

Original Article

Risk factors for chronic transplant dysfunction and cardiovascular disease are related to accumulation of advanced glycation end-products in renal transplant recipients

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Abstract

Background. Accumulation of advanced glycation end-products (AGEs) has been implicated in the pathogenesis of chronic transplant dysfunction and cardiovascular disease in renal transplant recipients. We aimed to investigate which factors are associated with tissue AGE accumulation in renal transplant recipients.

Methods. The AGE accumulation was assessed using a validated skin-autofluorescence reader (AFR) in 285 consecutive renal transplant recipients (57% male, aged 50 ± 12 years) visiting the outpatient clinic at a median (interquartile range) time of 73 (32–143) months after transplantation. Furthermore, various transplant- and recipient-related factors of interest were collected.

Results. Average skin-autofluorescence of lower arm and leg was 2.7 ± 0.8 a.u. Skin-autofluorescence was positively determined by recipient age, systolic blood pressure, smoking, high-sensitivity C-reactive protein, duration of pre-transplant dialysis, and negatively by plasma vitamin C levels, creatinine clearance at baseline, and change in creatinine clearance since one year after transplantation in linear multivariate regression analysis. Together, these factors explained 41% of the variance of skin-autofluorescence.

Conclusions. Skin-autofluorescence was associated with several risk factors for cardiovascular disease and chronic renal transplant dysfunction. These results are in line with the hypothesis that AGEs play a role in the pathogenesis of these conditions in renal

transplant recipients. Prospective studies are required to investigate whether the AFR can be used as a simple, non-invasive tool to identify and monitor patients at risk for chronic renal transplant dysfunction and cardiovascular disease.

Keywords: advanced glycation end-products; cardiovascular disease; chronic renal transplant dysfunction; renal transplantation; risk factors; skin-autofluorescence

Introduction

Transplantation is currently the best renal replacement therapy for patients with end-stage renal disease. Graft loss due to cardiovascular mortality and chronic transplant dysfunction is a major concern in renal transplant medicine. Due to the introduction of new immunosuppressive medication short-term renal allograft survival has improved substantially. These improvements led to expectations of improved long-term survival rates. These so far have been minor and long-term survival rates still strongly lag behind [1]. One of the challenges in transplant research is to obtain insight into the factors associated with long-term allograft survival.

In transplant recipients, death rates from cardiovascular disease exceed those of the general population [2]. Most likely, this is the consequence of the high prevalence of cardiovascular risk factors in transplant recipients. The notion is emerging that the development of chronic renal transplant dysfunction constitutes, at least to a certain extent, a manifestation of cardiovascular disease [3,4]. This is supported by the

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fact that there is a great overlap between risk factors for cardiovascular disease and risk factors for chronic renal transplant dysfunction [3,4]. The latter include recipient age, impaired renal function, hypertension, the presence of diabetes, proteinuria, hyperlipidaemia, obesity, transplant ischaemia and use of calcineurin inhibitors [3]. Interestingly, these same risk factors also overlap factors with associated with accumulation of advanced glycation end-products (AGEs). This led to the hypothesis that AGEs are involved in the development of cardiovascular disease and chronic renal transplant dysfunction after transplantation [3,4].

Advanced glycation end-products (AGEs) originate from reactions between sugar and lipid adducts with proteins [5]. AGE accumulation has been shown to increase with aging, renal function impairment and presence of diabetes in non-transplant populations. With respect to renal transplant recipients, AGE accumulation might be further influenced by transplantation techniques, donor characteristics, human leucocyte antigen (HLA) mismatching, use of immunosuppressive medication and deteriorating renal function. We aimed to investigate the factors associated with tissue AGE accumulation in renal transplant recipients.

Subjects and methods

Study population and design

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 were monitored after transplantation in the outpatient clinic in accordance with American Transplantation Guidelines [2], i.e. ranging from twice a week just after discharge from hospital to twice a year long-term after transplantation. All adult patients (aged ≥ 18 years) who survived the first year after transplantation with a functioning allograft were eligible to participate (1 year post-transplant was considered *baseline*). From December 2001 to March 2003, we invited 471 consecutive renal transplant recipients to participate in this study at their next visit to the outpatient clinic (*index date*). A total of 324 (69%) patients signed written informed consent. From this group, 12 patients were excluded from the analysis because of non-Caucasian ethnicity as the skin-autofluorescence reader (AFR) used in this study to measure AGE accumulation has not yet been validated in populations with increased skin pigmentation, and 27 patients because of missing data, leaving a total of 285 patient for analyses. All measurements were performed after an 8 h overnight fast.

