Chapter 6

Association of skin autofluorescence levels with kidney function decline in patients with peripheral artery disease


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Abstract

Objective
Skin autofluorescence (SAF), a measure of advanced glycation end product accumulation, is associated with kidney function. We investigated the association of SAF with rate of kidney function decline in a cohort of patients with peripheral artery disease.

Approach and Results
We performed a post hoc analysis of an observational longitudinal cohort study. We included 471 patients with peripheral artery disease and SAF was measured at baseline. Primary end point was rate of estimated glomerular filtration rate (eGFR) decline. Secondary end points were incidence of eGFR <60 and <45 mL/min per 1.73m² and rapid eGFR decline, defined as a decrease in eGFR of >5 mL/min per 1.73m² per year. During a median follow-up of 3 years, the mean change in eGFR per year was -1.8 ± 4.4 mL/min per 1.73m²/year. No significant difference in rate of eGFR decline was observed per 1 arbitrary unit increase in SAF (-0.1 mL/min per 1.73m² per year; 95% confidence interval -0.7 to 0.5; \( P=0.8 \)). Analyses of the secondary end points showed that there was an association of SAF with incidence of eGFR <60 and <45 mL/min per 1.73m² (hazard ratio: 1.54; 95% confidence interval 1.13-2.10; \( P=0.006 \) and hazard ratio: 1.76; 95% confidence interval 1.20-2.59; \( P=0.004 \), respectively), but after adjustment for age and sex significance was lost. There was no association of SAF with rapid eGFR decline.

Conclusions
In conclusion, in this cohort of patients with peripheral artery disease, elevated SAF was associated with lower baseline eGFR. Although SAF has previously been established as a predictor for cardiovascular disease and mortality, it did not predict the rate of kidney function decline during follow-up in this study.
Introduction

Advanced glycation end products (AGEs) are irreversibly glycated proteins with injurious effects on tissues. AGE formation is promoted by hyperglycemic environments and oxidative stress, and has previously been associated with aging, diabetes mellitus, chronic kidney disease (CKD) and cardiovascular disease. Accumulation of AGEs on long-lived proteins such as collagen occurs in all types of tissue, including the skin, vascular tissue, and glomerular and tubular basement membranes. Increased levels of AGEs contribute to tissue injury by activating pro-inflammatory and pro-oxidative pathways, which can cause kidney damage. In turn, reduced glomerular filtration rate causes a lower rate of AGE excretion, leading to a vicious cycle of further AGE accumulation and more kidney and cardiovascular damage. AGE accumulation has been and is currently being investigated as a treatment target using different approaches such as diet, improved glucose control, and specific AGE lowering drugs.

Skin autofluorescence (SAF), measured noninvasively with an optical device, is a validated technique to assess AGE levels in the skin. Peripheral artery disease (PAD) is associated with an increased risk of CKD. Previously, we showed that SAF is elevated in subjects with PAD and is associated with mortality, cardiovascular events and amputation during follow-up. The association of SAF and CKD has also previously been demonstrated. SAF was also found to be associated with development of albuminuria in a cohort of patients with type 2 diabetes mellitus. SAF may therefore be a noninvasive measure to predict kidney disease progression. Since both CKD and diabetes mellitus increase SAF, there may be an interaction between CKD and diabetes mellitus in the relationship with SAF. Therefore, we investigated the associations of the presence of impaired kidney function and diabetes mellitus with SAF at baseline. Furthermore, we assessed, the association of baseline SAF with rate of kidney function decline during follow-up. For this study we used data of an observational cohort study that included patients with PAD. We hypothesized that increased SAF levels are independently associated with impaired kidney function and diabetes mellitus at baseline, and with accelerated kidney function decline during follow-up.
Materials and Methods

Materials and Methods are available in the online-only Data Supplement. In short, we performed a post hoc analysis of a single-center prospective cohort study of 471 patients with established PAD. Deposition of tissue AGEs was noninvasively measured by SAF with the AGE Reader™. Five-year follow-up data were analyzed. The primary end point was rate of estimated glomerular filtration rate (eGFR) decline, defined as the change in eGFR in mL/min per 1.73m² per year. For the longitudinal data analysis of the primary end point, we performed a linear mixed model analysis with the repeated measurements of eGFR as the outcome and a random effect for time, to allow for individual deviations to the overall population slope. Patients with at least two eGFR measurements were included in this analysis. All models included SAF and an interaction term between SAF and time, to allow for assessment of differences in change in eGFR over time across SAF levels (SAFxtime). Covariates that were significant in the cross-sectional multivariable analysis with SAF were included in the linear mixed model. Three models were tested: model 1, crude model including SAF, time, and their interaction term; model 2, as model 1 plus age and sex; and model 3, as model 2 plus smoking status and diabetes status. Secondary end points were incidence of eGFR <60 mL/min per 1.73m², <45 mL/min per 1.73m² and rapid eGFR decline, defined as an eGFR decline of >5 mL/min per 1.73m² per year. A Cox proportional hazards regression analysis was used to assess the association of SAF with incidence of eGFR <60 mL/min per 1.73m² and <45 mL/min per 1.73m², and logistic regression analysis assess the association between SAF and rapid eGFR decline (coded as categorical variable).

