Skin Autofluorescence – A Non-invasive Measurement for Assessing Cardiovascular Risk and Risk of Diabetes

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Abstract

The results of the Diabetes Control and Complications Trial-Epidemiology of Diabetes Interventions and Complications (DCCT-EDIC) study have strengthened the 'glycaemic memory' concept, postulating that the quality of metabolic control over several years predicts the development of diabetic complications. To mirror long-term metabolic control, the degree of glycated haemoglobin (HbA$_{1c}$) might not represent the optimal biomarker. Other substances with a longer persistence, like the so-called advanced glycation end-products (AGEs), which probably form the substrate of the glycaemic memory, might perform better. Newly developed methods such as the assessment of skin autofluorescence (SAF), enable fast, uncomplicated and non-invasive AGEs assessment. SAF was validated for diabetes screening and shows a good predictive value for the development of diabetic and cardiovascular complications. This article deals with the theoretical background and with available clinical data on this new variable.

Theoretical Background

Advanced glycation end-products (AGEs) and oxidative stress (OS) are two main contributors to the development of diabetic complications.[1,2] AGEs represent a heterogeneous substance class, produced through non-enzymatic glycation of proteins, lipids or nucleic acids within the so-called Maillard reaction (MR). Intermediate products of the MR are Schiff's bases and Amadori products (e.g. the glycated haemoglobin [HbA$_{1c}$]).[3] The MR is important for nutrient preparation, explaining the browning of foods during heating causing specific colour and taste. Food AGEs are partially absorbed from the gut,[4] but AGEs are also produced endogenously under physiological conditions. This process is exacerbated by hyperglycaemia, hyperlipidaemia or increased OS.[3]

Endogenous and exogenous AGEs are partially degraded in the body or eliminated via the kidneys. Modification of the aminoacid(s) in the active centre of the enzyme by the AGEs is responsible, for example, for the alterations of enzyme activity (non-receptor-dependent mechanisms). In addition, AGEs bind to specific receptors thus inducing intracellular processes such as OS or inflammation (receptor-dependent mechanisms). An increased endogenous and exogenous AGEs supply and accumulation contributes to the development of vascular complications in both patients with and without diabetes,[3] thus playing a central role in the 'glycaemic memory' concept.

The Diabetes Control and Complications Trial (DCCT) demonstrated that improved metabolic control in patients with type 1 diabetes mellitus (T1DM) reduces microvascular and neuropathic complications.[5] The fact that glycated proteins form stable AGEs, which accumulate in tissues affected by diabetes complications[6] led to the establishment of the previously mentioned concept of glycaemic memory.[7] The assumption is that AGEs represent the substrate of the glycaemic memory and cause diabetes complications.[8] Measuring AGEs might therefore represent an integrated variable, reflecting tissue glucose exposure over several years and thus more accurately predicting the risk of diabetes complications than the HbA$_{1c}$ that mirrors glycaemic control over 8–12 weeks only.[8,9]

Circulating or tissue-bound AGEs can be measured using enzyme-linked immunosorbent assay (ELISA), fluorescence spectroscopy, fluid chromatography and gas chromatography with mass spectrometry.[10] While the measurement of circulating AGEs shows high physiological fluctuations (e.g. postprandial),[4] the measurement of tissue-bound AGEs is more stable. In particular, tissues with a slow turnover (e.g. dermis, vascular walls and eye lenses) display a high AGEs accumulation and are therefore most suitable for AGEs assessment.[11]

The skin represents the most accessible tissue for AGEs measurements, with the biochemical analysis of skin biopsies constituting the golden standard.[11,12] However, this method is invasive, expensive, requires analysis in specialised centres and results are available within days to weeks. A growing interest in the non-invasive assessment of AGEs existed lately and non-invasive methods were developed which measure the AGEs-related

autofluorescence (AF) of the skin (AGE Reader, DiagnOptics, Groningen, the Netherlands, or SCOUT DS®, VeraLight, Albuquerque, New Mexico, USA), of the cornea or the lens (Fluorotron™, OcuMetrics, Mountain View, California, USA), allowing for a quick, reproducible and relatively low-cost measurement of AGEs accumulation.[12–14] Interestingly, not all devices were developed from the very beginning with the purpose of measuring AGE-related fluorescence. The Fluorotron device primary purpose, for example, was to assess the retinal leakage by measuring fluorescein fluorescence (the excitation light it emits is of 440–480 nm and detector is capturing 531–634 nm, fluorescein ex/em of 490/520 nm) in the eye after intravenous injection. However, the instrument was demonstrated to be useful in the assessment of eye-related AGEs-like fluorescence.

