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Skin Autofluorescence as a Measure of Advanced Glycation End Product Deposition Is Elevated in Peripheral Artery Disease

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Objective—Evidence for an important role of advanced glycation end products (AGEs) in the development of atherosclerosis and cardiovascular disease beyond diabetes mellitus and renal disease is growing. Skin autofluorescence (SAF) is a validated noninvasive measure of tissue AGEs. We hypothesized that SAF is elevated in peripheral artery disease (PAD).

Methods and Results—A case-control study was performed in 492 patients with PAD and 164 controls, matched for age (mean 66 ± 10 years) and presence of diabetes mellitus. Cardiovascular risk factors and comorbidity (coronary artery disease, cerebrovascular disease, abdominal aortic aneurysm) were assessed. SAF was measured with the AGE Reader. SAF was higher in patients compared with controls: geometric mean 2.77 (95% confidence interval [CI], 2.71–2.83) versus 2.44 (95% CI, 2.35–2.53) arbitrary units, $P=0.4 \times 10^{-8}$. In logistic regression, the adjusted odds ratio for the presence of PAD was 2.47 (95% CI, 1.66–3.69) per 1 unit increase of SAF. PAD patients with cardiovascular comorbidity had a higher SAF compared with those without: geometric mean 2.93 (95% CI, 2.85–3.02) versus 2.63 (95% CI, 2.55–2.71) arbitrary units, $P=0.4 \times 10^{-6}$, also after correction for confounders. Regression analysis showed that age, smoking, diabetes mellitus, chronic kidney disease, and a history of cerebrovascular disease or abdominal aortic aneurysm were independently associated with SAF in the patients with PAD.

Conclusion—Accumulation of tissue AGEs is increased in patients with PAD, independent of cardiovascular risk factors and comorbidity, although these conditions are associated with a further increase. These findings underscore the importance of AGEs in PAD, irrespective of the presence of diabetes mellitus and renal insufficiency. (*Arterioscler Thromb Vasc Biol.* 2013;33:XXX-XXX.)

Key Words: advanced glycation end products ■ atherosclerosis ■ oxidative stress ■ peripheral vascular disease ■ skin autofluorescence

Patients with peripheral artery disease (PAD) are inflicted with a large burden of atherosclerosis, associated with a substantially increased risk for cardiovascular events and premature mortality.^{1–3} Advanced glycation end products (AGEs) are formed by nonenzymatic glycation and oxidative reactions to form stable structures accumulating on long-lived proteins and to promote cellular stress responses by engagement of the receptor for AGEs.¹ Although formerly only implicated in diabetes mellitus (DM) and renal disease, evidence for an important role of AGEs in cardiovascular disease beyond these conditions is growing. AGEs have been immunohistochemically localized in human atherosclerotic lesions, and lowering AGEs or blocking receptor for AGEs in murine models attenuates plaque formation, supporting a causal role of AGEs in the development of atherosclerosis.^{4–6} Indeed, in patients with PAD, elevated levels of plasma AGEs have been documented.⁷

Skin autofluorescence (SAF) is a validated measure of tissue AGEs that can be used in subjects with a skin pigmentation up to

Fitzpatrick type V.^{8–10} Earlier, we reported that SAF was increased and was a strong predictor of mortality in DM and end-stage renal disease.^{9,11,12} The aim of the present study was to compare SAF in PAD patients with controls. We hypothesized that SAF is increased in PAD. Furthermore, we explored the relationship of SAF with the traditional cardiovascular risk factors, as well as with additional cardiovascular damage expressed by a history of cerebrovascular or coronary artery disease (CAD) or an abdominal aortic aneurysm (AAA) in these patients.

Materials and Methods

Study Population

We performed a case-control study. Men and women at least 18 years of age were eligible to participate. PAD was ascertained by a resting ankle-brachial index (ABI) ≤ 0.90 or a toe-brachial index ≤ 0.70 in case of noncompressible calf arteries. PAD was confirmed by evidence of obstructive disease on computed tomographic angiography, magnetic resonance angiography, catheter angiography, and duplex ultrasonography. An age-matched control group was selected from

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Table 1. Baseline Characteristics

