

## Research: Complications

# Skin autofluorescence and risk of micro- and macrovascular complications in patients with Type 2 diabetes mellitus—a multi-centre study

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## Abstract

**Aims** Skin autofluorescence is a non-invasive marker of advanced glycation end product accumulation. In a previous study, skin autofluorescence correlated with and predicted micro- and macrovascular complications in Type 2 diabetes in a primary care setting. The present cross-sectional study aims to confirm the association between skin autofluorescence and diabetic complications in patients with Type 2 diabetes in a multi-centre secondary care setting.

**Methods** We analysed 563 subjects with Type 2 diabetes mellitus from five Dutch hospitals.

**Results** Median age was 64 years, median duration of diabetes 13 years and median HbA<sub>1c</sub> 58 mmol/mol (7.5%). Sixty-one per cent of patients had microvascular complications (38% nephropathy, 36% retinopathy, 35% neuropathy) and 42% had macrovascular complications. Median UK Prospective Diabetes Study 10-year risk for coronary events was 19%. Median skin autofluorescence was elevated compared with age-matched healthy control subjects: 2.77 (interquartile range 2.39–3.28) vs. 2.46 (2.08–2.84) arbitrary units. Skin autofluorescence was particularly increased in patients with complications: no complications, median 2.56 (2.26–2.90); microvascular complications, 2.79 (2.38–3.29); macrovascular complications, 2.85 (2.41–3.41); both micro- and macrovascular complications, 2.96 (2.56–3.60) arbitrary units,  $P < 0.001$ . Logistic regression analysis showed that age, duration of diabetes, renal function, gender, atrial fibrillation and skin autofluorescence were independently associated with macrovascular complications. Multiple regression analysis identified age, smoking, renal function, macrovascular complications and the number of microvascular complications as the determinants of skin autofluorescence.

**Conclusions** This study confirms that skin autofluorescence is increased in patients with Type 2 diabetes in a secondary care setting. Skin autofluorescence was associated with macrovascular complications in patients with diabetes and this association was independent of classical risk factors.

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## Introduction

Advanced glycation end products accumulate in tissues with a low turnover during a lifetime [1,2]. This is regarded as a process of normal ageing. The accumulation of accelerated advanced glycation end products results from combinations of hyperglycaemia, hyperlipaemia, oxidative/carbonyl stress and also decreased renal clearance of advanced glycation end

product precursors [1,2]. Highly accelerated advanced glycation end product formation and accumulation is seen in diabetes mellitus [1]. This is considered one of the important pathogenetic mechanisms causing end organ damage in diabetes [1]. Cross-linking of proteins by advanced glycation end products and receptor-mediated cellular activation contribute to loss of elasticity of the vascular wall and to cellular inflammation, resulting in micro- and macrovascular complications [1,3]. The contents of the advanced glycation end products in skin biopsies predicted long-term diabetic complications in a large cohort of patients with Type 1 diabetes, even after adjustment for HbA<sub>1c</sub> [4,5].

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Skin autofluorescence has a strong correlation with the specific advanced glycation end product content in skin biopsies, as shown by multiple validation studies [6–8]. Not only fluorescent advanced glycation end products (pentosidine), but also non-fluorescent advanced glycation end products such as *N*-carboxymethyl-lysine and *N*-carboxyethyl-lysine in the skin biopsies correlated with skin autofluorescence [6]. We therefore consider skin autofluorescence a non-invasive marker of tissue accumulation of advanced glycation end products. Earlier studies showed that skin autofluorescence is increased in Type 2 diabetes compared with healthy control subjects [9]. Skin autofluorescence showed a strong association with the severity of diabetes-related complications [9,10]. Furthermore, it also predicted both micro- and macrovascular complications in patients with Type 2 diabetes [10]. In a previous prospective follow-up study, skin autofluorescence had an additional value to the UK Prospective Diabetes Study (UKPDS) risk score for estimating mortality in Type 2 diabetes mellitus [11]. However, these findings concern one single-centre cohort of well-controlled patients with Type 2 diabetes in a primary care setting. Our study aims to confirm the relation between skin autofluorescence and diabetic complications and possibly broaden our insight into the role of skin autofluorescence in predicting micro- and macrovascular complications. We evaluated skin autofluorescence in a group of patients with Type 2 diabetes in a secondary medical care setting. Furthermore, we chose a multi-centre approach to possibly support the generalizability wider use of the results. Here, we present the baseline cross-sectional data. Prospective follow-up data on the predictive value of skin autofluorescence on macrovascular complication will be presented in the future.

