Clinical update

Redox biomarkers in cardiovascular medicine

Keyvan Karimi Galougahi1,2, Charalambos Antoniades3, Stephen J. Nicholls4,5, Keith M. Channon3, and Gemma A. Figtree1,2*

1Oxidative Signalling Group, Department of Cardiology, Kolling Institute, University of Sydney, Royal North Shore Hospital, St Leonards, NSW 2065, Australia; 2Department of Cardiology, Royal North Shore Hospital, Sydney, Australia; 3Cardiovascular Medicine, University of Oxford, Oxford, UK; 4South Australian Health and Medical Research Institute, University of Adelaide, Adelaide, Australia; and 5Department of Cardiology, Royal Adelaide Hospital, Adelaide, Australia

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The central role of oxidative signalling in cardiovascular pathophysiology positions biometric measures of redox state as excellent markers for research and clinical application. However, despite this tantalizing biological plausibility, no redox biomarker is currently in widespread clinical use. Major recent insights into the mechanistic complexities of redox signalling may yet provide the opportunity to identify markers that most closely reflect the underlying pathobiology. Such redox biomarkers may, in principle, quantify the integrated effects of various known and unknown pathophysiological drivers of cardiovascular disease processes. Recent advances with the greatest potential include assays measuring post-translational oxidative modifications that have significant cellular effects. However, analytical issues, including the relative instability of redox-modified products, remain a major technical obstacle. Appreciation of these challenges may facilitate future development of user-friendly markers with prognostic value in addition to traditional risk factors, and which could be used to guide personalized cardiovascular therapies. We review both established and recently identified biomarkers of redox signalling, and provide a realistic discussion of the many challenges that remain if they are to be incorporated into clinical practice. Despite the current lack of redox biomarkers in clinical application, the integral role of reactive oxygen species in pathogenesis of cardiovascular disease provides a strong incentive for continued efforts.

Keywords
Redox signalling • Biomarker • Cardiovascular disease • Reactive oxygen species • Caveolae

Introduction

The impact of cardiovascular disease is well recognized. In most cases, whether it is atherosclerosis resulting in stroke and heart attack, or myocardial pathology with subsequent heart failure, the driving pathophysiological factors have been present and acting for years. Dysregulated oxidative signalling serves, along with neurohormonal abnormalities and inflammation, as one of the common drivers of disease progression. Detection of the pathobiological processes with an aim to target preventative strategies prior to irreversible organ damage is a major unaccomplished goal. Redox biomarkers have great promise to assist in this quest.

The role of reactive oxygen species and redox signalling in cardiovascular disease

Reactive oxygen species (ROS) are generated during regulated physiological processes, and play a critical function as signalling molecules in control of cell homeostasis. However, the dysregulated generation of ROS contributes to the pathogenesis of cardiovascular disease. Superoxide anion (O2−), and the product of its dismutation, hydrogen peroxide (H2O2), and hydroxyl (·HO) constitute the ROS (as shown in Supplementary material online, Figure S1). In addition to quenching nitric oxide (NO), ROS directly impair the function of proteins, mostly via oxidative post-translational modifications. This results in changes in many cellular processes such as dysregulation of membrane transport, altered Ca2+ handling, increased cell proliferation, and accelerated fibrosis and atherosclerosis.1–6 Reactive nitrogen species (RNS), specifically NO and peroxynitrite (ONOO−), which is derived from the reaction between NO and O2−, also participate in redox signalling. Reactive oxygen species and RNS are generated by a variety of cellular sources during the evolution of both vascular and myocardial disease (Supplementary material online, Figure S1). These include dysfunctional mitochondrial electron transport chain, xanthine oxidoreductase (XOR) involved in metabolism, as well as nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX) and ‘uncoupled’ endothelial nitric oxide synthase (eNOS).

