin C, macrophage colony stimulating factor 1, α−1 microglobulin, CD40 antigen, erythropoietin, apolipoprotein A-IV, clusterin, kidney injury molecule-1, neutrophil gelatinase-associated lipocalin, osteopontin, Tamm-Horsfall protein, tissue inhibitor of metalloproteinases 1, trefoil factor-3, vascular endothelial growth factor, and thrombomodulin.

CTOT14 MULTICENTER SERIAL COHORT

The CTOT14 study prospectively enrolled 202 LT recipients at seven U.S. centers to identify biomarkers of several LT complications. Enrollment of new recipients commenced in October 2012, and last patient visit was in December 2015. Adult LT recipients were enrolled if they had undergone primary deceased or live donor LT and excluded if they had received previous or multiorgan transplants or were human immunodeficiency virus (HIV)

infected. The serial sample collection schedule post-LT was planned as follows: week 2 and month 1, 2, 3, 6, 9, 12, 15, 18, 21, and 24. We limited this analysis to year 1 given that only a small percentage of the study was followed to month 24. For all analyses, we used CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) estimated GFR for our group definitions and serial analyses.[17] All CTOT14 data were collected serially into an electronic database, MEDIDATA RAVE, managed by the Data Coordinating Center (DCC; Rho Federal Systems). Oversight by the DCC included development of the study protocol, review of clinical site visits, classification of clinical phenotypes at the sample level, validation of analyses, and manuscript review.

Given that there are no standard definitions for serial decline of GFR or progression to CKD *over time*, we used a definition supported by the published literature that a >10% persistent decline in GFR over a shorter time period such as 12 months can be predictive of future progression to CKD.[18,19] This definition was used to delineate the trajectory of GFR in CTOT14 given its shorter follow-up, with use of additional Kidney Disease: Improving Global Outcomes (KDIGO) CKD criteria in the longer-term BUMC cohort below. In CTOT14, we identified the subset of patients with baseline 3 months posttransplant eGFR >60 mL/min/1.73 m^2 (n = 97; Supporting Fig. S1). This time point was used to allow for any recovery of perioperative kidney injury and also to match up with the month 3 BUMC collections for validation. We then applied the following criteria to define two groups: (1) Diminished GFR (CTOT14-D) was defined as >10% decline in eGFR from baseline to year 1, with the majority (50%) of the intervening eGFR values lower than baseline eGFR. (2) Preserved GFR (CTOT14-P) was defined as 10% decline in eGFR, with the majority (50%) of the intervening eGFR values within 10% of the baseline eGFR. These criteria were chosen to account for intermittent improvements or declines in eGFR >10% so as to define phenotypes with sustained decline versus preservation of renal function.

BUMC COHORT

Once the CTOT14 model was developed, it was validated in this independent BUMC cohort. The BUMC biorepository has been collecting blood/tissue samples and accompanying prospective clinical data from LT recipients since 1985 under an institutional review board–approved protocol that included mGFR determinations (iothalamate) and eGFR (CKD-EPI) at 3 months and annually following LT. The unique aspects are the protocol-based assessments of simultaneous eGFR and mGFR as well as longitudinal follow-up beyond 1 year. The majority (two-thirds) of the recipients included in this study were transplanted after the year 2000.

We excluded 1,324 subjects with 1 missing GFR values and, similar to the CTOT14 cohort, second or multiorgan transplants and HIV-infected recipients. Thus, we identified 220 LT recipients with preserved GFR (mGFR and eGFR 60 mL/min/1.73 m^2 by CKD-EPI), normal liver tests, and sera available at month 3 (Supporting Fig. S2). The same 3- to 12-month criteria as CTOT14 were then applied, using an electronic software algorithm implemented in the BUMC data, followed by manual (J.L., S.A.) confirmation of the same group criteria as CTOT14 above. In addition, given that the BUMC cohort had longer follow-up, we continued to stratify patients by those with both 1-year deterioration and

progression to more advanced CKD by year 5 (mGFR <45 mL/min/1.73 m^2 or KDIGO CKD stage 3b)[20] versus those who had 1-year and up to 5-year GFR preservation. This validation cohort was used to determine whether the 3-month CTOT14 model was also predictive of late renal outcomes, which may be more clinically relevant. The two groups were defined as follows: (1) diminished GFR (BUMC-D): CTOT14-D criteria above *and* mGFR <45 mL/min/1.73 m^2 every year up to and including year 5; (2) Preserved GFR (BUMC-P): CTOT14-P criteria above *and* (3) mGFR >60 mL/min/1.73 m^2 every year up to and including year 5. Given that the BUMC repository does not store plasma like the CTOT14 samples, we used serum samples, which was deemed acceptable by the manufacturer (Myriad RBM, Inc.)

