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Endothelial Dysfunction and 6-Year Risk of Mortality in Kidney Transplant Recipients

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Background. Endothelial dysfunction is an early and potentially reversible stage in the atherosclerotic process. We assessed endothelial dysfunction noninvasively in kidney transplant recipients (KTRs) and evaluated the association with mortality and graft outcomes. **Methods.** Flow-mediated dilation (FMD) was measured in arteria brachialis by ultrasound, with baseline diameters obtained at rest and maximal diameters obtained during reactive hyperemia occurring after 5 min of forearm occlusion. FMD% is the percentage difference of flow-mediated dilation relative to baseline. Endpoints on mortality and graft outcomes were collected from The Norwegian Renal Registry. The distribution of risk according to FMD levels was assessed in Cox regression using a restricted cubic spline function. FMD was dichotomized using receiver operating characteristic analysis to identify optimal cut points at maximal sensitivity and specificity. **Results.** From a total of 269 KTRs in 2012, 152 (56.5%) were eligible and examined 10 wk after transplantation, and 145 had successful FMD measurements. During a mean follow-up of 6.5 y, 26 patients died, 11 lost their graft, and 34 experienced either graft loss or death. Mortality increased with lower FMD levels until about 5% dilation and did not change with further reduction in FMD% (P for nonlinearity <0.01). An optimal cut point of FMD ≤5.36% defined impaired endothelial function and FMD% below this level, was associated with fatal outcome, hazard ratio (HR), 9.80 (1.29–74.62), P = 0.03, uncensored graft loss, HR, 7.80 (1.83–33.30), P = 0.01, but an association with death-censored graft loss was lost after adjusting for pulse pressure, HR, 4.58 (0.55–37.92), P = 0.16. **Conclusions.** We found that impaired FMD is strongly associated with mortality in KTRs.

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Kidney transplant recipients (KTRs) have an elevated risk of cardiovascular disease and mortality.[1] Although the cardiovascular mortality in KTRs is lower than in dialysis patients, it remains 3- to 5-fold higher than in the general population.[2] This increased risk is not completely explained by traditional cardiovascular risk factors, such as hypertension, diabetes, blood lipids, and smoking.[3] Novel risk factors may contribute, such as chronic inflammation, mineral-bone disorder, malnutrition, and endothelial dysfunction.[4,5]

Endothelial dysfunction is identified early in the atherosclerotic process[6] and is associated with cardiovascular events in nontransplanted patients.[7,8] It is prevalent both in patients with chronic kidney disease[9,10] and kidney transplant recipients[11] and is possibly associated with graft loss.[12] Flow-mediated dilatation (FMD) is a validated functional measure of endothelial function, in which endothelial-dependent vasodilatation is measured by ultrasound, usually in a conduit (eg, the brachial) artery.[8,13] Briefly, the endothelium releases nitrogen monoxide (NO) when exposed to increased blood flow; this NO diffuses to surrounding vascular smooth muscle and causes relaxation and vasodilatation.[14] In endothelial dysfunction, there is impaired NO bioavailability, and the test reveals less vasodilatation. Of note, NO has several antiatherogenic properties (eg, limiting leukocyte[15] and platelet[16] adhesion and proliferation of vascular smooth muscle cells).[17] Thus, impaired FMD indicates poor vascular health.

We previously described the clinical correlates of FMD in KTRs.[18] In common with studies from the general population,

FMD was negatively correlated with age and blood pressure, especially pulse pressure.[19,20] In addition, we found impaired FMD to be related to time in renal replacement therapy.[18] The aim of the present study is to assess if FMD is associated prospectively with patient and graft outcomes.