## Subjects and methods

### Subjects

We recruited 616 subjects with Type 2 diabetes mellitus from five hospitals in different regions of the Netherlands. The University Medical Centre Groningen (UMCG), Medical Centre Leeuwarden, Onze Lieve Vrouwe Gasthuis Amsterdam, Sint Franciscus Gasthuis Rotterdam and Het Diaconessenhuis Meppel participated in this study. Patients were included from April 2007 to November 2009. Informed consent was obtained from all participants. The study was approved by the Medical Ethical Committee.

### Assessment of skin autofluorescence

Skin autofluorescence was measured with the advanced glycation end product (AGE) Reader™ (DiagnOptics Technologies BV, Groningen, the Netherlands). The AGE Reader is a desktop device that uses the characteristic fluorescent properties of some advanced glycation end products to estimate the level of advanced glycation end product accumulation in the skin. Technical and optical details of this non-invasive method have

been described more extensively elsewhere [8]. In short, the AGE Reader illuminates a skin surface of 4 cm<sup>2</sup>, guarded against surrounding light, with an excitation light source with a peak excitation of 370 nm. This wavelength is in the UVA spectrum. Emission light in the wavelength range of 420–600 nm (fluorescence) and excitation light that is reflected by the skin with a wavelength range of 300–420 nm from the skin is measured with a spectrometer. Skin autofluorescence was determined from the ratio between the emission light and the reflected excitation light, using the AGE Reader software, version 2.2. Each participating medical centre was equipped with an AGE Reader and given user instructions for skin autofluorescence measurement. Measurements were performed by a diabetes nurse or research assistant. Skin autofluorescence was measured at room temperature while patients were at rest in a seated position. In the current series of experiments, the forearm was positioned on top of the device in the usual manner, as described by the manufacturer. Measurements were not specifically performed in a fasting state. For each skin autofluorescence value, three consecutive measurements were carried out at three different skin sites of the same forearm, within a total test period of approximately 2 min. The mean of these three consecutive measurements was used in the analyses. Skin pigmentation influences the measurement of skin autofluorescence and its influence has been extensively studied and reported earlier [12]. For a reliable skin autofluorescence measurement, skin reflectance had to be above 12% with the hardware and software (version 2.1) of the AGE Reader that was used. Patients with a dark-coloured skin with a reflectance below 12% were excluded from the analysis.

### Study protocol

Clinical data from the participating subjects were gathered from the electronic medical data system of the different hospitals. Data on age, gender, diabetes duration, blood pressure and macrovascular events were collected from the medical records. HbA<sub>1c</sub>, a fasting lipid profile, serum creatinine and urinary microalbumin were received from the laboratories. The standard creatinine assays of the individual hospitals laboratories were used and they were all well standardized with validated assays. Glomerular filtration rate (GFR) was estimated by the abbreviated Modification of Diet in Renal Disease (MDRD) equation:  $186 \times (\text{creatinine}/88.4)^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if black})$ . The standard stages of chronic kidney disease (1–5) were used to classify renal insufficiency. Diabetic nephropathy was scored as an albumin:creatinine ratio > 3.5 in women and > 2.5 in men on two successive samples, or at least once in the previous year while using an angiotensin-converting enzyme (ACE) inhibitor. Diabetic retinopathy was scored according to the report of the yearly evaluation by the patient's ophthalmologist with retinal photography or fundoscopy. Diabetic neuropathy was scored by testing with a 5.07 Semmes–Weinstein monofilament applied 10 times on three