STATISTICAL ANALYSIS

We tested for these 16 proteins in all samples collected early after LT (month 3 time point) when GFR was preserved (60 mL/min/1.73 m^2) and samples were available. We employed Pearson's chi-square test or Fisher's exact test for categorical variables and *t* test or Wilcoxon rank-sum test for continuous variables to compare baseline demographics between the two groups. Association of protein abundance with GFR status were assessed using logistic regressions with and without the adjustment of baseline covariates. Using the CTOT14 as the discovery cohort, we developed a month 3 prediction model for GFR deterioration by year 1 based on multivariable logistic regression with step-wise variable selection and Bayesian information criterion, which started with seven key clinical and 16 multianalyte protein variables. Internal validation was performed using C-statistic with 1,000 bootstrap samples. We then fixed the prediction model and externally validated its performance in the BUMC cohort. In a subanalysis, we tested the performance of the model in the non-HCV (hepatitis C virus) cohorts.

In CTOT14, because we collected serial samples over the first transplant year, we performed an additional analysis of change of protein levels over time in relation to GFR change. This analysis began at month 1 through year 1 to give the full spectrum of serial changes in proteins over time, starting from eGFR > 60 (60 mL/min/1.73 m^2) at month 1 and then divided by diminished or preserved eGFR by the same criteria above. For the statistical analysis, we used a generalized linear mixed-effect model to examine whether the serial protein levels over time differed between the diminished and preserved GFR groups. We also estimated the average protein change from month 1 to 12 for each individual by the slope from a linear regression model. All analyses were done using R: A language and environment for statistical computing (Version 3.3.2; R Foundation for Statistical Computing, Vienna, Austria).

Results

CLINICAL CHARACTERISTICS

Figure 1 displays the percentage change in eGFR from the month 3 baseline to year 1 in all CTOT14 subjects, divided by preserved and diminished cohorts. Table 1 displays the clinical characteristics of both the CTOT14 discovery and BUMC validation cohorts with month 3 samples, divided by those developing eGFR deterioration versus preservation. In the

CTOT14 cohort, the only statistically significant difference was lower eGFR at year 1 in the CTOT14-D versus CTOT14-P group, which was expected. There was a trend toward a lower percentage of HCV infection in the CTOT14-D group. In the BUMC cohort, the BUMC-D group was also less HCV positive, older, more on late mammalian target of rapamycin (mTOR)-inhibitor therapy at year 5, and also as expected, had lower measured and estimated GFRs at years 1 and 5. Other clinical parameters were not statistically different.

PREDICTIVE MODEL GENERATION FROM THE CTOT14 DISCOVERY COHORT

Table 2 displays the month 3 plasma protein level comparison in the CTOT14 plasma discovery cohort, divided by renal outcomes at year 1. We initially performed a univariate logistic regression analysis that included the 16 renal injury proteins and seven key clinical variables from Table 1 (age, sex, race, HCV as listing diagnosis, hypertension, diabetes, and baseline month 3 eGFR) known to be clinically associated with changes in GFR (Supporting Table S1). From these data, we used a step-wise logistic regression approach with variable selection to develop a model at 3 months that was most predictive of 1-year diminished eGFR (Fig. 2A). This resulted in a model containing two proteins (β−2 microglobulin, CD40) and one clinical (HCV positivity) variable that had high prediction of eGFR deterioration (area under the curve [AUC], 0.81). We achieved similar results after internal bootstrapping (ordinary resampling method): AUC, 0.82; SD, 0.05. We named this model PRESERVE (Prediction of REnal outcomeS aftER liVEr transplant) with the formula: log it(p) = −1.1445231 +0.8616163b2 microglobulin −1.4749716cd40–2.0455445hcv+. Performance characteristics of this model are displayed in Supporting Table S2. The overall accuracy (0.75), sensitivity (0.71), specificity (0.78), positive predictive value (PPV; 0.74), and negative predictive value (NPV; 0.76) were most optimal at a probability threshold of 0.5.

PREDICTIVE MODEL VALIDATION FROM THE BUMC COHORT

Table 2 also displays the month 3 serum protein level comparison in the BUMC validation cohort. The PRESERVE model was then tested in the BUMC cohort, to provide external independent model validation. Similar to CTOT14, the PRESERVE model had high prediction of eGFR deterioration (AUC, 0.80) at 1 and 5 years (Fig. 2B). Applying the 0.5 threshold from the PRESERVE model led to excellent performance characteristics (Supporting Table S3): accuracy (0.80), sensitivity (0.67), specificity (0.88), PPV (0.75), and NPV (0.82).