* Corresponding author. Tel: +61 2 99264915, Fax: +61 2 99266521, Email: gemma.figtree@sydney.edu.au

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Reactive oxygen species generation and interaction with other signalling molecules is shown to occur in a compartmentalized manner.\(^2,7\) The caveolae are membrane invaginations that provide the structural basis for membrane ‘signalling microdomains’, including facilitating the interaction of ROS with redox-regulated proteins.\(^8\) A prime example is angiotensin II (Ang II) receptor-coupled signalling.\(^9\) Abnormal activation of the renin–angiotensin system is central in the pathogenesis of a multitude of cardiovascular disease states. In endothelial cells, Ang II causes NADPH oxidase activation, and NADPH oxidase-derived ROS initiate a maladaptive ‘feed-forward’ loop, where ROS generation is amplified by the resulting uncoupling of eNOS, mediated by its S-glutathionylation, a reversible modification of reactive cysteine residues.\(^3\) This molecular cascade, akin to ‘kindling of a bonfire’\(^10,11\), can result in modification of redox-sensitive caveolar proteins (e.g. the Na\(^+\)–K\(^+\) ATPase). Providing accurate quantitative insight into these sub-cellular compartmentalized molecular processes by biomarkers detected in circulation is essential for their validity, and presents a major analytical challenge (Figure 1). This conundrum is important to consider in the search for the novel redox biomarkers.

**Measurement of redox state and oxidative modifications**

As well as functioning as a putative ‘integrator’ of the cellular effects downstream of many known, and likely unknown, cardiovascular risk factors (as illustrated schematically in Figure 2), redox biomarkers may also provide an estimate of effects of pathophysiological processes that are otherwise difficult to accurately quantitate, e.g. neurohormonal activation in heart failure. The very short half-life of ROS makes their quantification, especially at the most relevant sites of generation, technically challenging. Other measures of cellular redox homeostasis such as the ratio of reduced to oxidized glutathione in the cytoplasm (GSH/GSSG), e.g. in red blood cells, used as a surrogate for endothelial cells or cardiac myocytes, may not fully reflect membrane redox signalling processes. A popular compromise has been the measurement of stable by-products, modified under conditions associated with elevated ROS, which have been released into the circulation.

Practically, redox biomarkers can be classified according to whether they reflect (i) sources of ROS, (ii) ROS levels, (iii) molecules that are modified by interactions with ROS, and (iv) molecules produced by cells in response to ROS, e.g. antioxidant enzymes, or as by-products (e.g. uric acid). The potential utility of these markers for clinical or research application depends on the ease of obtaining and preparing biological specimens, their stability, and the simplicity and reproducibility of the assay. Moreover, in contrast to a biomarker like troponin, the specificity of which for myocardial necrosis is *sine qua non* for its utility, redox biomarkers are not required to have specificity for individual disease processes. Instead, the precision and accuracy with which the assays reflect the redox homeostasis in critical cellular signalosomes should be sought. Combining markers

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**Figure 1** Compartmentalized redox signalling and their relationship to circulating biomarkers. The biomarkers measured in the blood are shown in boxes. The levels of oxidation products, cellular antioxidant enzymes, and sources of reactive oxygen species measured in circulation depend on the kinetics of release, retention and clearance, thus may not fully reflect redox homeostasis at the level of cells and tissues, in particular in membrane signalling microdomains (caveolae, inset). As an example of a strategy to circumvent such issues, neurohormone-mediated S-glutathionylation of Na\(^+\)–K\(^+\) pump in red blood cells is shown. Based on our preliminary data, these pathways reflect the redox signalling in cardiac myocytes, with the levels of pump S-glutathionylation closely paralleling the levels in myocytes in heart failure. MPO, myeloperoxidase; SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; OxLDL, oxidized low-density lipoprotein; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; sNOX2-dp, soluble NOX2-derived peptide.
specific for individual disease processes with the less specific but pathophysiological relevant redox biomarkers can provide more disease-specific diagnostic and prognostic information.

Discovery strategies for redox biomarkers have traditionally been candidate based. With the advent of non-biased ‘biomics’ (e.g. genomics, proteomics, transcriptomics, and metabolomics), new molecules reflective of redox state are much more likely to be identified a priori, providing a unique opportunity for development of novel biomarkers. The currently used redox biomarkers and their developmental milestones en route to clinical utility are discussed below and summarized in Figure 3. Further detail is provided in Supplementary material online, Table S1 and Figure S2.

