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For diabetic type 1 patients, the skin autofluorescence predicts ulcers and amputations

Saad Rojubally, Amélie Simoneau, Marie Monlun, Ninon Foussard, Laurence Blanco, Frédéric Domenge, Kamel Mohammedi, Eric Ducasse, Caroline Caradu, Vincent Rigalleau.

Bordeaux CHU and University, Endocrinology-Diabetology-Nutrition and Vascular Surgery 33000 Bordeaux, FRANCE

Address correspondence to Pr vincent rigalleau, email: vincent.rigalleau@chu-bordeaux.fr

Endocrinology-Nutrition, CHU Bordeaux

Hospital Haut-Lévêque, Avenue de Magellan, 33604 Pessac, FRANCE ☎ Phone: 33 5.57.65.60.78

Fax: 33 5.57.65.63.79

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ABSTRACT :

We searched whether the accumulation of Advanced Glycation End-products (AGEs), reflected by the skin autofluorescence (SAF), could predict diabetic foot ulcers (DFUs) during the long-term follow-up of people with type 1 diabetes.

During year 2009, we measured the SAF with an AGE-Reader in 206 subjects with type 1 diabetes. DFU and amputations were registered during the 10 following years. The relation between the SAF and later DFU was analyzed by Cox model regression, adjusted for vascular risk factors.

The 206 participants were mainly men (55.8%), 51±15 years old, with a 22±13 years diabetes duration. Twelve subjects presented a DFU. Their SAF were higher: 2.61±0.89 AU vs 2.11 ±0.53 for the others (p=0.003), related to the risk of DFU (OR:3.69; 95%CI:1.06-12.79) after adjustment for age, gender, diabetes duration, initial HbA1c, arterial hypertension, history of smoking, blood lipids and use of a statin. Five subjects were amputated, also related to the initial SAF: OR: 11.28 (95%CI:1.76-79.97) after adjustment for age, gender , duration of diabetes, and HbA1c.

The SAF has already been related to diabetic neuropathy and peripheral arterial disease. It predicts DFU in type 1 diabetes, which suggests that AGEs play a role in this highly specific and feared complication."

Introduction

During their lifetime, about 25% of subjects with diabetes present a diabetic foot ulcer (DFU) (1). This important complication exposes them to the risks of amputation, recurrence, and reduces their life expectancy (2) and quality of life (3). Its economic burden is considerable, mainly due to the cost of hospitalizations and amputations (4).

The general risk factors for DFU are those that predispose to the underlying diabetic peripheral neuropathy and peripheral arterial disease, namely arterial hypertension, dyslipidemia and tobacco use (5). More specifically, poor long-term glucose control increases the risk of DFU (6). In randomized controlled trials that tested glucose control in type 2 diabetes, the amputations were reduced by -35% by intensive glucose control (7). About 95% of DFUs occur in subjects with type 2 diabetes, but the prevalence is higher among type 1 diabetes, probably due to more prolonged hyperglycemia (5). One major mechanism by which long-term hyperglycemia may favor long-term vascular complications is the generation of Advanced glycation End-products (AGEs) (8). Their role in the diabetic foot disease is therefore suspected (9), but clinical arguments for this hypothesis are lacking.

The accumulation of AGEs can now be indirectly estimated due to their fluorescent properties, by the measurement of the skin autofluorescence, that has been related to their concentrations in skin biopsies (10). In cross-sectional studies, a high skin autofluorescence has been reported in subjects with diabetic neuropathy (11), peripheral arterial disease (12), and subjects with DFUs (13). But it is not known whether the skin autofluorescence can predict a later DFU.

The objective of our work was to determine whether the occurrence of a diabetic wound or amputation during follow-up was associated with the initial measure of skin auto-fluorescence. In 206 subjects with type 1 diabetes, we measured the skin autofluorescence, and conventional risk factors for diabetic complications (Age, gender, HbA1c, duration of diabetes, blood lipids, arterial hypertension and smoking history) during the year 2009, and thereafter we registered DFU and amputations during a 9-year follow-up. By Cox regression analysis, we searched whether the skin autofluorescence could be related to DFU, adjusted to conventional risk factors.

Methods And Subjects

During the year 2009, 206 subjects with type 1 diabetes were consecutively included during their visit to the Diabetology unit of Bordeaux's University Hospital. Inclusions stopped in December 2019, the 1st. The only exclusion criterion was a reflectance index <10% during the measurement of the skin autofluorescence, which made the analysis impossible for subjects with type V-VI phototypes.

On the same day as the measure of skin autofluorescence (SAF), we registered medical informations: age, gender, Body Mass Index (BMI), duration of diabetes, arterial hypertension, history of smoking. We measured the HbA1c, blood lipids, serum creatinine, and urinary albumin excretion.