PREDICTIVE MODEL ASSESSMENT IN THE NON-HCV RECIPIENTS

Given the declining prevalence of HCV infection in LT recipients, we tested the accuracy of the PRESERVE model in the non-HCV cohorts. These were CTOT14 and BUMC recipients with no history of HCV or were HCV+, but nonviremic attributable to previous antiviral therapy. This had a lower AUC in the CTOT14 group (0.75), but in the independent external BUMC validation cohort, the AUC (0.79) was nearly equivalent to the AUC (0.80 above) of the overall cohort. In addition, at the same 0.5 threshold, the performance characteristics were similar to the overall cohort: accuracy (0.77 vs. 0.80), sensitivity (0.71 vs. 0.67), specificity (0.83 vs. 0.88), PPV (0.80 vs. 0.75), and NPV (0.75 vs. 0.82).

CTOT14: SERIAL ANALYSIS OF PROTEIN LEVEL AND GFR CHANGE FROM MONTH 1 TO 12

We also examined the trajectory of each protein level over time in relation to change in eGFR, providing support for the validity of these biomarkers and a more robust understanding of mechanisms of renal injury in this population. Serial protein and eGFR values were available only in the CTOT14 prospective study. In requiring a minimum of four samples tied to eGFR, 31 CTOT14-D and 24 CTOT14-P subjects had 306 serial proteins tested. The following proteins were higher over time in the CTOT14-D versus CTOT14-P group: α−1 microglobulin, vascular endothelial growth factor, CD40 antigen, kidney injury molecule-1, neutrophil gelatinase-associated lipocalin, tissue inhibitor of metalloproteinases-1, β−2 microglobulin, cystatin C, and trefoil factor-3 (Supporting Table S4). Tamm-Horsfall protein (uromodulin) demonstrated the opposite effect, decreasing with time in the CTOT14-D group. We then combined the groups and analyzed specifically for an association between protein levels and eGFR over time in a linear mixed model. The following varied significantly with eGFR (Fig. 3; $P < 0.05$): α−1 microglobulin, β−2 microglobulin, cystatin C, trefoil factor-3, thrombomodulin (negative correlation), and Tamm-Horsfall protein (positive correlation). Those that trended toward significance were apolipoprotein A-IV (0.06), vascular endothelial growth factor (0.06), CD40 ($P = 0.07$), and kidney injury molecule-1 (0.08).

Discussion

Several reports of both blood and urine biomarkers have shown correlations with acute or chronic kidney injury in the general population.[11,21–25] Proteins such as albumin, kidney injury molecule-1, neutrophil gelatinase-associated lipocalin, and β−2 microglobulin, are associated with future GFR deterioration, but mainly in patients with abnormal baseline renal function. In LT recipients, causes of native kidney injury are similar to the general population, with the main difference being the use of nephrotoxic CNI therapies. High levels of cystatin C, neutrophil gelatinase-associated lipocalin, α−1 microglobulin, β−2 microglobulin, and others correlate with established CKD in liver recipients.[7–10,26–33] Given that many recipients have kidney injury at LT that does not resolve, biomarkers post-LT in this setting would be merely diagnostic, not predictive, and have little advantage over the standard creatinine-based GFR estimates. Validation of a *predictive* model could stratify future recipients for early, more aggressive renal preservation strategies, such as CNI minimization or withdrawal, rather than applying such strategies more broadly to all recipients where they might not be necessary and/or carry a higher risk of rejection. As an example, one previous study attempted to withdraw tacrolimus 3 months after LT in favor of everolimus.[34] That arm of the study was stopped early because of a higher rate of rejection, although those successful had significantly better GFR at 1 year than tacrolimus-maintained groups. An early predictive model could guide such efforts to identify and intervene on only those at higher risk of GFR deterioration and thus provide a personalized approach to LT management.

Our study was therefore designed to test the potential of a predictive biomarker model (PRESERVE) to identify LT recipients at risk of developing future CKD. We examined all

patients with preserved renal function at 3 months after LT, but still at risk for *de novo* future CKD.[13] Important in our model development were the preliminary studies we performed analyzing a large set of unbiased discovery proteins in both established post-LT CKD and early after transplant before CKD. From this panel, it was clearly identified that renal injury proteins were elevated in both settings, the latter (early) being most relevant for our current model generation, given the potential to proactively identify patients at higher CKD risk. Thus, we developed the PRESERVE model from the clinical and previously identified renal injury markers in the prospective CTOT14 cohort and validated it in the independent BUMC cohort having longer follow-up, measured GFRs, and established CKD.