Results

Baseline Characteristics

Baseline characteristics are presented in Table 1, for the overall cohort and for participating subjects stratified according to SAF tertiles (<2.5, 2.5-3.1, and >3.1 arbitrary units [AU]). Mean SAF was 2.8 ± 0.7 AU and mean age was 65.9 ± 10.6 years, 70% were men and 31% had diabetes mellitus. Baseline eGFR was 76.9 ± 21.5 mL/min per 1.73m². Patients with higher SAF levels were older; more frequently had diabetes mellitus, a history of coronary artery disease and cerebrovascular disease; used β-blockers and diuretics more often; had higher HbA1c levels and lower eGFR (all P<0.05; Table 1).
Patients with higher SAF levels were older; more frequently had diabetes mellitus, 70% were men and 31% had diabetes mellitus. Baseline eGFR was 76.9 ± 21.5 mL/min per 1.73 m². Mean SAF was 2.8 ± 0.7 AU and mean age was 65.9 ± 10.6 years.

Three models were tested: model 1, crude model including SAF, time, and their interaction term; model 2, as model 1 plus age and sex; and model 3, as model 2 plus an interaction term between SAF and time, to allow for assessment of differences in eGFR decline of >5 mL/min per 1.73 m² per year. A Cox proportional hazards regression model was performed with the primary end point, we performed a linear mixed model analysis with the two eGFR measurements were included in this analysis. All models included SAF and allow for individual deviations to the overall population slope. Patients with at least repeated measurements of eGFR as the outcome and a random effect for time, to

Table 1. Baseline characteristics of patients with peripheral artery disease

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Overall</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>471</td>
<td>156</td>
<td>158</td>
<td>157</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>65.9 ± 10.6</td>
<td>63.0 ± 11.4</td>
<td>65.6 ± 9.9</td>
<td>69.0 ± 9.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>328 (70)</td>
<td>101 (65)</td>
<td>115 (73)</td>
<td>111 (71)</td>
<td>0.3</td>
</tr>
<tr>
<td>Current smoker</td>
<td>243 (52)</td>
<td>74 (48)</td>
<td>84 (53)</td>
<td>85 (54)</td>
<td>0.4</td>
</tr>
<tr>
<td>Body mass index, kg/m²*</td>
<td>26.6 ± 4.5</td>
<td>26.8 ± 4.7</td>
<td>26.7 ± 4.4</td>
<td>26.4 ± 4.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Serum glucose, mmol/L†</td>
<td>6.3 ± 1.4</td>
<td>6.1 ± 1.4</td>
<td>6.6 ± 2.6</td>
<td>6.5 ± 2.1</td>
<td>0.07</td>
</tr>
<tr>
<td>HbA1c, mmol/mol‡</td>
<td>44.3 ± 10.9</td>
<td>41.0 ± 6.5</td>
<td>45.4 ± 10.9</td>
<td>48.6 ± 12.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>144 (31)</td>
<td>29 (19)</td>
<td>50 (32)</td>
<td>65 (41)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose-lowering drugs</td>
<td>103 (22)</td>
<td>15 (10)</td>
<td>38 (24)</td>
<td>50 (32)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin</td>
<td>43 (9)</td>
<td>4 (3)</td>
<td>12 (8)</td>
<td>27 (17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oral glucose-lowering drugs</td>
<td>76 (16)</td>
<td>12 (8)</td>
<td>31 (20)</td>
<td>33 (21)</td>
<td>0.002</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>146 ± 25</td>
<td>147 ± 22</td>
<td>143 ± 26</td>
<td>147 ± 26</td>
<td>0.2</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>79 ± 14</td>
<td>80 ± 12</td>
<td>79 ± 16</td>
<td>79 ± 15</td>
<td>0.9</td>
</tr>
<tr>
<td>Hypertension</td>
<td>434 (92)</td>
<td>145 (93)</td>
<td>143 (91)</td>
<td>146 (93)</td>
<td>0.6</td>
</tr>
<tr>
<td>Antihypertensive drugs</td>
<td>395 (84)</td>
<td>131 (84)</td>
<td>127 (80)</td>
<td>137 (87)</td>
<td>0.3</td>
</tr>
<tr>
<td>RAAS inhibitor</td>
<td>309 (66)</td>
<td>106 (68)</td>
<td>99 (63)</td>
<td>104 (66)</td>
<td>0.6</td>
</tr>
<tr>
<td>α-Blocker</td>
<td>24 (5.1)</td>
<td>7 (4.5)</td>
<td>7 (4.4)</td>
<td>10 (6.4)</td>
<td>0.7</td>
</tr>
<tr>
<td>β-Blocker</td>
<td>232 (49)</td>
<td>65 (42)</td>
<td>78 (49)</td>
<td>89 (57)</td>
<td>0.03</td>
</tr>
<tr>
<td>Calcium-channel blocker</td>
<td>117 (25)</td>
<td>35 (22)</td>
<td>34 (22)</td>
<td>48 (31)</td>
<td>0.1</td>
</tr>
<tr>
<td>Diuretic</td>
<td>149 (32)</td>
<td>40 (26)</td>
<td>48 (30)</td>
<td>61 (39)</td>
<td>0.04</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L§</td>
<td>4.7 ± 1.3</td>
<td>4.8 ± 1.2</td>
<td>4.6 ± 1.3</td>
<td>4.5 ± 1.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>405 (86)</td>
<td>136 (87)</td>
<td>131 (83)</td>
<td>138 (88)</td>
<td>0.4</td>
</tr>
<tr>
<td>Statin</td>
<td>354 (75)</td>
<td>116 (75)</td>
<td>113 (72)</td>
<td>125 (80)</td>
<td>0.2</td>
</tr>
<tr>
<td>Serum creatinine, µ/L</td>
<td>88.4 ± 35.4</td>
<td>79.6 ± 26.5</td>
<td>88.4 ± 35.4</td>
<td>97.2 ± 44.2</td>
<td>0.009</td>
</tr>
<tr>
<td>eGFR, mL/min per 1.73m²</td>
<td>76.9 ± 21.5</td>
<td>81.4 ± 19.5</td>
<td>76.2 ± 20.7</td>
<td>73.0 ± 23.4</td>
<td>0.002</td>
</tr>
<tr>
<td>Fontaine stage ≥III</td>
<td>63 (13)</td>
<td>16 (10)</td>
<td>19 (12)</td>
<td>28 (18)</td>
<td>0.2</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>142 (30)</td>
<td>35 (22)</td>
<td>53 (34)</td>
<td>54 (34)</td>
<td>0.04</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>69 (15)</td>
<td>14 (9)</td>
<td>23 (15)</td>
<td>32 (20)</td>
<td>0.02</td>
</tr>
<tr>
<td>Skin autofluorescence, AU</td>
<td>2.8 ± 0.7</td>
<td>2.1 ± 0.3</td>
<td>2.8 ± 0.2</td>
<td>3.6 ± 0.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as number (%), or mean ± standard deviation. AU indicates arbitrary units; eGFR, estimated glomerular filtration rate (by CKD-EPI formula); RAAS, renin-angiotensin-aldosterone system.

