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ORIGINAL ARTICLE

Urinary liver-type fatty acid-binding protein is independently associated with graft failure in outpatient kidney transplant recipients

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Urinary liver-type fatty acid-binding protein (uL-FABP) is a biomarker of kidney hypoxia and ischemia, and thus offers a novel approach to identify early kidney insults associated with increased risk of graft failure in outpatient kidney transplant recipients (KTR). We investigated whether uL-FABP is associated with graft failure and whether it improves risk prediction. We studied a cohort of 638 outpatient KTR with a functional graft ≥1-year. During a median follow-up of 5.3 years, 80 KTR developed graft failure. uL-FABP (median 2.11, interquartile range 0.93–7.37 μg/24"/>h) was prospectively associated with the risk of graft failure (hazard ratio 1.75; 95% confidence interval 1.27–2.41 per 1-SD increment; P = .001), independent of potential confounders including estimated glomerular filtration rate and proteinuria. uL-FABP showed excellent discrimination ability for graft failure (c-statistic of 0.83) and its addition to a prediction model composed by established clinical predictors of graft failure significantly improved the c-statistic to 0.89 (P for F-test <.001). These results were robust to several sensitivity analyses. Further validation studies are warranted to evaluate the potential use of a risk-prediction model including uL-FABP to improve identification of outpatient KTR at high risk of graft failure in clinical care.

KEYWORDS
clinical research/practice, graft survival, kidney transplantation/nephrology, outpatient care, risk assessment/risk stratification

Abbreviations: AIC, akaike information criterion; AUC, area under the curve; CI, confidence interval; CKD-EPI, chronic kidney disease-epidemiology collaboration equation; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HLA, human leukocyte antigen; HR, hazard ratio; hs-CRP, high-sensitivity C-reactive protein; IQR, interquartile range; KTR, kidney transplant recipients; LDL, low-density lipoprotein; ROC, receiver operator characteristic; SBP, systolic blood pressure; SD, standard deviation; SQUASH, short questionnaire to assess health-enhancing physical activity; uL-FABP, urinary liver-type fatty acid-binding protein.

1 | INTRODUCTION

Short-term outcomes of kidney transplantation have seen great improvements over the last decades.[1,2] In contrast, improving long-term kidney graft survival continues to be a major challenge with no comparable achievements during the same time frame.[3,4] Improvement of risk-prediction tools is the first step in advancing early risk management strategies post–kidney transplantation.[5,6] However, current clinical parameters[7–9] are of limited potential to allow improvement of long-term outcomes, because their alteration usually reflects already advanced structural damage.[10]

Liver-type fatty acid-binding protein (L-FABP) is an intracellular lipid chaperon[11] that in the kidney is exclusively expressed in the proximal tubule.[12] Early in the pathophysiology of chronic rejection, attenuated blood flow due to arterial intimal fibrosis leads to hypoxic challenge and graft ischemia.[13] It has been described that upon detection of lipid peroxidation increments, an hypoxia-responsive element upregulates L-FABP synthesis, which then allows binding of lipid peroxides for their urinary excretion[11] and both expression and urinary excretion of L-FABP have been shown to be increased under tubular hypoxic conditions.[12,14] Because kidney tubular epithelial cells are very rich in mitochondria, and therefore particularly vulnerable to hypoxic challenge, L-FABP may offer a novel interesting approach to identifying early graft tissue insult.[11]

In the kidney transplantation setting specifically, L-FABP measurement during hypothermic machine perfusion showed to be inversely associated with graft function at 6 months post-transplantation.[15] Furthermore, an elegant study by Yamamoto et al. showed that urinary L-FABP (uL-FABP) is directly correlated with graft ischemia time.[12] No study to date, however, has been devoted to investigating the biologically plausible association between uL-FABP and risk of graft failure in outpatient KTR.

In the current study, we aimed to investigate the prospective association of uL-FABP and graft failure in outpatient KTR. Furthermore, we aimed to explore its risk-predictive ability and whether addition of uL-FABP into a model of established risk factors could improve risk-predictive ability and model fit for kidney graft failure.

2 | MATERIALS AND METHODS

2.1 | Study design and population

In this prospective cohort study, adult KTR who visited the outpatient clinic at the University Medical Center Groningen (the Netherlands) between November 2008 and May 2011 and had a functioning graft for at least 1-year were invited to participate. The invitation was restricted to patients with 1-year functional graft because the objective of the TransplantLines study (NCT03272841) was to identify risk factors that impacted long-term graft survival, where, contrary to the first-year post-transplantation, little improvement has been seen in the last decades.[3,4] Seven hundred and seven patients signed a written informed consent at a median of 5.8 (interquartile range 2.0–12.2) years post-transplantation. We excluded patients in whom uL-FABP measurements were missing (n = 69), resulting in 638 KTR, of whom the data are presented here. The current study was approved by the institutional review board (METc 2008/186) and adhered to the Declarations of Helsinki and Istanbul.