The final PRESERVE model included one clinical variable (HCV as the primary indication for LT) and two renal injury proteins, β−2 microglobulin and CD40 antigen. The accuracy and AUC of PRESERVE for both cohorts were high (~0.8), with a somewhat higher specificity and NPV than sensitivity and PPV, suggesting that it may be slightly better at predicting future GFR preservation over deterioration. In addition, several of these proteins increased with declining GFR in our serial analysis and were present in both the early and late LT CKD protein profile we previously reported,[10,16] supporting an evolution of protein expression preceding full CKD. The model also performed well in both plasma and serum in the two cohorts. In recent reports, we demonstrated similar findings by discovering a model using plasma samples and validating it with serum samples.[35,36] In this instance, two pretransplant proteins, tissue inhibitor of metalloproteinase 1 and osteopontin, along with age and diabetes (REVERSE model) were predictive of renal recovery within weeks following transplantation. Thus, serum and plasma appear interchangeable in both models, which can facilitate clinical applications and laboratory testing with more flexibility.

The proteins identified correlate with pathways of both native renal injury and protection, best observed in the serial analysis linking protein levels and GFR changes over time. Trefoil factor-3 and thrombomodulin have been reported as sensitive markers of early and reversible renal injury.[10,37,38] β−2 microglobulin is the light chain of major histocompatibility complex expressed on all nucleated cells and, like α−1 microglobulin and cystatin C, is filtered by the glomerulus and reabsorbed/catabolized by the proximal tubule.[39,40] Similar to our findings, a recent nontransplant study found associations between β−2 microglobulin, cystatin C, and future CKD, even in patients with baseline eGFR >60 mL/min/1.73 m^2.[19] Although it is not clear whether β−2 microglobulin levels are related to decreased glomerular filtration or tissue injury itself, they appear predictive at preclinical renal injury stages regardless of the mechanism. CD40 levels also correlate with GFR changes, but may be more related to inflammation. CD4$^+$ T cells and platelets express CD40 ligand and mediate proinflammatory events in proximal tubular epithelial cells through interaction with CD40 antigen.[41] Both CD40 and β−2 microglobulin are related to costimulation pathways and the major histocompatibility structure, respectively, providing some evidence for immune-mediated renal injury. Even with these potential pathways, without renal biopsies, we cannot determine the exact cause of GFR deterioration in our cohorts; it is likely that multiple factors in addition to CNI nephrotoxicity are involved.[13] Finally, in the serial analysis, we found a direct correlation between better GFR and plasma Tamm-Horsfall protein levels (also known as uromodulin). This fits well with previous studies showing uromodulin being protective and inversely associated with CKD.[42,43]

Interestingly, HCV as a listing diagnosis was the only clinical variable remaining in the model after regression. In both CTOT14 and BUMC cohorts, HCV was less prevalent in the patients who had diminished GFR over time, and the absence of HCV was associated with GFR deterioration in the PRESERVE model. This is somewhat surprising given that HCV infection has been shown to correlate with CKD in both general and transplant populations. [3,44] As a potential explanation, given that the study enrollment took place in eras in which patients were transplanted with active HCV viremia, GFR improvement may have been related to practices such as minimizing CNI exposure in efforts to limit HCV recurrence. This is entirely speculative given that we do not have enough serial data on immunosuppression management and drug levels to confirm this hypothesis. Another explanation could be that the non-HCV populations, particularly those transplanted for nonalcoholic steatohepatitis (NASH) with metabolic syndrome, have known high rates of CKD after LT and thus the model (absence of HCV correlating with diminished GFR) may be reflecting this risk and useful for future populations.[13]

Given that our model includes HCV as a key clinical variable, it was reassuring that it performed well in both partially treated (6 nonviremic of 15 HCV[+]) CTOT14 discovery and untreated viremic BUMC validation cohorts. However, the current practice is to eradicate HCV before or soon after transplantation with oral antiviral therapy, and thus LT populations are increasingly non-HCV (e.g., NASH, alcoholic cirrhosis) or nonviremic.[45–48] To ensure that the PRESERVE model would still be usable for future LT populations, we performed additional analyses testing its accuracy in the non-HCV cohorts. The PRESERVE model had nearly equivalent AUC, accuracy, and other performance characteristics (sensitivity, specificity, PPV, and NPV) in the non-HCV population. Thus, even with a dwindling HCV prevalence, we believe the model should perform well and be applicable to future recipients, both HCV and non-HCV.