**Sources of reactive oxygen species**

Nicotinamide adenine dinucleotide phosphate oxidases are a family of pro-oxidant enzymes, which are mainly defined by their membrane-bound NOX subunit into four main subtypes in cardiovascular system. NOX1 forms an active complex with p22phox, p47phox, Noxa1, and Rac1 subunits, mainly in the vascular smooth muscle cells (VSMCs), and is a major source of O2•− in the vascular wall. NOX2 forms a complex with p22phox, p47phox, p67phox, p40phox, and Rac1/2, and is present in endothelial cells, VSMCs, and cardiomyocytes. NOX2 acts as a master regulator of cardiovascular redox state.12 NOX5 is a calcium-dependent isoform of NADPH oxidase in the endothelium, while NOX4 selectively increases OH−, important signalling molecule with a possibly protective role.12

Of special interest in cardiovascular biology is the enzyme eNOS, which is normally a source of NO in the endothelium, a molecule with antioxidant, anti-inflammatory, and anti-atherogenic properties. However, under conditions of elevated ROS, eNOS co-factor tetrahydrobiopterin (BH4) is oxidized and that results in enzymatic uncoupling of eNOS, to generate O2•− instead of NO.13 Although estimating the ratio of BH4 to its oxidized forms (dihydrobiopterin and biopterin) could serve as a marker of systemic redox state, the complex regulation of BH4 synthesis (that is induced by inflammation14) limits its value as a biomarker. This is also explained by the fact that despite the inability of oral BH4 administration to improve vascular redox state,15 indirect strategies that restore vascular BH4 bioavailability (e.g. using folates16 or statins) have major impacts on vascular oxidative signalling. This could either mean that vascular BH4 (and its oxidation status) is an excellent biomarker reflecting the overall vascular redox state, and/or that it is critically involved in regulation of the vascular redox state.

Although NADPH oxidase and uncoupled eNOS play a key role in ROS generation, they are localized in the vessel wall and myocardium, making quantitation of their expression impractical. A recently developed surrogate, soluble NOX2-derived peptide (sNOX2-dp) that is released from circulatory cells, and potentially other cell types, into the serum has been shown to correlate well with NOX activity in cardiovascular system. sNOX2-dp has been used as a measure of NOX activity in a number of disease states,17,18 including microvascular obstruction post primary percutaneous intervention for myocardial infarction.17,19
**Figure 3** Biomarkers and their respective developmental milestones on their path from discovery to clinical application. The progress of each biomarker is represented by the relative location of the schematic runners. The strength of the current evidence for each biomarker in cardiovascular disease is discussed in the adjacent text boxes. Despite great progress in redox biology field, no biomarker is currently in widespread clinical use.
Myeloperoxidase (MPO), a haem enzyme that is abundant in granules of human inflammatory cells, is an important source of ROS that directly participates in the pathophysiology of atherogenesis, and its circulatory levels are shown to be of clinical relevance. Myeloperoxidase catalyses the conversion of \( \text{H}_2\text{O}_2 \) to reactive species including \( \text{HO}^\cdot, \text{ONOO}^- \), hypochlorous acid, and \( \text{NO}_2^- \). The MPO-derived ROS cause endothelial dysfunction, oxidize LDL, modify HDL to impair its function in cholesterol efflux, and thus promote atherosclerosis. \(^{20}\) Myeloperoxidase concentration can be quantified in biological samples using commercially available enzyme-linked immunosorbent assay (ELISA) plates, with reliability dependent on sample collection and handling. \(^{21}\) Alternatively, MPO function can be measured by spectrophotometric-based peroxidase activity assays such as those detecting guaiacol oxidation product formation. \(^{22}\) Myeloperoxidase levels have been shown to independently predict cardiovascular events in patients presenting to emergency with chest pain, \(^{23}\) as well as to predict the development of coronary artery disease (CAD) in healthy individuals. \(^{24}\) Furthermore, increasing MPO levels are associated with accelerated atheroma progression in diabetic patients, with a greater benefit of statin therapy observed in diabetic patients with lower compared with higher MPO levels at baseline. \(^{25}\) The results of these relatively large, prospective studies, as well as the availability of commercial assays, make MPO one of the most promising redox biomarkers for clinical application. \(^{26}\)

**Reactive oxygen species levels**

Historically, there has been great interest in measurement of ROS levels as redox biomarkers. Although the generation of these free radicals is at the epicentre of redox signalling in health and disease, their functional effects occur through specific modifications on a variety of cellular targets, making ROS levels less pathophysiologically relevant as biomarkers. Furthermore, measurements of ROS levels in circulation by standard chemical approaches, such as spin-trapping, is problematic. Short half-life and restricted diffusion of most ROS, the problem with their quantification very challenging. \(^{27}\) Measurement of ROS effects on tissues is currently limited by the requirement for invasive biopsy, although advances in ROS-sensitive contrast agents and imaging techniques \(^{28}\) have the potential to partially overcome this limitation.