AGE measurement by skin-autofluorescence

The AGE accumulation was assessed by measuring skin-autofluorescence using a validated skin-autofluorescence reader (AFR; patent PCT/NL99/00607; DiagnOptics BV, Groningen, The Netherlands) as described previously [6].

In short, the AFR illuminates a skin surface of approximately 1 cm^2 , 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 300–600 nm range, using 200 μm glass fibre (Avantes UV/VIS, 200–750 nm, Avantes Inc., Eerbeek, The Netherlands). As a measure of autofluorescence the ratio between emission and excitation was calculated in arbitrary units (a.u.) by dividing the area under the curve between 420 and 600 nm by the area under the curve between 300 and 420 nm, and multiplying by 100 [7]. All measurements were performed at room temperature in a dark environment. Autofluorescence of the skin was measured at the volar side of the lower arm, and the posterior side of the lower leg at approximately 10–15 cm below the elbow fold and the hollow of the knee, respectively. Skin-autofluorescence measurements in an individual patient consisted of 75 measurements with an integration time of 75 ms. Care was taken to perform the measurement at normal skin site, i.e. without visible vessels, scars, lichenification or other skin abnormalities. Intra-observer variation of repeated AFR measurements on one day was 6%. For data analysis we calculated the average autofluorescence of arm and leg.

Recipient and transplant characteristics

Relevant recipient and transplant characteristics were partially extracted from the Groningen Renal Transplant Database. This database holds information of all renal transplantations that have been performed at our centre since 1968. Extracted were recipient and donor age, gender, primary renal disease, duration of pre-transplant dialysis, date of transplantation, ischaemia time, number of HLA mismatches, acute rejection treatment and 24 h urinary creatinine clearance at one year after transplantation. Current medication was extracted from medical records. History of cardiovascular disease and smoking status were obtained from a self-report questionnaire. Smoking was defined as current use of cigarettes. Patients were grouped as having experienced an episode of rejection, when drugs were used to treat rejection. History of cardiovascular disease was based on patient self-report of myocardial infarction, angina pectoris, cerebrovascular accident, transient ischaemic attack or intermittent claudication in the medical history of the patient. Standard immunosuppression consisted of the following: from 1968 until 1989 prednisolone and azathioprine; from January 1989 until February 1993 ciclosporin standard formulation (Sandimmune, Novartis) combined with prednisolone; from March 1993 until May 1996 ciclosporin microemulsion (Neoral, Novartis Pharma b.v., Arnhem, The Netherlands) and prednisolone; from May 1997 to date mycophenolate mofetil (Cellcept, Roche b.v., Woerden, The Netherlands) was added.

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 the supine position. Height and weight were assessed as well. Body mass index (BMI) was

calculated as weight (kg) divided by the square of height (m). Obesity was defined as BMI of 30 kg/m² or higher, according to the guidelines of the World Health Organization [8]. According to the 2003 guidelines of the European Society of Hypertension, patients with a systolic blood pressure 140 mmHg, a diastolic blood pressure 90 mmHg, or patients using anti-hypertensive drugs, were considered to be hypertensive [9].

Laboratory assessments

Blood was drawn at the outpatient clinic and 24 h urine samples were collected. Using standard laboratory techniques urine was assessed for concentrations of protein and creatinine, and blood was analysed for concentrations of creatinine, glucose and total cholesterol. Vitamin C and E were determined by HPLC (Knauer K-1001, Wissenschaftliche Gerätebau, Berlin, Germany; Waters 717 PLUS, Milford, MA, USA; Shimadzu RF 551, Shimadzu Scientific Instruments Inc., Maryland, Columbia, USA). HbA1c was determined by HPLC as well (VARIANT™ HbA1c Program with Bio-Rad CARIANT Hb Testing System, Bio-Rad, Hercules, CA, USA). Serum was assessed with a high-sensitivity (hs) CRP ELISA assay. Both intra- and inter-assay variation coefficients were 5%. Creatinine clearance was calculated from 24 h urine by the $U \times V/P$ formula. Delta creatinine clearance was calculated by subtracting creatinine clearance at index date from creatinine clearance at baseline. Hypercholesterolaemia 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 (NCEP) criteria [10]. 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 anti-diabetic medication or insulin [11].

