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Skin autofluorescence as tool for cardiovascular and diabetes risk prediction

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Purpose of review
Advanced glycation endproducts (AGE) have an important role in the development of chronic complications in diabetes mellitus and in renal failure. Skin autofluorescence (SAF) is a simple noninvasive optical technique to estimate AGE levels in the dermis. SAF increases with age, but rises more rapidly in diabetes and renal failure, and is also associated with, and a predictor of their complications.

Recent findings
In recent large population studies, SAF is a strong predictor of development of type 2 diabetes (T2D), and in persons with known diabetes of its complications. SAF also predicts new cardiovascular disease (CVD) and mortality not only in individuals with known type 2 diabetes but also in the general population.

Summary
SAF is a simple, powerful and independent predictor for development of type 2 diabetes (T2D), and also for cardiovascular disease and mortality in both persons with diabetes, and in the general population.

Keywords
advanced glycation endproducts, chronic kidney disease, end-stage renal disease, receptor for advanced glycation endproducts, skin autofluorescence

INTRODUCTION

Skin autofluorescence (SAF) has been developed 17 years ago as a noninvasive, optical tool to assess levels of advanced glycation endproducts (AGE) in dermal tissue [1]. SAF uses the fluorescent properties of some of the AGE with distinct excitation–emission wavelengths. AGE are formed in a multistep process by glycation and oxidation of free amino groups of proteins, lipids and nucleic acids. In addition to the classical Maillard reaction (involving glycation of proteins), AGE are also formed through the reaction of amino groups with α-dicarbonyls, such as 3-deoxyglucosone, methylglyoxal and glyoxal. Furthermore, AGE can be formed during lipid peroxidation of fatty acids in the presence of peptides or proteins. These distinct metabolic processes are complex and heterogeneous, yielding different AGE adducts, such as pentosidine, Nε-(carboxymethyl)lysine (CML), and several others.

Accumulation of AGE in long-lived tissues such as the dermis increases with calendar age but is accelerated in conditions like diabetes mellitus, renal failure and inflammatory diseases. Biochemical assays of several specific AGE (such as pentosidine, carboxymethyllysine and the others mentioned above, in blood and tissues) show increased levels in diabetes mellitus and in renal failure, and more so in long-lived tissues. Both for AGE assessed biochemically, and by use of SAF, a strong relation between high AGE/SAF levels and (development of) clinical complications in diabetes and renal failure has been reported.

TECHNIQUE OF SKIN AUTOFLUORESCENCE: SKIN AUTOFLUORESCENCE AND AGE

SAF is a fast (<10 s), noninvasive optical technique to assess the (slow) accumulation of AGE in skin tissue, using fluorescent properties of some of the AGE. The so called 'AGE Reader' performs SAF measurements by irradiating an area of less than 4 cm^2 of skin on the volar side of the forearm, guarded against ambient

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KEY POINTS

- Advanced glycation endproducts have a key role in the development of type 2 diabetes and its vascular complications.
- Skin autofluorescence is a simple, noninvasive technique for assessing levels of advanced glycation endproducts.
- A skin autofluorescence-based decision tree is effective for detection of impaired glucose tolerance and diabetes.

light, using excitation light in the near ultraviolet (UV) range (350–420 nm); it measures the amount of autofluorescence, and also the direct reflectance, in the UV and visible range coming back from the irradiated skin. The device immediately generates and presents a SAF value in arbitrary units, based on the ratio of the measured skin fluorescence and skin reflectance. The SAF value is skin type-independent; however, its use is limited to skin color Fitzpatrick type 1–4. This means that no reliable measurements can be obtained in very dark skin, or on tattoo or scar sites (the device then warns that no reliable measurement can be made).

MECHANISMS OF AGE ACCUMULATION AND PATHOGENIC EFFECTS

Several mechanisms are involved in the accumulation rate of AGE. First, endogenous AGE formation, and subsequent accumulation in long-lived tissues, is part of the natural metabolic processes in the (human) body. In healthy persons, accumulation of AGE linked to long-lived tissues like the dermis, eye lens, cartilage, vessel wall or brain will occur over decades. This slow natural accumulation of AGE, however, will be accelerated and increased when not only hyperglycaemia/diabetes is present but also during (episodes of) increased oxidative stress, for example, because of inflammatory diseases. On the other hand, an inhibiting effect on AGE accumulation is seen as a result of the detoxification of α-dicarbonyls by the glyoxalase system. Exposure to dietary AGE in highly processed food may also play a role.