Measurement of the skin autofluorescence

The accumulation of AGEs in the skin was measured with an AGE-reader (DiagnOptics BV, Groningen, the Netherlands) and expressed in arbitrary units (AU). The measurement was performed at the forearm, at a normal skin site without scars, lichenification or other abnormalities.

Outcome

Searching for new DFUs and amputations, we examined the medical files registered by the University Hospital of Bordeaux until September 2019. Follow-up was stopped at the last visit to our institution.

Statistical analysis

The results are presented as mean \pm standard deviation (SD) for continuous variables, median and interquartile ranges for the serum triglycerides and the albumin excretion rates, and as percentages for categorical variables. The characteristics of the subjects who developed a DFU were compared to the remaining subjects free from DFUs by t-tests for continuous variables, non-parametric test (Mann-Whitney) for triglycerides and Chi-2 test for categorical variables. The freedom from DFU survival curves were compared between subjects categorized as having a skin autofluorescence higher than the median, by Kaplan-Meier survival analysis, and we performed a cox regression analysis to adjust for potential confounders: age, gender, duration of diabetes, HbA1c, arterial hypertension, blood lipids and use of statins, and tobacco.

Results

Characteristics of the population at inclusion

Participants were mainly men (55.8%), 51±15 years old, with a diabetes duration of 22±13 years. Their BMI was 24.7±3.9 kg/m², with a HbA1c level of 7.5±0.9% and eGFR of 87±25 ml/min/1.73m². Among them, 25.3% had a history of smoking, 27.3% were treated by a statin, 44.2% had arterial hypertension, 20.9% had a diabetic kidney disease. Their SAF was 2.14±0.56 AU.

Subjects with diabetic foot ulcers during the follow-up (Table 1).

Twelve patients presented a DFU during the follow-up. The only significant difference, as compared to subjects without a DFU, was the duration of diabetes (+9 years, $p<0.05$), but there were also tendencies for higher age, higher triglycerides, more arterial hypertension and higher HbA1c at inclusion. The SAF was 2.61±0.89 AU in patients who presented a DFU during the follow-up vs 2.11 ±0.53 for the others ($p=0.003$).

Baseline SAF and appearance of diabetic foot ulcers (figure 1)

Patients were separated into two groups according to their baseline median SAF (≤ 2.1 vs >2.1). According to the Kaplan-Meier analysis, patients with higher baseline SAF more frequently developed a DFU during follow-up compared to patients with a lower baseline SAF (LogRank:5.48; $p=0.019$). Of note, a similar tendency was observed for the five amputations which occurred during follow-up (LogRank:2.97; $p=0.084$).

The baseline SAF was still related to the risk of DFU according to a Cox-model regression analysis (HR:3.69; 95%CI:1.06-12.79) with adjustment to the other risk factors: age, gender, diabetes duration, initial HbA1c, arterial hypertension, history of smoking, blood lipids and use of a statin. Of note, in that model, the only variable which remained related to a DFU was the initial HbA1c (HR: 2.15; 95%CI: 1.07-4.29). As for the risk of amputation, the HRs were 11.28 (95%CI:1.76-79.97) for SAF and 1.84 (95%CI:0.98-3.48; $p=0.058$) for the HbA1c, after adjustment for age, gender and the duration of diabetes.

Discussion

During the 9-year follow-up, 12 of our 206 patients presented with a DFU, and 5 required an amputation. As could be expected, these patients were older, more often male, with a longer duration of type 1 diabetes, and they had more risk factors such as arterial hypertension or dyslipidemia (blood lipids did not differ significantly but patients presenting with DFUs were more often treated by statins). Their baseline SAF was 23% higher. After adjustment for the other risk factors, the SAF was related to later DFU (HR: 3.69; 95%CI: 1.06-12.79) and to amputation (HR: 11.28; 95%CI: 1.76-79.97). Accordingly, higher SAFs of patients with DFUs were already reported in two cross-sectional studies that did not select patients with Type 1 Diabetes (13,14). Amputations were not registered in these studies, but interestingly, the SAF tended to relate to the healing time in Vouillarmet's report (14), which seems in line with the relation to amputation that we detected. The longitudinal design of our study further argues for a causal relation between a high SAF, and DFU and amputation. Multiple mechanisms can contribute to the risk of DFU in subjects with a high SAF.