The primary endpoint of the current study was death-censored graft failure, defined as restart of dialysis or re-transplantation. Follow-up was performed according to the American Society of Transplantation guidelines[16] until June, 2016. Collection of data was ensured by the continuous surveillance system of the outpatient clinic of our university hospital and close collaboration with affiliated hospitals. We contacted general practitioners or referring nephrologists in cases where the status of a patient was unknown. No participants were lost to follow-up.

2.2 | Data collection

Baseline data were collected during a visit to the outpatient clinic, following a detailed protocol described elsewhere.[17,18] Anthropometric measurements were taken while participants wore indoor clothing without shoes. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a semiautomatic device (Dinamap1846; Critikon) every minute for 15 minutes.[17] Relevant donor, recipient and transplant information was extracted from the Groningen Renal Transplant Database, which has been described in detail before.[19]

2.3 | Laboratory measurements and calculations

At first visit, blood samples were taken after a fasting period of approximately 8 h. Plasma glucose was measured by the glucose oxidase method (YSI 2300 Stat Plus Analyzer; Yellow Springs Instruments); total cholesterol by the cholesterol oxidase-phenol aminophenazone method (MEGA AU510; Merck Diagnostica); high-density lipoprotein (HDL) cholesterol by the cholesterol oxidase-phenol aminophenazone method on a Technicon RA-1000 (Bayer Diagnostics); triglycerides by the glycerol-3-phosphate oxidase-oxidase method (YSI 2300 Stat Plus Analyzer; Yellow Springs Instruments), and serum creatinine was determined by using an enzymatic, isotope dilution mass spectrometry–traceable assay on a Roche P-Modulator automated analyzer (Roche Diagnostics). Low-density lipoprotein (LDL) cholesterol was calculated by using the Friedewald equation.[20] Estimated glomerular filtration rate (eGFR) was calculated by the serum creatinine-based Chronic Kidney Disease EPIdemiology collaboration equation (CKD-EPI).[21] The cumulative dose of prednisolone was calculated as the sum of the maintenance dose of prednisolone from transplantation until baseline.

According to a strict protocol, all KTR were asked to collect a 24-hour urine sample during the day before the same visit. uL-FABP was measured with an enzyme-linked immunosorbent assay

(human uL-FABP assay kit 96 test; CMIC holdings Co). The test has a detection limit of 0.036 μg/L. The intra-assay variability calculated based on four replicate measurements on urine samples with uL-FABP concentrations of 2 and 40 μg/L, were 3.8% and 2.5%, respectively. Inter-assay variabilities, as assessed with repeated measurements in the same samples were 10.4% and 7.3%, respectively.

2.4 | Statistical analyses

Data analyses, computations, and graphs were performed with SPSS version 25.0 software (IBM Corporation), Stata version 13.1 (StataCorp), R version 3.2.3 (R Foundation for Statistical Computing) and GraphPad Prism version 8 software (GraphPad Software). Descriptive statistics are presented as mean ± SD for normally distributed data, and median (interquartile range [IQR]) for skewed variables. Categorical data are expressed as number (percentage). For uL-FABP, values below the detection limit were set to the detection limit and the natural log transformation was used for all Cox regression analyses. Differences in characteristics at baseline among subgroups of KTR according to tertiles of uL-FABP were tested by one-way ANOVA for continuous variables with normal distribution, Mann-Whitney *U* test for continuous variables with skewed distribution and χ^2 test for categorical variables. For all statistical analyses, a statistical significance level of $p < .05$ (two-tailed) was used. Further statistical modeling consisted of several steps:

2.4.1 | Generation of a reference model based on prespecified traditional risk factors

First, multiple univariable Cox proportional-hazards regression analyses were performed to individually assess the prospective association of prespecified (literature-based) established risk factors of graft failure with this outcome.[5,22,23] Hazard ratios (HR) and 95% confidence intervals (CI) were calculated per 1-SD relative increment (risk factors of continuous nature) or per change compared with the implied reference group (risk factors of categorical nature). Then, a reduced model with the stronger predictors was obtained by means of backwards selection ($\alpha = 0.05$). This reduced model was, hereafter, used and referred to as Reference Model.

2.4.2 | Association of uL-FABP with risk of graft failure

A restricted cubic spline regression, with three knots located at the 10th, 50th, and 90th percentile, was performed and graphed to visualize the association of uL-FABP with graft failure. Nonlinearity was tested by using the likelihood ratio test, comparing models with linear or linear and cubic spline terms. The association of uL-FABP with risk of graft failure was then analyzed using Cox proportional-hazards regression analyses. In model 1 we performed multivariable-adjusted analyses according to the Reference Model (determined as explained in the preceding section). Thereafter, we computed further models, with additive adjustments to Model 1 to avoid inclusion of too many variables for the number of events. Thus, we additionally adjusted for donor and transplantation characteristics (donor age, donor type [living, deceased after brain dead and deceased after cardiac dead], donor height, donor weight, donor diabetes and hypertension; and time since transplantation; Model 2); inflammation and immunosuppressive therapy (high-sensitivity C-reactive protein, use of calcineurin inhibitors, use of proliferation inhibitors, and cumulative prednisolone dose; Model 3); blood pressure and metabolism-related variables (systolic blood pressure, use of antihypertensive medication, fasting plasma glucose, plasma HDL cholesterol, and triglycerides; Model 4) and a combination of the prior (systolic blood pressure, diastolic blood pressure, high-sensitivity C-reactive protein, and plasma HDL cholesterol; Model 5).