* Data missing for 5 patients; † Data missing for 28 patients. ‡ Data missing for 68 patients.
§ Data missing for 59 patients. ‖ Data missing for 28 patients.
Cross-Sectional Analysis of Covariates Associated with SAF

Evaluation of covariates possibly associated with SAF is shown in Table 2. Univariable and multivariable linear regression analyses showed significant associations of SAF with several covariates. In the multivariable analysis, higher levels of SAF were independently associated with being older, smoking, having diabetes mellitus, and having impaired kidney function. Figure 1 shows the association of baseline diabetes status and impaired kidney function (eGFR above and below 60 mL/min per 1.73 m²) with SAF and indicates that SAF levels are dependent on presence of diabetes mellitus and impaired kidney function (P<0.001). No significant interaction was found between diabetes status and impaired kidney function versus SAF level (P for interaction 0.6), suggesting that diabetes and impaired kidney function are truly independently associated with SAF. A sensitivity analysis replacing hypertension, hypercholesterolemia, obesity, impaired kidney function, and diabetes status with baseline systolic blood pressure, total cholesterol, eGFR and HbA1c levels is shown in Table I in the online-only Data Supplement. This analysis showed similar associations of SAF with age, smoking status, HbA1c, and eGFR. In the univariable analysis, the P value for total cholesterol met the threshold for entering the multivariable analysis (P=0.06 for total cholesterol versus P=1.0 for hypercholesterolemia in the original analysis). However, in the multivariable analysis, no significant association of cholesterol with SAF was found.

Table 2. Linear regression analyses of SAF in patients with peripheral artery disease

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age</td>
<td>0.251</td>
<td>0.164-0.338</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.078</td>
<td>-0.550-0.212</td>
</tr>
<tr>
<td>Current smoking</td>
<td>0.113</td>
<td>-0.009-0.235</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.017</td>
<td>-0.211-0.245</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>-0.002</td>
<td>-0.179-0.175</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.305</td>
<td>0.174-0.435</td>
</tr>
<tr>
<td>Impaired kidney function</td>
<td>0.275</td>
<td>0.118-0.431</td>
</tr>
<tr>
<td>Obesity</td>
<td>-0.026</td>
<td>-0.178-0.126</td>
</tr>
</tbody>
</table>

Beta is expressed per 1 standard deviation increase for continuous variables and versus the reference category for dichotomous variables. Impaired kidney function is defined as an estimated glomerular filtration rate <60 per mL/min per 1.73 m². Obesity is defined as a body mass index >30 kg/m². CI indicates confidence interval; and SAF, skin autofluorescence.
Longitudinal Analysis of SAF and eGFR Decline

During a median follow-up of 3.0 years; interquartile range 1.5-4.7 years, patients had on average 7.4 eGFR measurements available; range 1-81 measurements, with a median interval of 0.5 years; interquartile range 0.3-1.1. Twenty-one patients without follow-up eGFR measurements were excluded from the longitudinal analysis. The overall eGFR slope for the remaining 443 patients was \(-1.8 \pm 4.4\) mL/min per 1.73m\(^2\)/year. Seventeen patients (4%) had a doubling of serum creatinine during follow-up.