Our study has limitations that need to be addressed. First, we recognize that although single-time point predictions like the PRESERVE model may be useful in clinical practice, they are not always definitive, particularly when other renal insults can occur over time. Even though month 3 likely represents a renal baseline or steady state, future studies will need to test the model serially or even earlier after LT. It is also not known whether the elevated kidney injury proteins at month 3 are related to recovery from earlier posttransplant renal injury or new-onset subclinical renal injury. We suspect the former given that there was a greater improvement in eGFR from the time of LT to month 3 in the CTOT14-D group (71.7 ± 28.8 to 81.2 ± 17.6 mL/min/1.73 m^2) than CTOT14-P (79.6 ± 27.7 to 81.3 ± 16.2 mL/min/1.73 m^2). Regardless of timing, this subclinical renal injury at month 3 appears to predict future deterioration. Second, our sample size was relatively small in relation to the overall cohort, and this was because we needed to identify "clean" phenotypes of patients who had distinct GFR trajectories over time, rather than testing all patients and trajectories that would limit model generation and validation. However, we acknowledge that exclusion of these patients with less pure phenotypes may reduce the generalizability of the model to the transplant population as a whole. Given that the PRESERVE model performed well in independent discovery and validation cohorts, it now needs to be more robustly tested in randomized, renal sparing interventional trials that include the larger LT population. Third, the validation cohort (BUMC) was more remotely transplanted and had differences in clinical

characteristics (Table 1) compared to CTOT14. However, the primary model was developed using the more contemporary CTOT14 group, and the fact that it had similar accuracy in the BUMC group strengthens its validity and external generalizability, as it appears agnostic to era. Finally, we defined 1-year renal deterioration by >10% persistent drop in eGFR, which may be considered modest in clinical impact. However, the literature supports that this early reduction predicts continued renal deterioration,[18,19] and the PRESERVE model prediction held up in our BUMC cohort who had more substantial, clinically relevant renal dysfunction by year 5 (CKD stage 3b).

In summary, we have identified and validated a clinical/protein model that can stratify recipients early after LT who are risk for future GFR deterioration. Based on these findings, we anticipate that nephroprotective strategies could be targeted toward LT recipients that are predicted to progress to CKD according to the PRESERVE model. Similarly, the pre-LT REVERSE model previously reported may allow for earlier personalized approaches.[35,36] We envision testing both models in imminent randomized controlled trials at the time of and weeks after LT to ultimately use them as personalized management strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations:

AUC	area under the curve
BUMC	Baylor University Medical Center
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CTOT	Clinical Trials in Organ Transplantation
CNIs	calcineurin inhibitors
CKD	chronic kidney disease
eGFR	estimated GFR
GFR	glomerular filtration rate
HCV	hepatitis C virus
LT	liver transplantation

mGFR	measured GFR
mTOR	mammalian target of rapamycin
NPV	negative predictive value
PPV	positive predictive value
RBM	Rules Based Medicine