2.4.3 | Discrimination power and model risk-prediction ability for graft failure

We explored uL-FABP risk-prediction ability by calculating the c-statistic of the Reference Model, and then the c-statistic after adding uL-FABP, to investigate whether adding uL-FABP to the Reference Model increased the model risk-prediction ability. We also performed an *F*-test to check whether the difference between both risk-prediction ability models was significant. Next, the Akaike information criterion (AIC) was used to evaluate model fit. Finally, a receiver operating characteristic (ROC) curve was generated for the reference model before and after inclusion of uL-FABP, the area under the curve was calculated for both curves.

2.4.4 | Secondary analyses and sensitivity analyses

In secondary analyses, we performed multivariable Cox proportional-hazards regression analyses, evaluation of model risk-prediction ability, and model fit analoguously to primary analyses, yet computing 24-hour urinary protein excretion and uL-FABP excretion as their indexed concentrations by urinary creatinine concentration.

Finally, as sensitivity analyses, we also evaluated the prospective association of uL-FABP with risk of graft failure, and model risk-prediction ability and model fit, with exclusion of patients (a) with eGFR <30 ml/min/1.73 m^2, (b) with proteinuria (urinary protein excretion >0.5 g/24-h), (c) who developed graft failure within the first year of follow-up, (d) patients with deceased donor, (e) patients with living donor (f) and who received preemptive transplantation; and finally, by (g) setting patients with uL-FABP below detection limit to half of the derection limit.

2.4.5 | Association of uL-FABP with secondary outcomes

Exploratory univariable and eGFR-adjusted linear regression (for continuous variables), logistic regression (for dichotomic variables), and Cox regression (for time-dependent outcomes) analyses were performed to evaluate the association between uL-FABP and other outcomes of clinical importance for KTR (progressive proteinuria, clinical episodes of rejection, graft loss, cardiovascular events, cardiovascular mortality, and all-cause mortality).

3 | RESULTS

3.1 | Baseline characteristics

Baseline characteristics of the study population are presented in Table 1. In total 638 KTR (57% men, 53 ± 13 years old, 99% Caucasian) were included in the analyses. Median (IQR) uL-FABP was 2.11 (0.93–7.37) µg/24 -h. Mean eGFR was 52 ± 20 ml/min/1.73 m^2, and median urinary protein excretion was 0.20 (0.02–0.39) g/24 h. Preemptive transplantation was performed in 102 (16%) patients. Two hundred and sixteen (34%) of the organs were obtained from living donors and mean donor age was 43 ± 15 years. Patients in the highest tertile of uL-FABP, when compared to the other two tertiles, were in their majority male ($p < .001$), had lower eGFR ($p < .001$), higher urinary protein excretion ($p < .001$), and received a kidney from an older donor ($p < .001$), whom where most usually female ($p = .02$). As for their immunosuppressive regimen they more frequently used tacrolimus ($p = .002$). Patients in the third tertile also had higher SBP and DBP ($p < .001$), more frequently used any antihypertensive medication ($p = .03$), had lower HDL cholesterol ($p < .001$), and had higher triglycerides ($p = .005$) and plasma glucose ($p = .01$). Finally, patients in the highest tertile had more apparent inflammation shown by higher hs-CRP concentration ($p = .01$).

3.2 | Reference model

During a median (IQR) follow-up of 5.3 (4.4–5.8) years, 80 (13%) patients developed graft failure at a median of 2.7 (1.4–4.3) years after enrollment. The most frequent cause of graft failure was chronic rejection (75%) followed by recurrence of primary disease (10%). Within patients whose values of uL-FABP were above the median, the most frequent cause also was chronic rejection (78%), followed by recurrence of primary disease (11%) and infection of the graft (4%); and within patients below the median the most common cause was chronic rejection, in a lower proportion (43%), followed by acute rejection (29%). The distributions of causes among subgroups was significantly different ($p < .001$; Table S1). In univariable Cox regression analyses of the associations between different literature-based established risk factors with the risk of graft failure, the presence of HLA antibodies class II showed the strongest association with outcome (HR 3.50; 95% CI 2.22–5.50; $p < .001$). Other variables significantly associated with the risk of graft failure and also included in the Reference Model computed by means of backwards selection were eGFR (HR 0.70; 95% CI 0.65–0.76 per 1-SD increment; $p < .001$), urinary protein excretion (HR 1.50; 95% CI 1.37–1.63 per 1-SD increment; $p < .001$), recipient age (HR 0.77; 95% CI 0.62–0.95 per 1-SD increment; $p = .01$), and preemptive transplantation (HR 0.39; 95% CI 0.17–0.89; $p = .03$) (Table S2).