PATIENTS AND METHODS

The study population and measurement of FMD have been described previously.[18] Briefly, KTRs from the national transplant center in Norway were examined by ultrasound about 10 wk after transplantation. Inclusion was during 2012, and 152 of 269 patients were included. Reasons for exclusion were lack of consent (n = 31), unavailable examinator (n = 30), earlier return to home hospital due to comorbid conditions (n = 37), early graft loss (n = 5), and other patient-related factors (n = 14). Such factors were carrier of resistant microbes (n = 5), participating in other study (n = 5), or intercurrent illness (n = 4). FMD was measured by 1 skilled examinator (D.O.D.) in arteria brachialis according to established guidelines.[13] Arteria brachialis was visualized before (baseline) and after 5 min of forearm blood stasis with a sphygmomanometer cuff (>200 mm Hg). After cuff release, the maximum vasodilatation during the following 90 s was used to calculate FMD. FMDmm is the difference between maximum diameter and baseline in millimeters, and percentage difference of flow-mediated dilation relative to baseline (FMD%) is the percentage difference relative to baseline. Thirteen patients had a repeat examination after 1 week; FMD% intrapatient SD was 1.8, intrapatient coefficient of variation was 0.34, and intraclass correlation was 0.66. Endpoints were collected from the Norwegian Renal Registry. Death-censored graft loss denotes return to dialysis or retransplantation, whereas uncensored graft loss includes death as an outcome. Cardiovascular and cerebrovascular events are reported annually to the registry and summarized as first nonfatal cardiovascular events in this analysis. Follow-up was censored on November 10, 2018. The study was approved by the Committees for Medical and Health Research Ethics in Health Region South-East in Norway and performed in accordance with the Declaration of Helsinki and the Declaration of Istanbul.

The distribution of risk according to levels of the risk factors was assessed by a restricted cubic spline function with 3 knots in R 3.1.0 (R Foundation for Statistical Computing, Vienna, Austria). To simplify the model and allow for uniform estimates of hazard ratios, FMD variables were dichotomized. Receiver operating characteristic analysis was used to optimize sensitivity and specificity for the dichotomized FMD variables (ie, cut points were defined at maximal Youden index [sensitivity + specificity–1]). Stata 12.1 (StataCorp, College Station, TX) was used to obtain Kaplan-Meier plots and Cox multivariable models. Covariates included the previously described clinical correlates of FMD (Tables 1–2),[18] with further selection to a multivariable model based on significant association with the outcome. A 2-sided P of 0.05 was considered statistically significant.

RESULTS

A total of 152 out of 269 (56.5%) eligible KTRs were examined approximately 10 wk after transplantation. The patients who were not examined had a similar age and gender distribution as those who were examined ($P > 0.78$) but experienced a higher crude mortality rate (hazard ratio [HR], 1.91, $P = 0.01$). Among the 152 patients examined, 145 had successful FMD measurements and constitute the present study sample. FMD could not be assessed in 7 patients because of poor ultrasound image quality.

Baseline characteristics are presented according to mortality outcome in Tables 1 and 2; variables with a known association with FMD[18] were included.

During a median follow-up of 6.5 y, 26 patients died, 11 patients lost their kidney graft, and 34 experienced either graft loss or death. The primary cause of death was coded as heart disease in 5 patients, infection in 7, malignancy in 10, and other or unknown in 2. The majority (7) of grafts lost were due to rejection. The association between FMD and death was nonlinear with a ceiling effect at low FMD levels, both in an unadjusted analysis (Figure 1 for FMD% and **Figure S1, SDC**, http://links.lww.com/TXD/A390 for FMDmm) and in multivariable models (Figures S2A–S2B; nonlinearity $P < 0.01$). Similarly, ceiling effects were seen in models of the association between FMD and uncensored renal graft loss (data not shown), although in these models, the nonlinearity component was not statistically significant (nonlinearity $P < 0.10$). The association between FMD and death-censored renal graft loss demonstrated no significant nonlinearity in unadjusted analysis (nonlinearity $P > 0.27$) and appeared to have no ceiling effect. This was not tested further in multivariable models due to few events. For the remaining covariates, associations were linear (no significant nonlinearity).

