

Skin-Autofluorescence Is an Independent Predictor of Graft Loss in Renal Transplant Recipients

Jasper W. L. Hartog,¹ Sascha Gross,^{1,2} Leendert H. Oterdoom,¹ Rutger M. van Ree,¹ Aiko P. J. de Vries,¹ Andries J. Smit,¹ Jan P. Schouten,³ Peter P. Nawroth,² Reinold O. B. Gans,¹ Willem J. van Son,¹ Angelika Bierhaus,² and Stephan J. L. Bakker^{1,4}

Background. Skin-autofluorescence (skin-AF) noninvasively measures the tissue accumulation of advanced glycation end products (AGEs). AGEs are nephrotoxic and potential effectors of cardiovascular mortality. We investigated whether skin-AF predicted graft loss after kidney transplantation.

Methods. A total of 302 renal transplant recipients were enrolled at a median time of 6.1 (2.6–12.1) years after transplantation and were subsequently followed up for first occurrence of graft loss (i.e., graft failure or all-cause mortality) for 5.2 (4.6–5.4) years. The association of baseline skin-AF with graft loss was investigated with univariable and multivariable Cox-regression and receiver-operator-characteristic curve analyses.

Results. Baseline skin-AF was 2.7 ± 0.8 arbitrary units. Skin-AF predicted graft loss in a univariable Cox regression analysis (Hazard ratios 2.40 [1.75–3.29], $P < 0.001$) and in a multivariable model (Hazard ratios 1.83 [1.22–2.75], $P = 0.003$), adjusted for other identified risk-factors, including patient age, creatinine clearance, protein excretion, high sensitivity C-reactive protein (hsCRP), and human leukocyte antigen-DR mismatching. The area under the receiver-operator-characteristic curve for skin-AF as predictor of graft loss was significantly different from 0.5. Skin-AF was also a significant predictor of graft failure and mortality as separate end points.

Conclusions. We conclude that skin-AF is an independent predictor of graft loss in kidney transplant recipients. Although skin-AF is not a direct measurement for AGEs, we believe that our results do support the hypothesis that accumulation of AGEs in renal transplant recipients contributes to the development of graft loss.

Keywords: Renal transplantation, Skin-autofluorescence, Advanced glycation end-products, Graft loss, Prognosis.

(*Transplantation* 2009;87: 1069–1077)

End-stage renal disease (ESRD) is an important medical problem in the Western world, which is expected to increase in the future (1). ESRD is preferably treated with kidney transplantation, because this treatment significantly enhances the quality of life and survival of patients in comparison with dialysis treatments (2, 3). Although the short-term success of kidney transplantations has improved steadily in recent years with efficient treatment protecting from acute rejection (4), the long-term success still needs improvement. Patients find themselves threatened by the enhanced risk for mortality, and sometimes even more by the risk of being readmitted to dialysis. As many as 60% of patients transplanted with a cadaveric donor kidney develop graft failure within 10 years after transplantation and age-adjusted rates of mortality are approxi-

mately 3 to 5 times higher in renal transplant recipients than in the general population (5, 6).

Both graft failure and patient mortality have been hypothesized to result at least partly from the pathogenic effects of oxidative stress and advanced glycation end-products (AGEs) (7, 8). Basically, oxidative stress causes protein damage such as protein glycation, the products of which can be recognized by a number of cellular receptors (9). Receptor activation then induces prolonged proinflammatory signaling, which might lead to vascular damage, and may finally result in graft failure and mortality (9).

The first two authors contributed equally to this work.

¹ Department of Internal Medicine, University Medical Center Groningen and University of Groningen, Groningen, The Netherlands.

² Department of Internal Medicine I and Clinical Chemistry, University of Heidelberg, Heidelberg, Germany.

³ Department of Epidemiology, University Medical Center Groningen and University of Groningen, Groningen, The Netherlands.

⁴ Address correspondence to: S. J. L. Bakker, M.D., Ph.D., Department of Internal Medicine, University Medical Center Groningen, P.O. Box 30001, 9700 RB Groningen, The Netherlands.

E-mail: s.j.l.bakker@int.umcg.nl

Received 8 September 2008. Revision requested 5 October 2008.

Accepted 27 November 2008.

Copyright © 2009 by Lippincott Williams & Wilkins

ISSN 0041-1337/09/8707-1069

DOI: 10.1097/TP.0b013e31819d3173

This work was further funded by a grant from the Dutch Kidney Foundation (Nierstichting Nederland C00.1877) and by a Clinical Research Fellowship from the Netherlands Organization for Scientific Research (NWO-AGIKO 920-03-181) (A.d.V.).

A. J. Smit is associated with DiagnOptics BV, which manufactures autofluorescence readers. This study was not financially supported by DiagnOptics BV, and final approval was always by the other authors who were not a member of DiagnOptics BV. None of the other authors declare any conflicts of interest.

This study was performed as part of the Graduertenkolleg (GRK) 880 a study exchange project of the University of Mannheim, the University of Heidelberg and the University of Groningen.

Skin-autofluorescence (skin-AF) measurement is a newly developed noninvasive technique that has been validated to measure the accumulation of AGEs (10). We previously found skin-AF to predict mortality in ESRD patients on dialysis (11). This study investigated whether skin-AF is an independent predictor of graft loss in kidney transplant recipients.

