Oxidative Stress in Cardiovascular Disease
Successful Translation From Bench to Bedside?

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Over the last decades, sound evidence has been generated that oxidative stress is one of the most potent inducers of endothelial dysfunction and is involved at all stages of atherosclerotic plaque evolution.1,2 Experimental and animal models provide a clear association between the amount of oxidative challenge and reversible vascular dysfunction that can be observed before permanent alterations of the vessel wall occur.

Important for cardiovascular biology is the consumption of nitric oxide (NO) by reactive oxygen species. Endothelium relaxant factor is a central molecule in vascular homeostasis as a modulator of endothelial tone and reactivity.3 It is produced by NO synthases and exerts pleiotropic positive effects on the cardiovascular system.4 Oxidative modification of NO not only leads to reduced bioavailability but also produces the toxic oxidant peroxynitrite, which further aggravates the imbalance of protective and aggressive factors. Because oxidative stress centrally contributes to atherothrombosis, sustained efforts have been undertaken to translate this knowledge into the characterization and identification of biomarkers that enable detection of oxidative stress and allow improved risk stratification by integration into cardiovascular risk stratification models.

Biomarkers of Oxidative Stress
Protagonists of oxidative stress have in common that they are highly active, short-lived agents that almost immediately react with surrounding molecules at the site of formation. However, oxidative species leave a detectable trace of modified oxidative products as is known for oxidized low-density lipoprotein at the site of atherosclerotic lesions. Among a multitude of oxidants that can be measured in vitro and in animal models, only comparatively few biomarkers have entered epidemiological investigations like homocysteine, nitrosated tyrosines, and the relatively unstable F2-isoprostanes, which have been related to endothelial function or cardiovascular outcome in cross-sectional and prospective investigations. Recently, myeloperoxidase, an endogenous generator of oxidants that is beneficial in host defense but detrimental for the vascular wall under long-term exposure, has joined the list of proposed biomarkers for oxidative stress.

As an alternative approach, investigators have tried to assess the capacity of antioxidative defense. Concentrations of glutathione, especially the ratio to oxidized glutathione, have been suggested as indicators of oxidative imbalance. In this pathway, erythrocyte glutathione-peroxidase-1 has been shown to be predictive of future cardiovascular events in patients with manifest coronary artery disease.5 The value of these biomarkers has been assessed mostly in cross-sectional or small prospective studies. Clearly, information about their predictive ability has to be achieved across population-based cohorts and different clinical settings. It could be demonstrated repeatedly that for risk indicators that performed well in initial studies, the overall gain of predictive information was rather modest, not meriting inclusion in general risk prediction models.6 In addition, the relevance of targeted intervention remains unclear.

Asymmetric Dimethylarginine
Asymmetric dimethylarginine (ADMA) is the product of endogenous L-arginine residue methylation of proteins. Because of its similarity of structure with L-arginine, the natural precursor for NO formation, it may act as a competitive inhibitor of endothelial NO synthase and thus reduce NO generation. It further interferes with the biological effects of NO and may finally lead to uncoupling of endothelial NO synthase. The reasons for elevated ADMA concentrations are not well established. ADMA concentrations are higher in patients with renal insufficiency and liver failure as a result of impaired metabolism and excretion. The enzyme dimethylarginine dimethylaminohydrolase (DDAH) is responsible for the degradation of ADMA. Interestingly, it is oxidant sensitive at its catalytic site, and conditions that lead to oxidative challenge like homocysteine or oxidative low-density lipoprotein application are related to elevated ADMA concentrations. On the other hand, antioxidant interventions restore DDAH activity, reduce ADMA overload, and normalize NO synthesis.

Therefore, it seems as if a variety of cardiovascular risk factors and disease conditions found to increase ADMA concentrations act by increasing oxidative stress. The presence of superoxide anions impairs the catalytic activity of DDAH, resulting in higher ADMA concentrations. ADMA might therefore be an indirect indicator of oxidative burden because it is more stable with a longer half-life and can be measured in peripheral blood.

The potential relevance of ADMA in the cardiovascular system was first described in the clinical setting in 1992.

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Intra-arterial infusion of ADMA significantly reduced forearm blood flow.7 In addition, elevated concentrations of ADMA inhibit forearm blood flow response to acetylcholine and vascular relaxation tested by flow-mediated dilation (FMD).8 In prospective analyses, small and moderate-size clinical studies could relatively uniformly demonstrate an association with adverse cardiovascular outcome, although the risks seem to be inflated in smaller studies.9–11 Large-scale studies are needed to better define the potential impact of ADMA in a clinical setting. In this respect, the article by Jou­nala et al12 in this issue of Circulation provides valuable additional information. Using a sufficiently large data set, the authors first investigated the distribution of ADMA concentrations among clinical variables in a young and healthy population. Although ADMA depends on a variety of clinical phenotypes like daily smoking, the inverse association with endothelial function assessed by FMD remained independently significant. This result adds to current knowledge in that ADMA concentration might be considered a risk factor even in the young. Nevertheless, the critical aspect is that the overall correlation between ADMA and FMD remains very modest although significant after inclusion of most potential confounders. Furthermore, the present cross-sectional results need to be proved in large-scale population-based prospective studies to elucidate whether determination of ADMA adds information beyond that obtained from simple contemporary risk factor models. To allow broader application and comparisons, measurement issues have to be solved. Determination of ADMA has been performed by laborious and costly mass spectrometry as the gold standard, by high-pressure liquid chromatography as in the present study, and by ELISA. None of the methods is applicable for clinical routine testing so far.