Due to few events, full nonlinear terms (ie, cubic spline functions) could not be included in the multivariable models. Instead, and to simplify the models, FMDmm and FMD% were recoded to dichotomous variables. For FMDmm, the optimal cut point with regard to sensitivity and specificity from receiver operating characteristic analysis was similar regardless of outcome (ie, ≤0.145 mm for death, ≤0.14 for uncensored graft loss, ≤0.145 mm for death-censored graft loss and, thus a dilatation ≤0.145 mm was used to indicate impaired FMDmm). For FMD%, the optimal cut point was ≤5.36 for both death and uncensored graft loss, whereas for death-censored graft loss a cut point ≤1.0 seemed slightly superior to ≤5.36 (ie, Youden index 0.389 versus 0.327). However, for simplicity, a dilatation ≤5.36% was used to indicate impaired FMD% in all analyses. Sensitivity and specificity using these cut points are shown in the Supplementary Material, **SDC**, http://links.lww.com/TXD/A390.

Kaplan-Meier plots of graft and patient outcomes in groups defined by normal or impaired FMD are shown in Figures 2A–2C for FMD% and in **Figure S3A–S3C, SDC**, http://links.lww.com/TXD/A390 for FMDmm. Survival analyses are shown in Tables 3–5. Both in relative (FMD%) and absolute (FMDmm) terms, an impaired FMD was independently associated with mortality ($P < 0.03$) and uncensored graft loss ($P < 0.01$). Additional adjustments for current smoking and cholesterol levels did not materially change these results (data not shown). Nonfatal cardiovascular events were reported in 22 patients. In a death-censored analysis, neither impaired FMD% nor impaired FMDmm was associated with this outcome, either in univariate (both $P > 0.09$) or in age- and gender-adjusted models (both $P > 0.57$). An association

TABLE 1.

Baseline variables according to 6-y mortality

	Overall	Dead	Alive	*P*
N	145	26	119	
Age (y)	54.9 ± 12.9	63.8 ± 8.1	52.9 ± 12.9	0.002
Female	50 (34.5)	10 (38.5)	40 (33.6)	0.64
Cardiovascular disease	37 (25.5)	10 (38.5)	27 (22.7)	0.10
DM or PTDM	41 (28.3)	15 (57.7)	26 (21.8)	<0.001
PP (mm Hg)	64.6 ± 21.8	77.1 ± 24.1	61.9 ± 20.3	0.52
TimeRRT (y)	3.7 ± 6.8	4.7 ± 8.7	3.5 ± 6.3	0.31
FMD%	4.4 ± 3.4	2.4 ± 1.7	4.8 ± 3.6	<0.001
FMD% range		0–6.0	0–14.3	
FMDmm	0.16 ± 0.12	0.10 ± 0.07	0.18 ± 0.12	<0.001
FMDmm range		0–0.25	0–0.53	

Variables correlating with FMD according to previous publication[18] by mortality outcome. Mean values ± SD or n (%). Cardiovascular disease includes cardiac or cerebral vascular disease. FMD% is the percentage difference of flow-mediated dilation relative to baseline. FMDmm is the difference between maximum diameter and baseline in millimeters.
DM, diabetes mellitus; FMD, flow-mediated dilation; PP, pulse pressure; PTDM, posttransplant diabetes mellitus; TimeRRT, time in renal replacement therapy.

TABLE 2.

Baseline variables according to endothelial function (FMD%)

	Overall	FMD% ≤ 5.36	FMD% > 5.36	*P*
N	145	88	57	
Age (y)	54.9 ± 12.9	58.1 ± 11.9	49.8 ± 12.8	<0.001
Female	50 (34.5)	27 (30.7)	23 (40.4)	0.23
Cardiovascular disease	37 (25.5)	29 (33.0)	8 (14.0)	0.01
DM or PTDM	41 (28.3)	34 (38.6)	7 (12.3)	0.001
PP (mm Hg)	64.6 ± 21.8	70.4 ± 22.2	55.6 ± 17.8	<0.001
TimeRRT (y)	3.7 ± 6.8	5.0 ± 7.9	1.7 ± 3.5	0.003

FMD% is the percentage difference of flow-mediated dilation relative to baseline.
DM, diabetes mellitus; PP, pulse pressure; PTDM, posttransplant diabetes mellitus; TimeRRT, time in renal replacement therapy.

with death-censored graft loss was lost after controlling for pulse pressure.

