Skin Autofluorescence, 5-Year Mortality, and Cardiovascular Events in Peripheral Arterial Disease

All That Glitters Is Surely Not Gold

Ann Marie Schmidt

The hunt for predictive biomarkers in diseases of the cardio-, cerebro-, and peripheral vascular systems is an ongoing effort and one whose importance cannot be overstated. The identification of subjects most at risk for death or major adverse cardiovascular events may provide a means to stratify diagnostic and therapeutic interventions based on validated predictive algorithms. In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, de Vos et al report that in 252 eligible subjects with documented peripheral arterial disease (PAD) studied at a single center, the measurement of skin autofluorescence (SAF) was independently associated with all-cause mortality and fatal or nonfatal major adverse cardiovascular events after a follow-up period of 5 years. Importantly, the authors excluded subjects with hemodialysis, kidney transplantation, recent myocardial infarction, or recent stroke and report that even after adjustment for cardiovascular risk factors and the use of lipid-lowering drugs, increased SAF remained associated with increased risk for death and major adverse cardiovascular events. A previous report from these authors compared subjects with PAD versus control PAD-free subjects and showed that increased SAF was significantly associated with PAD in a manner independent of traditional cardiovascular risk factors, although such risk factors were associated with further increases in SAF.

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The AGE reader was used as a biological marker of advanced glycation endproducts (AGEs) in the skin and is usually tested in the forearm. AGEs are the products of nonenzymatic glycation and oxidation of proteins and lipids. AGEs are increased not only in diabetes mellitus but also in natural aging, oxidative stress, and inflammatory conditions, and in renal failure. AGEs form on lysine and arginine residues and are heterogeneous; some AGEs are associated with crosslinking (such as pentosidine), and some AGEs do not fluorose, such as the carboxy methyl lysine AGEs. Based on defined fluorescent wavelengths of excitation and emission, SAF is reflective of skin levels of AGEs. Although few studies to date have compared directly the measures of SAF with the skin levels of AGEs, Meerwaldt et al measured specific skin AGEs in diabetic and nondiabetic subjects in the same arm as that used to measure the SAF. They reported that SAF correlated with measured levels of collagen-linked fluorescence, pentosidine, carboxy methyl lysine, and carboxy ethyl lysine AGEs. In a second study, this same group examined specific AGEs in subjects with end-stage renal disease and reported that SAF correlated with measured levels of collagen-linked fluorescence, pentosidine, carboxy ethyl lysine, and carboxy methyl lysine AGEs in the skin. Hence, in these 2 reports, evidence suggests that SAF did correlate with measured levels of a variety of crosslinked and non-crosslinked AGEs in the skin. The broader question is, of course, to what extent SAF measures in the skin forearm correlate with AGE levels at the site of vascular pathology. Although the present study by de Vos et al measured SAF and the authors proposed links to the pathogenesis of PAD, they did not directly measure the vascular AGE content. Hofmann et al sought to address this question experimentally. In their study, they related SAF to AGE intrinsic fluorescence measured in collagenase-digestible collagen fraction retrieved from discarded excess vein tissue explanted for bypass graft material. The authors reported that SAF and pulse wave velocity (a measure of vessel stiffness) both correlated with AGE measurements in the collagenase-digestible collagen fraction.

A major question is to what degree SAF, as a surrogate measure for AGE content or, perhaps, yet-to-be-identified specific mediators of vascular damage, reflects underlying vascular perturbation. Although de Vos et al do not assess measures beyond traditional risk factors, other studies have suggested that SAF correlates with such factors as high-sensitivity C-reactive protein (in subjects with hemodialysis), impaired high-density lipoprotein antioxidative capacity, and endothelial dysfunction. Beyond their ability to cause vascular damage by virtue of basement membrane thickening, vascular leakiness, and other pathologies, AGEs interact with specific cellular receptors, the best characterized of these is the receptor for AGE (RAGE). The extracellular domain of RAGE, composed of 1 V-type and 2 C-type immunoglobulin domains, acts as a ligand decoy in cultured cells exposed to RAGE ligands and in vivo in diabetic or aged animals. In human subjects, accruing evidence suggests that circulating soluble RAGEs (sRAGEs) correlate with the degree of diabetic or inflammatory pathologies, although studies differ, intriguingly, on whether high or low sRAGE levels reflect protection against or vulnerability to complications and tissue stress. Skrha et al measured SAF in subjects with type 1 or type 2 diabetes mellitus versus control subjects and reported that SAF levels were higher in the diabetic

From the Diabetes Research Program, New York University Langone Medical Center, New York, NY.
Correspondence to Ann Marie Schmidt, MD, Diabetes Research Program, Department of Medicine, NYU Langone Medical Center, 550 First Ave, Smilow 901C, New York, NY 10016. E-mail annmarie.schmidt@nyumc.org

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groups versus controls and correlated positively with levels of sRAGE. Significantly higher SAF also correlated with indices of endothelial dysfunction such as levels of von Willebrand factor, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 in that study. Levels of sRAGE were not measured in the study by de Vos et al, but others measured sRAGE in human subjects with coronary artery disease and PAD and found lower levels of sRAGE in subjects with both sites of disease. Clearly, the relationships between ligand, sRAGE levels, and affected vascular beds are complex and require further study to determine whether SAF combined with measures such as sRAGEs or distinct markers of oxidative or endothelial stress may be a superior biomarker panel to add predictive value for long-term outcomes. Certainly, one solution to the quandary may be to measure multiple putative biomarkers in all study subjects, where feasible.