Statistical analysis

Analyses were performed using SPSS version 12.01 (SPSS Inc., Chicago, IL, USA). Parametric variables are expressed as mean \pm SD, whereas non-parametric variables are expressed as median (interquartile range). Nominal variables are expressed as n (%). To gain insight into which risk factors are associated with AGE accumulation, we first performed univariate analyses for trend over quartiles of skin-autofluorescence. P -value for trend was determined by linear regression for continuous variables, and by chi-square and Jonckheere–Terpstra tests for nominal and ordinal variables, respectively. Second, univariate linear regression analyses were performed for factors that showed at least a trend ($P \leq 0.20$) with skin-autofluorescence in trend analyses (model 1). Adjustments were consecutively performed for age (model 2) and renal function parameters (model 3). Third, to analyse which of the factors were independently associated with skin-autofluorescence, a multivariate linear regression analysis was performed with skin-autofluorescence as Creative protein (CRP) dependent variable. Next to age and renal function parameters, co-variables with a P -value ≤ 0.20 in model 3 were included in the analysis. Variables which have not retained significance in this multivariate analysis were subsequently removed from the model (so-called *backward selection*). This method is most suitable for

cross-sectional data. To test whether the model is appropriate and whether the assumptions for linear regression are met, the model has been tested for overall regression, collinearity, interaction terms and lack-of-fit with ANOVA. Residuals were tested for normality of distribution. A P -value ≤ 0.05 was considered to indicate statistical significance.

Results

Results are presented for a total of 285 transplant recipients (163 male, 122 female). Mean age of transplant recipients was 50 ± 12 years. Index date was 73 (32–143) months after transplantation. At index date 96.8% of patients had hypertension, 15.8% had diabetes mellitus, 13.0% were obese and 66.0% had hypercholesterolaemia. Creatinine clearance at index date was 62 ± 22 ml/min. Skin-autofluorescence of lower arm and leg at index date were 2.6 ± 0.7 a.u. and 2.9 ± 1.0 a.u., respectively, with an average of 2.7 ± 0.8 a.u.

Tables 1 and 2 show recipient and transplant characteristics grouped according to quartiles of skin-autofluorescence. Significant associations with skin-autofluorescence were present for recipient sex, recipient age, systolic blood pressure, HbA1c, plasma vitamin C levels, hs-CRP, smoking, history of cardiovascular disease, donor age, duration of pre-transplant dialysis, creatinine clearance at baseline, creatinine clearance at index date and delta creatinine clearance. No effect of immunosuppressive treatment and the use of ACE inhibition (AII receptor antagonists or ACE inhibitors) was found.

Using univariate linear regression analysis we calculated standardized regression coefficients (β) and P -values for the variables that at least showed a tendency ($P \leq 0.20$) to be associated with skin-autofluorescence in trend analyses (model 1). Adjustments were consecutively performed for age (model 2), and renal function parameters (model 3). The effect of adjustments can be judged by comparing standardized regression coefficients and P -values of an association before and after adjustment (Table 3). Adjustments for age and renal function parameters did not substantially affect the association between plasma vitamin C and skin-autofluorescence. The association between smoking and skin-autofluorescence strengthened after adjustment for age. The associations of BMI, total time of ischaemia, HbA1c and history of cardiovascular disease with skin-autofluorescence were less strong after adjustment for age. The associations of durations of dialysis prior to transplantation, and donor age became less strong after adjustment for renal function parameters. The associations of recipient sex, systolic blood pressure and hs-CRP were (partially) dependent on both age and renal function parameters.

Using multivariate linear regression analysis we determined which factors were independently associated with skin-autofluorescence. Variables that showed significant association—or at least a tendency to be significant ($P \leq 0.20$)—with skin-autofluorescence

Table 1. Recipient characteristics grouped according to quartiles of skin-autofluorescence