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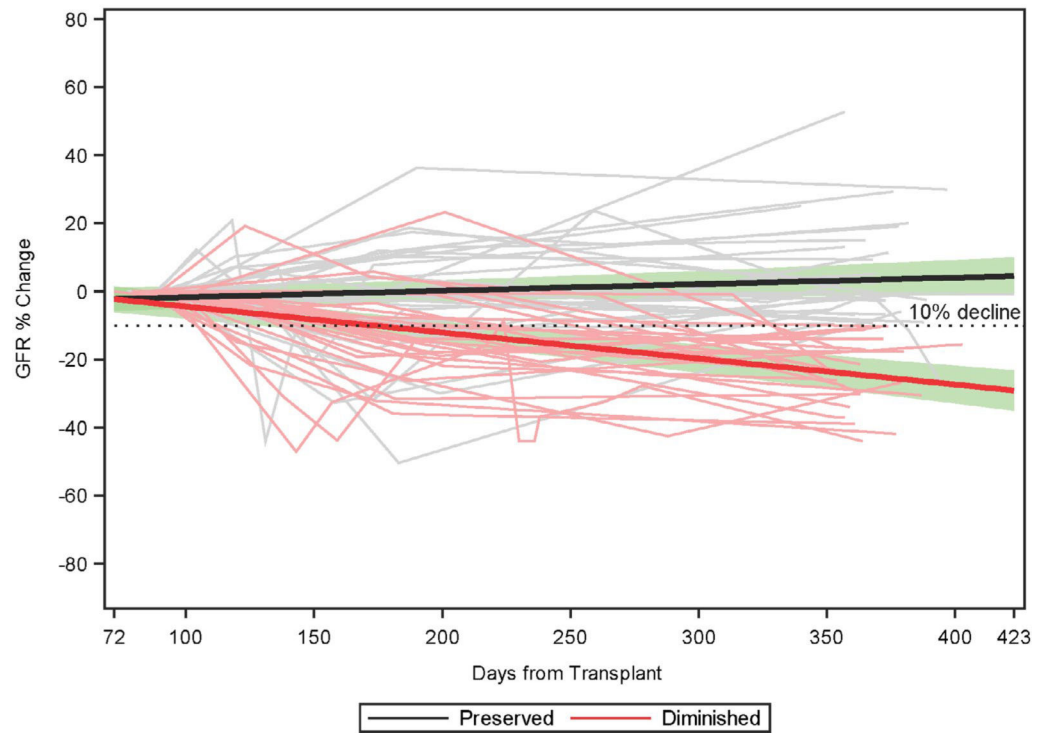
FIG. 1.
Percentage change in eGFR from baseline month 3 to year 1 in the preserved (CTOT-P) versus diminished (CTOT-D) GFR groups. The horizontal dashed line represents a 10% decline from baseline. Individual subject trajectories are shown in gray (preserved) and light red (diminished) with the mean predicted lines (black for preserved, red for diminished) overlaid. The green bands reflect the associated 95% confidence interval.
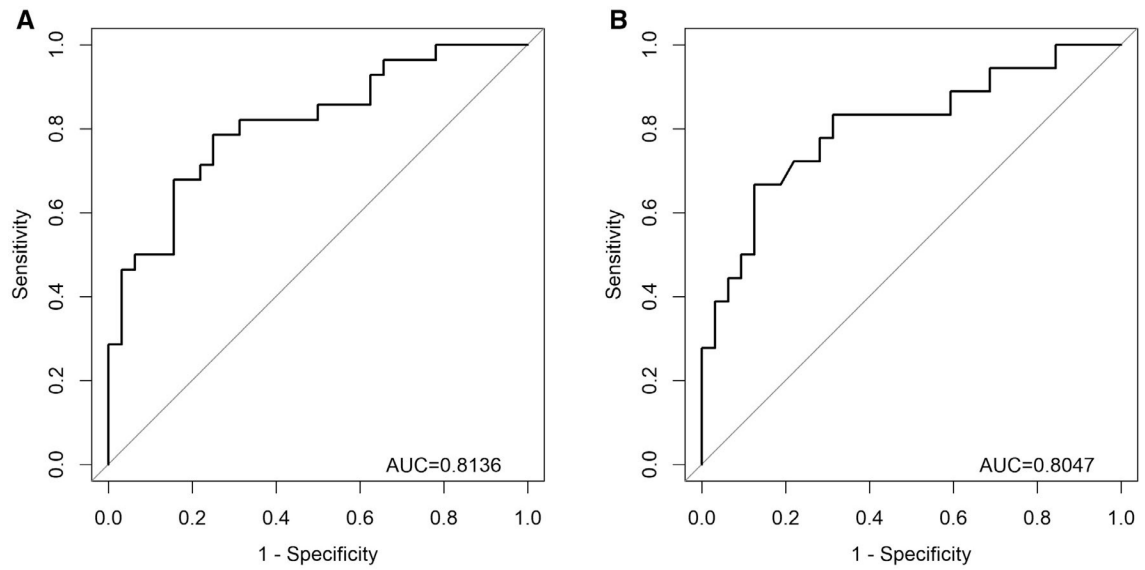
FIG. 2.
(A) Receiver operating curve for the PRESERVE step-wise logistic regression model predicting GFR deterioration at 1 year post-LT in the initial CTOT14 plasma discovery cohort. Variables included in the PRESERVE model: HCV+ (yes/no), β−2 microglobulin protein level, and CD40 protein level. (B) Receiver operating curve for the same PRESERVE step-wise logistic regression model predicting GFR deterioration at both 1 and 5 years post-LT in the BUMC serum validation cohort.
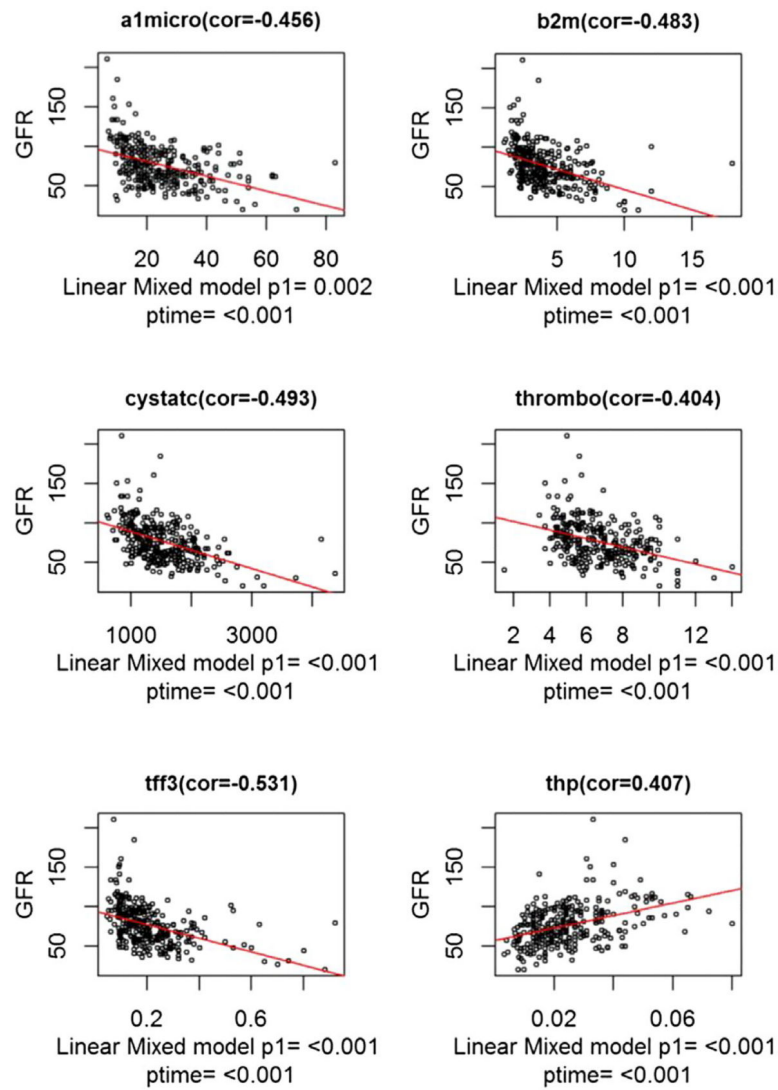
FIG. 3.
This figure highlights the specific plasma proteins that significantly correlated with serial eGFR changes over the first year following LT: CTOT14 cohort. Abbreviations: a1micro, α −1 microglobulin; b2m, β−2 microglobulin; cor, correlation; cystatc, cystatin C; tff3, trefoil factor-3; thp, Tamm-Horsfall protein; thrombo, thrombomodulin.