AGE accumulation also strongly depends on tissue turnover, because AGE are mainly irreversibly linked to tissue proteins. Therefore, in the above-mentioned tissues with slow turnover, AGE accumulate gradually over decades. In tissues with fast turnover (e.g. epidermis, mucosa and erythrocytes), AGE accumulation is limited. Finally, renal excretion of AGE influences AGE accumulation: in chronic kidney disease (CKD) class 3–4, AGE levels are moderately to strongly increased, and even more so in end-stage renal disease (ESRD).

AGE induce damaging effects to cells and tissues in several ways, including crosslinking of extracellular matrix proteins, activation of the receptor for AGEs (RAGE) and direct glycation of intracellular proteins, lipids, and cellular damage of mitochondria and DNA. AGE-crosslinking of structural proteins in the extracellular matrix leads to reduced elasticity, stiffness of vessels and resistance to proteolytic digestion. AGE-RAGE interaction activates multiple cell-signalling pathways leading to increased production of reactive oxygen species, adhesion molecules and pro-inflammatory cytokines. Activated RAGE upregulates its own receptors, providing an increased number of binding sites for RAGE-ligands. So, sustained RAGE expression sites in proximity to their ligands will further enhance chronic inflammation, and subsequent tissue damage. Intracellular AGE binding induces structural changes modifying the function of intracellular proteins, including enzymes and regulatory proteins. Furthermore, intracellular AGEs can directly bind to electron transport chain mitochondrial proteins, leading to inhibition of oxidative ATP synthesis and increased superoxide formation by mitochondria. This may also have deleterious effects by inducing increased oxidative stress.

SAF is associated with, and a predictor of (especially) cardiovascular complications, in diabetes and renal failure patients, but also in the general population. We will briefly mention some early articles but focus on recent evidence (published 2020–2022) for SAF as independent and strong predictor of cardiovascular complications and mortality; special attention will be given on the relation between SAF and clinical events in the so-called Lifelines population study, performed in the northern area of the Netherlands since approximately 15 years.

RELATION BETWEEN SKIN AUTOFLUORESCENCE AND MICROVASCULAR AND MACROVASCULAR COMPLICATIONS IN DIABETES MELLITUS

In early studies, the predictive value of SAF was described for diabetes complications: SAF predicted microvascular and macrovascular/cardiovascular diabetic complications in diabetes type 2 [2,3], and later also in diabetes type 1 [4]. More recently Boersma *et al.* [5▪▪] confirmed that SAF predicts new cardiovascular disease (CVD) and mortality in a larger group of 2349 people with type 2 diabetes from the so-called Lifelines population cohort. SAF even showed a stronger association with future

cardiovascular events (CVEs) and mortality than cholesterol or blood pressure levels.

In an early study [6], SAF also provided additional information on CVEs to the UKPDS risk engine, with risk reclassification in a substantial number of patients with diabetes mellitus type 2. In persons with type 2 diabetes identified in the Lifelines population cohort [7], the same was found. This study will be discussed in more detail below.

SAF was reported in a recent study from China to be independently associated with CVEs in type 2 diabetes [8▪▪]. In 3806 Chinese adults with type 2 diabetes, during a median follow-up of 1.8 years, 172 participants developed a CVE. Independent associations of SAF existed with CVE (hazard ratio 1.18/SD, 95% CI 1.02–1.37), coronary heart disease (hazard ratio 1.29/SD, 95% CI 1.02–1.63), and congestive heart failure (hazard ratio 1.53/SD, 95% CI 1.14-2.05). In another article about this same cohort, SAF was associated with progression of kidney disease (source for both studies: A prospective cohort study from the Hong Kong diabetes biobank), as reported by Jin *et al.* [9▪].

Increased SAF levels predicted development of foot ulcers and amputations in a small cohort of type 1 diabetes patients [10▪]: in 2009, SAF had been measured in 206 patients with type 1 diabetes. During 10 years of follow-up, 12 patients presented with a diabetic foot ulcer (DFU), and amputations occurred in 5 persons. In the DFU patients, SAF levels were higher: 2.6 ± 0.9 vs. 2.1 ± 0.5 AU for the others (OR: 3.69; $P = 0.003$). In five patients, amputations were performed, with amputation risk also strongly related to the initial SAF: OR: 11.3 (95% CI: 1.76-79.97) after adjustment for age, gender, duration of diabetes, and HbA1c.