Characteristics		Controls	Peripheral Artery Disease (PAD) Patients	P Value Controls vs PAD Patients
n	Total group	164	492	
No CV comorbidity			252	
With CV comorbidity			240	
Age, y	Total group	65.8 (10.4)	65.9 (10.5)	N/A
No CV comorbidity			63.0 (11.1)	
With CV comorbidity			68.8 (8.9)	
Male sex	Total group	88 (54%)	345 (70%)	0.0001
No CV comorbidity			155 (62%)	
With CV comorbidity			190 (79%)	
Current smoker	Total group	37 (23%)	251 (51%)	0.2×10 ⁻⁹
No CV comorbidity			150 (60%)	
With CV comorbidity			101 (42%)	
Body mass index, kg/m ² *	Total group	27.8 (27.1–28.5)	26.3 (25.9–26.7)	0.0002
No CV comorbidity			25.8 (25.2–26.4)#	
With CV comorbidity			26.9 (26.4–27.4)	
Diabetes mellitus	Total group	42 (26%)	127 (26%)	N/A
No CV comorbidity			54 (21%)**	
With CV comorbidity			73 (30%)	
Systolic BP, mm Hg†	Total group	149 (23)	146 (25)	NS
No CV comorbidity			147 (25)	
With CV comorbidity			145 (25)	
Diastolic BP, mm Hg‡	Total group	82 (11)	79 (14)	0.002
No CV comorbidity			81 (15)**	
With CV comorbidity			78 (14)	
Hypertension	Total group	67 (41%)	403 (82%)	0.5×10 ⁻²³
No CV comorbidity			181 (72%)¶	
With CV comorbidity			222 (93%)	
Hypercholesterolemia	Total group	48 (29%)	372 (76%)	0.9×10 ⁻²⁶
No CV comorbidity			173 (69%)¶	
With CV comorbidity			199 (83%)	
Oral anticoagulant therapy	Total group	53 (32%)	436 (89%)	0.1×10 ⁻⁴⁵
No CV comorbidity			209 (83%)¶	
With CV comorbidity			227 (95%)	
eGFR, mL/min per 1.73 m ² §	Total group	72 (69–75)	77 (74–79)	0.02
No CV comorbidity			83 (80–87)¶	
With CV comorbidity			71 (67–74)	
Ankle–brachial index	Total group	N/A	0.57 (0.14)	N/A
No CV comorbidity			0.57 (0.14)	
With CV comorbidity			0.57 (0.15)	
Coronary artery disease (CAD)	Total group	23 (14%)	168 (34%)	0.9×10 ⁻⁶
No CV comorbidity			0 (0%)¶	
With CV comorbidity			168 (70%)	
Cerebrovascular disease (CVD)	Total group	27 (17%)	76 (15%)	NS
No CV comorbidity			0 (0%)¶	
With CV comorbidity			76 (32%)	

(Continued)

Table 1. (Continued)

Characteristics		Controls	Peripheral Artery Disease (PAD) Patients	P Value Controls vs PAD Patients
Abdominal arterial aneurysm (AAA)	Total group	0 (0%)	61 (12%)	0.2×10 ⁻⁵
No CV comorbidity			0 (0%)¶	
With CV comorbidity			61 (25%)	

Data are presented as numbers of patients (%), mean (SD), or as geometric mean (95% CI). Cardiovascular (CV) comorbidity defined as a history of CAD, CVA, or AAA. Patients and controls were matched on age and presence of diabetes mellitus. BP indicates blood pressure; NS, nonsignificant; N/A, not applicable; eGFR, glomerular filtration rate by MDRD formula.

*Data missing for 1 control and 5 PAD patients; BMI was natural log-transformed for analysis.

†Data missing for 1 control and 2 PAD patients.

‡Data missing for 1 control and 4 PAD patients.

§Data missing for 13 controls and 6 PAD patients; eGFR was natural log-transformed for analysis.

||Data missing for 91 PAD patients.

PAD patients with versus without CV comorbidity, ¶ $P < 0.001$, # $P < 0.01$, ** $P < 0.05$.

patients without a history of PAD who visited the outpatient clinic for preoperative evaluation for orthopedic or minor surgical procedures. Because DM and end-stage renal disease strongly increase AGE levels, PAD patients with DM were matched with diabetic controls, and patients with end-stage renal disease (chronic kidney disease¹³ stage 5; estimated glomerular filtration rate [eGFR] <15 mL/min per 1.73 m²) were excluded from both groups. Additional exclusion criteria for patients and controls were recent myocardial infarction, stroke, or sepsis (all within the past 3 months), cancer, and solid organ transplantation. Patients and controls were not matched by sex, because no difference in SAF was found between men and women in previous studies.^{8,9,11,12} We designed the study to have 3:1 matching (patients:controls) to have sufficient patients for additional analyses (see Statistical Analysis section). For each set of 3 patients with approximately the same age (maximum deviation of 1 year from their mean age), 1 age-matched control was selected. The study was approved by the local institutional review board, and all participating subjects provided informed consent.