The linear mixed model analysis of SAF as continuous variable with the repeated eGFR measurements as outcome showed that although patients with higher SAF levels had on average a lower eGFR at baseline (-6.5 mL/min per 1.73m\(^2\) per 1 AU increase in SAF; 95% confidence interval -9.4 to -3.6; \(P<0.001\)), the interaction term between SAF and time was not significant (\(P=0.8\); Table 3). This indicates that eGFR slopes during follow-up across SAF levels at baseline did not differ. Across SAF tertiles, there was also no difference in eGFR slope: for tertile 1 (SAF <2.5 AU) -1.8 ± 2.2; for tertile 2 (SAF 2.5-3.1 AU) -1.7 ± 2.4; and for tertile 3 (SAF >3.1 AU) -2.1 ± 2.0 mL/min per 1.73m\(^2\) per year (\(P=0.6\) from linear mixed model analysis for both tertiles 2 and 3 versus tertile 1; Figure 2). The sensitivity analyses showed that patients with diabetes mellitus did not have a faster decline in eGFR than patients without diabetes mellitus (\(P=0.1\)) (Table II in the online-only Data Supplement). Additionally, the interaction between SAF, diabetes
mellitus, and time was also not significant ($P=0.5$), meaning that patients with diabetes mellitus and high levels of SAF did not have faster eGFR decline than patients without diabetes mellitus and lower levels of SAF. Lastly, to show the robustness of our results, we performed a linear regression analysis studying SAF levels across tertiles of eGFR change. No differences in SAF levels were found ($P=0.1$ for trend, Table III in the online-only Data Supplement).

Table 3. Linear mixed models for the association of baseline SAF with change in eGFR during follow-up, per unit increase of SAF

<table>
<thead>
<tr>
<th>Model</th>
<th>SAF</th>
<th>Standard error</th>
<th>95% confidence interval</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>SAF</td>
<td>-6.532</td>
<td>1.477</td>
<td>-9.427 to -3.637</td>
</tr>
<tr>
<td></td>
<td>SAF x time</td>
<td>-0.093</td>
<td>0.326</td>
<td>-0.731 to 0.545</td>
</tr>
<tr>
<td>Model 2</td>
<td>SAF</td>
<td>-2.576</td>
<td>1.276</td>
<td>-5.078 to -0.074</td>
</tr>
<tr>
<td></td>
<td>SAF x time</td>
<td>-0.119</td>
<td>0.326</td>
<td>-0.759 to 0.521</td>
</tr>
<tr>
<td>Model 3</td>
<td>SAF</td>
<td>-2.917</td>
<td>1.324</td>
<td>-5.513 to -0.322</td>
</tr>
<tr>
<td></td>
<td>SAF x time</td>
<td>-0.121</td>
<td>0.327</td>
<td>-0.762 to 0.520</td>
</tr>
</tbody>
</table>

The coefficient for SAF represents the association of SAF (per unit) with eGFR at baseline. The coefficient for SAF x time represents the association of baseline SAF (per unit) with eGFR slope during follow-up. Model 1: crude model including SAF, time, and an interaction term for SAF x time; Model 2: as model 1 + age and sex; Model 3: as model 2 + smoking status and diabetes mellitus status. Random effect in all models is time (years). Beta coefficient for SAF is the overall decrease in eGFR per unit increase in SAF. The beta coefficient for the interaction term of SAF with time represents the annual change in eGFR in mL/min per $1.73m^2$ per unit increase in SAF. eGFR indicates estimated glomerular filtration rate; SAF, skin autofluorescence.

Figure 2. Mean estimated glomerular filtration rate (eGFR) slope during follow-up by skin autofluorescence (SAF) tertile at baseline with 95% confidence intervals

$P$ values are provided for linear mixed model analysis with the lowest tertile as reference. AU indicates arbitrary units.
For the secondary outcomes (incidence of eGFR <60 and <45 mL/min per 1.73m²), 107 and 42 patients, respectively, already had eGFR below these thresholds at baseline and were excluded. During follow-up, 91 patients (25%) reached an eGFR <60 mL/min per 1.73m² and 59 patients (14%) reached an eGFR <45 mL/min per 1.73m². Kaplan-Meier figures for both end points are shown in Figure I in the online-only Data Supplement. The figures show significant differences in event-free survival for both incident eGFR <60 mL/min per 1.73m² (log-rank \( P=0.02 \)) and incident eGFR <45 mL/min per 1.73m² (log-rank \( P=0.002 \)). The hazard ratios for all Cox regression models tested for both end points are listed in Table IV in the online-only Data Supplement. Per unit increase in SAF, patients had a 54% higher risk of developing eGFR <60 mL/min per 1.73m² (hazard ratio: 1.54; 95% confidence interval 1.13-2.10; \( P=0.006 \)) and a 76% higher risk of developing eGFR <45 mL/min per 1.73m² (hazard ratio: 1.76; 95% confidence interval 1.20-2.59; \( P=0.004 \)). However, this association lost significance for both end points after adjusting for age and sex in model 2. The associations between SAF and both end points remained not significant in model 3.