Recipient characteristics	Quartiles of skin-autofluorescence (a.u.)				P for trend
	1.2–2.2 (n = 71)	2.2–2.6 (n = 71)	2.6–3.1 (n = 72)	3.1–5.2 (n = 71)	
Recipient demographics					
Recipient sex (male)	44 (62.0)	48 (67.6)	38 (52.8)	33 (46.5)	0.02
Recipient age (years)	44 ± 11	47 ± 11	52 ± 12	58 ± 10	<0.0001
Primary renal disease, n (%)					
Primary glomerulopathy	26 (36.6)	20 (28.2)	21 (29.2)	15 (21.1)	>0.2
Tubulointerstitial/pyelonephritis	11 (15.5)	13 (18.3)	9 (12.5)	11 (15.5)	
Cystic renal disease	10 (14.1)	9 (12.7)	8 (11.1)	13 (18.3)	
Vasculitis/autoimmune	6 (8.5)	7 (9.9)	4 (5.6)	3 (4.2)	
Other	18 (25.3)	22 (30.9)	30 (41.6)	29 (40.9)	
Clinical measurements					
Systolic blood pressure (mmHg)	144 ± 19	147 ± 18	153 ± 21	165 ± 26	<0.0001
Diastolic blood pressure (mmHg)	89 ± 10	89 ± 9	88 ± 11	92 ± 10	0.11
Body mass index (kg/m ²)	25.1 ± 3.7	25.5 ± 3.7	25.7 ± 4.9	26.5 ± 4.6	0.06
Laboratory assessments					
HbA1c (%)	6.2 ± 0.9	6.3 ± 1.1	6.5 ± 1.0	6.8 ± 1.2	<0.0001
Fasting glucose (mmol/l)	4.7 ± 1.0	5.0 ± 1.3	5.0 ± 1.3	4.9 ± 1.1	0.20
Total cholesterol (mmol/l)	5.5 ± 0.9	5.3 ± 1.0	5.6 ± 1.0	5.7 ± 1.1	0.14
Serum albumin (g/l)	41 ± 2.8	41 ± 3.8	40 ± 4.2	40 ± 3.5	0.07
Plasma vitamin C (µmol/l)	50 ± 18	46 ± 20	48 ± 21	37 ± 21	0.001
Plasma vitamin E (µmol/l)	35 ± 10	38 ± 12	37 ± 11	37 ± 12	>0.2
hs-CRP (µg/ml)	1.4 [0.6–2.8]	1.2 [0.7–3.5]	1.7 [0.7–4.1]	3.2 [1.2–7.3]	0.01
Proteinuria (g/24 h)	0.2 [0.0–0.4]	0.2 [0.1–0.5]	0.2 [0.0–0.5]	0.3 [0.0–0.7]	>0.2
Questionnaire results					
Smoking, n (%)	9 (12.7)	12 (16.9)	15 (20.8)	20 (28.2)	0.02
History of CVD, n (%)	6 (8.5)	4 (5.6)	10 (13.9)	12 (16.9)	0.05

Parametric parameters are expressed as mean ± SD; non-parametric parameters are expressed as median (25–75% IQR); ordinal parameters are expressed as n (%). CVD, cardiovascular disease.

after adjustments for age and renal function parameters (model 3) were entered in to our model. A summary of the multivariate regression model is given in Table 4. In our model, 41% of the variation of skin-autofluorescence was positively determined by recipient age, systolic blood pressure, smoking, hs-CRP, duration of pre-transplant dialysis, and negatively by plasma vitamin C levels, creatinine clearance at baseline and change in creatinine clearance since 1 year after transplantation. Although no association was observed for time elapsed beyond 1 year after transplantation in univariate analysis, we evaluated its influence in the multivariate model. However, time elapsed since baseline did not significantly contribute to the model. Furthermore, as diabetes mellitus is a potent trigger to AGE formation we evaluated its influence on our results. However, exclusion of patients with diabetes mellitus did not materially affect the results of our analyses.

Discussion

We found recipient age, systolic blood pressure, smoking, hs-CRP, duration of pre-transplant dialysis, plasma vitamin C levels, creatinine clearance at baseline and change in creatinine clearance since 1 year after transplantation to be independently associated with AGE accumulation after renal transplantation. To the best of our knowledge no other investigators have

systematically analysed determinants of AGE accumulation in transplant recipients. We showed that AGE accumulation is associated with multiple cardiovascular risk factors in transplant recipients. Furthermore, we showed that AGE accumulation is related to transplant-specific factors. The latter include baseline renal function, decrease in renal function over time, donor age and duration of dialyses prior to transplantation.