MATERIALS AND METHODS

Study Design and Patients

The study protocol was approved by the Institutional Review Board of the University Medical Center Groningen (METC 01/039). All renal transplant recipients transplanted at the University Medical Center Groningen are monitored in accordance with the American Transplantation Guidelines (12) in the outpatient clinic. Between August 2001 and July 2003, all adult allograft recipients who survived the first year after transplantation with a functioning allograft were eligible to participate at their next visit to the outpatient clinic. The aim of our study was to investigate AGE accumulation as a potential determinant of long-term transplant survival. In the first year after transplantation, graft loss is frequently related to acute rejection, urological problems, and infections. To avoid confounding by such events, we only considered patients eligible for participation in the study who were 1 year after transplantation or beyond. A total of 606 of 847 eligible renal transplant recipients signed written informed consent. Skin-AF was measured in a subpopulation consisting of 309 consecutive patients, because the AGE-reader measurement was not yet available at study initiation. From this subpopulation, seven non-white patients were excluded, because the skin-AF measurement has not yet been validated for measurements in patients with pigmented skin. The group that did not sign informed consent was comparable with the group that signed informed consent with respect to age, sex, time since transplantation, creatinine clearance, and proteinuria (13). Furthermore, no significant differences existed in donor age, recipient age, donor sex, recipient sex, diabetes, baseline creatinine clearance, and urinary protein excretion between the 302 patients in whom skin-AF was recorded and the 304 patients in whom skin-AF was not recorded. All measurements including blood sampling were performed after an 8 to 12 hr overnight fasting period for food and medication.

Follow-Up

Patients were enrolled at a median time of 6.1 (2.6–12.1) years after transplantation and were subsequently followed up for first occurrence of graft loss. Graft loss was considered to have occurred if patients were readmitted to dialysis, if they were retransplanted, or if they died. Up-to-date and complete information on patient status was ensured by our outpatient program, which operates in close collaboration with referral hospitals in our area. In the whole population, 137 (22.6%) patients experienced graft loss of whom 95 (15.7%) patients died and 42 (6.9%) experienced graft failure during follow-up for 5.3 (4.7–5.7) years. In the subpopulation of patients in whom skin-AF was measured, 53 (17.5%) patients reached the endpoint of graft loss of whom 34 (11.3%) died and 19 (6.3%) experienced graft failure during follow-up for 5.2 (4.6–5.4) years.

Skin-AF Measurements

The AGE accumulation was assessed by measuring skin-AF using a validated autofluorescence reader (AGE Reader, DiagnOptics b.v., Groningen, The Netherlands) as it was described previously (10). In short, the autofluorescence reader illuminates a skin surface of 1 cm², guarded against surrounding light, with an excitation light source between 300 and 420 nm (peak excitation ~370 nm). Light from the skin is measured with a spectrometer (AVS-USB2000, Avantes Inc., Eerbeek, The Netherlands) in the 300 to 600 nm range, using a 200- μ m glass fiber (UV/VIS 200–750 nm, Avantes Inc., Eerbeek, The Netherlands). Skin-AF measurements in an individual patient consisted of 75 measurements, each with an integration time of 75 msec. Skin-AF was measured as the ratio between emission and excitation calculated in arbitrary units (AU) by dividing the intensity of the fluorescent light coming from the skin (measured as area under the curve [AUC] of fluorescent wave lengths between 420 and 600 nm) by the intensity of the emitted light (measured as AUC of wave lengths between 300 and 420 nm) multiplied by 100. All measurements were performed at room temperature in a dark environment. Skin-AF was measured at the volar side of the lower arm at approximately 10 to 15 cm below the elbow fold and the hollow of the knee, respectively. The average of both measurements was calculated for further analyses. Care was taken to perform the measurements at normal skin site, that is, without visible vessels, scars, lichenification, or other skin abnormalities. Intraobserver variation of repeated autofluorescence measurements on 1 day was 6%.

Recipient and Transplant Characteristics

Relevant donor, recipient, and transplant characteristics were extracted from the Groningen Renal Transplant Database. Extracted were age and sex from both donors and recipients, duration of pretransplant dialysis, date of transplantation, transplantation type, ischemia time, human leukocyte antigen (HLA) mismatches, renal function at baseline, and type of acute rejection treatment. History of cardiovascular disease (CVD) and smoking status were obtained from a self-report questionnaire. Smoking was defined as current use of cigarettes. History of CVD was based on patient self-report of myocardial infarction, angina pectoris, cerebrovascular accident, transient ischemic attack, or intermittent claudication in medical history of patient. Current medication was extracted from medical record. Patients were defined as having experienced an episode of rejection, when drugs were used to treat rejection. Acute rejection was treated with corticosteroids in case of borderline or interstitial rejection. Steroid resistant and vascular rejections were treated with antithymocyte globulin or OKT3. Standard immunosuppression consisted of the following: from 1968 until 1989 prednisolone and azathioprine; from January 1989 until February 1993 cyclosporine standard formulation (Sandimmune, Novartis) combined with prednisolone; from March 1993 until May 1996 cyclosporine microemulsion (Neoral, Novartis Pharma b.v., Arnhem, The Netherlands) and prednisolone; and from May 1997 to date mycophenolate mofetil (Cellcept, Roche b.v., Woerden, The Netherlands).

Clinical Measurements

During the visit to the outpatient clinic, blood pressure was measured using an automated oscillometric blood pressure device (Omron M4, Omron Europe b.v., the Netherlands) as the average of three consecutive measurements with 1-min intervals after a 6 min rest in supine position. During this visit, height and weight were also assessed and the body mass index was calculated. According to the 2003 guidelines of the European Society of Hypertension, patients were considered to be hypertensive if they had a systolic blood pressure over 140 mm Hg or a diastolic blood pressure over 90 mm Hg.

Laboratory Measurements

Blood was drawn at the outpatient clinic and 24-hr urine samples were collected. Urine was assessed for concentrations of protein and creatinine, and blood was analyzed for concentrations of glucose, and total cholesterol using standard laboratory techniques. HbA1c was determined by high-performance liquid chromatography (VARIANTTm HbA1c Program with Bio-Rad CARIANT Hb Testing System, Bio-Rad, Hercules, CA). Serum CRP was assessed with a high-sensitivity CRP ELISA assay as described before (14). Serum triglycerides were determined with the GPO-PAP method (MEGA AU 510, Merck Diagnostica Darmstadt, Germany). Creatinine clearance was determined from 24-hr urine samples. High-density lipoprotein-cholesterol was determined using the CHOD-PAP method (Technikon RA-1000, Bayer Diagnostics b.v., Mijdrecht, The Netherlands). Low-density lipoprotein-cholesterol was calculated using the Friedewald formula (15). Hypercholesterolemia was defined as a total cholesterol higher than 6.2 mmol/L or use of lipid lowering drugs (statins), according to the National Cholesterol Education Program criteria (16). Diabetes mellitus was classified according to the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus as a fasting glucose higher than 6.9 mmol/L or the use of antidiabetic medication or insulin (17).