TABLE 1.

Clinical Characteristics: Discovery (CTOT14) and Validation (BUMC) Cohorts

Variable	CTOT14-D* N = 28	CTOT14-P* N = 32	P Value	BUMC-D† N = 18	BUMC-P† N = 32	P Value
At LT						
Age at enrollment (years)	52.5 (12.2)	54.1 (8.7)	0.575	54.33 (9.3)	44.31 (11.0)	0.002
Sex (% male)	17 (60.7%)	25 (78.1%)	0.142	10 (55.6)	22 (68.8)	0.531
Race (% white)	22 (78.6%)	27 (84.4%)	0.562	17 (94.4)	26 (81.2)	0.386
HCV as listing diagnosis‡	4 (14.3%)	11 (34.4%)	0.073	1 (5.6%)	14 (43.8%)	0.005
Hypertension	6 (21.4%)	8 (25.0%)	0.744	1 (5.6)	2 (6.2)	>0.999
Diabetes	7 (25.0%)	7 (21.9%)	0.775	2 (11.1)	4 (12.5)	>0.999
Month 3 post-LT						
CNI therapy, n (%)	24 (85.7%)	31 (96.9%)	0.175	18 (100)	32 (100)	>0.999
Alanine aminotransferase (U/L)	44.1 (77.8)	45.5 (46.71)	0.304	39.5 (24.5)	39.5 (29.1)	0.997
Alkaline phosphatase (U/L)	132.6 (177.1)	139.0 (121.1)	0.740	105.1 (35.2)	90.4 (33.1)	0.131
Total bilirubin (mg/dL)	0.9 (0.9)	0.7 (0.89)	0.733	0.54 (.31)	0.58 (0.33)	0.708
mGFR (mL/min/1.73 m²)	n/a	n/a		80.2 (16.74)	82.1 (26.8)	0.363
eGFR (mL/min/1.73 m²)	81.2 (17.6)	81.3 (16.23)	0.941	80.2 (16.7)	85.9 (24.5)	0.776
Year 1 post-LT						
CNI therapy, n (%)	22 (78.6%)	28 (87.5%)	0.491	18 (100%)	32 (100%)	>0.999
Mycophenolic acid, n (%)	9 (32.1%)	8 (25.0%)	0.540	8 (44.4%)	16 (50%)	0.706
mTOR-inhibitor therapy, n (%)	4 (14.3%)	7 (21.9%)	0.449	3 (16.7%)	1 (3.1%)	0.127
mGFR (mL/min/1.73 m²)	n/a	n/a		54.7 (16.1)	98.3 (20.7)	<0.001
eGFR (mL/min/1.73 m²)	62.5 (16.7)	84.6 (16.0)	<0.001	47.0 (11.6)	91.0 (24.2)	<0.001
At year 5 post-LT						
CNI therapy, n (%)	n/a	n/a		16 (88.8%)	32 (100%)	0.125
Mycophenolic acid or azathioprine, n (%)	n/a	n/a		9 (50%)	15 (83.3%)	0.831
mTOR-inhibitor therapy, n (%)	n/a	n/a		6 (33.3%)	0 (0%)	0.0012
mGFR (mL/min/1.73 m²)	n/a	n/a		33.9 (7.6)	94.7 (22.7)	<0.001
eGFR (mL/min/1.73 m²)	n/a	n/a		39.3 (14.1)	84.6 (28.9)	<0.001

*
Defined by GFR > 60 at month 3 and then >10% deterioration in GFR (Diminished) *vs.* ≤10% (Preserved) at year 1.