Measurement of the Skin Autofluorescence

The AF represents the natural light emission of substrates, after they have absorbed light.[12] The spectroscopic measurements of AGEs in the skin are based on the autofluorescent properties of some (e.g. pentosidine) but not of all (e.g. carboxymethyllysine) AGEs.[15] In addition, some redox regulated fluorophores (such as nicotine amide adenine dinucleotide reduced [NADH], flavin-adenine-dinucleotide [FAD] and porphyrins) display AF.[16,17] However, about 76 % of the variability of the SAF can be explained by the variability of pentosidine (a major AGE) concentration in the skin.[18] It is worth mentioning that fluorescence properties of pentosidine are: excitation 325–335 nm and emission 375 385 nm. Thus the AGE Reader is targeting only part of the pentosidine fluorescence spectrum.

Overall, Skin Autofluorescence is Regarded as a Surrogate Variable of Skin Advanced Glycation End-product Accumulation

The AGE Reader (DiagnOptics) non-invasively assesses SAF. Subjects lay their arm on a special arm rest with a window in the middle (see *Figure 1*). A skin surface of around 4 cm², guarded against surrounded light is illuminated with an excitation light source consisting of multiple light-emitting diodes (LEDs) with various intensities (maximum intensity at a wave length of approximately 370 nm) in a frequency range of 300 to 420 nm. The autofluorescent light is captured by multiple photo diodes, analysed and SAF is expressed in arbitrary units (AUs). The resultant SAF is calculated as the ratio of the light intensity in the 420–600 nm wavelength range and the light intensity in the 300–420 nm wavelength range. Further technical details were published by Meerwaldt et al.[19] Results are displayed within seconds, together with age-corrected reference values.

SAF measurements were validated against results of biochemical analyses of skin biopsies in healthy subjects, subjects with type 1 or type 2 diabetes mellitus (T2DM) and normal renal function,[12] as well as in subjects on haemodialysis.[18,20] AGEs accumulate in a comparable manner within various organs, and for SAF measurement the sun exposure seems not to be a major confounder. Indeed, SAF measured at the forehead (sun-exposed skin) and at the buttocks correlate well.[16] SAF correlates with the corneal and the lens AF[21] as well as with the AGEs concentration in material from bypass surgery.[22]

In persons with diabetes, SAF has an intra-individual, intra-day variability of 4.2–5.0 % and a seasonal variability of 5.9 %.[9,12,23] Our own study reported an intra-individual variability of 6.9 % and 10.9 % at 6 and 12 weeks, respectively.[24] A modest postprandial increase in SAF was described in some,[25,26] but not in all,[23] studies – a fasting measurement is therefore not mandatory.

The SAF shows a linear increase of 0.023 AU/year for persons up to 70 years.[27] Further important contributors to SAF are: smoking status, skin

Figure 1: AGE Reader

With an AGE Reader (DiagnOptics, Groningen, the Netherlands), the subject lays his/her forearm on the blue field, so that no outside light can penetrate the 'measurement window'. Light is directed through this window onto the skin for fractions of a second and then the autofluorescence of the skin is measured. The result is displayed on the screen within seconds, together with its age-adjusted reference values. The figure depicts the 'mu' iteration of the DiagnOptics flag product. In contrast to older versions, it utilises light-emitting diode (LED) light source. A shortcoming of the mu reader is the lack of the ability to adjust the measurement time in cases of darker skin pigmentation (low reflectance).