areas of each foot in a random order. An absent sensation of the monofilament of three or more times (i.e. a score of lower than 8 of 10) was considered neuropathy. Microvascular disease was defined by either the presence of microalbuminuria and/or retinopathy (at least background retinopathy) and/or neuropathy. The presence of macrovascular disease was defined as a known history of coronary disease, cerebrovascular disease or peripheral vascular disease. Cardiovascular disease was defined as a history of ischaemic heart disease [International Classification of Diseases (ICD) codes I20–25] and/or a history of percutaneous coronary intervention or coronary bypass surgery. Cerebrovascular disease was defined as a history of an ischaemic cerebrovascular accident (ICD codes 63–64). Peripheral vascular disease was defined as a history of claudication with abnormal ankle–arm index ( $< 0.8$  on a single measurement or  $< 0.9$  on repeated measurements) or a history of surgical intervention of peripheral artery disease. Future cardiovascular risk of the individual patient was calculated by the UKPDS risk score. The following variables are used for risk calculation: age, duration of diabetes, gender, atrial fibrillation, ethnicity, smoking behaviour, HbA<sub>1c</sub>, systolic blood pressure, total cholesterol and HDL cholesterol. These provided a measure of the 10-year risk of fatal and non-fatal coronary and cerebrovascular events for each patient. As the UKPDS score incorporates these 10 cardiovascular risk factors, we used the UKPDS score as a measure of cardiovascular risk.

### Statistical analysis

We calculated sample size with a power of 80%,  $\alpha = 0.05$ . We adopted the standard deviation of 0.5 from the literature [13]. We wanted to be able to demonstrate a difference in skin autofluorescence of 0.2 arbitrary units between groups with no diabetic complications, microvascular complications, macrovascular complications and combined micro- and macrovascular complications. Power analysis showed that 77 subjects were needed in each group. Data were gathered in a database (SPSS 15-0; SPSS Inc., Chicago, IL, USA). Normal distribution of variables was assessed by Kolmogorov–Smirnov tests. Descriptive statistics are presented as mean with standard deviation in the case of normal distribution, otherwise as median with interquartile range or as number of patients. A paired Student *t*-test was used for normally distributed variables and a paired Mann–Whitney *U*-test was used for variables with a skewed distribution. Multiple linear regression analysis was used to determine independent relations between skin autofluorescence and clinical data, including age, duration of diabetes, gender, atrial fibrillation, ethnicity, smoking behaviour, HbA<sub>1c</sub>, systolic blood pressure, cholesterol and the presence of micro- and macrovascular complications. Logistic regression analysis was used to determine which variables determined presence of macro- and microvascular disease. Analysis of variance (ANOVA) was applied to compare differences between groups. For cross tabulation,  $\chi^2$  analysis was used.

## Results

### Subject characteristics

A total of 616 patients were measured. Skin autofluorescence measurements proved to be unreliable with the software version used in this study in 53 patients, mainly because of low reflectance caused by dark pigmented skin. Therefore, 563 patients with Type 2 diabetes were included in our analysis. Baseline characteristics are presented in Table 1. The median diabetes duration was 13 years and glycaemic control was suboptimal. Blood pressure and cholesterol were reasonably well controlled. A number of 126 patients (24%) had renal insufficiency with a chronic kidney disease stage 3 or more. As expected, the number of subjects with diabetic complications was high in this secondary medical care setting: 61% had microvascular complications and 42% macrovascular complications.

### Skin autofluorescence

Skin autofluorescence values for the total group and different subgroups are given in Table 2. Median skin autofluorescence was 2.77 with an interquartile range of 2.39–3.28 for the total group. This is elevated and, specifically, in the 79th percentile

**Table 1** Baseline characteristics presented as mean (standard deviation), median (interquartile range) or as number of patients (%)