Evaluation

For data collection, history was taken and medical records were reviewed. Traditional cardiovascular risk factors were assessed: age, sex, smoking status, body mass index (BMI), DM, hypertension, and hypercholesterolemia. BMI was calculated as weight (kg) divided by

squared height (m). Patients were classified as having hypertension based on the use of blood pressure-lowering drugs, and hypercholesterolemia was assigned in case of current use of lipid-lowering drugs. The American Diabetes Association criteria were used to diagnose DM.¹⁴ Serum creatinine was determined, and renal function was estimated by calculating the eGFR.¹⁵ Renal insufficiency was classified according to chronic kidney disease stage.¹³ Anticoagulant therapy was defined as the use of antiplatelet or vitamin K antagonist therapy. CAD was evaluated by clinical history of myocardial infarction, angina pectoris, coronary artery surgery, and percutaneous coronary intervention. Cerebrovascular disease (CVD) was ascertained by history of symptoms of transient ischemic attack and stroke. An AAA was defined as a previously documented maximum infrarenal aortic diameter of ≥30 mm. For PAD patients without CAD, 10-year risk of a coronary event was estimated according to the Framingham risk score. This score depends on age, total cholesterol, high-density lipoprotein cholesterol, systolic and diastolic blood pressure, presence of DM, and smoking.¹⁶ Patients were classified into 3 risk categories depending on sex and Framingham risk score: low (<10%), intermediate (10%–20%), or high (>20%) 10-year risk of having a coronary event. Because this score is validated for subjects aged <75 years, all patients aged ≥75 years were excluded for this calculation.

Skin Autofluorescence

SAF was measured with the AGE Reader (DiagnOptics Technologies BV, Groningen, the Netherlands). The AGE Reader is a desktop device that uses the characteristic fluorescent properties of certain AGEs to estimate the level of AGE accumulation in the skin. Technical details of this noninvasive device concerning the optical technique have been described more extensively elsewhere.⁸ In short, the AGE Reader illuminates a skin surface of 4 cm² guarded against surrounding light with an excitation light source with a peak excitation of 370 nm (ultraviolet A). Emission light (fluorescence in the wavelength of 420–600 nm) and reflected excitation light (with a wavelength of 300–420 nm) from the skin are measured with a spectrometer. SAF is calculated as the ratio between the emission light and reflected excitation light, multiplied by 100 and expressed in arbitrary units (AU). In previous validation studies using skin biopsies, we showed a strong correlation between SAF and the skin contents of the fluorescent AGE pentosidine, as well as with the nonfluorescent AGEs Ne-(carboxymethyl)-lysine and Ne-(carboxyethyl)lysine.^{8,9} Also, a strong correlation between SAF measurements of the arm and leg was found in a validation study. In the present study, the right forearm was positioned on top of the device, which is the standard and most practical measuring site for SAF. A series of 3 consecutive measurements was carried out, taking less than a minute. The mean SAF was calculated from these 3 measurements and used in the analyses. An earlier validation study showed an intraindividual Altman error percentage of 5.03%, with SAF measurements taken over 1 single day, and an Altman error percentage of 5.87% for seasonal variation.⁸

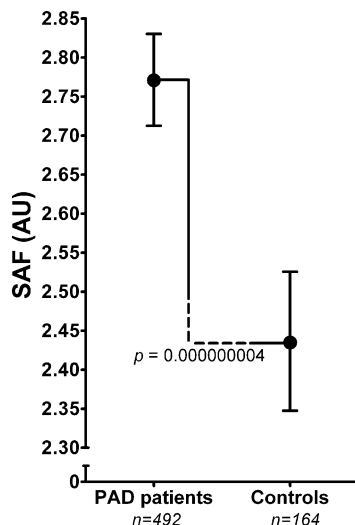


Figure 1. Skin autofluorescence (SAF) in peripheral artery disease patients and controls. Data are shown as geometric mean (95% CI). AU indicates arbitrary units; PAD, peripheral artery disease.

Statistical Analysis

All data are shown as number (percentage) for categorical variables, as mean (\pm SD) for variables with a normal distribution, and as geometric mean (95% CI) in case of a non-normally distributed parameter that was normalized by logarithmic transformation. Normal distribution was tested by a 1-sample Kolmogorov-Smirnov test. Characteristics of patients and controls were compared using the χ^2 test for categorical variables and Student independent *t* test for continuous variables.

SAF between patients and controls was compared by Student independent *t* test. Within the group of patients with PAD, the effect of the presence of each traditional cardiovascular risk factor, the presence of additional cardiovascular damage (expressed by a history CAD, CVD, or AAA), and the effect of ABI and Framingham risk score category on SAF were tested in a univariate analysis by Student independent *t* test or 1-way ANOVA where applicable. A $P < 0.05$ was considered statistically significant. Backward linear regression was used

