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Skin Autofluorescence

A tool to identify type 2 diabetic patients at risk for developing microvascular complications

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OBJECTIVE — Skin autofluorescence is a noninvasive measure of the level of tissue accumulation of advanced glycation end products, representing cumulative glycemic and oxidative stress. Recent studies have already shown a relationship between skin autofluorescence and diabetes complications, as well as the predictive value of skin autofluorescence for total and cardiovascular mortality in type 2 diabetes. Our aim was to investigate the predictive value of skin autofluorescence for the development of microvascular complications in type 2 diabetes.

RESEARCH DESIGN AND METHODS — At baseline, skin autofluorescence of 973 type 2 diabetic patients with well-controlled diabetes was noninvasively measured with an autofluorescence reader. The aggregate clinical outcome was defined as the development of any diabetes-associated microvascular complication of 881 surviving patients, which was assessed at baseline and at the end of follow-up. Single end points were the development of diabetes-associated retinopathy, neuropathy, and (micro)albuminuria.

RESULTS — After a mean follow-up period of 3.1 years, baseline skin autofluorescence was significantly higher in patients who developed any microvascular complication, neuropathy, or (micro)albuminuria but not in those who developed retinopathy. Multivariate analyses showed skin autofluorescence as a predictor for development of any microvascular complication along with A1C, for development of neuropathy along with smoking, and for development of (micro)albuminuria together with sex, A1C, and diabetes duration. Skin autofluorescence did not have predictive value for the development of retinopathy, albeit diabetes duration did.

CONCLUSIONS — Our study is the first observation of skin autofluorescence measurement as an independent predictor of development of microvascular complications in type 2 diabetes.

RESEARCH DESIGN AND METHODS — Between May 2001 and May 2002, 973 primary care type 2 diabetic patients were included in the study cohort and had a skin autofluorescence measurement. The included patients were all participating in a shared-care project of the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) Study and have also been described elsewhere (11). During follow-up, data of 967 patients were analyzed for this study (6 patients were lost to follow-up). Eighty-six patients died before the end of follow-up, and this subgroup will be addressed separately from the surviving 881 patients. Patients with a Fitzpatrick class V–VI skin type were excluded because of the autofluorescence reader’s limitation to measure accurately in dark skin types (13–15). All participating patients visited the outpatient clinic at least once a year. Follow-up ended in January 2005. All of the included patients had given their informed consent, and approval by the local ethics committee had been obtained.

Skin autofluorescence
The autofluorescence reader (prototype of the current AGE Reader; DiagnOptics, Groningen, the Netherlands) illuminates a skin surface of ~4 cm², guarded against surrounding light, with an excitation light source with peak intensity at ~370 nm. Emission light and reflected excitation light from the skin is measured with a spectrometer in the 300–600 nm range, using a glass fiber. Autofluorescence was computed by dividing the average light intensity of the emission spectrum 420–600 nm by the average light intensity of the excitation spectrum 300–420 nm, multiplied by 100 and expressed in arbitrary units (AU). Skin autofluorescence of all patients was assessed at the volar side of the arm, 10 cm below the elbow fold. Six diabetes specialist nurses did the autofluorescence measurements with two identical autofluorescence reader devices. The autofluorescence reader has been validated and more extensively been described in previous studies (9,11).

Data collection
Clinical data and laboratory results were obtained at the time of the baseline skin autofluorescence measurement. Serum creatinine, nonfasting lipids (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides), and urinary albumin and creatinine were measured according to the standard laboratory procedures. A1C was measured with a Primus CLC-385 using boronate affinity chromatography and high-performance liquid chromatography (reference value 4.0–6.0%). Blood pressure measurement was a single measurement obtained after a 5-min rest with the patient in seated position, using an aneroid device. At each visit to the outpatient clinic and at the end of follow-up, the absence or presence of retinopathy, neuropathy, and (micro)albuminuria was assessed.

Statistical analysis
One-way ANOVA using post hoc multiple comparisons (with Bonferroni correction) was used to compare mean skin autofluorescence between subgroups of microvascular complications in the 881 surviving patients. Subgroups are as follows: 1) no microvascular complication at baseline or at follow-up, 2) no microvascular complication at baseline but a microvascular complication at follow-up, and 3) a microvascular complication at baseline and at follow-up. Univariate and multivariate multinominal regression analyses were performed to determine the relationship of skin autofluorescence to the presence or development of microvascular disease. Patients without signs of microvascular complications at baseline or at follow-up formed the reference categories in these calculations. In the multivariate analyses, we controlled for potential confounding risk factors for the development of microvascular complications, which were derived from the UKPDS findings, including sex, diabetes duration, A1C, current smoking, systolic blood pressure, HDL cholesterol, LDL cholesterol, and triglycerides, with the addition of BMI (4). Odds ratios (ORs) (95% CI) for skin autofluorescence were calculated in the univariate and multivariate analyses. P values <0.05 were considered statistically significant.
Skin autofluorescence (total group) (AU) 2.74