DISCUSSION

To our knowledge, this is the first study that reports the association between FMD and survival in KTRs. Impaired FMD was independently associated with uncensored graft loss and mortality, although not with death-censored graft loss. The association with mortality was consistent for vasodilation relative to baseline diameter (FMD%) and in absolute terms (FMDmm) and FMD had a high sensitivity (>0.85) for mortality, which is also visualized in the survival plots (ie, almost all deaths were in the group defined by impaired FMD). These findings highlight that there may be strong protective effects of a normal FMD and a "healthy" endothelium, which is consistent with previous studies in other cohorts.[7]

The lack of association between impaired FMD and nonfatal cardiovascular events may at first seem counterintuitive. Furthermore, only 5 of the 26 (19%) deaths were coded as cardiac. We speculate that nonatherosclerotic cardiovascular mechanisms may have contributed to the increased mortality risk seen in our cohort. It is well known that patients with end-stage kidney disease are prone to arrhythmia and heart failure,[1] which can predispose to death when experiencing noncardiac intercurrent illness. Alternatively, our rather limited sample size leaves our study prone to type 2 errors.

Impaired FMD was also associated with a combined endpoint of graft loss and death, although an association with death-censored graft loss was lost after adjustment for pulse

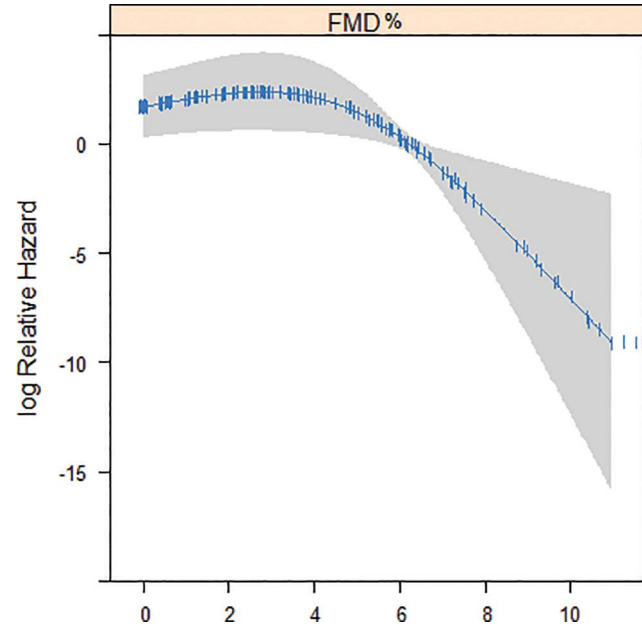

FIGURE 1. Cox regression model of mortality with FMD% represented by a spline transformation. FMD% is the percentage difference of flow-mediated dilation relative to baseline. The association between FMD% and risk of mortality increased with lower FMD levels until about 5% and did not change with further reduction in FMD%, indicating a "ceiling effect" at low levels of FMD% (nonlinearity $P = 0.01$). Similar ceiling effects were found for the absolute value of FMD (FMDmm; **Figure S1**, **SDC**, http://links.lww.com/TXD/A390) and in multivariable models (**Figure S2A–S2B, SDC**, http://links.lww.com/TXD/A390). FMDmm is the difference between maximum diameter and baseline in millimeters. FMD, flow-mediated dilation.