Table 1: Skin Autofluorescence Measured with the AGE Reader

Age Group (Years)	SAF in Healthy Adults (AU)	SAF in Type 2 Diabetes (AU)
40–49	1.84±0.4 (n=64)	2.17±0.5* (n=44)
50–59	2.12±0.3 (n=199)	2.57±0.7* (n=47)
60–69	2.50±0.6 (n=278)	2.69±0.7 (n=32)
70–79	2.76±0.6 (n=307)	3.04±0.8 (n=37)
>80	2.89±0.5 (n=108)	3.17±0.7 (n=7)

*AU = arbitrary unit; SAF = skin autofluorescence. Mean ± standard deviation in various age categories in healthy subjects and patients with type 2 diabetes (*p<0.01 versus healthy subjects). Adapted from Lutgers HL et al., Skin autofluorescence as a noninvasive marker of vascular damage in patients with type 2 diabetes, Diabetes Care, 2006; 29:2654–59.*

tone, renal function and average HbA$_{1c}$ value.[9] For measurements with the AGE Reader reference values exist for Caucasians[15,27] (see *Table 1*) and Asians.[28] Klenovics et al.[29] recently reported slight differences in SAF in a Slovakian population compared with a Dutch population suggesting that for precise clinical interpretation specific population-derived reference data should be obtained. The same study group suggested that persons exerting increased physical activity show lower SAF values.

In patients with T1DM, the age-adjusted SAF correlates significantly with the long-term HbA$_{1c}$ (ca. 15 years) but not with the current HbA$_{1c}$.[30,31] Therefore, due to their long persistence, AGEs assessment might be superior to the measurement of HbA$_{1c}$ with regard to the prediction of diabetes risk, the risk of development of cardiovascular and diabetes complications and mortality.

There are several drawbacks of SAF measurements, such as the impossibility to use SAF in persons with a very dark skin and the possibility that some external factors (e.g. creams, sunscreens, self-browning creams, extreme hyperaemia or vasoconstriction, etc.) can influence SAF measurements as reported by Noordzij et al.[32]

Skin Autofluorescence for the Diagnosis of Diabetes Mellitus

Fasting plasma glucose (FPG), glucose excursions during an oral glucose tolerance test (OGTT) and the HbA$_{1c}$ are established biomarkers

Figure 2: Algorithm for the Diagnosis of Impaired Glucose Tolerance and Diabetes
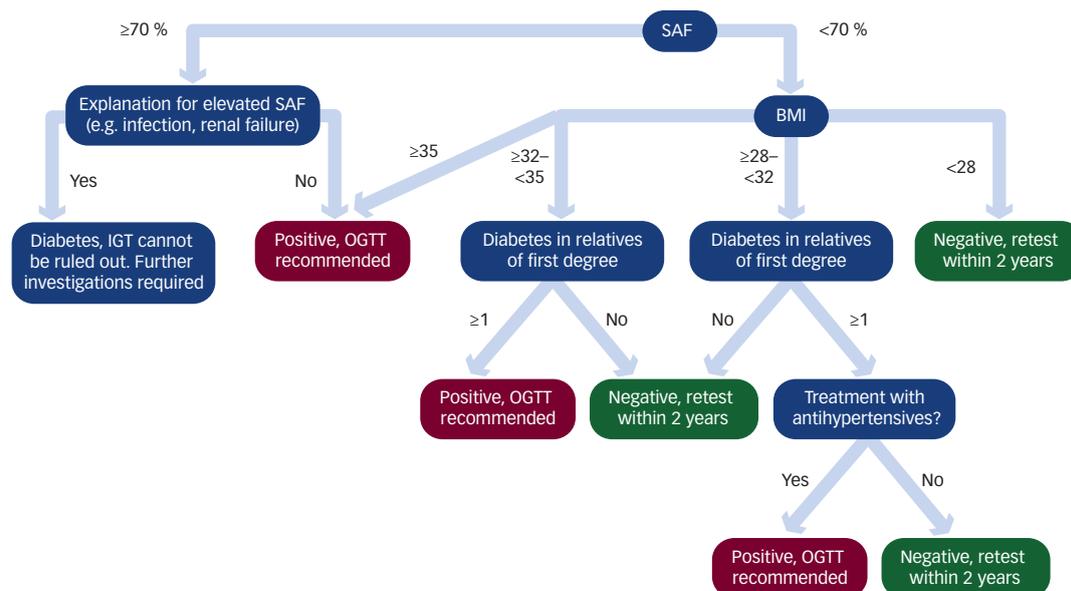
The algorithm is based on skin autofluorescence (SAF) normal ranges (in this example for people >50 years of age, the 70th age percentile was defined as the normal range; for persons <50 years of age the 80th age percentile is recommended to be used as a normal range). Adjusted according to reference 34. BMI = body mass index; IGT = impaired glucose tolerance; OGTT = oral glucose tolerance test.

