Oxidative Stress in Kidney Transplantation: Malondialdehyde Is an Early Predictive Marker of Graft Dysfunction

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Background. Oxidative stress is one of the most important components of the ischemia-reperfusion process after kidney transplantation (KTx) and increases with graft dysfunction.

Methods. This prospective study was conducted on 40 consecutive KTx recipients to evaluate time-dependent changes in oxidative stress-related parameters within the first week after KTx and to assess their performance in predicting delayed graft function (DGF=dialysis requirement during initial posttransplant week) and graft function at 1 year. Blood samples were collected before (day 0) and after KTx (days 1, 2, 4, and 7). Total antioxidant capacity, plasma levels of malondialdehyde (MDA), and activities of glutathione peroxidase, glutathione reductase and superoxide dismutase were measured. Multivariable linear mixed and linear regression models, receiver-operating characteristic (ROC), and areas under ROC curves (AUC-ROC) were used.

Results. At all time points after KTx, mean MDA levels were significantly higher in patients developing DGF (n=18). Shortly after KTx (8–12 hr), MDA values were higher in DGF recipients (on average, +0.16 μmol/L) and increased further on following day, contrasting with prompt functioning recipients. Day 1 MDA levels accurately predicted DGF (AUC-ROC=0.90), with a performance higher than SCr (AUC-ROC=0.73) and similar to cystatin C (AUC-ROC=0.91). Multivariable analysis revealed that MDA levels on day 7 represented an independent predictor of 1-year graft function. Antioxidant enzyme activities were not significantly changed during the study period and were not predictors of 1-year graft function.

Conclusions. Increased MDA levels on day 1 after KTx might be an early prognostic indicator of DGF, and levels on day 7 might represent a useful predictor of 1-year graft function.

Keywords: Oxidative stress, Malondialdehyde, Kidney transplantation, Kidney graft dysfunction.

(*Transplantation* 2014;97: 1058–1065)

Ischemia-reperfusion (I/R) injury is a complex phenomenon in kidney transplantation (KTx) that can cause graft dysfunction and determine both the early and long-term outcomes of transplant recipients. Oxidative stress is one of the most important components of I/R process (*1–3*). Reactive oxygen species (ROS) are products of normal cellular metabolism that are completely inactivated by antioxidant defense mechanisms during physiological conditions.

This study was funded by Astellas Farma Portugal. Astellas Farma Portugal contributed with financial funding sources for reagents acquisition and had no role in the design or conduct of the study, collection, management, analysis, interpretation of the data or in the preparation, review, or approval of the manuscript.

The authors declare no conflicts of interest.

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I.F. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: I.F. Acquisition of data and patient recruitment: I.F., M.A., L.D., S.P., J.S., A.C.H. Technical support and laboratorial analysis: H.R., I.F. Statistical analysis: I.F., D.M. Analysis and interpretation of data: I.F., D.M. Drafting of the manuscript: I.F. Critical revision of the manuscript for important intellectual content: D.M., H.R., A.C.H., L.L. All authors approved the final version. Obtained funding: A.C.H. Study supervision: A.C.H., D.M., L.L.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantjournal.com).

Received 27 August, 2013. Revision requested 14 September 2013.
Accepted 4 November 2013.

ISSN: 0041-1337/14/9710-1058
DOI: 10.1097/01.TP.0000438626.91095.50

The antioxidant defense system can be predominantly divided into endogenous enzymes, such as superoxide dismutases (SOD), catalases, glutathione reductases (GR) and peroxidases (GPx), and exogenous small molecules, such as carotenoids and vitamins A, C, and E (*4*). In some pathologic conditions, an imbalance between ROS generation and antioxidant capacity can result in enhanced ROS activity and oxidative stress (*5*).

Markers of oxidative stress, including elevated levels of malondialdehyde (MDA) and reduced antioxidant activity,

TABLE 1. Summary of baseline and clinical characteristics in kidney transplant donors and recipients (total sample and categorized by delayed or prompt graft function)