In their pioneer work, Meerwaldt et al. related the SAF to diabetic peripheral neuropathy (11), which was later confirmed in type 2 diabetes by the longitudinal study of the ZODIAC cohort (15). In type 1 diabetes, the skin intrinsic fluorescence has also been related to neuropathy, both peripheral and autonomic (16). In our patients, we have previously shown that a high SAF predicted neuropathy four years later (17). Of note, the SAF was related to a later high vibration perception threshold, which is a good predictor of DFU (18). Higher SAF have been reported in patients with DFU, as compared to neuropathic controls without any wound, which suggests that they may not only relate to neuropathy in the patients with DFU (14).

SAF has also been related to clinical and subclinical atherosclerosis (12), carotid atherosclerotic plaque burden (19) and plaque vulnerability (20), outside diabetes context. In subjects with peripheral arterial disease, the skin autofluorescence predicts amputations (21) and mortality (22). In type 2 diabetes, it is related to the carotid Intima-Media-Thickness (23), the pulse wave velocity and the flow-mediated vasodilation (24). In type 1 diabetes it relates to indexes of early-stage atherosclerosis (25). We have previously reported that the SAF predicted cardiovascular events in our patients with type 1 diabetes (26).

Whether other mechanisms than neuropathy and peripheral arterial disease may play a role in the relation between SAF and DFU and amputation seems more speculative. We are not aware of any report suggesting that a high SAF may favor a foot infection, but it has been related to more post-operative complications, including systemic infections, after colorectal procedures (27). It has also been linked to

lower osteo-sono assessment at the calcaneum, suggesting that AGEs may alter bone strength (28). AGEs are also suspected to alter wound healing (9), and SAF tended to relate to delayed healing of DFU in Vouillarmet's study (14).

SAF can be considered as a surrogate marker of hyperglycemic memory, related to ancient glucose and HbA1c values in the general elderly population (29), and previous HbA1c in type 1 diabetes (30). Its relation to late DFU however kept significant after adjustment for age, diabetes duration and initial HbA1c in our patients. Tobacco is a known source of exogenous AGEs, and smoking is associated with higher autofluorescence (31), and favors DFUs (32). However, the relation between autofluorescence and DFU remained significant after adjustment for history of smoking in our patients. This relation seemed independent of conventional cardiovascular risk factors, which suggest a specific role for accumulated AGEs in DFUs, as previously hypothesized (9).

Some limitations to our study must, however, be kept in mind. The foot structure and presentation of foot deformities were not systematically registered for all the participants, which is an important confounder. The SAF does not directly measure AGEs: spectroscopic measurements of AGEs in the skin are based on the autofluorescent properties of some AGEs like pentosidine, a major AGE that explains most of the variability of SAF, but not of all AGEs. In addition, some redox regulated fluorophores such as NADH - nicotinic amide adenine dinucleotide reduced, FAD - flavin-adenine-dinucleotide and porphyrins also display autofluorescence. Thus, SAF represents a surrogate parameter of skin AGEs accumulation and reflects overall a state of increased oxidative stress and enhanced glycation." It is, however, correlated to their concentrations in the skin (10), and in type 1 diabetes it has further been related to corneal autofluorescence, hemoglobin-AGEs, and systemic vascular resistance assessed by pulse wave analysis (33). Circulating levels of AGEs (Carboxymethyllysine) and their soluble receptor (sRAGE) have been related to peripheral neuropathy in type 2 diabetes (34). We did not measure vascular and peripheral nerve functions, which would have helped distinguish whether high autofluorescence predisposes to neuropathic vs arteriopathic DFU, but the number of DFUs during our follow-up was small: 12/206 (5.8%). It was, however, higher than the 4.5% prevalence of DFUs among subjects with type 1 diabetes (5), which could be expected, as the mean duration of diabetes was 20 years in our patients. The number of DFU among our participants was 12/206 for a 10 year follow-up, which corresponds to a 5.8/1000 patient years, identical to a recent report from Rasmussen et al (35). Further work will be necessary to confirm whether SAF may also be related to DFUs in patients with type 2 diabetes, who account for 95% of cases (5). The relation between the initial HbA1c and latter DFU was significant for

our patients, but significance was not reached for initial HbA1c (7.6%) and latter amputation: this seems well-accorded to the recent meta-analysis from Lane et al, who reported that only HbA1c higher than 8% predicted amputations of DFU (36).

In summary, we found that SAF, a non-invasive marker of the accumulation of AGEs in tissues and metabolic memory, predicts the occurrence of DFU in subjects with type 1 diabetes, independent of conventional risk factors: diabetes duration and control, arterial hypertension, blood lipids, and smoking. This could be expected as skin autofluorescence has previously been related to diabetic neuropathy and peripheral arterial disease. Higher SAF years before the occurrence of a DFU suggest that AGEs play a role in this long-term, specific and feared complication.