Statistical Analyses

Analyses were performed with SPSS version 14 (SPSS Inc., Chicago, IL). Parametric variables are expressed as mean \pm standard deviation, nonparametric variables are given as median (25%–75% interquartile range), and nominal variables are given as n(%). Hazard ratios (HR) and areas under the receiver-operator-characteristic (ROC) curves are displayed with 95% confidence intervals. For all analyses, a *P* value less than or equal to 0.05 was considered to indicate statistical significance. All baseline variables were stratified for skin-AF tertiles and tested for difference over the respective tertiles. Tertile 1 was defined as skin-AF between 1.2 and 2.3 AU, tertile 2 as skin-AF between 2.3 and 2.9 AU, and tertile 3 as skin-AF between 2.9 and 5.2 AU. Parametric variables were tested using one-way analysis of variance, nominal variables using the chi-square test, and nonparametric variables using the Jonckheere-Terpstra test. A survival plot for graft loss stratified for tertiles of skin-AF was constructed from an unadjusted Cox-regression model. Cox regression analysis was used to construct a model for the prediction of graft loss. First, all baseline variables depicted in Table 1 were entered in univariable Cox regression analyses. All continuous variables showed a linear trend in the estimated HR and were thus

introduced in the Cox-regression analyses as continuous variables. A correlation matrix was constructed to detect potential existence of a multicollinearity problem for the baseline variables in the Cox-regression analyses. Correlations between all variables in the final model were less than 0.5, so no variables had to be excluded for reasons of potential multicollinearity. Subsequently, variables that at least showed a trend ($P \leq 0.10$) were entered in a multivariable Cox regression analysis. Variables that did not retain significance were removed from the model, which resulted in the first multivariable model. Next, we (re)introduced known risk factors for graft loss to validate the multivariable model. This resulted in the final multivariable model that was tested for interaction terms. Diabetes and HbA1c were separately tested for potential interaction. Furthermore, in subanalyses, we investigated the association of skin-AF with all-cause mortality and death-censored graft loss as separate end-points. Log-minus-log survival curves and time-dependent covariates were used to evaluate adherence of the Cox proportional hazard assumptions. No violations of the proportional hazard assumption were identified. ROC curves were plotted for skin-AF, urinary protein excretion, patient age, hsCRP, and creatinine clearance. For the ROC analyses, censoring was ignored by using a fixed follow-up time of 4.4 years for which complete follow-up data were available, as has been described by Mandel et al. (18).

RESULTS

A total of 302 outpatients (age 50 ± 12 years, 45% women, creatinine clearance 63 ± 23 mL/min) participated at a median time of 6.1 (2.6–12.1) years after transplantation. Baseline characteristics are summarized in Table 1 stratified for tertiles of skin-AF. Fifty-one patients (17%) were identified as having diabetes mellitus and 215 patients (71%) as having hypertension. Skin-AF of the leg was slightly higher than skin-AF of the arm (2.9 ± 1.0 vs. 2.6 ± 0.7 AU, $P < 0.001$). Average skin-AF was 2.7 ± 0.8 AU. Trend analysis showed that skin-AF was positively associated with patient age, female sex, donor age, dialysis duration, hypertension, smoking, CVD history, systolic blood pressure, glucose concentration, HbA1c, hsCRP, statin use, and inversely with creatinine clearance and hypercholesterolemia (Table 1).

Follow-up was performed for a median (interquartile range) time of 5.2 (4.6–5.4) years, during which 53 patients reached the endpoint of graft loss (19 graft failures and 34 deaths). Graft survival stratified for skin-AF tertiles is shown in Figure 1. Results of univariable and multivariable Cox-regression analyses are summarized in Table 2. Skin-AF significantly predicted graft loss in a univariable Cox regression analysis (HR 2.40 [1.75–3.29], $P < 0.001$). Other factors that univariately predicted graft loss included patient age, smoking, systolic blood pressure, HbA1c, hsCRP, serum creatinine, creatinine clearance, and urinary protein excretion. Furthermore, a trend ($P \leq 0.10$) for an association with graft loss existed for donor age and hypertension. Variables with at least a trend ($P \leq 0.10$) for an association with graft loss were entered into a multivariable Cox regression analysis. Variables that did not retain significance were subsequently removed from the model, which resulted in a multivariable model for prediction of graft loss consisting of skin-AF (HR 2.34 [1.70–3.24], $P < 0.001$), protein excretion (HR 1.51