	Total (n=40)	DGF (n=18)	Non-DGF (n=22)	*P*
Donor				
Age (yr)	51.2±11.4	51.1±13.4	51.2±9.9	0.172
Male sex	26 (65)	14 (78)	12 (54.5)	0.125
Living donor	11 (27.5)	3 (16.7)	8 (36.4)	0.165
Expanded criteria donors	3 (7.5)	1 (5.6)	2 (9.1)	0.541
Serum creatinine (mg/dL)	0.81±0.18	0.85±0.21	0.78±0.16	0.318
Donor-recipient				
HLA mismatches	3.39±1.24	3.38±1.07	3.41±1.46	0.941
Cold ischemia time (hr)	12.1±7.9	15.2±7.8	9.6±7.3	0.035*
Living donor	2.8±0.5	2.5±0.5	3.0±0.5	0.204
Deceased donor	16.2±5.9	18.1±5.1	14.1±6.2	0.088
Recipient				
Age (yr)	49.2±15.2	56.3±10.9	43.3±15.9	0.006*
Male sex	26 (65)	11 (61)	15 (68)	0.641
White	40 (100)	18 (100)	22 (100)	—
BMI (kg/m^2)	24.8±4.9	26.2±4.4	23.6±5.0	0.091
Previous transplant	2 (5)	0 (0)	2 (9.1)	
Time on dialysis (yr)	4.4±4.7	5.6±6.2	3.4±2.3	0.135
Pretransplant therapy				
Dialysis	38 (95)	18 (100)	20 (90.9)	0.296
Preemptive transplantation	2 (5)	0 (0)	2 (9.1)	
Cause of kidney disease				
IgA nephropathy	7 (17.5)	2 (11.1)	5 (22.7)	—
Glomerulonephritis	6 (15.0)	4 (22.2)	2 (9.1)	—
Diabetic nephropathy	5 (12.5)	3 (16.7)	2 (9.1)	—
Autosomal dominant polycystic kidney disease	3 (7.5)	3 (16.7)	0 (0)	—
Unknown	4 (10.0)	1 (5.6)	3 (13.6)	—
Others	15 (37.5)	5 (27.8)	10 (45.5)	—
Peak PRA (%)	5.5 ± 15.1	5.0 ± 15.0	5.9 ± 15.5	0.853
0	29 (72.5)	14 (77.8)	15 (68.2)	—
1–25	8 (20.0)	3 (16.7)	5 (22.7)	—
26–75	3 (7.5)	1 (5.6)	2 (9.0)	—
Current PRA (%)	2.3 ± 8.6	3.1 ± 11.7	1.6 ± 4.9	0.585
0	34 (85)	15 (83.3)	19 (86.4)	—
1–25	5 (12.5)	2 (11.1)	3 (13.6)	—
26–50	1 (2.5)	1 (5.6)	0 (0)	—
Induction regimen				
Antithymocyte globulin (ATG-F)	4 (10)	1 (5.6)	3 (13.6)	0.613
Basiliximab/Daclizumab	30 (75)	14 (77.8)	16 (72.7)	0.789
Immunosuppression at time of discharge				
Steroids	38 (95.0)	18 (100)	20 (90.9)	0.296
Tacrolimus	38 (95.0)	17 (94.4)	21 (95.5)	0.886
Cyclosporine A	2 (0.05)	1 (5.6)	1 (5.6)	0.884

Values are expressed as mean±standard deviation or absolute numbers and percentages. Comparisons between groups of continuous variables were done using parametric (*t* test) or nonparametric (Mann-Whitney) tests; associations between categorical variables were analyzed using the χ^2 test and Fisher's exact test as appropriate; *$P<0.05$.

HLA, human leukocyte antigen; BMI, body mass index; PRA, panel reactive antibody.

have been reported in renal patients (*6–9*). The restoration of kidney function after KTx can improve oxidative stress (*10*), but certain studies (*11, 12*) have reported increased systemic biomarkers of oxidative stress in KTx recipients, particularly in the early phase (*13, 14*) and thereafter, coinciding with chronic allograft dysfunction (*11, 15–18*). Despite a significant amount of literature on oxidative stress and renal disease, data regarding KTx in the early stages remain limited. Therefore, we investigated the time-dependent changes in the antioxidant defense system during the first week after transplantation by measuring the overall antioxidant status (TAS) and the activity of the predominant antioxidant enzymes as a response to lipid peroxidation evaluated by MDA levels.

The purposes of this study were as follows:

to assess whether oxidative markers differ between patients (pretransplant and 1 week posttransplant) and control subjects (healthy blood donors);
to evaluate longitudinal changes of MDA, TAS, SOD, GPx, and GR within the first week after KTx and identify factors associated with these changes;
to investigate the association of MDA/antioxidant parameters with DGF (defined as dialysis requirement within the first posttransplant week) and their accuracy in predicting DGF; and
to examine the relationship between any of the oxidative markers measured during the first week posttransplant and the 1-year allograft function, evaluated by serum creatinine (SCr) levels.