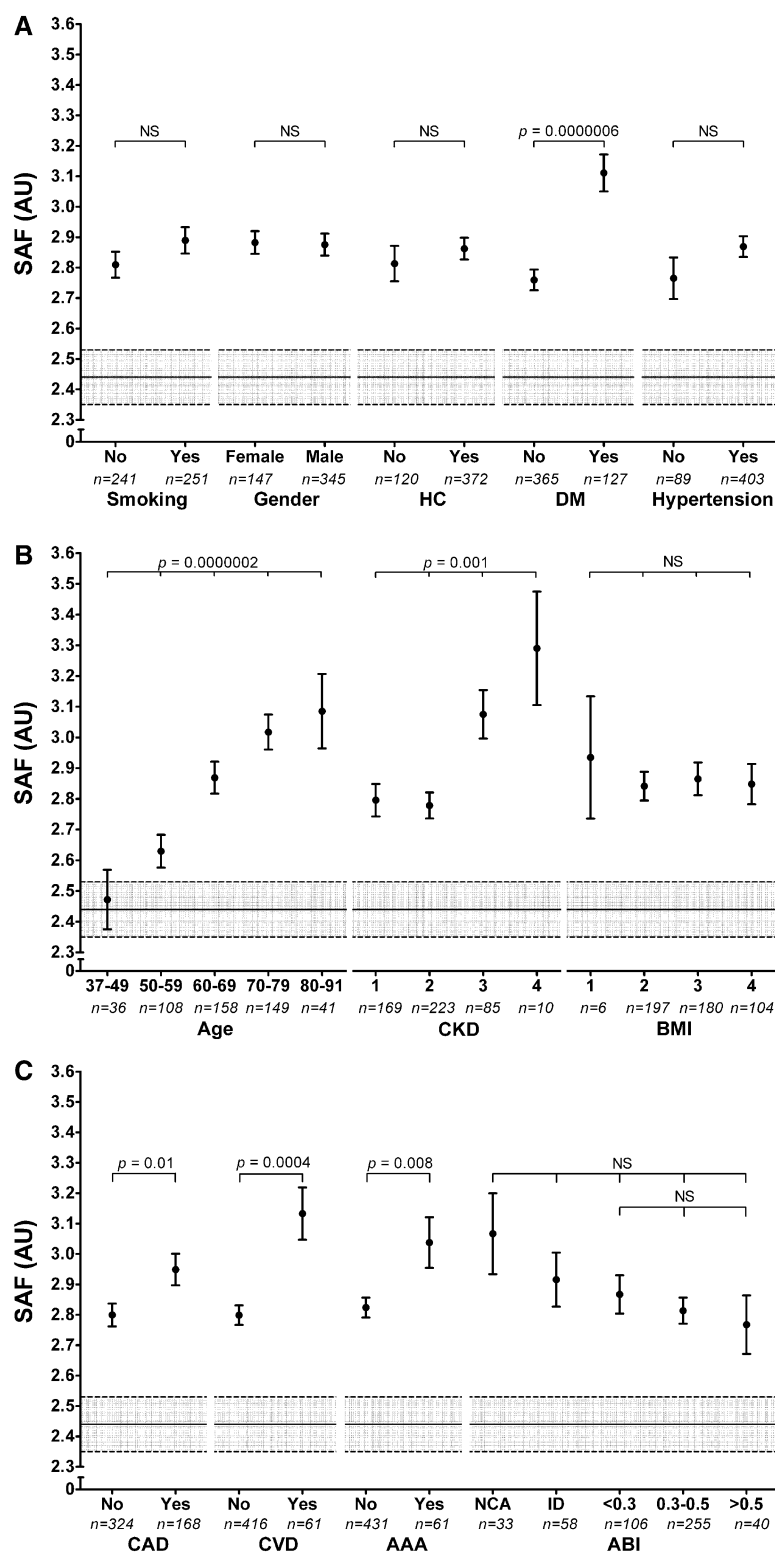


Figure 2. Association of skin autofluorescence (SAF) with cardiovascular risk factors (dichotomous variables are shown in **A**: smoking, sex, hypercholesterolemia, diabetes mellitus, and hypertension; categorical variables are shown in **B**: age, chronic kidney disease [CKD] class, and body mass index [BMI] class) and cardiovascular morbidity and ankle-brachial index (**C**: coronary artery disease [CAD], cerebrovascular disease [CVD], abdominal aortic aneurysm [AAA], and ankle-brachial index) in patients with peripheral artery disease (PAD). Data are shown as geometric mean (95% CI). The gray area represents the geometric mean (95% CI) SAF of the control group. Age category by decade, except for the lowest category consisting of all patients aged <50 years and the highest category consisting of all patients aged ≥ 80 years. Patients without kidney disease are combined with patients in CKD 1. CKD data are missing for 6 PAD patients. Class 1=underweight, BMI<18.5 kg/m²; class 2=normal weight, 18.5≤BMI<25.0 kg/m²; class 3=overweight, 25.0≤BMI<30 kg/m²; class 4=obesity, BMI≥30 kg/m². BMI data are missing for 5 PAD patients. ABI indicates ankle-brachial index; NCA, group with noncompressible arteries; ID, group with directly invasively diagnosed PAD without ABI measurement; AU, arbitrary units; NS, not significant; HC, hypercholesterolemia; DM, diabetes mellitus.