Finally, we studied the association between baseline SAF and rapid kidney function decline during follow-up. A total of 104 patients had an eGFR decline of >5 mL/min per 1.73m² per year. Results of the logistic regression analysis are listed in Table V in the online-only Data Supplement. The logistic regression showed no association between SAF and rapid kidney function decline in all models \( (P>0.05) \).

**Discussion**

Our results indicate that in patients with PAD, both diabetes mellitus and impaired kidney function were associated with increased AGE accumulation, measured as SAF. In a multivariable analysis of the cross-sectional data, higher SAF levels were significantly associated with lower eGFR independent of age, smoking status and diabetes status. However, in longitudinal analyses, we found no independent association of baseline SAF levels with eGFR decline during follow-up, incidence of eGFR <60 mL/min per 1.73m² or eGFR <45 mL/min per 1.73m², or rapid eGFR decline. Furthermore, we found no interaction between SAF and diabetes mellitus on eGFR decline.

The finding that SAF is cross-sectionally associated with impaired kidney function confirms the findings of earlier studies. However, the longitudinal analyses in our cohort showed no association between SAF and eGFR decline. Only one study has
previously investigated the relationship between SAF and kidney function outcome. That study showed, in a cohort of 449 Japanese pre-dialysis CKD patients with a 32% diabetes prevalence, that SAF predicted the rate of kidney function decline and incidence of end-stage kidney disease.\textsuperscript{11} The discrepant findings in our study versus those in the study by Tanaka et al. may be because of population differences between the cohorts. For Caucasians, the reference value of SAF in healthy individuals is 2.46 ± 0.57 for the age group of 60 to 70 years, and SAF has been shown to increase with ≥0.023 AU per year.\textsuperscript{22} Our cohort had a mean SAF of 2.8 AU versus 2.1 AU in the other cohort, suggesting that our cohort was in poorer health. In addition, the other study included only Japanese patients, whereas we included Dutch patients. Others have shown in a large cohort of CKD patients from 3 different ethnic origins (white, Oriental Asian, and South Asian), that both Asian groups had faster progression toward end-stage kidney disease than the white group.\textsuperscript{23} In addition, the subjects from the Tanaka cohort were selected to have CKD. In line, average baseline eGFR was 55.8 mL/min per 1.73m\textsuperscript{2}, whereas in our study it was 76.9 mL/min per 1.73m\textsuperscript{2} although age was similar in the two cohorts (64.0 and 65.9 years, respectively). Subjects with lower eGFR are known to have a higher risk for adverse kidney outcomes during follow-up.\textsuperscript{24} Indeed, in the Tanaka study, 11% reached end-stage renal disease or doubling of serum creatinine during follow-up, and this figure was considerably lower in our cohort. The more rapid rate of eGFR decline in the Asian cohort may have increased the sensitivity for finding significant associations between SAF and kidney end points. Furthermore, it is known that AGEs accumulate in case of impaired kidney function, creating a vicious cycle of AGE accumulation, leading to further kidney damage with even more reduced excretion of AGEs, and further AGE accumulation.\textsuperscript{7} Given the difference in baseline eGFR between our cohort and the Japanese cohort and our discrepant findings, it is possible that this vicious cycle only occurs in established CKD with eGFR levels below a certain threshold.

Our cohort had a high prevalence of (severe) cardiovascular comorbidity, which is independently associated with SAF, but most patients had preserved kidney function.\textsuperscript{10,25} Furthermore, others have shown that SAF serves as a marker of metabolic memory, reflecting glycemic and renal status during the past 10 years.\textsuperscript{26} We found a significant cross-sectional association of SAF with eGFR but did not find an association of SAF with eGFR decline. Additionally, there was no interaction between SAF and diabetes mellitus on eGFR decline. It is therefore possible that SAF is merely a marker of impaired kidney function and metabolic memory and not a risk factor for further kidney function decline.
A limitation of our study is that SAF was measured only at baseline. Furthermore, data on albuminuria were not available. However, it may be expected that additional adjustment for albuminuria could only have weakened the association between SAF and kidney function decline in our analyses. Lastly, creatinine was only measured on indication by regular medical caregivers of the patients included in our cohort. This may have resulted in bias, with relatively more information on eGFR during follow-up being available in patients at highest risk for kidney function decline. However, because this bias would lead to a higher chance of finding a significant association of SAF with kidney function decline, this only strengthens our negative findings. Strengths of this study were that we investigated a relatively large cohort, and that longitudinal associations of SAF with eGFR slopes have not been investigated before in a white cohort.

Given the presently available findings, the future of SAF as a potential risk factor for adverse renal outcome and as a screening tool to identify subjects at risk for accelerated kidney function decline remains unclear. We found no association of SAF with eGFR decline in patients with PAD, which conflicts with earlier findings that showed a significant association of SAF with eGFR decline in patients with established CKD. Therefore, the use of SAF as a predictor of kidney function decline may be limited to patients without cardiovascular comorbidity or SAF may only be a predictor in patients with established CKD. Further research is needed to clarify if, and in which populations, SAF can predict kidney function decline. Such studies should preferably include mixed populations to allow within study subgroup analyses. In addition, to our knowledge, it has not been investigated whether serial measurements of SAF may further improve the calculation of cardiovascular or kidney risk. It may be that changes in SAF are more strongly associated with eGFR decline than a single SAF measurement.