TABLE 1. Recipient and transplant characteristics

Characteristics	Tertiles skin-AF			P
	1.2–2.3 AU (n=100)	2.3–2.9 AU (n=101)	2.9–5.2 AU (n=101)	
Patient demographics				
Age (yr)	45.0±11.3	49.3±11.9	57.4±10.4	<0.001
Sex (male)	67 (67.0)	57 (56.4)	48 (47.5)	0.02
Dialysis duration (mo)	20.5 (10.0–37.5)	28.0 (14.0–48.0)	29.0 (12.0–57.0)	0.01
Donor demographics				
Age (yr)	36.8±15.1	35.1±15.6	40.6±14.9	0.03
Sex (male)	59 (59.0)	52 (52.5)	51 (50.5)	0.45
Risk-factors CVD				
Diabetes mellitus, n (%)	13 (13.0)	16 (15.8)	22 (21.8)	0.24
Hypertension, n (%)	57 (57.0)	75 (74.3)	83 (82.2)	<0.001
Hypercholesterolemia, n (%)	65 (65.0)	49 (48.5)	62 (61.4)	0.05
Smoking, n (%)	13 (13.0)	17 (16.8)	27 (26.7)	0.04
CVD history, n (%)	7 (7.0)	12 (11.9)	20 (19.8)	0.02
Physical examination				
Systolic blood pressure (mm Hg)	143.7±18.7	148.9±18.9	162.1±26.7	<0.001
Diastolic blood pressure (mm Hg)	88.1±9.7	88.6±9.2	91.0±11.2	0.11
Body mass index (kg/m ²)	25.1±3.6	26.1±4.6	26.0±4.5	0.15
Laboratory examinations				
Glucose (mmol/L)	4.5 (4.1–4.9)	4.6 (4.2–5.1)	4.7 (4.3–5.3)	0.04
HbA1c (%)	6.1±0.9	6.4±1.1	6.7±1.2	<0.001
Total cholesterol (mmol/L)	5.6 (4.8–6.1)	5.3 (4.8–5.9)	5.6 (5.0–6.3)	0.14
Triglycerides (mmol/L)	1.6 (1.2–2.4)	1.9 (1.5–2.6)	1.9 (1.4–2.7)	0.21
hsCRP (mg/L)	1.3 (0.6–3.4)	1.5 (0.6–3.3)	3.0 (1.2–7.4)	<0.001
Creatinine (μmol/L)	133 (118–153)	135 (114–172)	139 (110–175)	0.45
Creatinine clearance (mL/min)	71.5±19.3	64.4±23.6	53.6±21.4	<0.001
Urinary protein excretion (g/24 h)	0.2 (0.0–0.4)	0.2 (0.0–0.4)	0.3 (0.0–0.6)	0.07
Skin-autofluorescence				
Skin-AF arm (AU)	2.0±0.3	2.6±0.4	3.1±0.6	<0.001
Skin-AF leg (AU)	1.9±0.5	2.7±0.4	3.9±0.8	<0.001
Average skin-AF (AU)	2.0±0.3	2.6±0.2	3.5±0.5	<0.001
Transplantation type				
Living, n (%)	19 (19.0)	14 (13.9)	14 (13.9)	0.51
Cadaveric, n (%)	79 (79.0)	83 (82.2)	82 (81.2)	0.84
Kidney/pancreas, n (%)	2 (2.0)	2 (2.0)	5 (5.0)	0.36
Kidney/liver, n (%)	0 (0.0)	2 (2.0)	0 (0.0)	0.14
Warm ischemia time (min)	36.5±10.9	40.9±17.6	39.4±13.5	0.09
Cold ischemia time (h)	19.6±10.4	20.5±10.1	21.4±10.2	0.42
HLA-AB mismatch, n (%)	75 (75.0)	66 (65.3)	69 (68.3)	0.31
HLA-DR mismatch, n (%)	41 (41.4)	38 (38.4)	35 (35.0)	0.65
Time since transplantation (yr)	6.0 (2.9–12.1)	6.5 (3.2–11.9)	6.0 (2.2–12.0)	0.89
Follow-up time (yr)	5.2 (4.8–5.4)	5.2 (4.6–5.4)	5.2 (4.2–5.4)	0.15
Acute rejection, n (%)				
No rejection	47 (47.0)	49 (49.0)	61 (52.2)	0.22
Steroid responsive	37 (37.0)	30 (30.0)	25 (30.5)	
ATG/OKT3 treated	16 (16.0)	21 (21.0)	15 (17.3)	
Drug-use				
RAAS blockade, n (%)	34 (34.0)	39 (38.6)	31 (30.7)	0.49
Beta-blocker, n (%)	62 (62.0)	61 (60.4)	71 (70.3)	0.29
Antidiabetic drugs, n (%)	11 (11.0)	11 (10.9)	17 (16.8)	0.36
Anti-platelet drugs, n (%)	16 (16.0)	22 (21.8)	23 (22.8)	0.44
Statines, n (%)	43 (43.0)	61 (60.4)	53 (52.5)	0.05
(Continued)				

(Continued)

TABLE 1. Continued

Characteristics	Tertiles skin-AF			P
	1.2–2.3 AU (n=100)	2.3–2.9 AU (n=101)	2.9–5.2 AU (n=101)	
Immunosuppressive drug				
Prednisolone day dose (mg)	10.0 (7.5–10.0)	10.0 (7.5–10.0)	10.0 (7.5–10.0)	0.55
Calcineurin inhibitors, n (%)	75 (75.0)	81 (80.2)	80 (79.2)	0.64
Mycophenolate mofetil, n (%)	43 (43.0)	37 (36.6)	41 (40.6)	0.65
Azathioprine n (%)	36 (36.0)	41 (40.6)	28 (27.7)	0.15

Parametric parameters are expressed as mean±SD; nonparametric parameters are expressed as median (25%–75% IQR); ordinal parameters are expressed as n(%).
AF, autofluorescence; CVD, cardiovascular disease; HLA, human leukocyte antigen; IQR, interquartile range; AU, arbitrary units.

[1.31–1.75], $P<0.001$), and hsCRP (HR 1.02 [1.01–1.04], $P=0.003$). To further validate this model, we (re)introduced known predictors of graft loss. No significant independent contribution was found for patient sex, use of calcineurin inhibitors, diabetes mellitus, HbA1c, glucose concentration, acute rejection, donor age, hypertension, hypercholesterolemia, body mass index, and ischemia times. We did not find a significant contribution of acute rejection to our model. This remained so also after subdivision of rejection episodes in steroid responsive and ATG/OKT3-treated rejection. We did, however, find additional contributions of patient age, creatinine clearance, and HLA-DR mismatching to our model, which resulted in a final model consisting of skin-AF (HR 1.83 [1.22–2.75], $P=0.003$), patient age (HR 1.03 [1.00–1.06], $P=0.04$), hsCRP (HR 1.02 [1.00–1.03], $P=0.03$), creatinine clearance (HR 0.99 [0.97–1.00], $P=0.05$), urinary protein excretion (HR 1.57 [1.34–1.83], $P<0.001$), and HLA-DR mismatching (HR 2.02 [1.14–3.61], $P=0.02$). No significant interaction of skin-AF with other predictors of graft loss, including patient age, creatinine clearance, proteinuria, diabetes mellitus, HbA1c, and hsCRP were identified. Finally,