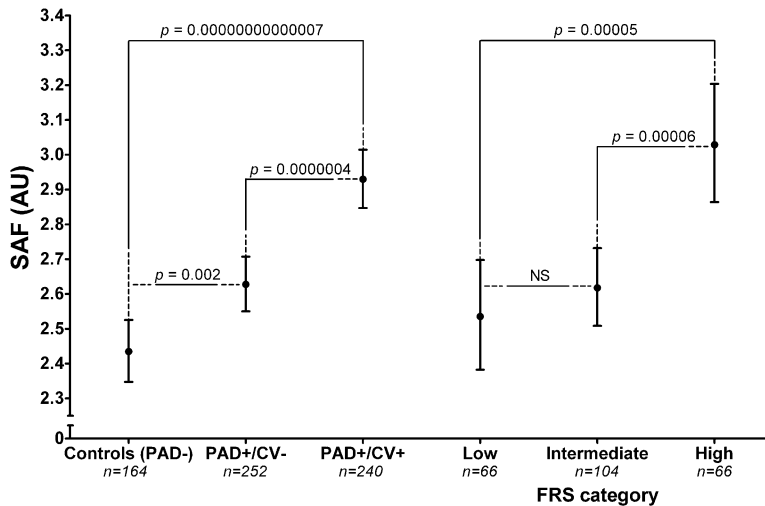


Figure 3. Data are shown as geometric mean and 95% CI. **Left,** Skin autofluorescence (SAF) in peripheral artery disease (PAD) patients with and without cardiovascular comorbidity (cardiovascular or cerebrovascular disease or abdominal aortic aneurysm) and controls (PAD-). PAD+/CV- = PAD patients without cardiovascular comorbidity. PAD+/CV+ = PAD patients with cardiovascular comorbidity. **Right,** SAF in PAD patients without coronary artery disease (n=324) with a low (<10%), intermediate (10%–20%), or high (>20%) 10-year risk of a coronary event according to the Framingham risk score (FRS). FRS could be calculated for 236 patients. For 88 patients, FRS could not be calculated because of a high age (>74 years) or missing blood pressure or cholesterol values. AU indicates arbitrary units; NS, not significant; CV, cardiovascular.

to identify the independent determinants of SAF within the group of patients with PAD. In the complete study group (patients and control subjects), logistic regression was performed to study the association between SAF and the presence of PAD. All statistical analyses were carried out with the Statistical Package for Social Science (SPSS, version 18.0).

Results

Characteristics of Patients and Controls

Five hundred ten patients with PAD were willing to participate. Eighteen patients were excluded, 7 patients because of renal failure and 11 patients because of a kidney transplantation. The remaining 492 PAD patients and 164 controls, with a mean age of 66 ± 10 years, were included. In 91 patients, PAD was not ascertained by a resting ABI ≤ 0.90 because of noncompressible arteries (33 patients) or because invasive angiography and surgery had directly been performed (58 patients). In all of these cases, PAD was confirmed by angiography. The characteristics of patients and controls are shown in Table 1. These characteristics are shown for the complete group of patients with PAD, as well as for the 2 subgroups of PAD patients with and without a history of cardiovascular comorbidity (CAD, CVA, or AAA). As expected, the traditional cardiovascular risk factors were more prevalent in patients with PAD than in the controls. Male sex, current smoking, hypertension, and hypercholesterolemia were more common in patients with PAD ($P \leq 0.001$ for all). Patients with PAD had a slightly but statistically significant higher eGFR (77 [95% CI, 74–79] versus 72 [95% CI, 69–75] mL/min per 1.73 m^2 ; $P=0.02$) and a lower BMI ($P < 0.001$). Diastolic blood pressure was lower in patients with PAD ($P < 0.01$), probably because of the more frequent use of blood pressure-lowering drugs. Obviously, patients with PAD used oral anticoagulants more often ($P < 0.001$). CAD and AAA were more prevalent in patients with PAD (both $P < 0.001$), but the prevalence of CVD did not differ between patients with PAD and controls.

Skin Autofluorescence

SAF was higher in patients with PAD compared with the controls: geometric mean 2.77 (95% CI, 2.71–2.83) versus 2.44

(95% CI, 2.35–2.53) AU ($P=0.4 \times 10^{-8}$; Figure 1). The univariate influence of the presence of traditional risk factors, cardiovascular comorbidity, and ABI on SAF is shown in Figure 2. Age, presence of DM, renal function, and a history of CAD, CVD, or AAA had a significant effect on SAF. Sex, current smoking, presence of hypertension and hypercholesterolemia, BMI, and ABI were not correlated with SAF. The results of the multivariate linear regression analysis with SAF as dependent variable are shown in Table 2. Age, current smoking, DM, chronic kidney disease class, and a history of CVD or AAA were independently associated with SAF. The variables sex, hypertension, BMI, hypercholesterolemia, and a history of CAD and ABI did not independently contribute to SAF.