EVIDENCE ON THE RELATION BETWEEN AGE AND SKIN AUTOFLUORESCENCE LEVELS IN RENAL FAILURE AND SUBSEQUENT CLINICAL EVENTS

Renal failure is another common condition, which is associated with AGE accumulation (partly by decreased renal excretion of AGE adducts). Renal failure is clinically also associated with a strongly increased rate of cardiovascular complications. Initial studies on SAF in renal failure reported increased SAF levels, ranging from (moderate) renal failure (CKD III) to haemodialysis patients, who also reported that SAF is a strong and independent risk predictor for mortality in patients with renal failure [11,12]; several other later studies on SAF in patients with renal failure reported that SAF is a predictive marker for cardiovascular events [13,14], also in renal transplant patients [15].

More recently, additional studies on the use of SAF in renal failure have been reported: Shardlow *et al.* [16▪] presented a prospective study in 1707 elderly (mean age 73 years) participants with CKD 3 at baseline, and with SAF readings at baseline; 319 deaths and 590 cardiovascular events occurred during a follow-up of 6 ± 1.5 years. Higher baseline SAF was an independent risk factor for CVEs.

Interestingly, Viramontes Hörner *et al.* [17] reported in a prospective cohort study, SAF and malnutrition as important predictors of mortality in persons receiving haemodialysis. She proposes to assess effects on reducing SAF through correction of malnutrition or dietary AGE restriction in prospective studies in these patients. In older studies in haemodialysis patients, Arsov *et al.* [18] in 2014 and Crowley *et al.* [19] in 2013 found in haemodialysis patients strongly increased SAF levels; in the later study, 64% higher than in age-matched controls, with a fall in SAF levels after renal transplantation; the degree of this fall in SAF was found to be related to the reduction not only in cardiovascular risk but also in the risk of transplant dysfunction in persons having received a kidney transplant.

RECENT EVIDENCE ON THE PREDICTIVE VALUE OF SKIN AUTOFLUORESCENCE FOR CARDIOVASCULAR EVENTS AND FOR DIABETES, BOTH IN HIGH-RISK GROUPS AND IN THE GENERAL POPULATION

As for the relation between SAF and major adverse coronary events (MACE), SAF levels were reported in a recent Japanese study to be associated with the incidence of MACE in patients with heart failure undergoing cardiac rehabilitation [20▪]. This article extends the previous reports on SAF as a predictor of secondary cardiovascular events in high cardiovascular risk patients, and may allow early identification of very high cardiovascular risk candidates for intensified secondary prevention. Further support on the predictive role of SAF for cardiovascular events, resulting from the Lifelines study, will be discussed in the following section.

REPORTS ON THE USE OF SKIN AUTOFLUORESCENCE IN THE GENERAL POPULATION IN THE DUTCH LIFELINES STUDY

Over the last few years, several articles have been published on studies using SAF in the so-called Lifelines cohort. Lifelines is a large, multidisciplinary, prospective, population-based cohort study that includes over 167 000 participants (10% from the

population of the northern Netherlands. Between 2007 and 2013, 72 880 participants of the Dutch Lifelines Cohort Study, who underwent baseline investigations between 2007 and 2013, had validated baseline SAF values available, and were not known to have diabetes or CVD (at baseline). In an early study in the Lifelines cohort, van Waateringe *et al.* assessed in 2016, the association of clinical and lifestyle parameters with SAF, as well as their interactions in a nondiabetic population ($n = 8695$), and performed the same analysis in a type 2 diabetic subgroup ($n = 314$). Mean SAF was 2.04 ± 0.44 AU in nondiabetic individuals, and 2.44 ± 0.55 AU in type 2 diabetic patients ($P < 0.0001$). Multivariate backward regression analysis showed that in the nondiabetic population, SAF was significantly and independently (from other associations) associated with age, BMI, HbA1c, creatinine clearance, a specific genetic polymorphism, current smoking, pack-years of smoking and coffee consumption. In the participants with known type 2 diabetes in Lifelines, a similar set of factors was associated with SAF, except for coffee consumption.