subanalysis revealed that skin-AF was significantly associated with both all-cause mortality (HR 2.50 [1.72–3.64], $P<0.001$) and death-censored graft loss (HR 2.42 [1.43–4.09], $P=0.001$). Further adjustment for the co-variables patient age, hsCRP, creatinine clearance, urinary protein excretion, and HLA-DR mismatching resulted in comparable point estimates for the HR for skin-AF as predictor of all-cause mortality (HR 1.86 [1.12–3.10], $P=0.017$) and death-censored graft loss (HR 1.79 [0.88–3.67], $P=0.11$).

ROC curves for graft loss are shown in Figure 2. The area under the ROC curve of skin-AF (0.73 [0.65–0.81]) was similar to the one for urinary protein excretion (0.69 [0.61–0.78]), patient age (0.66 [0.57–0.75]), hsCRP (0.66 [0.56–0.75]), and creatinine clearance (0.71 [0.63–0.79]). All areas under the ROC curve were significantly different from 0.5.

DISCUSSION

This study showed for the first time that skin-AF, a validated marker for the accumulation of AGEs, is a strong predictor of graft loss in renal transplant recipients. The as-

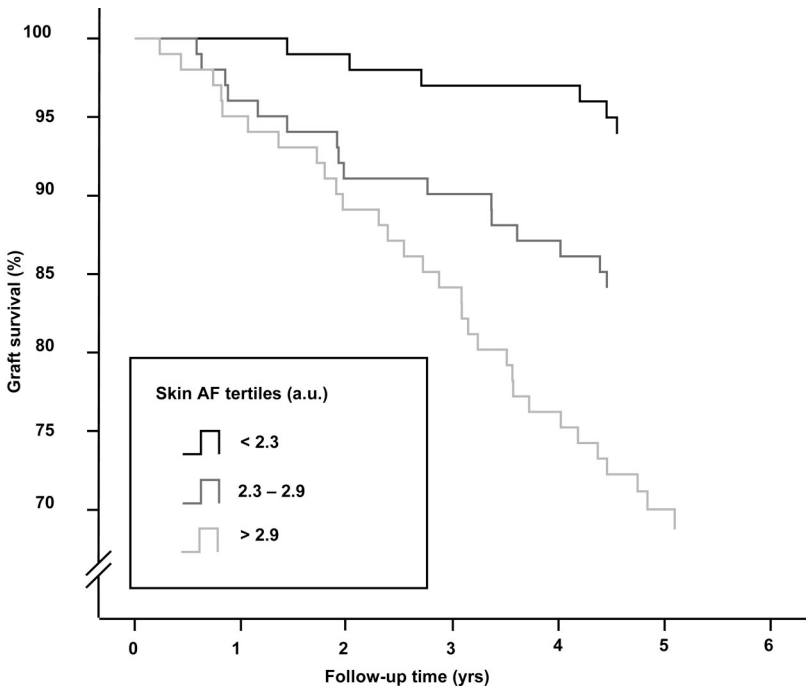


FIGURE 1. Graft survival stratified for skin-AF tertile. Shown are unadjusted Cox-survival curves stratified on tertiles of skin-AF.

TABLE 2. Results of univariable and multivariable Cox regression analysis

Characteristics	Univariable		Multivariable	
	HR (95% CI)	P	HR (95% CI)	P
Patient demographics				
Age (yr)	1.04 (1.01–1.06)	0.003	1.03 (1.00–1.06)	0.04
Sex (male)	0.98 (0.57–1.69)	0.94		
Dialysis durations (mo)	1.03 (0.46–2.27)	0.95		
Donor demographics				
Age (yr)	1.02 (0.99–1.04)	0.07		
Sex (male)	1.31 (0.75–2.26)	0.34		
Risk factors CVD				
Diabetes mellitus (yes)	1.31 (0.67–2.54)	0.43		
Hypertension (yes)	1.85 (0.93–3.67)	0.08		
Hypercholesterolemia (yes)	1.03 (0.46–2.27)	0.95		
Smoking (yes)	2.45 (1.39–4.33)	0.002		
CVD history (yes)	1.03 (0.46–2.27)	0.95		
Physical examination				
Systolic blood pressure (mm Hg)	1.02 (1.01–1.03)	0.003		
Diastolic blood pressure (mm Hg)	1.03 (0.99–1.04)	0.33		
Body mass index (kg/m ²)	1.02 (0.95–1.08)	0.64		
Laboratory values				
Glucose (mmol/L)	1.00 (0.79–1.27)	0.99		
HbA1c (%)	1.30 (1.05–1.59)	0.01		
Total cholesterol (mmol/L)	0.93 (0.70–1.23)	0.61		
Triglycerides (mmol/L)	1.08 (0.91–1.29)	0.38		
hsCRP (mg/L)	1.03 (1.02–1.04)	<0.001	1.02 (1.00–1.03)	0.03
Creatinine (μmol/L)	1.01 (1.01–1.01)	<0.001		
Creatinine clearance (mL/min)	0.97 (0.96–0.98)	<0.001	0.99 (0.97–1.0)	0.05
Protein excretion (g/24 hr)	1.54 (1.33–1.78)	<0.001	1.57 (1.34–1.83)	<0.001
Skin-AF (AU)	2.40 (1.75–3.29)	<0.001	1.83 (1.22–2.75)	0.003
Transplantation type				
Living (yes)	1.14 (0.55–2.33)	0.73		
Cadaveric (yes)	1.02 (0.51–2.03)	0.96		
Kidney/pancreas (yes)	0.59 (0.08–4.27)	0.60		
Kidney/liver (yes)	n/a	n/a		
Warm ischemia time (min)	1.00 (0.98–1.02)	0.95		
Cold ischemia time (hr)	0.99 (0.97–1.02)	0.65		
HLA-AB mismatch (yes)	1.01 (0.56–1.82)	0.96		
HLA-DR mismatch (yes)	1.28 (0.74–2.20)	0.38	2.02 (1.14–3.61)	0.02
Time since transplantation (yr)	0.98 (0.94–1.03)	0.39		
Acute rejection, n (%)				
No rejection	1			
Steroid responsive	1.36 (0.76–2.43)	0.30		
ATG/OKT3 treated	0.81 (0.35–1.86)	0.61		
Drug-use				
RAAS blockade (yes)	0.67 (0.36–1.24)	0.20		
Beta-blocker (yes)	0.82 (0.48–1.43)	0.49		
Antidiabetic drugs (yes)	1.19 (0.56–2.52)	0.66		
Antiplatelet drugs (yes)	1.02 (0.52–1.97)	0.97		
Statins (yes)	1.04 (0.61–1.78)	0.90		
Immunosuppressive drug				
Prednisolone day dose (mg)	1.18 (0.93–1.50)	0.18		
Calcineurin inhibitors (yes)	0.93 (0.49–1.77)	0.82		
Mycophenolate mofetil (yes)	0.89 (0.51–1.55)	0.69		
Azathioprine (yes)	0.81 (0.45–1.45)	0.47		