RESULTS

Study Cohort

During recruitment, 42 patients were consecutively enrolled. Two recipients had primary graft failure and were excluded during the first 2 days. Therefore, the final study sample included 40 patients. Baseline demographical and transplant data are shown in Table 1.

Oxidative Stress Markers

We initially compared oxidative markers evaluated in the 40 ESRD patients scheduled for KTx with those of 30 healthy subjects with similar ages (a control group of blood donors). Before KTx, the patients presented with significantly increased mean (SD) MDA levels (0.40 [0.12] vs. 0.26 [0.09] μmol/L, $P<0.01$), TAS (1.79 [0.19] vs. 1.39 [0.53], $P<0.001$), SOD (1971 [630] vs. 1208 [254] U/g Hb, $P<0.001$) and GR (63 [12] vs. 52 [7.0] U/L, $P<0.001$) compared with controls. No significant differences were detected in GPx.

The evolution of oxidative parameters during the first posttransplant week is summarized in Table 2. Compared with before transplant, mean (SD) MDA levels significantly decreased at first day (0.40 [0.12] vs. 0.36 [0.12] μmol/L, $P=0.031$), and a reduction of approximately 28% was observed on the seventh posttransplant day (0.40 [0.13] to 0.28 [0.13] μmol/L, $P<0.001$). Levels of TAS, SOD, GPx, and GR did not exhibit any significant changes within the first posttransplant week.

None of the oxidative stress markers differed significantly between male and female patients at any time point. Mean MDA levels were increased in deceased donor recipients at all time points, although the increases were only statistically significant on second and fourth days. No significant differences were found in antioxidant parameters.

Recipient age was positively correlated with MDA levels at days 4 and 7 (respectively, $r=0.46$, $P=0.004$; and $r=0.39$, $P=0.013$). Time on dialysis, donor age, and cold ischemia time were not correlated with MDA levels or with any antioxidant marker. Levels of MDA and SCr, but not of the antioxidant markers, were positively correlated at most of time points (data not shown).

TABLE 2. Time-course of oxidative stress biomarkers within the first week after kidney transplantation

		Prior-KTx	1st day*	2nd day	4th day	7th day
		Mean (SD)	(n=40)	(n=40)	(n=40)	(n=40)
MDA (μmol/L)	Overall	0.40 (0.12)	0.36 (0.12)	0.28 (0.10)	0.29 (0.13)	0.28 (0.13)
	DGF (n=18)	0.42 (0.12)	0.45 (0.10)	0.33 (0.10)	0.40 (0.13)	0.37 (0.13)
	Non-DGF (n=22)	0.39 (0.12)	0.29 (0.09)	0.24 (0.08)	0.23 (0.07)	0.19 (0.05)
TAS (mmol/L)	Overall	1.79 (0.19)	1.74 (0.21)	1.68 (0.35)	1.73 (0.37)	1.77 (0.30)
	DGF (n=18)	1.80 (0.16)	1.73 (0.24)	1.70 (0.25)	1.82 (0.21)	1.89 (0.24)
	Non-DGF (n=22)	1.78 (021)	1.76 (0.19)	1.74 (0.22)	1.75 (0.28)	1.66 (0.32)
SOD (U/g Hb)	Overall	1966 (638)	1894 (596)	1984 (578)	1947 (457)	1997 (580)
	DGF (n=18)	1842 (559)	1837 (622)	1876 (538)	1928 (439)	2158 (604)
	Non-DGF (n=22)	2068 (691)	1943 (584)	2071 (608)	1959 (478)	1944 (413)
GR (U/L)	Overall	63 (12)	50 (14)	51 (17)	56 (16)	62 (14)
	DGF (n=18)	66 (12)	54 (19)	57 (20)	63 (18)	69 (15)
	Non-DGF (n=22)	61 (12)	47 (9)	48 (7)	53 (10)	56 (10)
GPx (U/g Hb)	Overall	58 (15)	59 (13)	62 (15)	62 (14)	60 (14)
	DGF (n=18)	58 (13)	59 (11)	62 (14)	64 (15)	60 (14)
	Non-DGF (n=22)	58 (17)	60 (15)	62 (17)	60 (14)	60 (14)

*1st day=8 to 12 hr after surgery; the values are the mean and standard deviation.