Studies reporting SAF may be complicated by reports suggesting that, not surprisingly, SAF and, by inference, AGES accumulate in diverse disorders and, at least in some cases, correlate with the extent of disease or its complications. Extensive evidence links elevated SAF not only to end-stage renal disease and mortality and the complications of types 1 and 2 diabetes mellitus but also to such disorders as renal disease and mortality, and the complications of types 1 and 2 diabetes mellitus, but also to such disorders as renal disease and mortality, and the complications of types 1 and 2 diabetes mellitus. These considerations reflect 2 key points: first, that SAF increases and AGES accumulate in metabolic, inflammatory, and oxidative stresses; and second, that studies reporting SAF must take care to exclude subjects displaying advanced stages of these disorders. In this context, de Vos et al excluded patients with hemodialysis or kidney transplantation.

Despite the promise of SAF as a biomarker for AGE deposition, there are caveats, some of which were noted by de Vos et al. It is known that in subjects with dark skin pigmentation, SAF may not be reliable. It is possible that intense vasodilation, vasoconstriction, certain skin creams used to induce skin browning, or high AGE meals might affect SAF, even if just temporarily. However, SAF is painless and is said not to be affected by short-term glycemic variations. Although it must be acknowledged that AGES may be surrogate markers for yet-to-be-identified pathogenic mediators being measured by SAF, this is not highly likely. Hence, although there are some caveats to measurements of SAF, careful study design and patient inclusion and exclusion criteria should aid in improving the predictive value of this marker.

Finally, it is intriguing to consider the fate of SAF after putative therapeutic interventions. In one study in subjects with heart failure, the AGE crosslink breaker alagebrium was administered for 36 weeks twice daily. In parallel with no changes in cardiac function or American Heart Association heart failure score in treated subjects, no changes in SAF were noted post-therapy. Issues such as dose, schedule, and, in the context of SAF, baseline AGE burden and character might have confounded the results. These findings underscore that future studies should focus on identifying the specific AGES that mediate tissue damage to develop targeted therapies. In that context, it would then be critical to determine whether/to what extent SAF might track the accumulation of disease-mediating specific AGES and whether and how they parallel vascular AGE burden.

**Figure.** Skin autofluorescence (SAF) mirrors advanced glycation endproduct (AGE) burden and the state of vascular dysfunction. Beyond hyperglycemia, oxidative and inflammatory stresses are independently linked to the generation of AGES. AGES production occurs in diverse disorders such as end-stage renal disease (ESRD), complicated by certain dialysis fluids, aging, chronic obstructive pulmonary disease (COPD), congestive heart failure, pre-eclampsia in pregnancy, cirrhosis of the liver, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and age-related macular degeneration, for example (see the Figure). These considerations reflect 2 key points: first, that SAF increases and AGES accumulate in metabolic, inflammatory, and oxidative stresses; and second, that studies reporting SAF must take care to exclude subjects displaying advanced stages of these disorders. In this context, de Vos et al excluded patients with hemodialysis or kidney transplantation.

**Figure.** Skin autofluorescence (SAF) mirrors advanced glycation endproduct (AGE) burden and the state of vascular dysfunction. Beyond hyperglycemia, oxidative and inflammatory stresses are independently linked to the generation of AGES. AGES production occurs in diverse disorders such as end-stage renal disease (ESRD), complicated by certain dialysis fluids, aging, chronic obstructive pulmonary disease (COPD), congestive heart failure, pre-eclampsia in pregnancy, cirrhosis of the liver, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and age-related macular degeneration, for example. AGES cause damage by multiple mechanisms such as vascular stiffness, vascular leakiness, and via receptor engagement. At least certain AGE formation is irreversible, and therefore AGES are linked to chronic tissue damage, organ dysfunction, and, as suggested by de Vos et al, mortality in peripheral arterial disease (PAD). Others linked SAF to mortality in ESRD. The study by de Vos et al suggests that simple and painless measures of the AGE burden, through the SAF surrogate, may predict long-term (5-year) mortality in subjects with PAD, even after correcting for usual cardiovascular risk factors. Studies are essential to correlate SAF with vascular AGE burden, receptor (RAGE) engagement, and the mechanisms of chronic vascular stress to bring full circle the AGE hypothesis to disease mechanism and to discover novel treatments for which measures of SAF may be a bona fide target engagement biomarker.
In conclusion, in the study by de Vos et al, meticulous attention to patient entry/exclusion criteria and the long-term follow-up of nearly all of the initial study entrants provided a formidable window into the association between SAF, AGE burden, and mortality and major adverse cardiovascular events in PAD and suggest that further research is needed to establish SAF and AGE content as a mechanism and validated biomarker in this disorder. Indeed, the work of de Vos et al supports that SAF highlights the pathogenic importance of glycation, oxidative and inflammatory stresses as these processes come of AGE as mechanisms of a diverse array of chronic diseases.

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References

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