for diabetes diagnosis, but have some drawbacks: high costs, time consumption or limited sensitivity.[33] Since the development of type 2 diabetes is preceded by years of subclinical metabolic disturbances potentially leading to AGEs accumulation, it is conceivable that skin AGEs might be used for diabetes screening. In a study including 218 persons with a medium diabetes risk, SAF measurement combined with an algorithm to diagnose diabetes (SAF-DM, see *Figure 2*), was superior to FPG and not inferior to HbA$_{1c}$ (sensitivity 82 %; specificity 89 %).[34] In two further studies, SAF displayed a higher sensitivity than FPG or HbA$_{1c}$ for diagnosing diabetes.[35,36] Nevertheless, the usage of SAF in the diagnosis of diabetes mellitus is highly controversial and taking into account the kidney function is paramount for an accurate interpretation of results.

Skin Autofluorescence and Diabetes Complications

In patients with T2DM (n=973) compared with 231 healthy, higher SAF in nearly all age categories was reported (see *Table 1*).[15] In patients with diabetes and microvascular and macrovascular complications, SAF was higher (mean: 3.12 AU) than in those with microvascular complications only (2.71 AU), or patients without complications (2.57 AU; p<0.001 for all). Even in patients with macrovascular complications only, SAF was higher (2.91 AU) than in those without complications. Similar results yield a study by Noordzij et al.[37]

In n=881 persons with T2DM and good metabolic control (mean HbA$_{1c}$ 6.6 %) observed on average for 3.1 years, SAF demonstrated a good predictive value (odds ratio [OR] 2.05; p>0.001) for the development of microvascular complications, in particular for neuropathy and nephropathy, but not for retinopathy.[38] The predicative value of SAF was higher than that of HbA$_{1c}$ and diabetes duration. These results were confirmed by another study in T1DM persons in whom an association of SAF with nephropathy or neuropathy was found, but not with retinopathy.[39] Sugisawa demonstrated a significant association between SAF and diabetic neuropathy and retinopathy.[31] In patients with T1DM, SAF was higher in those with peripheral (PNP) and autonomic (ANP) neuropathy (p<0.0001 for both); and each SAF increase by a standard deviation was

associated with a 2.6 times higher probability of ANP.[40] Furthermore, Meerwaldt and colleagues demonstrated that SAF increases with the severity of ANP and PNP even at subclinical stages.[41]

Skin Autofluorescence, Cardiovascular Risk and Mortality

SAF correlates with both early vascular changes (e.g. vascular stiffness [VS], intima-media thickness [IMT] and overt atherosclerosis). AGEs contribute to the development of VS (a predictor for total as well as cardiovascular mortality).[42,43] SAF correlated with the VS in healthy (especially younger) persons,[44,45] in patients with T1DM[21] as well as in persons who underwent coronary bypass surgery.[22] A correlation of SAF with the IMT of the carotid artery (an established cardiovascular risk marker[46]) was found in healthy non-smokers.[47]

SAF was elevated (2.66 AU) in subjects with carotid artery stenosis and further increased (3.28 AU) with the additional presence of peripheral artery disease (PAD).[48] Conversely, SAF was increased in people with PAD versus without PAD (2.77 versus 2.44 AU; p<0.001).[49] The adjusted OR for the presence of PAD was 2.47 per unit elevation of SAF. In 252 PAD patients, SAF was independently associated with all-cause mortality and fatal or non-fatal major adverse cardiovascular events within 5 years.[50]

In patients with T1DM, SAF was associated with the calcification of coronary arteries[51] and was higher in persons with overt coronary heart disease (CHD) (p<0.0001), correlating significantly with the CHD (OR 3.5).[13] For patients with T2DM, Lutgers et al.[52] reported that adding SAF to the United Kingdom Prospective Diabetes Study (UKPDS) risk score calculation improves risk stratification in both low- and high-risk groups.