KTx, kidney transplantation; MDA, malondialdehyde; TAS, total antioxidant status; SOD, superoxide dismutase; GR, glutathione reductase; GPx, glutathione peroxidase; SD, standard deviation.

Delayed Graft Function and Acute Rejection

Eighteen (45%) and 22 (55%) patients had DGF and prompt graft function, respectively. The DGF rate was higher in grafts from deceased donors, but this difference was not statistically significant (51.7% vs. 27.3%, *P*=0.286). In terms of traditional DGF predictors and except for cold ischemia time, no significant differences were found between DGF/non-DGF in relation to baseline characteristics and induction therapy (Table 1). The mean age was significantly higher in patients with DGF (56 [11] vs. 43 [16] yr, *P*=0.006).

Ten recipients had an acute rejection episode during inpatient hospitalization for transplantation, and acute rejection was more frequently diagnosed in patients with DGF than in those with prompt function (44% vs. 9%, *P*=0.025).

DGF and Longitudinal Changes in Oxidative Stress Markers

Before transplantation, no significant differences were found between patients with DGF or non-DGF regarding any of the evaluated oxidative stress markers. After transplantation, mean MDA levels were consistently higher in DGF patients at all time points, compared with non-DGF recipients (Table 2). No differences were found between DGF and non-DGF recipients in relation to antioxidant parameters.

Longitudinal Changes in MDA Levels According to Graft Function

A linear mixed-effects model was used to analyze the longitudinal changes in MDA of the 2 groups of patients (DGF/non-DGF), by controlling for variables found to be associated with MDA by bivariate analysis (donor status and recipient age) and confirmed the independent association of DGF with and MDA levels. Donor status and recipient age lost their statistical significance and were removed from the final model. Time measurements of MDA and DGF were the only independent factors associated with MDA levels (Table 3). Delayed graft function was significantly associated with MDA levels: recipients with prompt function presented reduced average MDA levels at all time points. According to our estimation, the first MDA values after transplantation were 0.16 μmol/L higher in DGF patients. A significant interaction between time of measurement and DGF confirmed that the pattern of longitudinal changes in MDA levels depend on whether the recipient had DGF.

Because DGF occurs more frequently in KTx from deceased donors, we performed the same analysis considering only deceased donor transplants, and the results were similar (see **SDC**, http://links.lww.com/TP/A919). According to our estimation and after excluding living donors, the first MDA levels after KTx were, on average, 0.144 μmol/L higher in DGF patients who underwent a deceased-donor transplant.

The effect of DGF on the progression of antioxidant parameters over time was not statistically significant, even when we considered only deceased-donors transplants.

Prognosis of DGF by Oxidative Stress Markers (ROC Analysis)

Receiver-operating characteristic (ROC) analyses were performed to assess the potential of oxidative markers to predict DGF. Only MDA levels predicted the need for dialysis within the first week. The MDA levels on day 1 represented an optimal predictor for the early diagnosis of DGF (AUC=0.90), as well as the changes in MDA levels between preoperative and first posttransplant day (AUC=0.84) (Fig. 1). The diagnostic performance of MDA on day 1 was better than diagnostic performance of SCr (AUC=0.73) and similar to that of cystatin C (CystC, AUC=0.91), which is considered a marker with greater sensitivity for the detection of impaired renal function. The reduction ratio in MDA levels between pretransplant and day 1 resulted in an AUC of 0.84 for identifying DGF, which was better than the reduction ratio of SCr on the same day (AUC=0.69). In analyzing

TABLE 3. Results of the final linear mixed model for dependent variable MDA levels (n = 194 observations derived from 40 patients)

	Estimate	*P*	95% CI	
Intercept	0.368	<0.001	0.318	0.418
Graft function				
DGF=0 (immediate graft function)	–0.170	<0.001	–0.238	–0.102
DGF=1 (with DGF - reference)	0	—	—	—
Time				
Time 0 (pretransplant)	0.053	0.089	–0.008	0.115
Time 1 (1st day)	0.079	0.012	0.018	0.141
Time 2 (2nd day)	–0.034	0.279	–0.095	0.028
Time 3 (4th day)	0.030	0.363	–0.036	0.097
Time 4 (7th day- reference)	0	—	—	—
Time*DGF				
Time 0*DGF=0	0.134	0.002	0.050	0.217
Time 1*DGF=0	0.013	0.257	–0.070	0.097
Time 2*DGF=0	0.077	0.295	–0.007	0.161
Time 3*DGF=0	0.029	0.039	–0.058	0.116
Time 4*DGF=0 (reference)	0	—	—	—

MDA, Malondialdehyde; DGF, delayed graft function.