Table 1 also shows the characteristics of patients with PAD in 2 subgroups: patients with and without additional cardiovascular comorbidity (history of CVD, CAD, or AAA). Patients with a history of cardiovascular comorbidity were older, more often men, less frequently smokers, had a higher BMI, more often DM, hypertension, and hypercholesterolemia, used oral anticoagulant therapy more often, had a lower eGFR, and a lower diastolic blood pressure compared with PAD patients without cardiovascular comorbidity. Systolic blood pressure and ABI did not differ between these groups. Figure 3 (left)

Table 2. Multivariate Model of (Natural Log of) Skin Autofluorescence in the Peripheral Artery Disease Patients: Result of Backward Linear Regression Analysis

Variable	Coefficient B	SE	Standardized Coefficient β	P
Age	0.005	0.001	0.227	0.000004
Smoking	0.097	0.022	0.195	0.00002
Diabetes mellitus	0.113	0.024	0.204	0.000003
Natural log eGFR	−0.061	0.030	−0.092	0.044
Cerebrovascular disease	0.073	0.029	0.109	0.011
Abdominal aortic aneurysm	0.070	0.032	0.095	0.028

Variables removed from the model include the following: sex, natural log BMI, hypertension, hypercholesterolemia, coronary artery disease, ankle-brachial index. eGRF indicates estimated glomerular filtration; BMI, body mass index.

shows the influence of an increasing burden of atherosclerosis on SAF, with a history of CVD, CAD, or AAA as marker of additional atherosclerotic burden. There was a significant difference in SAF between patients with PAD without cardiovascular comorbidity and patients with PAD and cardiovascular comorbidity: 2.63 (95% CI, 2.55–2.71) versus 2.93 (95% CI, 2.85–3.02) AU, $P=0.4\times 10^{-6}$. The difference in SAF between controls and PAD patients without cardiovascular comorbidity was significant, as well as with PAD patients with cardiovascular comorbidity: 2.44 (95% CI, 2.35–2.53) versus 2.63 (95% CI, 2.55–2.71) AU, $P=0.002$ and 2.44 (95% CI, 2.35–2.53) versus 2.93 (95% CI, 2.85–3.02) AU, $P=0.7\times 10^{-13}$, respectively. After correction for sex and age, SAF remained significantly different between PAD patients with and without cardiovascular comorbidity ($P=0.0005$), as well as between controls and PAD patients without cardiovascular comorbidity ($P=0.0001$) and between controls and PAD patients with cardiovascular comorbidity ($P=0.1\times 10^{-11}$). Figure 3 also shows the relationship between Framingham risk score category and SAF (right) in PAD patients without CAD and <75 years of age. Patients with a high risk had a higher SAF compared with patients with an intermediate or low risk: 3.03 (2.86–3.20) versus 2.62 (2.51–2.73) and versus 2.54 (2.38–2.70), $P=0.00006$ and $P=0.00005$, respectively.

Table 3 shows the results of the logistic regression models that describe the association between SAF and the presence of PAD in the complete group (patients and controls). In the crude model, each 1 AU increase of SAF was associated with a 2.34-fold (95% CI, 1.73–3.17) increased chance of having PAD. The strength of this association was unaffected by correction for cardiovascular risk factors (model 1, odds ratio 2.36 [95% CI, 1.56–3.50]), history of CAD and CVD (model 2, odds ratio 2.27 [95% CI, 1.66–3.09]), and cardiovascular risk factors and history of CAD and CVD (model 3, odds ratio 2.47 [95% CI, 1.66–3.69]).

Discussion

Our study in patients with PAD shows that SAF, as a measure of tissue AGE accumulation, is considerably increased compared with controls, independent of cardiovascular risk factors and cardiovascular comorbidity. Still, the presence of cardiovascular risk factors known to be strongly associated with PAD further increases SAF in patients with PAD. Multivariate analysis shows that age, current smoking, presence of DM, eGFR, and a history of CVD or AAA are independent determinants of SAF in patients with PAD. This is in agreement with our previous studies. Furthermore, a 1 unit increase in SAF is associated with a 2.45-fold higher chance of having PAD, independent of cardiovascular risk factors and cardiovascular comorbidity in the complete group.