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Figure 1 : Kaplan-Meier estimates of freedom from diabetic foot ulcers according to the median Skin autofluorescence

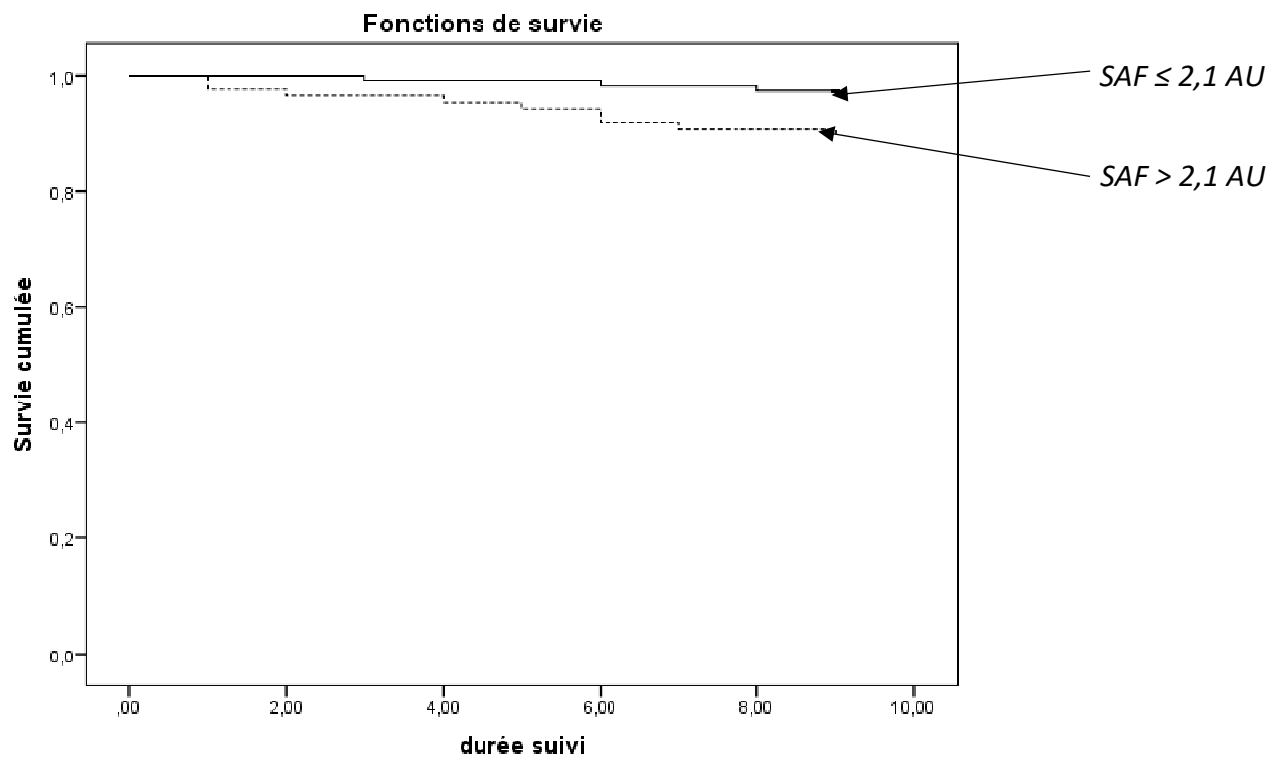


Table 1. Characteristics of the population and Diabetic Foot Ulcers cases.

	All subjects	Subjects remaining free of Diabetic Foot Ulcers	Subjects with Diabetic Foot Ulcers	p
N	206	196	12	
Gender (% women)	44.2%	44.8%	33.3%	0.436
Age (years)	51±15	50±15	59±14	0.075
Body Mass Index (kg/m ²)	24.7±3.9	24.8±3.8	24.2±5.0	0.658
History of smoking (%)	25.3%	25.3%	25.0%	0.983
Arterial hypertension (%)	44.2%	42.8%	66.7%	0.106
Triglycerides (mg/dL)	71 (54-108)	70 (53-108)	85 (69-137)	0.069
HDL-cholesterol (mg/dL)	64±17	64±17	62±17	0.738
LDL-cholesterol (mg/dL)	104±29	104±29	110±31	0.466
Treated by a statin (%)	27.3%	26.3%	41.7%	0.248
Duration of diabetes (years)	22±13	21±13	30±19	0.027
HbA1c (%)	7.6±0.9	7.5±0.9	7.9±1.3	0.119
Skin autofluorescence (AU)	2.14±0.56	2.11±0.52	2.61±0.89	0.003