Characteristic	Type 2 diabetes
Age (years)	64 (11.3)
Male sex	269 (47.9%)
Caucasian	517 (91.3%)
Atrial fibrillation	41 (7.3%)
Current smoker	97 (17%)
Diabetes duration (years)	13.0 (7–19)
HbA <sub>1c</sub> (mmol/mol; %)	58 (51–67); 7.5%
Systolic blood pressure (mmHg)	140 (130–150)
Total cholesterol (mmol/l)	4.2 (3.6–4.9)
HDL cholesterol (mmol/l)	1.2 (1.0–1.4)
GFR (ml/min)	75 (61–92)
Chronic kidney disease	
None	83 (16%)
Stage 1	57 (11%)
Stage 2	253 (49%)
Stage 3	117 (22.5%)
Stage 4	9 (2%)
Stage 5	0
Diabetic nephropathy	195 (38%)
Diabetic retinopathy	189 (36%)
Diabetic neuropathy	161 (35%)
Microvascular disease	346 (61%)
Coronary artery disease	169 (30%)
Cerebrovascular disease	74 (13%)
Peripheral artery disease	85 (15%)
Total macrovascular disease	238 (42%)
Risk coronary events UKPD	19% (12–30)
Risk cerebrovascular events UKPDS	13% (6–29%)

compared with reference values in healthy control subjects, where for this median age a median skin autofluorescence of 2.46 (interquartile range 2.08–2.84) would be expected [13]. Table 2 shows that skin autofluorescence slowly rises with increasing age, which is regarded as a physiological phenomenon of ageing [13]. Skin autofluorescence levels increased significantly with increasing diabetic complications ( $P < 0.001$ ). In subjects without any diabetic complications, median skin autofluorescence was 2.56 (interquartile range 2.26–2.90), which is the 65th percentile for healthy control subjects of the same age. With microvascular complications, median skin autofluorescence was 2.79 (interquartile range 2.38–3.29 and 80th percentile). In a univariate analysis, no differences in skin autofluorescence values were found between patients with one, two or three microvascular complications (ANOVA,  $P = 0.12$ ). With macrovascular complications, median skin autofluorescence was 2.85 (interquartile range 2.41–3.41 and 82th percentile) and, in those with both micro- and macrovascular complications, median skin autofluorescence was 2.96 (interquartile range 2.56–3.60, 88th percentile). The differences in skin autofluorescence between patients without diabetic complications, with microvascular complications and with both micro- and macrovascular complications remained significant after correction for age ( $P = 0.008$ ).

#### Association between skin autofluorescence, micro- and macrovascular complications

Multiple linear regression analysis was performed to evaluate which variables were associated with skin autofluorescence. Up to 19% in the variance of skin autofluorescence could be explained by age ( $\beta = 0.28$ ,  $P < 0.000$ ), smoking ( $\beta = 0.18$ ,  $P < 0.000$ ), renal function (eGFR) ( $\beta = -0.13$ ,  $P < 0.006$ ), macrovascular complications ( $\beta = 0.11$ ,  $P = 0.009$ ) and number of microvascular complications ( $\beta = 0.081$ ,  $P = 0.045$ ).

**Table 2** Skin autofluorescence according to micro- and macrovascular complications and in different age groups (median with interquartile range)

	Skin autofluorescence	<i>n</i>
Total of group with Type 2 diabetes	2.77 (2.39–3.28)	563
Type 2 diabetes without any complications	2.56 (2.26–2.90)	141
Type 2 diabetes with microvascular complications	2.79 (2.38–3.29)	181
Type 2 diabetes with macrovascular complications	2.85 (2.41–3.41)	71
Both micro- and macrovascular complications	2.96 (2.56–3.60)	169
< 50 years	2.37 (2.00–2.76)	55
50–60 years	2.63 (2.30–3.03)	120
60–70 years	2.77 (2.36–3.20)	195
70–80 years	3.08 (2.61–3.50)	142
> 80 years	3.09 (2.57–3.87)	51

Medical centre, gender, duration of diabetes, HbA<sub>1c</sub>, systolic blood pressure and lipid profile did not significantly influence skin autofluorescence. When we entered nephropathy, neuropathy and retinopathy separately in the regression analysis, none of them were significant.

Subsequently, we analysed which variables were associated with the presence of macrovascular disease. Logistic regression analysis showed age (odds ratio 1.03,  $P = 0.05$ ), renal function (eGFR) (odds ratio 0.98,  $P = 0.008$ ), diabetes duration (odds ratio 1.03,  $P = 0.01$ ), gender (odds ratio 0.51,  $P = 0.001$ ), atrial fibrillation (odds ratio 0.39,  $P = 0.02$ ) and skin autofluorescence (odds ratio 1.45,  $P = 0.023$ ) to be independently associated with the presence of macrovascular disease. Medical centre, HbA<sub>1c</sub>, lipid profile, smoking, systolic blood pressure and microvascular disease, however, were not independently associated with the presence of macrovascular disease.