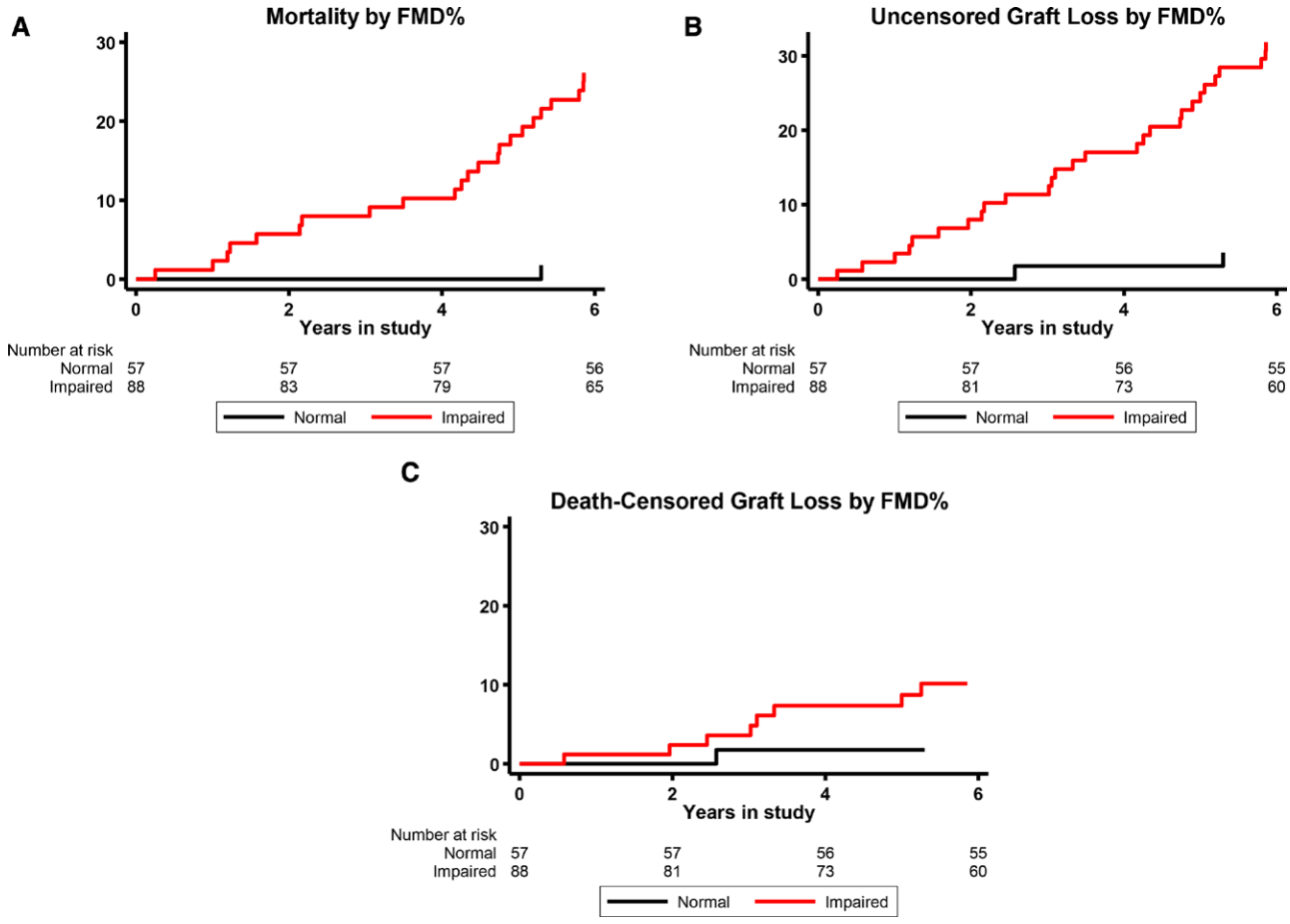

FIGURE 2. Kaplan-Meier plots of graft and patient outcomes in groups defined by normal or impaired flow-mediated dilation (FMD%) in the brachial artery. FMD% is the percentage difference of flow-mediated dilation relative to baseline. Corresponding plots for FMD in absolute terms (FMDmm) are shown in **Figure S3A–S3C**, **SDC**, http://links.lww.com/TXD/A390. FMDmm is the difference between maximum diameter and baseline in millimeters.

pressure. This finding is apparently at odds with a previous study from our group on endothelial function measured in skin microvasculature by laser Doppler flowmetry.[12] In that study, several projects were pooled and an association with graft loss was seen in studies undertaken later (3 mo or more) after transplantation. The present study used a more homogeneous sample and interrogated a conduit artery, which may explain the difference. Noteworthy, both studies have relatively small sample size and need verification.