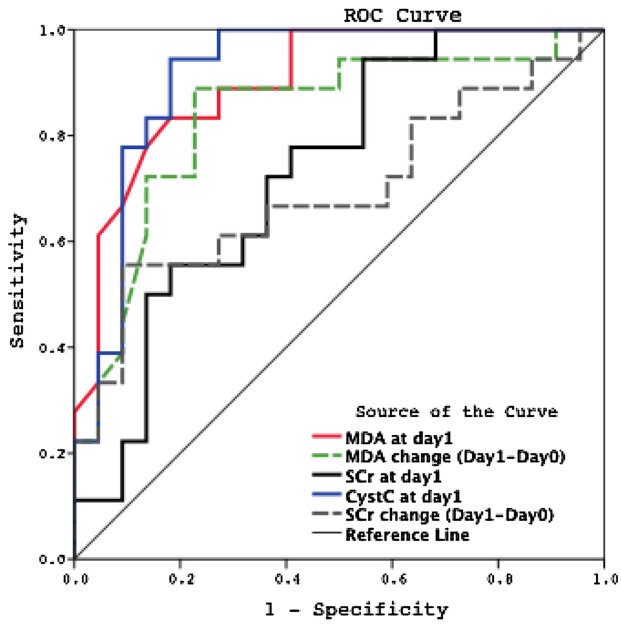

FIGURE 1. Receiver-operating characteristic curves for plasma MDA, serum creatinine, and cystatin C levels measured at the first day after KTx and changes in MDA and serum creatinine levels from pretransplant to day-1 after KTx for predicting delayed graft function. The table lists the areas under the ROC curves of MDA, serum creatinine, and serum cystatin C for predicting DGF, as well as the AUCROC of MDA and serum creatinine changes between baseline and first day after transplant. MDA, malondialdehyde; SCr, serum creatinine; Cyst, serum cystatin C; MDA or SCr change (day 1–day 0), Serum creatinine or MDA reduction rate between pretransplant and the first day after transplant (the difference between MDA or SCr on day 1 and day 0, divided by MDA or SCr on day 0, multiplied by 100); AUC, area under the ROC curves.

the ROC curve of MDA on day 1, the optimal sensitivity and specificity occurred at a value of 0.365 μmol/L (sensitivity, 83%; specificity, 82%; positive and negative predictive value, 82 and 71, respectively).

During the First Year After KTx

Within the first year after KTx, 10 KTx recipients were rehospitalized, accounting for a total of 19 hospital admissions. The causes of rehospitalization were infection in five admissions (predominantly urinary tract infections), renal dysfunction in six, and nonrenal causes in the remaining eight admissions (suicidal ideation, acute pulmonary edema, and neutropenia). Records from the acute rejection episodes throughout the first posttransplant year were reviewed, and only one patient was rehospitalized at 1 month after KTx with an acute rejection episode. At 1 year, all of the patients were alive, but two grafts of DGF recipients were lost.

Predictive Value of MDA Levels on 1-Year Allograft Function

At 1 year after KTx, the median (IQR) SCr was significantly higher in patients with DGF (1.58 [1.20–2.52] vs. 1.26 [1.05–1.52] mg/dL, P=0.049) and a correlation with MDA at day 7 was found (Fig. 2). The prognostic value of early MDA values on long-term allograft function (1 year after KTx) was tested using multivariable analysis, including all patients (DGF and non-DGF). In multivariable linear regression models for 1-year SCr, MDA levels measured on day 7 were independent predictors of 1-year graft function after controlling for established variables that generally affect graft function, including acute rejection and rehospitalizations occurring during the first posttransplant year (Table 4). Levels of MDA before KTx and on remaining posttransplant days were not significant predictors of 1-year SCr.

DISCUSSION

In this prospective cohort study, we report the independent association of high levels of plasma MDA with DGF with poor 1-year allograft function. To the best of our knowledge, this study is the first to demonstrate this association in KTx recipients.