AGEs in PAD

This is the first report demonstrating an increased SAF in patients with PAD, although we earlier reported that increased SAF in patients with carotid artery disease was confined to those with PAD.¹⁷ The role of AGEs in the pathogenesis of PAD has recently been suggested, which is in line with the more established concept of AGEs as important players in the

Table 3. Logistic Regression Models for the Association Between PAD and SAF

Variable	B	SE	Odds Ratio (95% CI)
Crude model			
SAF	0.85	0.16	2.34 (1.73–3.17)
Constant	−1.17	0.41	0.31
Model 1			
SAF	0.86	0.20	2.36 (1.59–3.50)
Male sex	0.71	0.24	2.03 (1.26–3.26)
Smoking	1.45	0.28	4.25 (2.48–7.23)
Hypertension	1.56	0.27	4.78 (2.82–8.09)
Hypercholesterolemia	1.64	0.25	5.13 (3.12–8.43)
BMI	−0.09	0.03	0.91 (0.87–0.96)
eGFR	0.01	0.01	1.01 (1.00–1.02)
Constant	−2.95	1.09	0.05
Model 2			
SAF	0.82	0.16	2.27 (1.66–3.09)
CVD	−0.40	0.26	0.67 (0.40–1.12)
CAD	1.08	0.25	2.95 (1.81–4.80)
Constant	−1.27	0.42	0.28
Model 3			
SAF	0.91	0.20	2.47 (1.66–3.69)
Male sex	0.73	0.25	2.07 (1.26–3.37)
Smoking	1.47	0.28	4.35 (2.53–7.47)
Hypertension	1.58	0.28	4.85 (2.80–8.39)
Hypercholesterolemia	1.66	0.27	5.28 (3.12–8.92)
BMI	−0.09	0.03	0.91 (0.86–0.95)
eGFR	0.01	0.01	1.01 (1.00–1.02)
CVD	−1.07	0.33	0.34 (0.18–0.65)
CAD	0.39	0.32	1.48 (1.80–2.75)
Constant	−2.85	1.10	0.06

Because PAD patients and controls were matched for age and presence of diabetes mellitus, these variables were not included in the model. For sex, female is the reference. AAA was excluded from the model; no estimation of the odds ratio is possible because there are no controls with an AAA. PAD indicates peripheral artery disease; SAF, skin autofluorescence; eGFR, estimated glomerular filtration rate by MDRD formula; BMI, body mass index; CVD, cerebrovascular disease; CAD, coronary artery disease; AAA, abdominal arterial aneurysm.

development of atherosclerosis in DM, renal insufficiency, and coronary artery disease.^{9,11,12,18}

The literature on AGEs in PAD, however, is relatively scarce. Previously, in a study of patients with end-stage renal disease, plasma levels of S100A12, which is a ligand for the receptor for AGEs, were elevated in patients with PAD compared with those without PAD.¹⁹ Furthermore, a direct inverse association between ABI and circulating levels of the AGE pentosidine was found in apparently healthy men.²⁰ However, no patients with documented PAD or an ABI <0.9 were included. In contrast, we included PAD patients with a mean ABI of 0.57 and did not find any relationship between SAF and ABI in the univariate analysis. Also, the multivariate model showed that in our PAD patients SAF was primarily depending on age,

smoking, DM, and renal function rather than the severity of PAD as assessed by the ABI. Interestingly, we did find that SAF was particularly increased in those patients with PAD who had additional cardiovascular morbidity, univariately as well as in the multivariate model. Therefore, in these patients, AGEs may reflect the burden of atherosclerosis with incremental AGE accumulation in those patients in whom atherosclerosis is not confined to the peripheral arteries but extends to the aorta, coronary, or cerebral arteries as well. In line with this concept of increasing AGEs with increased manifestation of atherosclerosis are the results of a postmortem study in patients with DM demonstrating a stronger expression of receptor for AGEs in patients with an increased necrotic plaque area in the coronary arteries.²¹

In our study, logistic regression analysis showed that each 1 AU increase of SAF was associated with a 2.34-fold increased chance of having PAD in the crude model. The independency of SAF as a determinant of the presence of PAD was underscored by the unchanged strength of their association in models that include an increasing number of known determinants of the presence of PAD such as smoking, hypertension, and the presence of CAD. The main outcome of our study that shows an increased SAF in PAD, as well as the additional analyses, further reinforces the concept of a key role for AGEs in the development of atherosclerotic disease, in particular PAD.

Limitations of the Study

The selection of patients with PAD and controls may have influenced the results. The controls were not selected on being healthy, but only on being free of symptoms or signs of PAD. However, if there have been controls with PAD at a subclinical level, this has resulted in an underestimation rather than an overestimation of the true difference in SAF between patients with PAD and controls. Furthermore, patients and controls were matched for age and the presence of DM, because in earlier studies these were the most important determinants of SAF.^{8,9,11,12,17,18} This, again, may have led to an underestimation of the true difference in SAF between PAD and controls. The same is true for the exclusion of patients with end-stage renal disease (chronic kidney disease class 5, on dialysis, after kidney transplantation). We had to exclude 18 patients and none of the controls because of this criterion, while end-stage renal disease is known to impair clearance of AGEs resulting in accelerated accumulation of AGEs.