**Noninvasive Endothelial Function Testing**

FMD measured in peripheral arteries13 has been used for more than a decade as a surrogate parameter of coronary endothelial function. It makes use of not-yet-completely understood mechanisms induced by peripheral arterial occlusion, including ischemia and hyperemic blood flow, which results in elevated shear stress activating endothelial NO synthase. This activation results in a measurable increase in vascular diameter in a healthy vessel, depending on the quality of current resolution of ultrasound techniques. Commercially available software packages allow the online evaluation of FMD. In a second step, most studies complemented FMD by assessing endothelium-independent vascular dilation through the administration of sublingual nitroglycerin medication that revealed the maximum capacity of vascular relaxation.

The impact of FMD measurement on outcome prediction is controversial. Some clinical trials have successfully applied FMD and revealed significant relations to therapeutic cardiovascular interventions. In addition, mostly smaller studies suggested an association between decreased FMD and cardiovascular outcome in secondary prevention, whereas no significant additional risk information for cardiovascular events was provided by FMD measurement in an elderly population-based cohort.14 Thus, the association with cardiovascular end points, especially in initially healthy subjects, has yet to be established. Of more practical concern, the correlation with invasively measured vascular response is only modest. Joint efforts have been undertaken to standardize noninvasive endothelial function measurement by FMD.15 Although a relatively high reproducibility can be achieved when the same subjects are scanned at short time intervals when environmental conditions are stable, multiple factors directly influence FMD under real-life conditions. Inaccuracies in FMD determination may be caused by diurnally differing responses of vascular reactivity and a large number of environmental and individual factors like temperature, season, ethnicity, food intake, menstrual cycle, sympathetic stimuli, and drugs, which are major drawbacks of the currently used method. These confounders, as far as they are known, can be controlled for under study conditions but are difficult to assess for their relevance in single measurements in clinical practice and limit the universal application of FMD measurement. In addition, no single method of FMD measurement has emerged that has led to comparability between centers. Results vary significantly only if the measurement site on the upper extremity is taken into account. Upper arm occlusion compared with forearm occlusion provides a more pronounced hyperemic response with the disadvantage of a more difficult transducer adjustment. Considering the above-mentioned facts, the still-missing definition of normal values, and the relevance of a point measurement for individual risk prediction, the usefulness for a broad application in clinical routine, apart from highly standardized study conditions, needs verification.

Although it has not been demonstrated that effects on endothelial function translate into outcome,16 FMD measurement has successfully been applied to prove the impact of various risk factors and vasoactive substances on endothelial performance for which this method serves as a valid surrogate end point.17 As noted above and as reported for the first time in a large population-based cohort in the article by Jounala and coworkers, circulating ADMA concentrations tend to be negatively correlated with endothelial function. The advantage of a competitive enzyme inhibition is the ability to outweigh the competitive inhibitor by increasing the concentration of the main substrate. Thus, the local or systemic application of l-arginine, the primary substrate of endothelial NO synthase, was shown to improve endothelial function and to normalize vascular reactivity.18 Whether l-arginine might lead to the prevention of cardiovascular events has to be further elucidated.

In conclusion, the assessment of oxidative stress by valid biomarkers or vascular function testing might emerge as an attractive approach to assess an individual’s response to oxidative challenges. The short-lived impact of acute oxidative stress, rapid biological changes in oxidative status, sophisticated measurement methods, unsolved preanalytic issues, and lack of specificity are among the factors that have so far prevented the establishment of a well-accepted single marker or distinct marker panel to measure oxidative stress that fulfill stringent validity criteria.19 The hope of finding surrogate markers of coronary endothelial function and cardiovascular disease burden has spurred further investigations into peripheral arteries, and promising other noninvasive and
simpler measurements of vascular reactivity addressing slightly different aspects of vasomotor function and arterial stiffness are under investigation.\textsuperscript{20,21} The ability to improve cardiovascular risk scores beyond traditional risk factors has to be proved in prospective studies for both oxidative stress biomarkers and new diagnostic tools. Further evidence is needed to establish the role of endothelial function testing and oxidative biomarkers in individuals to identify new targets for intervention and to guide medical decision making. With currently available tools, we cannot yet provide a reliable answer for oxidative status and its preventive and therapeutic impact. Until now, the translation to bedside has not been successful.

**Disclosures**

None.

**References**


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