AF, autofluorescence; CVD, cardiovascular disease; HLA, human leukocyte antigen; HR, hazards ratio; CI, confidence interval; AU, arbitrary units.

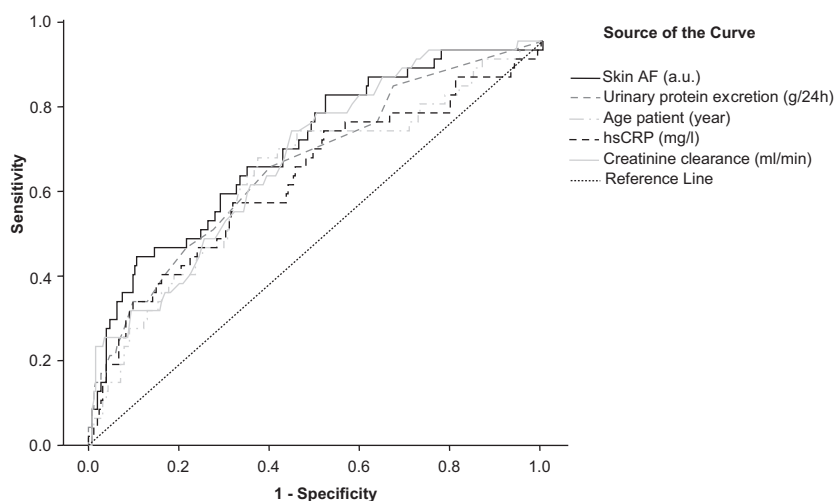


FIGURE 2. Receiver-operator-characteristic (ROC) curves for graft loss. Shown are the ROC curves of skin-AF, urinary protein excretion, age patient, hsCRP, and creatinine clearance.

sociation of skin-AF with graft loss was independent from other identified risk factors, including patient age, hsCRP, creatinine clearance, protein excretion, and HLA-DR mismatching. The prognostic value of skin-AF for graft loss was comparable with the prognostic value of the other significant predictors of graft loss as was concluded from the AUCs found by the ROC curve analyses.

So far, no prospective study existed which investigated the association of AGEs with graft failure or mortality in kidney transplant recipients. However, some data exist of studies that investigated associations of oxidative stress and AGEs in kidney transplantation. Raj et al. (19) investigated levels of circulating AGEs and markers of oxidative stress in patients who developed chronic renal transplant dysfunction. Patients with biopsy-proven chronic renal transplant dysfunction had higher levels of AGEs and markers of oxidative stress when compared with transplant recipients with normal renal function and patients with chronic renal failure of their native kidneys. Recently, data from our own center showed that inhibition of AGE formation is renoprotective in a Fischer 344 to Lewis (F-L) allograft rat model of experimental chronic renal transplant dysfunction (20).

Several studies did investigate the association of AGEs with outcome in ESRD. Overall, the findings of these studies have been inconsistent (21–23). Wagner et al. (21) and Roberts et al. (22) reported that high levels of AGEs are a risk factor for mortality, whereas Schwedler et al. (23) reported a potential protective effect of serum AGEs for mortality. In a nondiabetic population, high-serum AGE levels were found to be a risk factor for mortality in women but not in men (24). In a type 2 diabetic population, serum AGEs were not found to be a risk factor for cardiovascular mortality (25). However, in all these studies serum levels of AGEs were measured, which are more prone to short-term variations than tissue AGE accumulation. Our group previously reported that AGE accumulation measured as skin-AF was associated with mortality in dialysis patients and diabetic patients independent from known risk factors (11, 26). This study confirmed this association in kidney transplant recipients.