The process of AGE accumulation is time-dependent and influenced by AGE production on the one hand and AGE breakdown and clearance by the kidneys on the other [3,5]. As expected the most compelling factors associated with AGE accumulation in our study were time-dependent as well. Strongly and independently associated with AGE accumulation was age. This finding confirms earlier observations in renal transplant recipients [12]. Our results also indicate that renal function is an important determinant of AGE accumulation. Renal function may be responsible for AGE accumulation, both because of disturbed clearance of AGEs and intermediate products, and due to increased oxidative stress [3]. The fact that AGE accumulation is strongly associated with baseline as well as index renal function suggests that the formation, accumulation, breakdown and clearance of tissue AGEs as measured by skin-autofluorescence in our study is a slow process. This idea is supported as well by the finding that duration of pre-transplant dialysis was independently associated with AGE accumulation

Table 2. Transplant characteristics grouped according to quartiles of skin-autofluorescence

Transplant characteristics	Quartiles of skin-autofluorescence (a.u.)				P for trend
	1.2–2.2 (n = 71)	2.2–2.6 (n = 71)	2.6–3.1 (n = 72)	3.1–5.2 (n = 71)	
Donor demographics					
Donor sex (male)	42 (59.2)	36 (50.7)	35 (48.6)	40 (56.3)	>0.2
Donor age (years)	36 ± 15	37 ± 16	36 ± 15	42 ± 15	0.04
Duration of pre-transplant dialysis (months)	28 ± 35	31 ± 26	40 ± 53	42 ± 32	0.01
Type of transplantation, n (%)					
Post-mortem donor	60 (84.5)	57 (80.3)	56 (77.8)	60 (84.5)	>0.2
Living donor	9 (12.7)	13 (18.3)	13 (18.0)	8 (11.3)	
Renal and pancreas	2 (2.8)	1 (1.4)	3 (4.2)	3 (4.2)	
HLA-AB mismatches, n (%)					
0	20 (28.2)	14 (19.7)	27 (37.5)	24 (33.8)	>0.2
1–2	44 (61.9)	52 (73.3)	32 (44.4)	39 (54.9)	
3–4	7 (9.9)	5 (7.0)	13 (18.1)	8 (11.3)	
HLA-DR mismatches, n (%)					
0	48 (67.6)	36 (50.7)	50 (69.4)	44 (62.0)	>0.2
1–2	23 (32.4)	35 (49.3)	22 (30.6)	27 (38.0)	
Total time of ischaemia (h)	23 ± 11	21 ± 11	23 ± 16	26 ± 17	0.18
Transplant follow-up					
Time elapsed since baseline (months)	62 [31–134]	60 [25–131]	71 [19–124]	58 [12–137]	>0.2
Acute rejection, n (%)	38 (53.5)	33 (46.5)	37 (51.4)	27 (38.0)	0.12
Creatinine clearance at baseline (ml/min)	69 ± 19	69 ± 20	65 ± 19	58 ± 18	<0.0001
Creatinine clearance at index (ml/min)	71 ± 19	67 ± 21	60 ± 24	52 ± 20	<0.0001
Delta creatinine clearance (ml/min)	2 ± 17	−2 ± 17	−6 ± 21	−6 ± 18	0.01
Drug-use of interest					
Use of prednisolon, n (%)	71 (100)	71 (100)	72 (100)	71 (100)	>0.2
dose (mg/day)	10 [7.5–10]	10 [8.8–10]	10 [7.5–10]	10 [8.8–10]	>0.2
Use of calcineurin inhibitors					
Ciclosporine, n (%)	44 (62.0)	42 (59.2)	47 (65.3)	47 (66.2)	>0.2
Trough-level (g/l)	108 ± 44	111 ± 47	107 ± 45	117 ± 50	>0.2
Tacrolimus, n (%)	10 (14.1)	13 (18.3)	13 (18.1)	9 (12.7)	>0.2
Trough-level (g/l)	9 ± 2	9 ± 4	8 ± 3	10 ± 8	>0.2
Use of proliferation inhibitors					
Azathioprine, n (%)	25 (35.2)	26 (36.6)	23 (31.9)	22 (31.0)	>0.2
Mycophenolate mofetil, n (%)	27 (38.0)	31 (43.7)	27 (37.5)	29 (40.8)	>0.2
Use of ACE inhibition, n (%)					
Angiotensin receptor antagonist, n (%)	3 (4.2)	7 (9.9)	5 (6.9)	5 (7.0)	>0.2
ACE inhibitors, n (%)	21 (29.6)	20 (28.2)	24 (33.3)	16 (22.5)	>0.2

Parametric parameters are expressed as mean ± SD; non-parametric parameters are expressed as median (25–75% IQR); ordinal parameters are expressed as n (%).

after transplantation. The relation between duration of pre-transplant dialysis and skin-autofluorescence was at least partially determined by renal function as can be concluded from the decreasing standardized regression coefficients after adjustment for renal function depicted in Table 3. A similar pattern occurs for donor age implicating that a lower creatinine clearance intrinsic to older kidneys partially explains the relationship of donor age with AGE accumulation.