In 2017, Fokkens *et al.* [21] and later van Waateringe *et al.* [7] reported in the Lifelines study that SAF improves the Finnish Diabetes Risk Score in the detection of diabetes: after the exclusion of participants with previously diagnosed diabetes, pregnant women and those using corticosteroids, 79 248 patients were eligible: diabetes was detected in 1042 participants. Skin autofluorescence improved the area under the receiver-operating characteristic (AUROC) curve of the FINDRISC model. More significantly, the addition of SAF to FINDRISC reclassified 8–15% of all participants into more accurate risk categories (NRI: 0.080, 95% CI: 0.052–0.110). The proportion of reclassified participants was especially high (>30%) in the intermediate (1 to <5%, and 5 to <10%) risk categories.

After a median follow-up time of 4 years in 72 880 participants in the Lifelines cohort with a SAF measured at baseline, and who were not known to have diabetes or CVD, van Waateringe *et al.* [7] examined in 2019 whether SAF can predict the 4-year risk of incident type 2 diabetes, CVD and mortality in the general population. A clear predictive effect of SAF for all these end points in the general population was found [7]. In 1056 of these participants (1.4%), type 2 diabetes had developed, 1258 individuals (1.7%) were diagnosed with CVD, whereas 928 (1.3%) had died (predictive effect of SAF for all three end points $P < 0.001$). The authors concluded that SAF is of clinical value for screening for future risk of type 2 diabetes (T2D), CVD and mortality, independent of glycaemic measures and the metabolic syndrome.

Recently, with a longer follow-up, Boersma *et al.* [5▪▪] reported again on the predictive value of SAF in the Lifelines cohort, in 2349 people with T2D, and baseline SAF measurements available, and a median follow-up of 3.7 years. They concluded that SAF predicts new CVD and mortality in individuals with known type 2 diabetes [5▪▪]. Importantly, SAF showed a stronger association with future CVD events and mortality than cholesterol or blood pressure levels.

AGE AND SKIN AUTOFLUORESCENCE LEVELS IN DIABETES SCREENING

Although type 1 diabetes mellitus usually becomes clinically evident soon, the onset of type 2 diabetes mellitus may not become clinically manifest for prolonged periods, often years. As discussed in the paragraphs above, strong evidence is now available that SAF is able to identify persons with increased risk of diabetes. The risk of developing vascular complications in this 'silent period' is already increased. Type 2 diabetes mellitus is a prevalent disease, involving approximately 500 million people worldwide. Detection of diabetes now still involves glycaemic tests [plasma glucose, HbA1c/glycated haemoglobin or even oral glucose tolerance tests (OGTT)], which are used for case finding (e.g. in symptomatic patients, or those presenting with CVD). Not only OGTT but also HbA1c tests are actually little used as a screening tool.

SAF has been proposed to be useful as a test for diabetes screening, based on earlier (2013) studies, at that time in smaller groups of patients at risk for diabetes. Newer studies using SAF now show that SAF is comparable or superior to HbA1c and fasting plasma glucose for detection of impaired glucose tolerance and diabetes as detected by the oral glucose tolerance test (OGTT). Superiority of a SAF-based decision tree for detection of impaired glucose tolerance and diabetes has been confirmed in the earlier mentioned large population study (Lifelines) [7,21]. So, these results indeed support the use of SAF as a first-line choice for (pre)diabetes screening.

LIMITATIONS OF THE ADVANCED GLYCATION ENDPRODUCT READER

The SAF value is skin type-independent; however, its use is limited to skin colour Fitzpatrick type 1–4. This means that no reliable measurements can be obtained in persons with very dark skin types, or on sites of tattoos or scars or on skin sites where sun blockers have been recently (<24 to 48 h) used (the device then warns that no reliable measurement can be made).

CONCLUSION

Skin autofluorescence, as a simple noninvasive optical tool for the assessment of AGE, is a valuable tool to identify persons with, or at high risk for developing diabetes, and especially for identifying those at high risk for vascular complications.

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Conflicts of interest

A.J.S. is founder and shareholder of Diagnoptics Technologies (Groningen, The Netherlands), the company, which developed and markets the AGE reader, the device which uses skin autofluorescence. The other authors have no conflict of interest.

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