Oxidative stress is involved in the pathophysiology of renal injury in I/R (*1, 2, 19*). As in other clinical conditions, if the kidney scavenging capacity is insufficient for an excess of ROS production, such an oxidative imbalance might trigger an inflammatory response within the transplanted organ, leading to tissue damage and graft dysfunction (*2, 13, 20*). Because of the composition of renal lipids, which predominantly comprise long-chain, polyunsaturated fatty acids, lipid peroxidation represents one of the most widespread hypothesized causes of ROS-mediated cell injury (*21*). Despite the controversy of whether lipid peroxidation is the cause or an epiphenomenon of injury, the fact is that increased lipid peroxidation is observed in I/R injury. Moreover, MDA is the principal product of polyunsaturated fatty acid peroxidation, reflecting the I/R stress of grafts (*5, 22*). In our study, recipients who developed DGF presented increased MDA levels during the first week after KTx, which seem to reflect the postischemic tissue damage of DGF kidneys. Compared with pretransplant, these patients presented higher MDA levels at 8 to 12 hr after KTx, in contrast to recipients with prompt graft function whose MDA levels continuously decreased throughout the

TABLE 4. Significant predictors of serum creatinine at 1 year after kidney transplantation

	Regression coefficient	P	95% CI
	Serum creatinine at 1-year posttransplantation (ln)		
Time on dialysis (ng/mL)	0.048	<0.001	0.024–0.072
MDA measured on day-7 (μmol/L)	1.338	0.003	0.475–2.201
Rehospitalizations (yes vs. no)	0.361	0.007	0.107–0.615

Results are given by multiple linear regression (a stepwise method) after including donor status, recipient and donor age, pretransplant time on dialysis, rehospitalizations and acute rejection episodes throughout the first year; serum creatinine (ln) at 1-year after transplant as the dependent variable.
MDA, malondialdehyde.

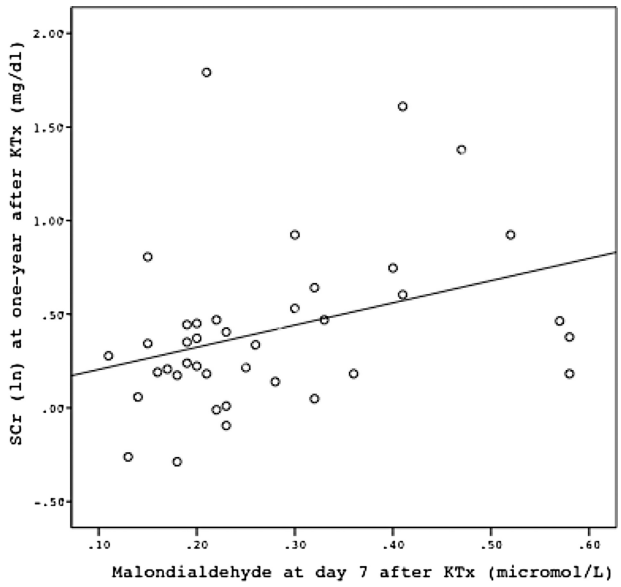

FIGURE 2. Relationship between serum creatinine and plasma malondialdehyde at day 7 after kidney transplantation (r=0.346, P=0.031). KTx, kidney transplantation; SCr, serum creatinine.

week. The independent association of DGF with longitudinal changes in MDA was confirmed using a general linear mixed model approach, which also corroborated that DGF can model the trajectory of MDA changes after KTx.

Our results not only establish MDA as an early marker of DGF but also demonstrate its predictive value as early as 8 to 12 hr after KTx in terms of the evolution of graft function and the need for dialysis during the first week. In regard to clinical application, a new biomarker should be more accurate in predicting DGF than the current SCr. After this, ROC analysis showed that MDA levels are better suited than SCr for predicting the need of renal replacement therapy within the first week after KTx. Similar to serum CystC, MDA levels on day 1 were highly accurate in predicting DGF and performed better than SCr. This emphasizes the clinical value of MDA levels as a diagnostic marker for the prediction of DGF, facilitating an earlier diagnosis compared with SCr.

Only a highly effective antioxidant system can counteract the deleterious hydroxyl radicals formed during lipid peroxidation. A wide range of protective substances, such as antioxidant enzymes, might potentially elicit a protective effect by limiting the production of ROS and the damage of oxidative stress after I/R injury of a kidney graft. Conflicting results have reported on the activities of antioxidant enzymes in KTx patients. Levels of antioxidant enzymes have been reported to increase (*23, 24*), decrease (*12, 16*), or remain unchanged (*25, 26*) after KTx. Because of this lack of consensus, we aimed to examine the changes of antioxidant activity during the early phase of KTx. Compared with healthy controls, our patients presented with significantly increased SOD and GR levels before KTx, likely in response to significant oxidative stress levels in ESRD patients. However, no significant changes were found after KTx, even when stratifying by graft function.