The independent relation of smoking with AGE accumulation is likely to be caused by reactive glycation adducts in cigarette tobacco and cigarette smoke. Glycation adducts in cigarette tobacco are able to form cross-links with proteins [13]. Furthermore, it has been demonstrated, that smokers have significantly more serum AGEs than non-smokers [13].

Oxidative stress and inflammation are believed to be involved in the pathogenesis of chronic renal transplant dysfunction, and are also intricately linked to AGE

formation [3,5]. Although we found that vitamin C and hs-CRP were related independently to AGE formation, these markers do not provide conclusive information on oxidative stress and inflammation in our patients.

While HbA1c was associated with AGE accumulation univariately, HbA1c was not independently associated with AGE accumulation in our group. In diabetic patient groups, HbA1c is known to be independently associated with AGE accumulation [3,5]. From Table 3 it can be concluded that the relation between HbA1c and skin-autofluorescence in our group was mainly determined by age. Probably, this is related to the small percentage of diabetics in our group (15.8% at index date).

The independent relationship between systolic blood pressure and AGE accumulation has been reported previously [14]. In contrast with most of the factors discussed above we assume that this factor mainly represents a consequence of AGE accumulation, rather than a cause. It suggests that AGEs might be involved

Table 3. Regression analysis with recipient- and transplant-related factors; influences of age and renal function on model relations

Determinants	Model 1		Model 2		Model 3	
	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>
Recipient sex (female)	0.14	0.02	0.10	0.08	0.05	>0.2
Recipient age (years)	0.44	<0.001	—	—	—	—
Duration of pre-transplant dialysis (months)	0.15	0.01	0.14	0.007	0.10	0.06
Systolic blood pressure (mmHg)	0.32	<0.001	0.20	0.001	0.16	0.003
Diastolic blood pressure (mmHg)	0.06	>0.2	0.08	0.13	0.06	>0.2
Body mass index (kg/m ²)	0.10	0.11	0.01	>0.2	0.08	0.14
HbA1c (%)	0.24	<0.001	0.12	0.04	0.10	0.05
Fasting glucose (mmol/l)	0.07	>0.2	0.00	>0.2	0.04	>0.2
Total cholesterol (mmol/l)	0.06	>0.2	0.04	>0.2	0.02	>0.2
Serum albumin (g/l)	−0.14	>0.2	−0.09	>0.2	−0.06	>0.2
Plasma vitamin C (μmol/l)	−0.23	<0.001	−0.23	<0.001	−0.22	<0.001
hs-CRP (μg/ml)	0.21	0.001	0.17	0.002	0.11	0.03
Smoking, <i>n</i> (%)	0.16	0.009	0.19	<0.001	0.18	<0.001
History of CVD, <i>n</i> (%)	0.12	0.04	0.06	>0.2	0.04	>0.2
Donor age (years)	0.19	0.001	0.16	0.002	0.08	0.14
Total time of ischaemia (h)	0.08	0.20	−0.01	>0.2	−0.04	>0.2
Acute rejection, <i>n</i> (%)	−0.06	>0.2	−0.03	>0.2	−0.04	>0.2
Creatinine clearance at baseline (ml/min)	−0.27	<0.001	−0.21	<0.001	—	—
Creatinine clearance at index (ml/min)	−0.39	<0.001	−0.34	<0.001	—	—
Delta creatinine clearance (ml/min)	−0.18	0.003	−0.18	0.001	—	—

β , standardized regression coefficients; CVD, cardiovascular disease.

Model 1 is the crude model; model 2 is corrected for age; model 3 is corrected for age and renal function parameters (baseline creatinine clearance, creatinine clearance at index and delta creatinine clearance).