Our findings are in line with a systematic review on FMD and cardiovascular disease and mortality,[7] which included 35 studies with 17280 nontransplanted participants; a majority were from the general population or patients with preexisting cardiovascular disease. Overall, FMD independently predicted cardiovascular events (HR, 0.88 [0.84–0.91], $P < 0.001$ per 1% increase in FMD), and a 1 SD deterioration in FMD could double the risk. FMD was a stronger predictor in patients with preexisting cardiovascular disease compared with studies in the general population. It is not known if this reflects true difference in biology or merely that the patient cohorts were smaller with a greater tendency for publication bias.[7]

Few studies have examined the association between FMD and outcomes in patients with kidney disease. Yilmaz et al[21] investigated FMD in 304 patients with CKD stages 1 to 5. FMD decreased with increasing stage of CKD and was independently associated with cardiovascular events. Two studies on hemodialysis patients did not find an association between FMD and mortality,[22,23] although sample sizes were moderate (n = 17 and n = 165). Lee et al[24] investigated FMD in 143 peritoneal dialysis patients and 32 controls. FMD was significantly lower in the patients (2.9% versus 6.2%, $P < 0.001$). After mean 42 mo of follow-up of the patients, 25 cardiovascular events occurred. Similar to our study, the risk seemed to plateau at lower levels of FMD, and an FMD below the median (FMD<2.9%) predicted outcomes with an HR of 2.73 ($P = 0.04$). It is not known if this ceiling effect reflects a biological phenomenon, such as a certain level of FMD below which no further harm can be imposed on the endothelium, or simply the fact that measurement error is increased at low levels of FMD, thus diluting the risk gradient. Finally, Kensinger et al[25] investigated FMD prospectively in 149 KTRs and found that values were stable at 1, 12, and 24 mo after transplantation, at 6.3%, 5.4%, and 5.6%, respectively. However, that study did not report on cardiovascular or mortality outcomes. In summary, our study is in line with most studies in populations with preexisting disease, finding FMD to be associated with cardiovascular disease or mortality.

Other measures of endothelial function have also been associated with outcomes in patients with kidney disease and may shed some light on the link with kidney function. Asymmetric dimethylarginine (ADMA) accumulates with declining kidney function and is a competitive inhibitor of NO synthase and thus reduces NO generation.[26] Increased

TABLE 3.

Cox regression models for all-cause mortality

	Univariable model		Multivariable model including FMDmm		Multivariable model including FMD%	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age	1.09 (1.04–1.13)	<0.001	1.07 (1.01–1.12)	0.02	1.07 (1.01–1.13)	0.02
Female	0.82 (0.48–1.40)	0.47				
DM or PTDM	1.87 (1.06–3.30)	0.03	2.48 (1.10–5.62)	0.03	2.08 (0.93–4.69)	0.08
Cardiovascular disease	1.71 (0.78–3.74)	0.18				
Pulse pressure	1.03 (1.01–1.04)	0.001	1.00 (0.98–1.02)	0.77	1.00 (0.98–1.02)	0.96
Dialysis before transplantation	1.81 (0.73–4.49)	0.20				
Time in RRT	1.02 (0.97–1.08)	0.36				
FMDmm impaired	6.62 (2.28–19.24)	0.001	3.57 (1.16–11.02)	0.03		
FMD% impaired	18.42 (2.50–136)	0.004			9.80 (1.29–74.62)	0.03

FMD% is the percentage difference of flow-mediated dilation relative to baseline. FMDmm is the difference between maximum diameter and baseline in millimeters.
DM, diabetes mellitus; HR, hazard ratio; PTDM, post-transplant diabetes mellitus; RRT, renal replacement therapy.

TABLE 4.

Cox regression models for uncensored graft loss

	Univariable model		Multivariable model including FMDmm		Multivariable model including FMD%	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age	1.07 (1.04–1.11)	<0.001	1.04 (1.00–1.08)	0.08	1.04 (1.00–1.09)	0.053
Female	0.93 (0.58–1.48)	0.76				
DM or PTDM	1.44 (0.85–2.47)	0.18				
Cardiovascular disease	1.69 (0.85–3.35)	0.13				
Pulse pressure	1.03 (1.01–1.04)	<0.001	1.01 (0.99–1.03)	0.24	1.01 (0.99–1.03)	0.17
Dialysis before transplantation	1.24 (0.59–2.58)	0.57				
Time in RRT	1.02 (0.98–1.07)	0.29				
FMDmm impaired	7.37 (2.85–19.06)	<0.001	4.47 (1.63–12.22)	0.004		
FMD% impaired	12.32 (2.95–51.45)	0.001			7.80 (1.83–33.30)	0.006