Macrovascular disease (%) 37

Microvascular disease (%) 50

Triglycerides in mmol/l 2.1 (1.4–2.9)

LDL cholesterol (mmol/l) 2.9

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Data are means ± SD of skin autofluorescence in AUs within the group or mean differences between groups (95% CI) (ANOVA with Bonferroni correction).

at baseline. However, skin autofluorescence was higher in the patient groups who developed neuropathy or (micro)albuminuria compared with that in patients without these complications. At follow-up, newly developed neuropathy was diagnosed in 7.5% and newly developed (micro)albuminuria in 10.1% of patients; 12.5% of the population developed at least one microvascular complication. Skin autofluorescence at baseline was also significantly higher in the patient groups that developed any microvascular complication or who already had a microvascular complication at baseline compared with patients who did not develop any microvascular disease.

Multinominal logistic regression analysis showed that skin autofluorescence was a strong predictor of the development of the aggregate of microvascular complications (OR 2.05 [95% CI 1.51–2.80], P < 0.001). Skin autofluorescence was significantly associated with the development of retinopathy (1.42 [1.01–1.99], P = 0.042), neuropathy (1.59 [1.15–2.19], P = 0.005), and (micro)albuminuria (1.73 [1.28–2.34], P < 0.001). After correction for the confounding risk factors, baseline skin autofluorescence still appeared to be significantly associated with the development of these end points, except for retinopathy (1.21 [0.83–1.74], P = 0.32) (Table 3). Diabetes duration at baseline was the only significant independent variable for development of retinopathy in this multivariate analysis (1.10 [1.06–1.15], P < 0.001). Surviving smokers less often developed neuropathy compared with nonsmokers. In the nonsurviving group (86 patients), 70% had a microvascular complication at baseline; there were 23 nonsurviving smokers. Seventy percent of the nonsurviving smokers already had a microvascular complication at baseline, and 13% of the nonsurviving smokers developed a microvascular complication before they died.

When baseline skin autofluorescence levels are categorized in subgroups of practically feasible levels of skin autofluorescence (three categories in rounded tertiles: skin autofluorescence < 2.35 AU, 2.35 ≤ skin autofluorescence < 3.00 AU, and skin autofluorescence ≥ 3.00 AU); those in the category skin autofluorescence ≥ 3.00 AU do have a higher chance to develop a microvascular complication compared with patients with a lower skin autofluorescence level (Table 4).

Table 1—Characteristics of the type 2 diabetic patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>Age (years)</th>
<th>Sex (male/female)</th>
<th>Smoking (%)</th>
<th>BMI (kg/m²)</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Diabetes duration (years)</th>
<th>A1C (%)</th>
<th>Creatinine (μmol/l)</th>
<th>Creatinine clearance (ml/min)</th>
<th>Total cholesterol (mmol/l)</th>
<th>HDL cholesterol (mmol/l)</th>
<th>Urinary albumin-to-creatinine ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>66 ± 11</td>
<td>406/475</td>
<td>19</td>
<td>29.4 ± 4.8</td>
<td>146 ± 20</td>
<td>4.0 (1.5–8.1)</td>
<td>6.6 (6.0–7.6)</td>
<td>95 ± 19</td>
<td>77 ± 27</td>
<td>5.2 ± 1.0</td>
<td>1.3 ± 0.3</td>
<td>2.1 (1.4–2.9)</td>
</tr>
</tbody>
</table>

Values are means ± SD or median (interquartile range) unless otherwise indicated. Reference values of the laboratory: A1C 4.0–6.0%, creatinine 70–110 μmol/l, creatinine clearance (Cockcroft-Gault formula) 80–120 ml/min, urinary albumin-to-creatinine ratio 0–2.5, total cholesterol 3.5–5.0 mmol/l, HDL cholesterol 0.9–1.7 mmol/l, LDL cholesterol 3.6–4.4 mmol/l, and triglycerides 0.6–2.2 mmol/l.