Table 4. Determinants for skin-autofluorescence in a multivariate regression model

Determinants	Regression coefficients ($R^2 = 0.41$, adjusted $R^2 = 0.39$, $P < 0.0001$)			
	β	B	CI of B	<i>P</i> -value
Constant	—	1.411	0.726–2.096	<0.0001
Recipient age (years)	0.36	0.022	0.016–0.029	<0.0001
Systolic blood pressure (mmHg)	0.17	0.006	0.002–0.009	0.002
Smoking	0.13	0.25	0.068–0.438	0.008
hs-CRP (μg/ml)	0.12	0.007	0.001–0.013	0.02
Duration of pre-transplant dialysis (months)	0.11	0.002	0.000–0.004	0.03
Plasma vitamin C (mol/l)	−0.15	−0.005	−0.009 to −0.002	0.003
Creatinine clearance at baseline (ml/min)	−0.24	−0.009	−0.013 to −0.005	<0.0001
Delta creatinine clearance (ml/min)	−0.24	−0.010	−0.014 to −0.005	<0.0001

β , standardized regression coefficients; B, unstandardized regression coefficient; CI, confidence interval.

in the development of vascular stiffness, resulting in hypertension. However, enhanced wall tension in blood vessels and cardiac tissue due to hypertension is thought to enhance oxidative stress and might thereby result in enhanced AGE accumulation.

Immunosuppressive drugs (mainly ciclosporin) and the use of ACE inhibition have previously been associated with oxidative stress and AGE accumulation. ACE inhibition with either ACE inhibitors or AII-receptor antagonists has been shown to decrease AGE accumulation [15,16]. Ciclosporin has been reported to aggravate oxidative stress, possibly leading to enhanced AGE accumulation [17]. We did not find a relationship between the use of ACE inhibition and/or immunosuppressive drugs and AGE accumulation. Caution should be used in the interpretation of the

lack of relation, because of the variability of duration of exposure of these drugs. The fact that tissue AGEs are thought to have a longer half-life than plasma AGEs may be another explanation for the lack of correlation found.

AGE accumulation was determined using our newly developed and validated AFR. This tool is based upon the principle of the fluorescent properties of several (but not all) AGEs. Collagen linked fluorescence has long been used as a single standard for measuring AGE accumulation. One limitation of the AFR is that not all AGEs exhibit fluorescent properties. Indeed, fluorescence is a group reactivity, which fails to provide quantitative information on concentrations of individual compounds. Another limitation of the AFR is that we cannot exclude the interference of other

fluorophores in our measurement. Changes in skin fluorescence may also occur as a consequence of light absorption by chromophores such as melanin and haemoglobin. Our findings are limited as well by the fact that it can not be concluded whether skin AGE levels are an independent risk factor for the development of cardiovascular disease as well as chronic transplant dysfunction in our group. On the one hand this is the consequence of the cross-sectional nature of our study. On the other hand this is due to the absence of a control group of the general population. A further limitation of our study is that we do not have pre-operative AGE level data. This would have been of interest as many facets changed post-transplant, particularly renal function (thus affecting AGE clearance) and the institution of highly potent anti-rejection medications.

In conclusion, increased accumulation of AGEs measured as skin-autofluorescence *in vivo* is associated with several risk factors for chronic renal transplant dysfunction and cardiovascular disease. Some of these relations are suggestive for a causative role of AGEs in chronic transplant dysfunction and cardiovascular disease in renal transplant recipients. Prospective studies and/or future intervention studies with AGE-lowering therapy may allow to more definitely determine the relative role of AGE accumulation in the development of chronic renal transplant dysfunction and cardiovascular disease after transplantation. The availability of a simple, non-invasive method to measure AGE accumulation in renal transplant recipients may be useful in identifying and monitoring patients at risk for AGE accumulation.

Acknowledgements. This research was funded by the Dutch Renal Foundation (Research grant C00.1877), the Netherlands Organization for Scientific Research (NWO-AGIKO 920-03-181), and the Dutch Diabetes Research Foundation (Research grant 2000-00-006).

Conflict of interest statement. A.J.S. and R.G. both are founders of DiagnOptics BV, which manufactures autofluorescence readers. This study was not financially supported by DiagnOptics BV, and final approval was always by the first author (J.W.L.H.) who is not a member of DiagnOptics BV. None of the other authors declare any conflicts of interest.

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Received for publication: 27.2.06

Accepted in revised form: 2.3.06