Predictors of the presence of microvascular disease were duration of diabetes (odds ratio 1.05,  $P = 0.002$ ), renal function (eGFR) (odds ratio 0.98,  $P < 0.000$ ) and skin autofluorescence (odds ratio 1.60,  $P = 0.004$ ). HbA<sub>1c</sub>, age, gender, smoking, systolic blood pressure and lipid profile did not contribute significantly.

#### Macrovascular diabetic complications by UKPDS risk score and skin autofluorescence

Regression analysis showed that skin autofluorescence was independently associated with the presence of macrovascular complications. Subsequently, we divided the patients into tertiles of UKPDS risk score and tertiles of skin autofluorescence. In these cross-sectional data, we used the UKPDS risk score as a measure of cardiovascular risk, integrating classical cardiovascular risk factors and glycaemic control. For the different tertiles, we analysed the prevalence of macrovascular complications. Results are shown in Table 3. Within each tertile of skin autofluorescence, the prevalence of macrovascular complications increased significantly. Macrovascular complications rose from 32% in the 1st skin autofluorescence tertile to 55% in the 3rd tertile ( $P < 0.000$ ). For the tertiles according to UKPDS risk score, the same phenomenon was seen: with each tertile, macrovascular complications were significantly higher ( $P < 0.000$ ). Interestingly, within each tertile of UKPDS risk score, skin autofluorescence appeared to further determine the presence of macrovascular complications. When focusing on the 1st UKPDS tertile, macrovascular complications were present in 20% in the 1st skin autofluorescence tertile, while this was 38% in the 3rd skin autofluorescence tertile ( $P = 0.04$ ).

#### Discussion

Skin autofluorescence was previously shown to predict micro- and macrovascular complications in one prospective study in a single-centre cohort of well-controlled patients with Type 2

**Table 3** Percentage of macrovascular complications according to different tertiles of skin autofluorescence and of UKPDS risk score

Percentage of macrovascular complications	Tertiles of skin autofluorescence				Total
		1st	2nd	3rd	
Tertiles of UKPDS risk score	1st	20.2	27.9	37.8	26.2
	2nd	35.6	34.9	50.7	40.7
	3rd	51.3	56.9	66.3	59.9
	Total	31.6	40.2	55.1	

diabetes in the primary care setting [9,10]. In this cohort, skin autofluorescence even proved to add predictive information on cardiovascular prognosis to the UKPDS risk score [11].

Our present multi-centre study in the secondary medical setting confirms that skin autofluorescence is indeed increased in patients with Type 2 diabetes mellitus. Furthermore, it confirms that skin autofluorescence levels have a graded relation with the presence of micro- and macrovascular complications. Our cohort had a relatively long duration of diabetes of 13 years and a reasonable, although not optimal, glycaemic control. The prevalence of micro- and macrovascular diabetic complications, as well as UKPDS risk score, were somewhat higher, but not much different than previously described in studies concerning skin autofluorescence in Type 2 diabetes mellitus [9–11]. The cohort we describe is surprisingly similar to the cohort in the primary care setting that Lutgers and co-workers previously presented [9–11]. Age, sex, smoking behaviour, creatinine clearance, micro- and macrovascular disease and skin autofluorescence values were similar in both populations. Our cohort had a longer diabetes duration (13 vs. 4 years), a marginally higher HbA<sub>1c</sub> [58 vs. 53 mmol/mol (7.5 vs. 7.0%)], a better controlled systolic blood pressure (140 vs. 146 mmHg) and better controlled total cholesterol (4.2 vs. 5.2 mmol/l).