Table 2—Mean ± SD skin autofluorescence at baseline and mean differences between groups

<table>
<thead>
<tr>
<th></th>
<th>A: t₀ absent/t₉₀ absent</th>
<th>B: t₀ absent/t₉₀ present</th>
<th>C: t₀ present/t₉₀ present</th>
<th>B vs. A</th>
<th>C vs. A</th>
<th>C vs. B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinopathy</td>
<td>2.69 ± 0.73</td>
<td>2.88 ± 0.74</td>
<td>2.91 ± 0.72</td>
<td>0.20</td>
<td>0.22</td>
<td>0.02</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>2.67 ± 0.72</td>
<td>2.93 ± 0.75</td>
<td>2.88 ± 0.75</td>
<td>0.26</td>
<td>0.21</td>
<td>0.05</td>
</tr>
<tr>
<td>(Micro)albuminuria</td>
<td>2.62 ± 0.68</td>
<td>2.91 ± 0.67</td>
<td>2.97 ± 0.83</td>
<td>0.28</td>
<td>0.34</td>
<td>0.06</td>
</tr>
<tr>
<td>Any</td>
<td>2.52 ± 0.69</td>
<td>2.86 ± 0.66</td>
<td>2.88 ± 0.75</td>
<td>0.34</td>
<td>0.36</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Data are means ± SD of skin autofluorescence in AUs within the group or mean differences between groups (95% CI) (ANOVA with Bonferroni correction). t₀, baseline; t₉₀, follow-up.
CONCLUSIONS — Our study provides the first evidence that skin autofluorescence is an independent predictor of development of microvascular complications in a population of patients with well-controlled type 2 diabetes. Separately, this also holds for the development of neuropathy and (micro)albuminuria (and in univariate analysis for retinopathy). This noninvasive marker of tissue AGE accumulation may reflect the deleterious effects of long-term glycemic and oxidative stress. Meerwaldt et al. (12) recently showed that skin autofluorescence is a predictor of 5-year coronary heart disease and mortality in diabetes. The present study shows that skin autofluorescence also has a predictive value for the development of microvascular complications that, in the analysis of this study, is superior to that of many other commonly used risk predictors, such as diabetes duration and A1C, in type 2 diabetes. This conclusion is applicable for primary care type 2 diabetic patients treated according to current standards, which is the large majority of type 2 diabetes patients in the Netherlands.

The DCCT/EDIC (Epidemiology of Diabetes Interventions and Complications) substudy already showed the predictive value for skin AGE levels obtained from skin biopsies for the progression of microvascular complications in patients with type 1 diabetes (8). Our study population consisted of type 2 diabetic patients with skin AGE level assessment by means of a noninvasive, rapid method. Another difference is that the DCCT/EDIC substudy investigated the development as well as the progression of microvascular complications. The limited follow-up period; the low rate of clearly classifiable progression of the microvascular complications, especially retinopathy; and the confounding role of introduced medication made us decide to restrict our study to the evaluation of the development of microvascular complications and not to address progression of these diabetes complications.

In retinopathy, skin autofluorescence turned out to have no prognostic value in the multivariate analysis. Possible explanations are the short follow-up period and the smaller amount of patients who developed retinopathy versus the other complications. Moreover, the different pathophysiologic mechanisms of microvascular damage in the different organs (retina, kidneys, and neurons) could play a role in the differences in incidence rates of outcomes. In particular, the pathobiology of retinopathy might be different from that of the kidney and neurologic system as a result of a different role of vascular endothelial growth factor as a possible mediator for proliferation (20).

(Micro)albuminuria is an early clinical sign of diabetic nephropathy; when left untreated, it predicts a high risk for the development of progressive renal damage, which eventually may lead to end-stage renal disease. Progressive renal disease is also associated with a vastly increased cardiovascular risk. This study defined (micro)albuminuria as a sign of microvascular complications with the intention to reflect early stages of diabetic nephropathy.

In the predictive analyses, the nonsurviving patients were excluded from the analyses. These nonsurvivors had markedly increased skin autofluorescence values, but they also had a very high prevalence of microvascular complications at baseline (70%), so this does not reduce the strength of the relation between skin autofluorescence and microvascular complications.

Ethnicity is one of the mentioned UKPDS confounding risk factors for the development of microvascular disease. Because of the limitation of measuring skin autofluorescence in dark skin types associated with the prototype of the AGE.
reader used in the present study, individuals with dark skin had to be excluded. Over 95% of the participants were Caucasian; therefore, ethnicity was not taken into account in the analyses. Further developments of the AGE reader may hopefully enable measurements in dark skin type in future investigations.

Lutgers et al. (11) previously described the other limitations of the autofluorescence reader as a marker of tissue AGE accumulation: nonfluorescent AGES will not be measured with the autofluorescence reader, and other tissue components that fluoresce in the same range of wavelength might be confounders.

In conclusion, our study confirms skin autofluorescence as a helpful clinical method to identify type 2 diabetic patients at risk for developing any microvascular complication, neuropathy, and (micro)albuminuria. Further investigation with longer follow-up needs to be done to assess whether skin autofluorescence is a factor in the development of diabetic retinopathy and to assess the relationship of skin autofluorescence and the progression of microvascular complications. Its noninvasive and time-saving application makes the autofluorescence reader an easy clinical tool that is useful in the outpatient clinic in risk assessment and for monitoring changes in accumulation of tissue AGES reflecting long-term glycemic stress.

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References