3.3 | uL-FABP and association with the risk of graft failure

uL-FABP was univariately associated with the risk of graft failure as shown in Cox regression analyses (HR 3.37; 95% CI 2.66–4.29 per 1-SD increment; $p < .001$; Table S2) and restricted cubic spline regression (Figure 1). Multivariable-adjusted analyses showed that this association was independent of adjustment for variables of the Reference Model (HR 1.75; 95% CI, 1.27–2.41 per 1-SD increment; $p = .001$; Model 1), and independent of additional adjustment for donor and transplantation characteristics (Model 2), inflammation and immunosuppressive therapy (Model 3), blood pressure and metabolism-related characteristics (Model 4) and a combination of the prior (Model 5; Table 2).

3.4 | uL-FABP and prediction of graft failure

The reference model had a c-statistic of 0.85 and a model fit, evaluated by the AIC, of 843 for risk prediction of graft failure. The risk prediction of the model was significantly improved by the addition of uL-FABP (c-statistic of 0.87 and AIC of 833; *F*-test for difference between models, $p < .001$; Table 3). ROC curves built to assess the prediction value of the reference model before and after the inclusion of uL-FABP for risk of graft failure are shown in Figure 2. The area under the curve (AUC) of the ROC curve for the reference model was 87 and improved to 89 after inclusion of uL-FABP.

Secondary analyses, in which concentrations of uL-FABP and urinary protein excretion were indexed by urinary creatinine excretion showed the same independent association (HR 2.03; 95% CI 1.50–2.77 per 1-SD increment; $p < .001$; Table S3). Our findings were also robust in several sensitivity analyses. Urinary L-FABP remained independently associated with the risk of graft failure in analyses performed after exclusion of patients (a) with eGFR <30 ml/min/1.73 m^2 (HR 2.42; 95% CI 1.59–3.69 per 1-SD increment), (b) with proteinuria (HR 2.43; 95% CI 1.33–4.44 per 1-SD increment), (c) who developed graft failure within the first year of follow-up (HR 2.03; 95% CI 1.42–2.92 per 1-SD increment), (d) patients with deceased donor (HR 2.51; 95% CI 1.24–5.07 per 1-SD increment), (e) patients with living donor (HR 1.61; 95% CI 1.11–2.33 per 1-SD increment), (f) who received preemptive transplantation up (HR 1.71; 95% CI 1.24–2.38 per 1-SD increment), and (g) after setting patients below the detection limit of uL-FABP to half of the detection limit (HR 1.75; 95% CI 1.27–2.42 per 1-SD increment). The improvement of risk prediction ability of the reference model also remained significant under the same sensitivity analyses (Table S4).