**Conclusion**

In conclusion, in this cohort of patients with PAD, elevated SAF was associated with lower eGFR levels at baseline. Although SAF has previously been established as a predictor for cardiovascular disease and mortality, it did not predict the rate of kidney function decline during follow-up.
Chapter 6

References


Supplemental Material and Methods

Study population
This study was performed as a post-hoc analysis using data of a single-center prospective cohort of 471 patients with established peripheral artery disease (PAD). Cross-sectional data from this cohort have been published previously. Patients were recruited at the outpatient clinic of the Department of Surgery (division of Vascular Surgery), University Medical Center Groningen, Groningen, The Netherlands. Patients were included from October 2007 to August 2011. Men and women older than 18 years old 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 non-compressible calf arteries. PAD was confirmed by evidence of obstructive disease on computed tomographic angiography, magnetic resonance angiography, digital subtraction angiography, or duplex ultrasonography. Exclusion criteria were recent myocardial infarction, stroke or sepsis (all within the previous 3 months), renal replacement therapy, active cancer and solid organ transplantation. The study was approved by the local medical ethics committee and all participating patients gave informed consent.

Clinical data and measurement of skin autofluorescence
Baseline measurements included age, sex, current smoking status, BMI, blood pressure, total serum cholesterol, glucose, HbA1c and diabetes mellitus status. Hypertension was defined as using blood pressure-lowering drugs and/or a blood pressure >140/90 mmHg, obesity as BMI >30 kg/m², hypercholesterolemia as using lipid-lowering drugs and/or a total cholesterol >5 mmol/L, and diabetes mellitus as having a HbA1c ≥6.5%, fasting plasma glucose level ≥7.0 mmol/L, a random plasma glucose ≥11.1 mmol/L and/or using glucose lowering drugs.

Skin autofluorescence was measured at baseline with the AGE Reader™ (DiagnOptics Technologies BV, Groningen, The Netherlands). Technical details of this noninvasive device concerning the optical technique have been described in detail elsewhere. In short, the AGE Reader™ illuminates a skin surface of 4 cm², guarded against surrounding light, with an excitation light source between 300 and 420 nm and 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). SAF was measured at the posterior side of the right forearm,
which is the standard and most practical measurement site for SAF. A series of 3 consecutive measurements was carried out, taken less than a minute apart. The mean SAF was calculated from these 3 measurements and used in the analyses. An earlier validation study showed an intra-individual Altman error percentage of 5.0%, with SAF measurements taken over 1 single day, and an Altman error percentage of 5.9% for seasonal variation. ³

Creatinine was measured for every patient at baseline, and obtained during follow-up from patient files. During follow-up, creatinine was measured when deemed clinically appropriate during regular medical care. Creatinine measurement was performed with an isotope dilution mass spectrometry (IDMS)-traceable enzymatic method on a Roche Modular analyzer (Roche Diagnostics, Mannheim, Germany). The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to estimate glomerular filtration rate (eGFR). ⁴ Creatinine measurements during acute kidney injury events and during hospitalization were excluded from the present analyses.

For the longitudinal analysis the primary end point was rate of eGFR decline, defined as change in eGFR in mL/min per 1.73m² per year. Secondary end points were incidence of eGFR <60 mL/min per 1.73m², <45 mL/min per 1.73m² and rapid eGFR decline, defined as eGFR decline of >5 mL/min per 1.73m² per year.⁵

Statistical analysis
Baseline characteristics are presented for all patients in the present analysis stratified in SAF tertiles. Continuous data are presented as mean with standard deviation (SD) or as median with interquartile range (IQR) in case of skewed distribution. Categorical data are presented as count and percentage. Differences in patient characteristics across SAF tertiles were tested with ANOVA and Chi-Square tests for continuous and categorical variables, respectively.

Patients were then classified into four groups, based on the presence or absence of diabetes mellitus and impaired kidney function at baseline (eGFR > or <60 mL/min per 1.73m²) to visually inspect the overall difference in SAF between the four groups. Again, ANOVA and Chi-Square tests were used to further assess differences between the four groups for continuous and categorical covariates, respectively.
Next, univariable and multivariable linear regression analyses were performed in a cross-sectional setting for SAF as dependent variable and various baseline covariates. Covariates were chosen a priori based on established risk factors for kidney function decline: age, sex, smoking, hypertension, hypercholesterolemia, obesity, diabetes mellitus and impaired kidney function. A $P$ value of $\leq 0.1$ was set as threshold for adding the covariate to the multivariable analysis. Two sensitivity analyses were performed. First, we replaced the categorical variables (hypertension, hypercholesterolemia, obesity, diabetes mellitus and impaired kidney function) with their continuous baseline counterparts (i.e., systolic blood pressure, total cholesterol, BMI, HbA1c and eGFR, respectively). Second, we tested for interaction between diabetes status and impaired kidney function, as well as between HbA1c and eGFR for their association with SAF.