†
Defined by GFR > 60 at month 3, >10% deterioration in GFR (Diminished) *vs.* ≤10% (Preserved) at year 1, and then GFR < 45 (Diminished) *vs.* GFR ≥ 60 mL/min (Preserved) up to 5 years.

‡
Two in the CTOT14-D group and 4 in the CTOT14-P group were HCV nonviremic in the study period. None of the BUMC patients were HCV nonviremic.

Abbreviation: n/a, not applicable.

TABLE 2.

Month 3 protein Level Comparison: Discovery (CTOT14) and Validation (BUMC) Cohorts

	CTOT14 (Predict 1 Year) Plasma			BUMC (Predict 1 and 5 Year) Sera		
	Diminished	Preserved	P Value	Diminished	Preserved	P Value
β-2 microglobulin (μg/mL)	3.75 [2.58; 5.32]	2.55 [2.20; 3.70]	0.011	4.25 [3.70; 5.42]	3.15 [2.35; 3.92]	0.002
Cystatin C (ng/mL)	1,325 [1,222; 1,872]	1,105 [1,028; 1,322]	0.004	1,435 [1,282; 1,578]	1,085 [896; 1,350]	0.001
Macrophage colony stimulating factor 1 (ng/mL)	0.94 [0.66; 1.60]	0.90 [0.66; 1.22]	0.694	2.15 [1.80; 2.92]	1.50 [0.83; 2.20]	0.007
α-1 microglobulin (μg/mL)	23.0 [15.8; 29.0]	18.5 [15.0; 24.0]	0.135	24.0 [21.2; 29.8]	21.0 [15.8; 24.0]	0.007
CD40 antigen (ng/mL)	0.94 [0.72; 1.50]	0.94 [0.76; 1.42]	0.911	1.50 [1.30; 1.70]	1.10 [0.98; 1.40]	0.001
Erythropoietin (mIU/mL)	18.0 [14.0; 32.2]	15.5 [9.67; 23.0]	0.099	33.0 [24.0; 38.5]	23.5 [19.8; 29.0]	0.041
Apolipoprotein A-IV (μg/mL)	3,850 [3,640; 5,520]	5,100 [4,052; 7,350]	0.113	49.5 [38.5; 64.2]	40.5 [33.8; 61.8]	0.189
Clusterin (μg/mL)	256 [234; 297]	271 [228; 349]	0.382	262 [208; 283]	212 [184; 237]	0.027
Kim-1 (ng/mL)	0.08 [0.05; 0.12]	0.12 [0.09; 0.23]	0.065	0.10 [0.07; 0.20]	0.08 [0.04; 0.11]	0.067
Neutrophil gelatinase-associated lipocalin (ng/mL)	280 [177; 425]	220 [172; 270]	0.095	391 [234; 484]	294 [223; 378]	0.176
Osteopontin (ng/mL)	42.0 [35.5; 62.5]	37.5 [34.0; 48.0]	0.187	25.5 [17.2; 30.0]	21.0 [11.8; 25.2]	0.056
Tamm-Horsfall protein (μg/mL)	0.03 [0.02; 0.04]	0.02 [0.02; 0.03]	0.588	0.03 [0.03; 0.04]	0.04 [0.03; 0.05]	0.117
Tissue inhibitor of metalloproteinases 1 (ng/mL)	114 [84.8; 153]	97.0 [84.8; 118]	0.187	154 [137; 184]	146 [123; 166]	0.122
Trefoil factor-3 (μg/mL)	0.20 [0.14; 0.26]	0.16 [0.13; 0.20]	0.045	0.15 [0.12; 0.20]	0.12 [0.10; 0.15]	0.037
Vascular endothelial growth factor (pg/mL)	94.0 [81.5; 133]	92.5 [75.8; 122]	0.386	256 [192; 355]	298 [188; 400]	0.864
Thrombomodulin (ng/mL)	7.15 [5.28; 8.90]	6.55 [4.80; 7.23]	0.157	7.20 [6.70; 9.10]	6.50 [5.15; 7.82]	0.037