TABLE 1 Baseline characteristics of the study population

Baseline characteristics	Overall KTR *n* = 638	Tertiles of uL-FABP			*p*
		Tertile 1	Tertile 2	Tertile 3	
		<1.20 µg/24 h	1.20–4.61 µg/24 h	>4.61 µg/24 h	
uL-FABP, µg/24 h	2.11 (0.93–7.37)	0.65 (0.35–0.93)	2.10 (1.59–3.03)	13.82 (7.32–28.86)	–
Demographics and anthropometrics					
Age, years	53 ± 13	53 ± 13	54 ± 13	52 ± 13	.14
Sex (male), *n* (%)	363 (57)	90 (43)	127 (60)	146 (69)	<.001
Caucasian ethnicity, *n* (%)	635 (99)	211 (99)	211 (99)	213 (100)	.37
Body mass index, kg/m^2	26.5 ± 4.8	26.3 ± 5.1	27.0 ± 4.7	26.3 ± 4.6	.22
Renal allograft function					
eGFR, ml/min/1.73 m^{2a}	52 ± 20	62 ± 18	55 ± 19	41 ± 18	<.001
Urinary protein excretion, g/24 h[b]	0.20 (0.02–0.39)	0.02 (0.02–0.18)	0.17 (0.02–0.28)	0.45 (0.24–1.03)	<.001
Kidney transplant characteristics					
Preemptive transplantation, *n* (%)	102 (16)	35 (17)	33 (16)	34 (16)	.96
Time since transplantation, years	5.8 (2.0–12.2)	6.3 (3.5–12.8)	5.4 (1.3–11.0)	5.1 (1.4–12.3)	.07
Primary kidney disease, *n* (%)					
Primary glomerulosclerosis	183 (29)	61 (29)	56 (26)	66 (31)	.10
Kidney cyst	131 (21)	38 (18)	53 (25)	40 (19)	
Tubulointerstitial nephritis and pyelonephritis	76 (12)	31 (15)	19 (9)	26 (12)	
Glomerulonephritis	47 (7)	19 (9)	18 (9)	10 (5)	
Renovascular disease	38 (6)	9 (4)	10 (5)	19 (9)	
Other	163 (25)	54 (25)	57 (26)	51 (24)	
Acute rejection, *n* (%)	176 (28)	57 (27)	50 (24)	69 (32)	.12
HLA class I antibodies positive, *n* (%)	97 (15)	29 (14)	34 (16)	34 (16)	.75
HLA class II antibodies positive, *n* (%)	106 (17)	28 (13)	35 (16)	43 (20)	.15
Kidney donor characteristics					
Status, *n* (%)					
Living	205 (32)	62 (29)	76 (36)	67 (32)	.52
Deceased after brain dead	319 (50)	112 (53)	102 (48)	105 (49)	
Deceased after cardiac dead	80 (13)	29 (14)	26 (12)	25 (12)	
Unknown	34 (5)	9 (4)	9 (4)	9 (4)	
Age, years[c]	44 (15)	38 (15)	46 (15)	47 (15)	<.001
Sex (male), *n* (%)[d]	322 (51)	124 (59)	102 (48)	96 (45)	.02
Height, m[e]	1.75 (0.16)	1.75 (0.18)	1.74 (0.13)	1.72 (0.16)	.67
Weight, kg[f]	76 (17)	75 (17)	77 (17)	75 (16)	.53
Hypertension, *n* (%)	50 (8)	14 (7)	16 (8)	20 (9)	.53
Diabetes mellitus, *n* (%)	6 (1)	0 (0)	2 (1)	4 (2)	.09
Immunosuppressive therapy					
Cumulative prednisolone dose, g	18.1 (5.5–36.2)	18.5 (10.2–37.7)	17.8 (4.2–34.7)	16.7 (4.7–37.1)	.16
Use of sirolimus or rapamune, *n* (%)[g]	9 (1)	4 (2)	1 (1)	4 (2)	.38
Use of calcineurin inhibitors					
Cyclosporine, *n* (%)	244 (38)	85 (40)	87 (41)	72 (34)	.26
Tacrolimus, *n* (%)	119 (19)	29 (14)	34 (16)	56 (26)	.002

(Continues)

Table 1 (Continued)

Baseline characteristics	Overall KTR *n* = 638	Tertiles of uL-FABP			
		Tertile 1 <1.20 µg/24 h	Tertile 2 1.20–4.61 µg/24 h	Tertile 3 >4.61 µg/24 h	*p*
Use of proliferation inhibitors					
Mycophenolic acid, *n* (%)	419 (66)	142 (67)	147 (69)	130 (61)	.20
Azathioprine, *n* (%)	113 (18)	37 (18)	35 (16)	41 (19)	.74
Cardiovascular history and lifestyle					
Systolic blood pressure, mm Hg[a]	136 ± 17	132 ± 15	137 ± 17	139 ± 19	<.001
Diastolic blood pressure, mm Hg[a]	83 ± 11	80 ± 10	83 ± 10	85 ± 12	<.001
Use of antihypertensive treatment, *n* (%)	559 (88)	177 (84)	185 (87)	197 (93)	.03
Alcohol intake >30 g/day, *n* (%)[h]	28 (4)	11 (5)	7 (3)	10 (5)	.35
SQUASH score, intensity × h	5040 (1811–7650)	5280 (2220–7470)	4470 (1470–6760)	5360 (1940–8705)	.67
Fasting lipids					
Total cholesterol, mg/dl[b]	199 ± 44	202 ± 43	196 ± 42	198 ± 46	.45
HDL cholesterol, mg/dl[i]	54 ± 19	58 ± 19	54 ± 19	49 ± 17	<.001
LDL cholesterol, mg/dl[i]	115 ± 36	117 ± 37	113 ± 36	116 ± 37	.23
Triglycerides, mg/dl[j]	148 (110–202)	139 (107–188)	143 (103–200)	164 (117–252)	.005
Diabetes and glucose homeostasis					
Diabetes mellitus, *n* (%)	168 (26)	51 (24)	51 (24)	66 (31)	.05
Plasma glucose, mg/dl[k]	95 (86–110)	94 (85–106)	95 (88–112)	95 (86–112)	.01
HbA_{1C}, %[l]	5.8 (5.5–6.3)	5.8 (5.5–6.2)	5.9 (5.5–6.3)	5.8 (5.5–6.2)	.29
Inflammatory biomarkers					
Leukocyte count, $10^9/L$[k]	8.2 ± 2.6	8.1 ± 2.4	8.3 ± 2.6	8.1 ± 2.8	.50
hs-CRP, mg/L[m]	1.6 (0.7–4.7)	1.4 (0.7–3.7)	1.5 (0.6–5.1)	1.9 (0.8–5.5)	.01

Abbreviations: KTR, kidney transplant recipients; uL-FABP, urinary liver-type fatty acid-binding protein; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; SQUASH, short questionnaire to assess health-enhancing physical activity; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; HbA_{1C}, glycated hemoglobin; hs-CRP, high-sensitivity C-reactive protein.