The evaluation of TAS has been used as a biological marker for monitoring oxidative stress. Measuring TAS allows for the detection of the overall antioxidant capacity, including the contribution of as of yet unknown antioxidants and the synergism between them (*27*). In our study, TAS levels did not exhibit any significant changes during the first posttransplant week, even when we stratified the patients according to DGF. Although oxidative stress expressed by MDA levels is most significantly pronounced in DGF patients, our study highlights the observation that during the first posttransplant week, the overall antioxidant status and potential protection exerted by the antioxidant enzymes are not enhanced to counteract the intensified oxidative stress, specifically in DGF patients.

Immunosuppressive therapy, particularly cyclosporine, represents an additional potential source of ROS generation and enhanced renal lipid peroxidation after KTx (*15, 28*). In our study, the effect of immunosuppression on plasma MDA and antioxidant parameters was not assessed, as only one patient was on a combined therapy with cyclosporine.

In various studies, it has been reported that oxidative stress occurring in KTx might be implicated in the pathophysiology of chronic transplant dysfunction (*1, 11, 15, 17, 29*). Djamali et al. (*30*) suggested that ROS represents an important fibrogenic factor in chronic allograft nephropathy because oxidative stress is increased in the presence of the interstitial fibrosis and tubular atrophy that generally precedes chronic allograft failure. In experimental models of chronic allograft tubular atrophy/interstitial fibrosis, increased intragraft MDA levels were detected, reflecting lipid peroxidation (*31*). Therefore, we verified the effects of MDA levels evaluated within the first week on 1-year posttransplant allograft function. Together with time on dialysis and rehospitalizations during the first year, MDA levels at day 7 were the best predictors of 1-year SCr. Higher MDA levels on day 7 were associated with worse graft function at 1 year, suggesting that oxidative damage reflected by increased MDA levels on day 7 will reflect long-term injury.

This study has several strengths. We used a prospective and longitudinal study design to determine the effects of DGF on the progression of oxidative markers over time. Most of the previous oxidative stress studies on KTx were cross-sectional and included only stable patients. Longitudinal studies are more helpful in understanding how subtle associations between factors of interest change over time, and we used this methodology to consider five measurements of each oxidative stress marker. Uncertainty remains concerning the determination of oxidative stress in KTx and the interpretation of the potential variability. However, our study highlights the importance of studying oxidative stress according to graft function because DGF can significantly modify the trajectory of MDA changes. To the best of our knowledge, this is the first study to demonstrate that MDA levels are strongly associated with DGF and with poorer 1-year graft function.

Regardless of its several mentioned strengths, this study has some limitations. This is a single centre study with a relatively small sample size. Despite the encouraging results found, the accuracy of MDA levels as a diagnostic marker of renal graft injury and prognostic value of MDA for DGF after KTx needs to be assessed in a larger cohort and in other centers and transplant recipients.

In KTx, numerous diagnostic biomarkers have been evaluated in the past decade, but, so far, evidence to support

their use in routine practice is limited. The discovery of novel biomarkers can be complex and costly. In this study, we demonstrated that a novel marker predicted who would develop DGF with about the same degree of accuracy of serum CystC and both with a diagnostic performance superior to serum creatinine. Undoubtedly, CystC displays several good characteristics that make it a viable biomarker for early detection of DGF. Nonetheless, and particularly during the first week when high doses of corticosteroids are used, glucocorticoid medication can be shortcoming in using serum CystC in KTx, and it is important to take this into account when interpreting this serum marker. Thus, a combination of biomarkers may be more valuable for the diagnosis of DGF and prognosis of graft function. Because DGF is a critical early insult to the renal allograft that augments the risk of long-term graft loss, and it is a complex process with multiple underlying pathogenic mechanisms and confounding risk factors, it can be prudent to predict DGF with more than a single biomarker, at least in some situations. MDA can be a valuable marker as an alternative or as a complement in the risk prediction, not only in relation to serum CystC and any other serum/plasma markers but also regarding urine biomarkers, like neutrophil gelatinase-associated lipocalin, that cannot be measured if a urine sample cannot be taken, particularly during transient anuria that commonly occurs after KTx.

In conclusion, intensified oxidative stress persists during the early phase of transplant, particularly in DGF recipients. The antioxidant enzymes did not counterbalance the overload of ROS by a compensatory increase in their activities. The present study showed that MDA is a novel and a reliable biomarker for the prediction of early and long-term graft damage.