In our study, we show that skin autofluorescence was independently associated with the presence of both micro- and macrovascular complications. Surprisingly, most classical cardiovascular risk factors and glycaemic control were not significantly associated with the presence of macrovascular disease. Macrovascular disease was predicted by age, diabetes duration, renal function (eGFR), gender, atrial fibrillation and skin autofluorescence. Specifically, HbA<sub>1c</sub>, blood pressure and lipid profile did not contribute. Earlier studies showed that HbA<sub>1c</sub> and skin autofluorescence have a limited relation [14], suggesting that skin autofluorescence is only partly determined by the components of the slow Maillard reaction.

When, in our present cohort, the UKPDS risk score and skin autofluorescence were both divided into tertiles, there was an increasing percentage of patients with macrovascular complications within each tertile of UKPDS risk score (as an integration of the classical riskfactors and glycaemic control), going from the 1st to the 3rd skin autofluorescence tertile. The association between prevalence of macrovascular complications, UKPDS risk score and skin autofluorescence, which we

present now on cross-sectional data, has no value in prediction of future cardiovascular events. The results, however, do show that the prevalence of macrovascular disease is not only associated with a high classical cardiovascular risk profile (estimated by the UKPDS risk score), but, in addition, is associated with an increased skin autofluorescence. The prospective follow-up data of our study on cardiovascular events are required to confirm that skin autofluorescence has additive value in predicting future cardiovascular disease events on top of the UKPDS risk score. These data will be presented in the future.

Our results also contribute to establish the generalizability of skin autofluorescence results in patients with Type 2 diabetes concerning the association between skin autofluorescence with diabetic complications. First of all, within our study, no differences in skin autofluorescence results were found between the five participating medical centres. Also, the levels of skin autofluorescence in these subgroups, according to diabetic complications, are very similar to the skin autofluorescence levels found in the population with Type 2 diabetes previously described by Lutgers *et al.* [9]. Their study group found a skin autofluorescence of 2.77 in a population of nearly the same age as our group. Without any complications, mean skin autofluorescence was 2.57, with microvascular complications mean skin autofluorescence was 2.71 and with both micro- and macrovascular complications mean skin autofluorescence was 3.12. This is exactly the same grading of skin autofluorescence according to diabetic complications that we found in the present study. Chabroux *et al.* [15] also found very high skin autofluorescence values in a cohort of patients with Type 1 diabetes with a median age of 30 years and a diabetes duration of 17 years. Without diabetic complications, subjects had a skin autofluorescence of 1.86 (which is the 93th percentile adjusted to age) and, for subjects with both retinopathy and nephropathy, skin autofluorescence was 2.94 (which is the 99.9th percentile). This study in young patients with Type 1 diabetes cannot directly be compared with results found in patients with Type 2 diabetes, but it does again show that skin autofluorescence is elevated in patients with diabetes and even significantly more when microvascular complications exist.

## Limitations

Our current results are based on cross-sectional data, which are by definition inferior to prospective data. This study has,



however, been designed as a prospective follow-up study. Follow-up data on complications and mortality will be presented in the future.

Macrovascular disease was defined as a known history of coronary disease, cerebrovascular disease or peripheral artery disease. We did not screen all patients for macrovascular disease by, for example, coronary angiogram or computed tomography cerebrum of ankle–arm index. Therefore, asymptomatic macrovascular disease may have been missed in our study.

The used version of the AGE Reader in this study was limited by an influence of skin pigmentation. Skin reflectance had to be above 12% for an accurate reading. This led to inaccurate skin autofluorescence readings in 53 patients who therefore could not be included in the analysis. Development of AGE Reader is still ongoing and adjustments in the device have in the meantime improved the accuracy of readings in patients with high skin pigmentation [12].

## Conclusions

This study confirms in a multi-centre secondary care setting that skin autofluorescence is increased in patients with Type 2 diabetes and has a graded relation with the presence of micro- and macrovascular complications. In Type 2 diabetes, skin autofluorescence appears to be independently associated with the presence macro- and microvascular complications, in addition to the well-known classical risk factors of the UKPDS risk score.

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None.

## Competing interests

AJS is founder and stockholder of DiagnOptics Technologies BV, the Netherlands, manufacturer of the AGE Reader, which has been used as the device for performing skin autofluorescence measurements discussed in this study. The other authors have nothing to declare.

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