Although our data limit us in being conclusive about causality, we can speculate about possible underlying pathways that may explain the prognostic value of AGEs found in

the present study. In kidney transplantation, oxidative stress may be a source for AGE accumulation (27). Oxidative stress itself may be a consequence of ischemia-reperfusion injury, chronic rejection, and immunosuppressive therapy (28–30). Oxidative stress damages DNA, proteins, and lipids by means of chemical reactions of oxygen and nitrogen radicals. It has been hypothesized that protein damage resulting from oxidative stress such as advanced glycation could be the main contributor for pathologic changes in ESRD (9). Certain damaged proteins may be recognized by proinflammatory receptors, because it is the case for AGEs and their receptor (RAGE) (31). Under healthy conditions, AGEs are cleared efficiently by the kidney without causing severe damage, but under uremic conditions AGEs may accumulate significantly potentially leading to enhanced receptor binding and prolonged proinflammatory signaling (32). The AGE-RAGE interaction stimulates second messenger pathways, among which the renin-angiotensin pathway, the Rac-Cdc42 pathway, the Jac-Stat pathway, and the production of reactive oxygen species by the NADPH oxidase pathway (7). In addition to the activation of these pathways, the AGE-RAGE interaction also up-regulates nuclear factor- κ B (NF- κ B) which subsequently up-regulates the production of inflammatory mediators, such as TNF and VCAM-1, and also RAGE itself (33, 34). The up-regulation of RAGE and the production of reactive oxygen species may finally lead to a vicious cycle and an amplified inflammatory response. In addition to the activation of receptor-mediated pathways, AGEs can also directly affect endogenous targets. AGEs can covalently bind other AGEs and form cross-links between matrix proteins such as collagen. Extensive cross-linking may then lead to, for example, myocardial stiffening and cardiac mortality (35).

Although skin-AF has been validated to represent accumulation of AGEs, it has to be taken into account that the fluorescence wavelength used to measure AGEs is not specific. In addition to AGEs other substances such as lipofuscin and ceroid exist in the human organism that can be detected using the same excitation and emission wavelengths (36). However, precursors for the formation of these so-called age pigments and AGEs both result from oxidative stress (36), which suggests that skin-AF measures the accumulation of oxidative-stress-derived metabolites in general rather than

AGEs in particular. Skin-AF might also represent susceptibility for chronic diseases in general rather than a specific susceptibility to renal or CVD. Finally, the skin-AF reader has to date only been validated in whites, limiting the implications of our results to white patients only.

We were also limited by the number of cardiovascular deaths and graft failures in our study, because these were too low to investigate a specific association of skin-AF with cardiovascular mortality or graft failure caused by chronic transplant dysfunction. Using cardiovascular mortality in stead of all-cause mortality and graft failure caused by chronic transplant dysfunction in stead of all-cause graft failure as end points would have been more supportive to the general theory of AGE pathology. However, in renal transplant patients most deaths were due to cardiovascular events, and most graft failures were due to chronic transplant dysfunction (37).

In the final model for graft loss some well-known predictors of graft outcome, such as acute rejection (38), donor age (39), hypertension (40), and diabetes (41) were not identified as independent predictors. In univariable analyses, however, some of these variables (donor age) or closely linked variables (systolic blood pressure and HbA1c) were significantly or borderline significantly associated with outcome. This suggests that sample size—and thus the power of our study—was only sufficient to detect strong predictors as independent predictors.

In univariable analyses, both HbA1c and skin-AF were significant predictors of graft loss, indicating stronger relations between these continuous variables with graft loss than the diagnosis of diabetes. Yet, in multivariable analyses, HbA1c disappeared from the model as well, whereas skin-AF was retained, which is consistent with the notion that skin-AF is a stronger determinant of graft loss, with some predictive properties shared with HbA1c, but also some predictive properties that are not held by HbA1c.

The predictive power of skin-AF is not stronger than that of proteinuria or creatinine clearance. However, the practical benefit of skin-AF is that it is a predictor independent of age, proteinuria, hsCRP, and creatinine clearance. Thus, it independently adds to the prognostication of individual patients. Another practical benefit is its simplicity. While proteinuria and creatinine clearance require 24-hr collection of urine and laboratory assessments, and hsCRP requires blood sampling and laboratory assessment, skin-AF can be measured directly at the outpatient clinic within a few minutes, without any inconvenience to the patient.

In conclusion, our data show for the first time that high skin-AF values are strongly and independently associated with the development of graft loss in kidney transplant recipients. Although we should keep in mind that skin-AF is no direct measurement of AGE accumulation, we do believe that our results are in line with results of other studies and they support the general concept that oxidative stress and AGE accumulation are pathophysiologically involved in the development of graft loss in renal transplant recipient. Skin-AF might be a useful method to estimate the risk for graft loss after kidney transplantation. Intervention studies are required to find out whether the association between skin-AF and graft loss that we observed implies a causal relation between AGE accumulation and graft loss.

ACKNOWLEDGMENTS

The authors thank all collaborators, especially the Deutsche Forschungsgesellschaft (DFG), which provided funding of this project. The authors also thank Gerhard Opelz for revising the manuscript before submission.