SUBJECTS AND METHODS

Study Design and Patient Population

Consecutive patients with end-stage renal disease (ESRD), undergoing living or deceased KTx at the Nephrology and Kidney Transplantation Department of the Centro Hospitalar do Porto between December 2010 and May 2011 were prospectively enrolled. Patients younger than 18 years or who required multiorgan transplants were not included. After transplant, recipients with primary graft failure related to surgical causes were excluded. The institutional review board of Centro Hospitalar do Porto approved the study. Each participant provided informed consent before enrollment.

Data Collection

At time of transplantation, several demographical and clinical parameters were collected. During the first posttransplant year, the rehospitalizations of KTx recipients were registered, as well as the length of the hospital stays and outcomes (functioning allograft or graft failure).

Sampling and Laboratory

Blood samples for determining oxidative stress parameters were collected as follows: 3 to 6 hr before transplant surgery (pretransplant); on the following morning, approximately 8 to 12 hr after graft reperfusion (day 1); and then at second (day 2), fourth (day 4), and seventh day (day 7) after transplant, for a total of five samples per patient. Blood samples were taken by conventional procedures and immediately centrifuged. All samples were aliquoted and frozen within 1 hr after collection and stored at –80ô¤C until further assay.

Measurements of SCr were performed by Jaffé method (Roche Diagnostics), and CystC was measured with a particle enhanced immunonephelometric method (Siemens Diagnostics) at the same time points as oxidative markers.

Plasma levels of MDA were measured using a commercial high-performance liquid chromatography kit (Chromsystems). superoxide dismutase levels were measured in erythrocytes according to a protocol previously described by Beauchamp and Fridovich (*32*) using the RANSOD kit; GR, GPx, and TAS levels were measured in plasma/serum using a Randox Laboratories kit.

Definition of Variables

Delayed graft function was defined by the need for dialysis during the first week. "Prompt" function (non-DGF) was considered if no dialysis was required during the first posttransplantation week.

Graft function at 1 year was evaluated by the average of the two SCr levels measured at 1 year posttransplant. Two grafts were lost at the seventh and eighth months, and the last SCr presented by these patients before the restart of dialysis was considered as being the 1-year SCr.

Statistics

Distributions of continuous variables were analyzed, and Kolmogorov-Smirnov tests were performed to assess their deviation from Normal distribution. Quantitative variables were summarized as the mean and standard deviation (SD), or as median and 25th and 75th quartiles (interquartile range [IQR]) for variables exhibiting skewed distributions. Categorical variables were reported as percentages.

Statistical analysis was performed in four steps. First, a cross-sectional bivariate analysis was performed to compare groups and to study the association between oxidative stress markers and demographic/clinical variables (*t* test). Correlations were assessed using Pearson correlation.

Second, a linear mixed-effects model was used to study the association of DGF with serial changes of each oxidative marker, controlling for variables associated by bivariate analysis. The interaction between DGF and the time-course measurement of oxidative markers were included in the model, as such a significant interaction would suggest that DGF affects the levels and trajectory of each marker.

Third, ROC analysis was performed to estimate the sensitivity and specificity of MDA levels (as well as SCr and CystC) to predict DGF. The optimal cutoff points were determined by maximizing the sum of sensitivity and specificity.

Fourth, multivariable stepwise linear regression was performed to assess the independent association of MDA levels with SCr at 1 year posttransplantation, including variables that generally predict graft function (donor status, recipient and donor age, pretransplant time on dialysis, rehospitalization, and acute rejection episodes throughout the first year). Linear regression models used log-transformed 1-year SCr levels as the dependent variable. To avoid collinearity, each time point of MDA was included separately in the different models.

Statistical analyses were performed using SPSS version 21.0, and a significance level of 0.05 was considered significant.

ACKNOWLEDGMENTS

The authors thank Astellas Pharma Portugal for their valuable contribution in supporting the acquisition of reagents for measuring malondialdehyde and all of the antioxidant parameters.

The authors also thank Dr. Maria Luís Cardoso for the technical assistance in laboratorial analysis and to the nursing staff for the commitment and crucial help in sample collection. The authors also thank Drs. António Cabrita and José Carlos Oliveira for allowing the study in their departments (the Nephrology and Kidney Transplantation and Chemical Chemistry Departments, respectively, of the Centro Hospitalar do Porto, Hospital de Santo António, Porto, Portugal).

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