In patients with diabetes (n=48 with type 1; n=69 with type 2) and healthy (n=43) observed over 5 years, SAF was strongly associated with CHD (OR 7.9) and mortality (OR 2.0).[53] Increased mortality in both T1DM and T2DM occurred when SAF exceeded 2.0 AU and further increased when SAF >3.0 AU. SAF was more strongly associated with CHD and mortality than HbA$_{1c}$, triglycerides or low-density lipoprotein

(LDL) cholesterol. This underlines the remarkable predicative value of SAF in patients at risk.

SAF was elevated in subjects with stable CHD compared with healthy[54] people and it seems to further increase during an acute event like ST-elevation myocardial infarction (probably due to exacerbated OS and inflammation) and decreases after the acute event (>200 days).[55] An SAF value above the median was predictive of further cardiovascular events (hazard ratio [HR] –HR 11.6), an important aspect for risk stratification.

Skin Autofluorescence and Chronic Kidney Disease

Elevated serum AGEs concentrations in persons with CKD are due to exacerbated OS, which accompanies uraemia, dyslipidaemia,[56] elevated AGEs production[57,58] and reduced AGEs renal clearance. For example, up to 30 % of exogenous AGEs are eliminated renally – this amount decreases to 5 % when CKD occurs.[59] It is therefore conceivable that patients with CKD stage 3,[60] terminal CKD or persons undergoing dialysis displayed higher SAF.[44,61] At the same time, SAF was found to be a strong and independent predictor of CKD progression.[62]

Renal transplantation reduces but does not normalise SAF, an effect dependent on the functional quality of the transplant[63] but probably also on the accompanying medication.[64] Another study in 302 renal transplant patients showed that elevated SAF was associated with a HR of 2.40 for transplant loss within 5.2 years (p<0.01).[65] Therefore, SAF was proposed to be used for the identification and monitoring of patients at risk of renal transplant dysfunction as well as of cardiovascular complications.[66]

In dialysis patients, SAF strongly predicts mortality within 3 years,[20] with a higher OR for the overall mortality than a pre-existing cardiovascular disease (OR 3.9 versus 3.1) and an OR of 6.8 for cardiovascular mortality. In line with this, Kimura et al. demonstrated in 128 patients on chronic haemodialysis observed over 6 years that SAF represents an independent predictor of cardiovascular mortality (adjusted HR 3.97).[67] Another study also found SAF to be an independent predictor of overall mortality (HR 1.83) but not of cardiovascular mortality.[68]

Summary

AGEs appear to play a major role in the development of diabetic and cardiovascular complications. Non-invasive SAF measurements allow for a quantification of the AGEs load and show a good predictive value (better than the HbA_{1c}) for the development of diabetic complications, microvascular and macrovascular disease, cardiovascular and overall mortality.

As mentioned, some limitations of SAF measurements are related to very dark skin and the influence of some external factors (creams, extreme vasoconstriction or vasodilatation, etc.) Furthermore, the validity of measurements in terms of diabetic and cardiovascular complications depends maybe not only on renal function but also on dietary factors like AGEs intake. Indeed, intake of dietary AGEs significantly increases circulating AGEs[69,70] and influences SAF in infants.[29] In adults, acute consumption of dietary AGEs with a meal inconsistently and only minimally increases SAF, therefore we discussed that it is not necessary to measure SAF in fasting state.[25,24] However, to our knowledge, evidence of effects of long-term dietary AGEs intake on SAF in adults are lacking.

Some reports suggest that SAF measurement constitutes a variable suitable for large-scale diabetes screening, but these data are controversial. Overall, further studies are warranted to strengthen the role of SAF measurements in clinical routine. ■

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