REFERENCES

- Gilbertson DT, Liu J, Xue JL, et al. Projecting the number of patients with end-stage renal disease in the United States to the year 2015. *J Am Soc Nephrol* 2005; 16: 3736.
- Pontou P, Rupolo GP, Marchini F, et al. Quality-of-life change after kidney transplantation. *Transplant Proc* 2001; 33: 1887.
- Wolfe RA, Ashby VB, Milford EL, et al. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N Engl J Med* 1999; 341: 1725.
- Merville P. Combating chronic renal allograft dysfunction: Optimal immunosuppressive regimens. *Drugs* 2005; 65: 615.
- Hariharan S, Johnson CP, Bresnahan BA, et al. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med* 2000; 342: 605.
- Arend SM, Mallat MJ, Westendorp RJ, et al. Patient survival after renal transplantation: More than 25 years follow-up. *Nephrol Dial Transplant* 1997; 12: 1672.
- Hartog JW, Smit AJ, van Son WJ, et al. Advanced glycation end products in kidney transplant patients: A putative role in the development of chronic renal transplant dysfunction. *Am J Kidney Dis* 2004; 43: 966.
- Stenvinkel P, Diczfalusy U, Lindholm B, et al. Phospholipid plasmalogen, a surrogate marker of oxidative stress, is associated with increased cardiovascular mortality in patients on renal replacement therapy. *Nephrol Dial Transplant* 2004; 19: 972.
- Galli F. Protein damage and inflammation in uraemia and dialysis patients. *Nephrol Dial Transplant* 2007; 22(Suppl 5): v20.
- Meerwaldt R, Links T, Graaff R, et al. Simple noninvasive measurement of skin autofluorescence. *Ann N Y Acad Sci* 2005; 1043: 290.
- Meerwaldt R, Hartog JW, Graaff R, et al. Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. *J Am Soc Nephrol* 2005; 16: 3687.
- Kasiske BL, Vazquez MA, Harmon WE, et al. Recommendations for the outpatient surveillance of renal transplant recipients. American Society of Transplantation. *J Am Soc Nephrol* 2000; 11(Suppl 15): S1.
- Oterdoom LH, de Vries AP, Gansevoort RT, et al. Determinants of insulin resistance in renal transplant recipients. *Transplantation* 2007; 83: 29.
- de Leeuw K, Sanders JS, Stegeman C, Smit A, et al. Accelerated atherosclerosis in patients with Wegener's granulomatosis. *Ann Rheum Dis* 2005; 64: 753.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18: 499.
- Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002; 106: 3143.
- Genuth S, Alberti KG, Bennett P, et al. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* 2003; 26: 3160.
- Mandel M, Galai N, Simchen E. Evaluating survival model performance: A graphical approach. *Stat Med* 2005; 24: 1933.
- Raj DS, Lim G, Levi M, et al. Advanced glycation end products and oxidative stress are increased in chronic allograft nephropathy. *Am J Kidney Dis* 2004; 43: 154.
- Waanders F, van den Berg E, Nagai R, et al. Renoprotective effects of the AGE-inhibitor pyridoxamine in experimental chronic allograft nephropathy in rats. *Nephrol Dial Transplant* 2008; 23: 518.
- Wagner Z, Molnar M, Molnar GA, et al. Serum carboxymethyllysine predicts mortality in hemodialysis patients. *Am J Kidney Dis* 2006; 47: 294.
- Roberts MA, Thomas MC, Fernando D, et al. Low molecular weight advanced glycation end products predict mortality in asymptomatic patients receiving chronic haemodialysis. *Nephrol Dial Transplant* 2006; 21: 1611.
- Schwedler SB, Metzger T, Schinzel R, et al. Advanced glycation end products and mortality in hemodialysis patients. *Kidney Int* 2002; 62: 301.

24. Kilhovd BK, Juutilainen A, Lehto S, et al. High serum levels of advanced glycation end products predict increased coronary heart disease mortality in nondiabetic women but not in nondiabetic men: A population-based 18-year follow-up study. *Arterioscler Thromb Vasc Biol* 2005; 25: 815.
25. Busch M, Franke S, Wolf G, et al. The advanced glycation end product N(epsilon)-carboxymethyllysine is not a predictor of cardiovascular events and renal outcomes in patients with type 2 diabetic kidney disease and hypertension. *Am J Kidney Dis* 2006; 48: 571.
26. Meerwaldt R, Lutgers HL, Links TP, et al. Skin autofluorescence is a strong predictor of cardiac mortality in diabetes. *Diabetes Care* 2007; 30: 107.
27. Hartog JW, de Vries AP, Bakker SJ, et al. Risk factors for chronic transplant dysfunction and cardiovascular disease are related to accumulation of advanced glycation end-products in renal transplant recipients. *Nephrol Dial Transplant* 2006; 21: 2263.
28. Perrea DN, Moulakakis KG, Poulakou MV, et al. Correlation between oxidative stress and immunosuppressive therapy in renal transplant recipients with an uneventful postoperative course and stable renal function. *Int Urol Nephrol* 2006; 38: 343.
29. Plotnikov EY, Kazachenko AV, Vyssokikh MY, et al. The role of mitochondria in oxidative and nitrosative stress during ischemia/reperfusion in the rat kidney. *Kidney Int* 2007; 72: 1493.
30. Djamali A, Sadowski EA, Muehrer RJ, et al. BOLD-MRI assessment of intrarenal oxygenation and oxidative stress in patients with chronic kidney allograft dysfunction. *Am J Physiol Renal Physiol* 2007; 292: F513.
31. Bierhaus A, Ritz E, Nawroth PP. Expression of receptors for advanced glycation end-products in occlusive vascular and renal disease. *Nephrol Dial Transplant* 1996; 11(Suppl 5): 87.
32. Bergmann R, Helling R, Heichert C, et al. Radio fluorination and positron emission tomography (PET) as a new approach to study the in vivo distribution and elimination of the advanced glycation endproducts N epsilon-carboxymethyllysine (CML) and N epsilon-carboxyethyllysine (CEL). *Nahrung* 2001; 45: 182.
33. Bierhaus A, Schiekofe S, Schwaninger M, et al. Diabetes-associated sustained activation of the transcription factor nuclear factor-kappaB. *Diabetes* 2001; 50: 2792.
34. Mohamed K, Bierhaus A. The role of oxidative stress and NF-kappaB activation in late diabetic complications. *Biofactors* 1999; 10: 157.
35. Aronson D. Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes. *J Hypertens* 2003; 21: 3.
36. Yin D. Biochemical basis of lipofuscin, ceroid, and age pigment-like fluorophores. *Free Radic Biol Med* 1996; 21: 871.
37. Kreis HA, Ponticelli C. Causes of late renal allograft loss: Chronic allograft dysfunction, death, and other factors. *Transplantation* 2001; 71: S55.
38. Opelz G, Dohler B. Influence of time of rejection on long-term graft survival in renal transplantation. *Transplantation* 2008; 85: 661.
39. Meier-Kriesche HU, Cibrik DM, Ojo AO, et al. Interaction between donor and recipient age in determining the risk of chronic renal allograft failure. *J Am Geriatr Soc* 2002; 50: 14.
40. Opelz G, Wujciak T, Ritz E. Association of chronic kidney graft failure with recipient blood pressure. Collaborative Transplant Study. *Kidney Int* 1998; 53: 217.
41. Miles AM, Sumrani N, Horowitz R, et al. Diabetes mellitus after renal transplantation: As deleterious as non-transplant-associated diabetes? *Transplantation* 1998; 65: 380.