For the longitudinal data analysis of the primary end point, we performed linear mixed model analysis with the repeated measurements of eGFR as outcome and a random effect for time, to allow for individual deviations to the overall population slope. Creatinine and thus eGFR were measured only when deemed clinically appropriate by the patients regular physicians, and thus the time intervals between measurements were irregular. Linear mixed models take into account the irregular and varied number of eGFR measurements as it weighs the individual slopes depending on the number of measurements available. This prevents skewing of the overall slope by patients with short follow-up duration and/or extreme positive/negative slopes. Patients with at least 2 eGFR measurements were included in this analysis. The covariance structure was unstructured. All models included SAF and an interaction term between SAF and time, to allow for assessment of differences in change in eGFR over time across SAF levels (SAFxtime, the corresponding coefficient is to be interpreted as change in eGFR in mL/min per 1.73m$^2$/year per unit increase in SAF). Covariates that were significant in the cross-sectional multivariable regression analysis with SAF were included in the linear mixed model. Three models were tested: model 1; crude model including SAF, time and their interaction term; model 2, as model 1 plus age and sex; model 3, as model 2 plus smoking and diabetes mellitus. In addition, we performed 2 sensitivity analyses to assess the role of diabetes mellitus in eGFR decline. We tested 2 additional mixed models: model 1; crude model including SAF, diabetes mellitus, time and their interaction terms; model 2, as model 1 plus a three-way interaction term between SAF, diabetes mellitus, and time.
The association of SAF with the secondary end points (incidence of eGFR <60 and <45 mL/min per 1.73m²) were tested using Cox proportional hazards regression analyses. Patients who at baseline had eGFR values below these thresholds were excluded from the analyses. Finally, the association between SAF and rapid progression of eGFR decline, defined as a rate of eGFR decline of more than -5 mL/min per 1.73m² per year was tested using logistic regression analysis for patients with at least two eGFR values available. Both the Cox regression and the logistic regression models used the same covariates as the linear mixed model analysis.

All statistical analyses were performed using IBM Statistical Package for Social Studies (SPSS, version 22.0) and Stata software (Version 13, StataCorp LP, College Station, Texas, USA). A P value of ≤0.05 was adopted to indicate statistical significance.
Supplemental References


Supplemental Data

Table I. Linear regression analyses of SAF in patients with PAD (sensitivity analysis)

<table>
<thead>
<tr>
<th></th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age</td>
<td>0.251</td>
<td>0.164 to 0.338</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.078</td>
<td>-0.550 to 0.212</td>
</tr>
<tr>
<td>Current smoking</td>
<td>0.113</td>
<td>-0.009 to 0.235</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>-0.014</td>
<td>-0.105 to 0.077</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-0.094</td>
<td>-0.189 to 0.003</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.264</td>
<td>0.167 to 0.360</td>
</tr>
<tr>
<td>eGFR</td>
<td>-0.206</td>
<td>-0.295 to -0.117</td>
</tr>
<tr>
<td>Body mass index</td>
<td>-0.020</td>
<td>-0.112 to 0.071</td>
</tr>
</tbody>
</table>

Beta is expressed per 1 standard deviation increase for continuous variables and versus the reference category for dichotomous variables. CI indicates confidence interval; eGFR, estimated glomerular filtration rate; PAD, peripheral artery disease; SAF, skin autofluorescence.

Table II. Linear mixed models for the association of diabetes mellitus with change in eGFR during follow-up (mL/min per 1.73m²/year), and a model including the interactions between SAF and time, diabetes mellitus and time, and the three-way interaction between SAF, diabetes mellitus and time, to assess whether the association of SAF with eGFR decline is stronger in patients with diabetes compared to patients without diabetes mellitus

<table>
<thead>
<tr>
<th></th>
<th>Beta</th>
<th>Standard error</th>
<th>95% confidence interval</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1 SAF</td>
<td>-6.122</td>
<td>1.513</td>
<td>-9.089 to -3.156</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SAF x time</td>
<td>-0.004</td>
<td>0.329</td>
<td>-0.649 to 0.641</td>
<td>1.0</td>
</tr>
<tr>
<td>DM</td>
<td>-1.801</td>
<td>2.206</td>
<td>-6.129 to 2.528</td>
<td>0.4</td>
</tr>
<tr>
<td>DM x time</td>
<td>-0.709</td>
<td>0.462</td>
<td>-1.614 to 0.197</td>
<td>0.1</td>
</tr>
<tr>
<td>Model 2 SAF</td>
<td>-6.120</td>
<td>1.513</td>
<td>-9.086 to -3.154</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DM</td>
<td>-1.807</td>
<td>2.209</td>
<td>-6.135 to 2.522</td>
<td>0.4</td>
</tr>
<tr>
<td>SAF x time</td>
<td>-0.152</td>
<td>0.402</td>
<td>-0.940 to 0.635</td>
<td>0.7</td>
</tr>
<tr>
<td>DM x time</td>
<td>-1.973</td>
<td>2.018</td>
<td>-5.928 to 1.981</td>
<td>0.3</td>
</tr>
<tr>
<td>DM x SAF x time</td>
<td>0.438</td>
<td>0.680</td>
<td>-0.896 to 1.772</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Model 1: crude model including SAF, DM, time, and interaction terms for SAF x time and DM x time;
Model 2: model including SAF, DM, time, and an interaction term between SAF x time and DM x time, plus a three-way interaction term between DM, SAF, and time.
Random effect in all models is time (years). Beta coefficient for SAF is the overall decrease in eGFR per unit increase in SAF. Beta coefficients for interaction terms between SAF and time represent the change in eGFR in mL/min per 1.73m²/year per unit increase of SAF. Beta coefficient for the interaction terms of DM and time represent the change in eGFR in mL/min per 1.73m²/year for patients with DM versus patients without DM. The beta coefficient for the interaction term of DM, SAF, and time represents the change in eGFR in mL/min per 1.73m²/year, per unit increase of SAF for patients with DM.
DM indicates diabetes mellitus; SAF, skin autofluorescence.
Table III. Mean skin autofluorescence levels for tertiles of eGFR change per year