FMD% is the percentage difference of flow-mediated dilation relative to baseline. FMDmm is the difference between maximum diameter and baseline in millimeters.
DM, diabetes mellitus; FMD, flow-mediated dilation; HR, hazard ratio; PP, pulse pressure; PTDM, posttransplant diabetes mellitus; RRT, renal replacement therapy.

TABLE 5.

Cox regression models for death-censored graft loss

	Univariable model		Multivariable model including FMDmm		Multivariable model including FMD%	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age	1.03 (0.98–1.08)	0.27				
Female	0.86 (0.37–1.97)	0.71				
DM or PTDM	0.96 (0.33–2.79)	0.94				
Cardiovascular disease	1.63 (0.48–5.57)	0.44				
Pulse pressure	1.04 (1.01–1.06)	0.001	1.03 (1.01–1.05)	0.01	1.03 (1.01–1.05)	0.006
Dialysis before transplantation	0.59 (0.18–1.94)	0.39				
Time in RRT	1.04 (0.96–1.12)	0.33				
FMDmm impaired	5.81 (1.25–26.94)	0.03	3.15 (0.60–16.39)	0.17		
FMD% impaired	7.79 (1.00–60.89)	0.051			4.58 (0.55–37.92)	0.16

FMD% is the percentage difference of flow-mediated dilation relative to baseline. FMDmm is the difference between maximum diameter and baseline in millimeters.
DM, diabetes mellitus; FMD, flow-mediated dilation; HR, hazard ratio; PTDM, posttransplant diabetes mellitus; RRT, renal replacement therapy.

levels of ADMA have been associated with outcomes (progression to dialysis and death) in nontransplant kidney disease patients.[27] Our group previously analyzed ADMA levels in 1847 stable KTRs and found significant associations between either graft failure or doubling of creatinine, and cardiac events, cerebrovascular events, or all-cause mortality.[28] We also found that a structural isomer of ADMA, symmetric dimethylarginine (SDMA), was associated with mortality and failing grafts.[29] Although SDMA does not directly inhibit NO synthase, it is cleared by renal filtration and can limit arginine supply for the NO synthase and stimulate inflammation.[30-33] Endothelial function is closely linked to inflammation,[6] and we previously found that C-reactive protein and interleukin-6 were associated with graft loss, cardiovascular events, and mortality in KTRs.[34,35] However, in the present study, C-reactive protein was not associated with FMD,[18] and we did not measure ADMA or SDMA levels. Further studies with longitudinal assessment of FMD and potential covariates are needed to better clarify determinants of FMD in KTRs.

Although FMD is a labor-intensive and operator-dependent test, we chose FMD of the brachial artery when investigating endothelial function in our KTRs because this test is both widely used in research studies and is noninvasive. A recent (2020) consensus paper from the European Society of Cardiology describes endothelial dysfunction as a "spectrum of phenotypic states" and highlights that although no ideal test currently exists, further study is recommended to characterize reference values and improve risk stratification.[8] Our study complements the literature in line with these recommendations.

Our findings may not have any immediate implication for clinical practice but add to our knowledge on mortality risk factors that may have a clinical application in the future. Our present knowledge indicates that hypertension and time in dialysis are factors known to affect endothelial function[18] and longevity in KTRs.[1] Thus, better treatment of blood pressure and increased access to transplantation are fundamental in the care of patients with kidney disease.

Strengths of this study include a homogeneous study sample, FMD measurements undertaken by a single examiner, and consistent reporting of outcomes to the Norwegian Renal Registry. Limitations include a relatively modest sample size, exclusion of patients with heavy comorbidity, lack of longitudinal FMD measurements, and a primarily White population.

In conclusion, we found that impaired FMD is strongly associated with mortality in KTRs.

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