Generalizability of the results may be limited to a part of all patients with PAD. The low mean ABI of 0.57 demonstrates that our group of patients with PAD suffered from severe PAD, especially when taking into account that the ABI was not measured in 58 patients because invasive angiography and surgery had directly been performed, necessitated by the clinical presentation of these patients with critical ischemia. The high prevalence of most cardiovascular risk factors in the PAD patients of our study may be expected in a cohort with established vascular disease. The high percentages of patients with hypertension or hypercholesterolemia in the patients with PAD can be explained by the fact that these conditions are known risk factors for PAD, but also by the definition we used for these conditions, that is, the use of anti-hypertensive or cholesterol-lowering drugs. These drugs may

have been prescribed as secondary cardiovascular prevention measures rather than because of the presence of hypertension or hypercholesterolemia.

Also, we did not perform skin biopsies in the present study to confirm the strong correlation between SAF and skin content of AGEs, as reported in earlier validation studies in different patient groups and healthy controls.^{8,9} Furthermore, we did not measure plasma AGEs to corroborate our findings. However, it is unclear whether sampling of plasma AGEs would have been useful, because blood and urine sampling of AGEs is hampered by the fact that these AGEs do not necessarily reflect tissue AGE levels.^{22,23}

A major limitation of the use of SAF by the AGE Reader to measure AGE level is that it cannot be used in all types of skin. For a valid measurement of SAF, the reflectance of excitation light has to be at least 6%.¹⁰ Strongly pigmented skin type absorbs too much excitation light, resulting in a reflectance of <6%. In practice, the AGE Reader can be used in subjects with a skin pigmentation up to Fitzpatrick skin type V. Therefore, the AGE Reader can be used in subjects with a white, Mediterranean, Hispanic, Asian, or Eastern Indian origin, but not in blacks.

Finally, we do not know the strength of the correlation between SAF and actual level of AGEs in the atheroma of affected arteries. A study on the agreement between SAF as measure of tissue AGEs in the skin and tissue AGEs or plaque size in arteries is yet to be performed. Still, in patients with type 1 DM, another research group has established the positive association between coronary artery calcification score and skin intrinsic fluorescence measurements that in essence use the same fluorescent properties of AGEs in the skin as the AGE Reader we used.²⁴ Also, a recently published study showed a correlation between SAF and the actual content of AGEs in residual bypass graft material.²⁵ SAF as a noninvasive method to quantify tissue accumulation of AGEs has made it possible to study 656 subjects in the present study relatively easily, not requiring skin biopsies or atheroma of affected arteries.

Clinical Implications

Notwithstanding these limitations, our observation of highly increased SAF in patients with PAD remains relevant, whereas the use of SAF as a measure of tissue AGE deposition has been validated earlier and used as such in a series of prospective clinical studies as well.⁸ In several of these studies, SAF was shown to be a strong predictor for cardiovascular morbidity and mortality and total mortality, independent of conventional cardiovascular risk factors.^{9,11,12,17,18} If such a predictive value can be established in patients with PAD, SAF may be used to identify PAD patients at highest risk for cardiovascular events. Our observation of a relationship between SAF and Framingham risk score category supports the possibility of the use of SAF for risk prediction in patients with PAD. However, this is yet to be shown in a prospective follow-up study. Similarly, whether PAD patients with a high SAF may benefit from more aggressive treatment (ie, tighter control of cardiovascular risk factors with lower low-density lipoprotein cholesterol and blood pressure targets), similar to patients with DM or renal insufficiency, should be subject to

an intervention study. Finally, inhibition of AGE formation, breakdown of AGE cross-links, and blocking the receptor for AGEs may become treatment targets in cardiovascular disease. Drugs that are currently prescribed to treat DM, hypertension, and hypercholesterolemia may have such properties to a certain extent, but also new drugs have specifically been designed to counter AGEs.²⁶ However, although some of these new drugs have already been tested in clinical studies, efficacy and safety concerns have prevented these drugs from their use in clinical practice.

Conclusions

SAF, as a measure of tissue AGE deposition, is considerably increased in patients with PAD compared with controls. This increase is independent of the presence of cardiovascular risk factors or other established cardiovascular disease, although these conditions are associated with a further increase of SAF. These findings underscore the importance of AGEs in PAD, irrespective of the presence of diabetes mellitus and renal insufficiency.

Disclosures

A.J.S. is founder of DiagnOptics BV, the Netherlands, manufacturing autofluorescence readers (<http://www.diagnoptics.com/>). The other authors have no conflicts to report.

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