<table>
<thead>
<tr>
<th>eGFR slope</th>
<th>Skin autofluorescence, AU</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; -3.4</td>
<td>2.9 ± 0.6</td>
</tr>
<tr>
<td>-3.3 - 0.0</td>
<td>2.7 ± 0.7</td>
</tr>
<tr>
<td>&gt; 0.1</td>
<td>2.9 ± 0.7</td>
</tr>
</tbody>
</table>

Data presented as mean ± standard deviation. AU indicates arbitrary units; eGFR slope, estimated glomerular filtration rate in mL/min per 1.73m^2/year.

Table IV. Cox regression analyses of baseline SAF and secondary end points

<table>
<thead>
<tr>
<th>eGFR &lt;60</th>
<th>Hazard ratio</th>
<th>95% confidence interval</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>1.54</td>
<td>1.13-2.10</td>
<td>0.006</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.18</td>
<td>0.85-1.65</td>
<td>0.3</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.15</td>
<td>0.97-1.67</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>eGFR &lt;45</th>
<th>Hazard ratio</th>
<th>95% confidence interval</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>1.76</td>
<td>1.20-2.59</td>
<td>0.004</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.43</td>
<td>0.96-2.13</td>
<td>0.08</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.31</td>
<td>0.87-1.97</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Model 1: crude model; Model 2: as model 1 + age and sex; Model 3: as model 2 + smoking and diabetes mellitus. eGFR indicates estimated glomerular filtration rate in mL/min per 1.73m^2/year; SAF, skin autofluorescence.

Table V. Logistic regression analyses of baseline SAF with rapid eGFR decline

<table>
<thead>
<tr>
<th>Odds ratio</th>
<th>95% confidence interval</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>1.36</td>
<td>0.99-1.88</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.34</td>
<td>0.97-1.88</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.10</td>
<td>0.75-1.59</td>
</tr>
</tbody>
</table>

Rapid eGFR decline is defined as > 5mL/min per 1.73m^2 decline per year. Model 1: crude model; Model 2: as model 1 + age and sex; Model 3: as model 2 + current smoking and diabetes mellitus. eGFR indicates estimated glomerular filtration rate in mL/min per 1.73m^2/year; SAF, skin autofluorescence.

Figure I. Kaplan-Meier survival curves with log-rank test for eGFR <60 (left) and eGFR <45 (right) according to median of SAF of the total group

eGFR indicates estimated glomerular filtration rate (by CKD-EPI) in mL/min per 1.73m^2; SAF, skin autofluorescence.
Table III. Mean skin autofluorescence levels for tertiles of eGFR change per year

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Table IV. Cox regression analyses of baseline SAF and secondary end points

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<td>0.3</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.15</td>
<td>0.97 - 1.67</td>
<td>0.5</td>
</tr>
</tbody>
</table>

| eGFR <45 |              |                         |         |
| Model 1  | 1.76         | 1.20 - 2.59             | 0.004   |
| Model 2  | 1.43         | 0.96 - 2.13             | 0.08    |
| Model 3  | 1.31         | 0.87 - 1.97             | 0.2     |

Model 1: crude model; Model 2: as model 1 + age and sex; Model 3: as model 2 + smoking and diabetes mellitus. eGFR indicates estimated glomerular filtration rate in mL/min per 1.73m²/year; SAF, skin autofluorescence.

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<td>0.08</td>
</tr>
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<td>Model 3</td>
<td>1.10</td>
<td>0.75 - 1.59</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Rapid eGFR decline is defined as > 5mL/min per 1.73m² decline per year. Model 1: crude model; Model 2: as model 1 + age and sex; Model 3: as model 2 + current smoking and diabetes mellitus. eGFR indicates estimated glomerular filtration rate in mL/min per 1.73m²/year; SAF, skin autofluorescence.
Chapter 7

Skin accumulation of advanced glycation end products is increased in patients with an abdominal aortic aneurysm.

Submitted

J. Boersema, L.C. de Vos, T.P. Links, A.J. Smit, C.J. Zeebregts, J.D. Lefrandt