[a]Data available in 635 patients.
[b]Data available in 637 patients.
[c]Data available in 620 patients.
[d]Data available in 625 patients.
[e]Data available in 521 patients.
[f]Data available in 522 patients.
[g]Data available in 597 patients.
[h]Data available in 590 patients.
[i]Data available in 628 patients.
[j]Data available in 629 patients.
[k]Data available in 636 patients.
[l]Data available in 609 patients.
[m]Data available in 600 patients.

3.5 | uL-FABP and prospective association with the risk of other outcomes

The exploration of the association of uL-FABP with secondary outcomes showed that it was significantly prospectively associated with, graft loss (HR 1.90; 95% CI 1.65–2.20 per 1-SD increment; $p < .001$), development of cardiovascular events (HR 1.54; 95% CI 1.26–1.89 per 1-SD increment; $p < .001$), cardiovascular mortality (HR 1.58; 95% CI 1.23–2.05 per 1-SD increment; $p < .001$), and all-cause mortality (HR 1.34; 95% CI 1.14–1.59 per 1-SD increment; $p < .001$). However, after adjustment for graft function, the associations with cardiovascular mortality and all-cause mortality were no longer significant (HR 1.35; 95% CI 1.00–1.82 per 1-SD; $p = .05$ and HR 1.19; 95% CI 0.98–1.44 per 1-SD; $p = .08$, respectively). No association was found between

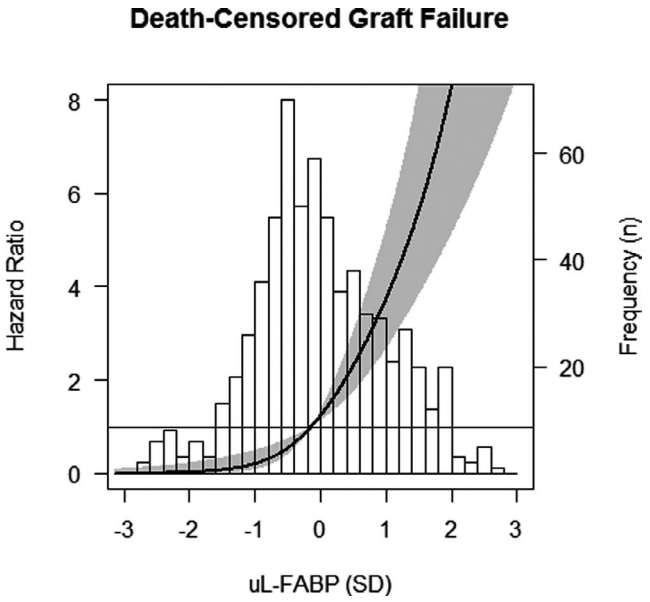

FIGURE 1 Restricted cubic spline regression of the association between uL-FABP and risk of death-censored graft failure. Data were fit by a Cox proportional-hazards regression model that was based on restricted cubic splines. The solid line represents the HR. The gray area represents the 95% CI

TABLE 2 Multivariable-adjusted association between uL-FABP and risk of graft failure in 638 KTR

	uL-FABP, per 1-SD increment		
Models	**HR**	**95% CI**	***P***
Model 1	1.75	1.27–2.41	.001
Model 2	1.84	1.27–2.67	.001
Model 3	1.90	1.34–2.67	<.001
Model 4	1.73	1.22–2.45	<.002
Model 5	1.80	1.25–2.50	.001

Note: Cox proportional-hazards regression analyses were performed to assess the association of uL-FABP with risk of graft failure (n_{events} = 80). Multivariable-adjusted model 1 included adjustment for age, estimated glomerular filtration rate, urinary protein excretion, preemptive transplantation, and human leukocyte antigen II mismatch (Reference Model). Additional adjustment was performed for donor and transplantation characteristics (Model 2), inflammation and immunosuppressive therapy (Model 3), blood pressure and metabolism-related characteristics (Model 4) and a combination of the prior (Model 5).

Abbreviations: uL-FABP, urinary liver-type fatty acid-binding protein; KTR, kidney transplant recipients; HR, hazard ratio; CI, confidence interval.

uL-FABP and progressive proteinuria during follow-up (Std B. 0.01; 95% −0.07 to 0.15 per 1-SD increment; p = .81) or clinical episodes of rejection (OR 1.37; 95% CI 0.92–2.05 per 1-SD increment; p = .12; Table S5).

4 | DISCUSSION

In stable KTR, this study shows that uL-FABP is positively and strongly associated with the risk of graft failure, independently of several established risk factors, including HLA mismatching, eGFR, and urinary protein excretion. Moreover we show that uL-FABP has a strong predictive value for this outcome and that inclusion of uL-FABP into a risk-prediction model composed by well-established risk factors of graft failure, seems to significantly improve risk-prediction value and model fit; although this findings would require validation in an external cohort.

Chronic graft failure remains a major challenge in kidney transplantation.[23] A main characteristic of this phenomenon is arterial intimal fibrosis, which generates a progressive luminal narrowing of graft vessels, and therefore progressive ischemia of the transplanted kidney[14] and loss of kidney allograft function (previously known as chronic allograft nephropathy).[24] Current clinically used biomarkers, such as eGFR and urinary protein excretion, even though they are strongly associated with graft failure, share the drawback of being a reflection of advanced structural damage.[7] Therefore, by the time an alteration is identified through outpatient monitoring of otherwise stable KTR, therapeutic interventional options are rather limited.[10]

Novel renal tubular biomarkers such as uL-FABP, may offer an alternative approach to overcome these limitations and act more anticipatory. Renal tubule epithelial cells are especially vulnerable and fast responding to hypoxic challenge, therefore early identification of this tubular insult has been proposed as a better approach to timely detect tissue injury.[11] L-FABP is a 14 kDa protein[24,25] part of a family of intracellular lipid chaperons,[11] which in the kidney it is exclusively expressed in the epithelial cells of the proximal tubule.[26] The role of L-FABP is to eliminate lipid peroxides, produced under circumstances of hypoxia-induced oxidative stress, by transferring them into the tubular lumen for further urinary excretion.[27]

Under hypoxic conditions, its synthesis is increased by the activation of an hypoxia-inducible factor 1α response element in the promotor region of the L-FABP gene and its enhanced genetic expression within the kidney has shown to be protective of ischemic injury in rat models.[12,26] This response to injury leads to an increase of uL-FABP, which is why it works as a marker of ongoing of renal hypoxia.[27] The same study showed that in the kidney post-transplantation setting during a short-follow up, uL-FABP was indeed increased by hypoxic conditions of the graft, with a direct correlation between uL-FABP and ischemia time during transplantation and also with outcomes with it being directly associated with longer hospital stay after the procedure.[12] Also, higher concentrations of L-FABP during hypothermic machine perfusion have been associated with lower eGFR in the short term after transplantation.[15] We show, for the first time, that uL-FABP is also a promising biomarker for long-term clinical outcomes in KTR, with a prospective independent association between uL-FABP and risk of graft failure. Remarkably, as for the predictive value of uL-FABP, it has shown consistent promising results in other clinical settings, that is, acute kidney injury and chronic kidney disease,[28–30] being able to improve discrimination when added to models of established risk factors.[31] In agreement with aforementioned studies, we found that uL-FABP had good prediction value for graft failure in this particular cohort, and more

TABLE 3 Risk-prediction ability of uL-FABP in addition to established risk factors of graft failure (reference model), in 638 KTR

		Multivariable-adjusted regression coefficients			Risk-prediction ability coefficients		
		HR	95% CI	*p*	c-statistic	AIC	*p**
Reference model	Age, per 1-SD increment	0.72	0.57–0.91	<.005	0.85	843	Ref.
	eGFR, per 1-SD increment	0.78	0.71–0.86	<.001			
	Urinary protein excretion, per 1-SD increment	1.18	1.04–1.33	.008			
	Preemptive transplantation	0.35	0.15–0.82	<.016			
	HLA class II antibodies, positive	2.37	1.48–3.78	<.001			
	+uL-FABP, per 1-SD increment	1.75	1.27–2.41	.001	0.87	833	<.001

Abbreviations: AIC, Akaike information criterion; CI, confidence interval; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; HR, hazard ratio; KTR, kidney transplant recipients; uL-FABP, urinary liver-type fatty acid-binding protein.

**p*-value of *F*-test for difference between the reference model and the model including uL-FABP.

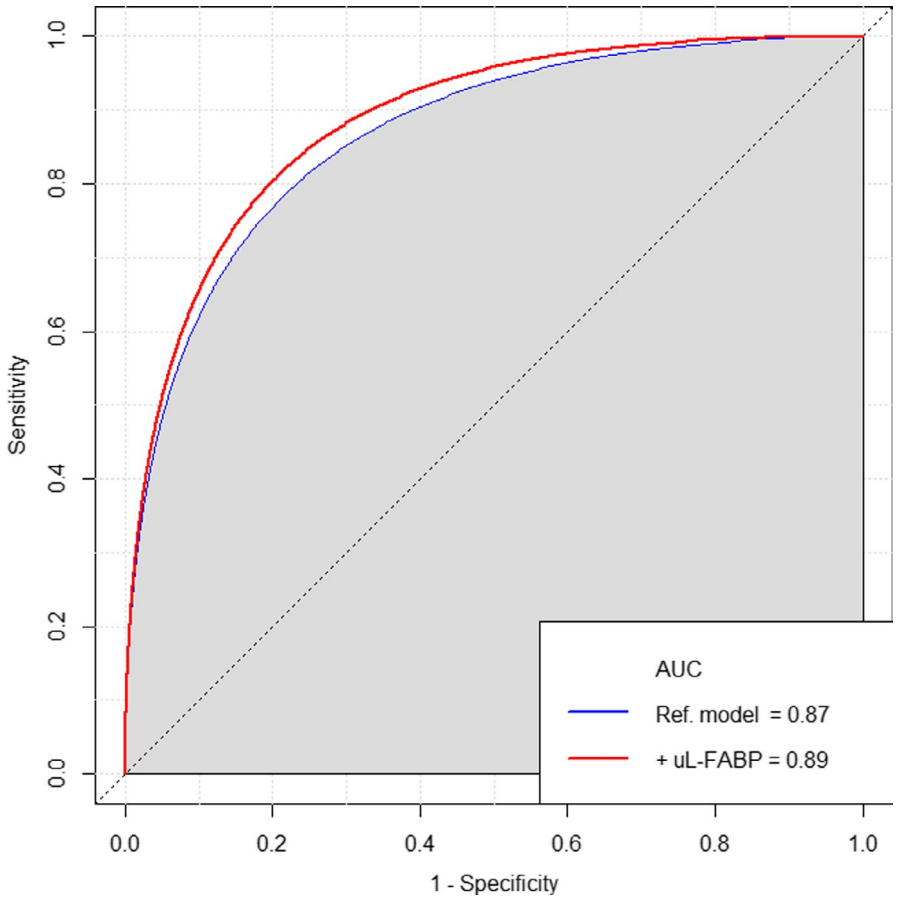

FIGURE 2 ROC curve of the reference model before and after addition of uL-FABP for prediction of death-censored graft failure. *F*-test for difference between models: $p < .001$. Blue line: ROC curve of a reference model composed by age, estimated Glomerular Filtration Rate, urinary protein excretion, preemptive transplantation, and human leukocyte antigen II mismatch. Red line: ROC curve of the reference model after addition of uL-FABP. AUC, area under the curve; ROC, receiver operator characteristic; uL-FABP, urinary liver-type fatty acid-binding protein

importantly, improved the prediction value achieved by currently used biomarkers for risk assessment.

To the date, no cut-off point for uL-FABP has been validated for clinical implementation. We consider that the value of adding uL-FABP into clinical monitoring would lie in the fact that elevated (or increasing) uL-FABP reflects in real time a graft suffering from ischemic injury, at a point when therapeutic strategies could avoid progression to graft failure. It should be realized that due to the sensitivity of tubular cells to hypoxia, elevation of uL-FABP is a very early phenomena[32–34] and could well occur before structural changes

are established, which is a significant advantage over markers like proteinuria and eGFR.[10] Although tubular ischemia is a multifactorial process,[35] the main recognized enhancer of this phenomenon is immunologic aggression of the host against the allograft.[35,36] Therefore, attention should be given to other strategies to salvage the graft such as the tailoring of immunosuppressive regimens.[36,37] However, we acknowledge that supporting these hypotheses requires further evidence. Our findings are a call for the performance of studies that allow for defining cut-off points for uL-FABP and assess its impact in real clinical practice.

A strength of this study is that collection of our data was ensured by the continuous surveillance system of the outpatient clinic of our university hospital and close collaboration with affiliated hospitals which provided us with complete information on endpoints during follow-up. Moreover, our extensively phenotyped cohort allowed us to evaluate several potential confounders, and the robustness of our findings was tested with multiple sensitivity analyses. Because of its observational design, our study does not allow hard conclusions on causality, and reversed causation or residual confounding may occur. Furthermore, we did not have data on de novo DSA and nonadherence, so we could not explore associations with these outcomes. Next, the current study was performed in a single center with over-representation of Caucasian subjects, which calls prudence to extrapolation of our results to different populations regarding ethnicity. Finally, although the main source of uL-FABP is kidney tubular production,[32,33] it is also produced in other organs and can be filtered into urine,[11] therefore it cannot be considered a completely kidney-specific biomarker; however, studies performed on this matter show that: (a) in a mice model of acute kidney injury, the magnitude of the urine increase after kidney injury was much higher than that of the plasma,[33] and (b) in a human clinical study performed in patients post–cardiopulmonary bypass surgery, uL-FABP only increased in patients that presented acute kidney injury afterwards.[34] These observations support the notion that uL-FABP concentration is mostly determined by proximal tubule production and excretion after kidney injury, even in the context of a systemic challenge.

In conclusion, this is the first study showing that uL-FABP, being a biomarker of hypoxic tubular injury, is independently associated with long-term graft failure in KTR and could offer a different pathophysiological-based approach to improve the prediction value of well-established risk factors of graft failure to allow earlier detection of kidney tissue insult and earlier identification of otherwise stable outpatient KTR at high risk of graft failure. The utility of a risk-prediction model for graft failure that additionally accounts for uL-FABP in clinical care of stable KTR requires validation in an external cohort before clinical application.

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DISCLOSURE

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. Takeshi Sugaya is a researcher of the company that developed the assay used to measure L-FABP in the current study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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