**Molecules undergoing oxidative modifications**

Lipids, proteins, carbohydrates, and DNA are all susceptible to oxidative modification depending on the level of ROS they are exposed to. Some modifications have direct functional effects, such as enzyme inhibition, with the remainder functionally silent indicators of increased ROS levels in the microenvironment. The direct impact of the molecular modifications on the cell, organ and system’s ability to adapt to the elevated levels of ROS is an important contributor to the plausibility and validity of the marker, and its likelihood of emerging as a robust prognostic tool. However, this is challenged by the high reactivity and short half-life of many of these oxidative products, as well as their variable specificity. \(^{29}\)

**Lipid oxidation**

The oxidation of lipids, or lipid peroxidation, is recognized as a crucial step in the pathogenesis of several cardiovascular disease states, particularly atherosclerosis. \(^{30,31}\) It results from ROS attack of the polyunsaturated fatty acids of the membrane and initiation of a self-propagating chain reaction. Lipids are particularly susceptible targets of oxidation because of their abundant reactive double bonds. \(^{32}\) Biophysical properties of the membrane are directly affected, resulting in altered fluidity and inactivation of critical membrane-bound receptors and enzymes. \(^{33}\) Furthermore, the end-products of lipid peroxidation, such as the highly reactive secondary aldehyde products isoketals from the isoprostane pathway, directly threaten the viability of tissues via their ability to covalently modify molecules that are critical to cell function. \(^{34}\)

The sensitivity of lipids to peroxidation, and its functional effects have made lipid peroxides good candidates as redox biomarkers. The most frequently studied markers of lipid peroxidation are isoprostanes, and malondialdehyde (MDA). Others include lipid hydroperoxides, fluorescent probes of lipid peroxidation, and oxysterols. \(^{35,36}\)

Isoprostanes are prostaglandin-like substances that are produced independently of cyclooxygenase enzymes by ROS-induced peroxidation of arachidonic acid. \(^{36}\) Reactive oxygen species from the mitochondria, P450 enzymes, lipoxygenase and transition-metal catalysis are involved in lipid peroxidation. The isoprostanes are initially formed esterified on phospholipids and are then released by phospholipases. \(^{36}\) The most commonly measured members of the family are the F2-isoprostanes, a group of 64 compounds isomeric in structure to prostaglandin F2α that have the greatest stability, thus are suitable for quantification. \(^{37}\) Although commercial immunoassay kits that are user-friendly and relatively cheap have been developed, they have shown variable performance compared with the gold standard mass-spectrometric techniques. \(^{37,38}\) In addition, artefactual generation of F2-isoprostanes in plasma ex vivo necessitates performing measurements within 24 h. \(^{39}\)

F2-isoprostanes are detectable in all biological fluids, reflecting baseline or ‘physiological’ levels of redox signalling. They are substantially elevated in animal models of oxidant injury, as well as human disease states characterized by elevated ROS. \(^{40,41}\) They also increase in association with well-recognized risk factors such as cigarette smoking, hypercholesterolaemia, and diabetes mellitus. \(^{38}\) Their causal role in human atherosclerosis is suggested by their effect to induced vasoconstriction, \(^{41}\) platelet aggregation, \(^{42}\) proliferation of VSMCs, \(^{43}\) and their increased levels in atherosclerotic lesions. \(^{44}\) The role of F2-isoprostanes in heart failure is less well studied, although levels in pericardial fluid have been shown to correlate with severity of heart failure and ventricular dilatation. \(^{45}\)

Malondialdehyde, generated via peroxidation of polyunsaturated fatty acids, is also widely used to examine redox state. Malondialdehyde-induced generation of lysine—lysine cross-links in apolipoprotein B fractions of oxidized low-density lipoprotein (OxLDL) has been proposed to play a role in atherogenesis via impairing the action of macrophages. \(^{46}\) However, despite its role in pathophysiology, reduced specificity is an issue, both in vivo where MDA can be produced independently of lipid peroxidation, as well as in the laboratory, with the most commonly used assay actually measuring thiobarbituric acid-reactive substances (TBARS). \(^{47}\) Several
commercially available ELISA kits demonstrate good performance when compared with high-performance liquid chromatography (HPLC)-based analysis. Malondialdehyde quantification therefore remains a useful biomarker in clinical research. Numerous studies have demonstrated the elevation of MDA in association with smoking and diabetes in both animals and humans. In a clinical study of a moderate size population, serum levels of TBARS predicted major cardiovascular events independently of traditional risk factors and inflammatory markers. There has been a paucity of prospective clinical studies on TBARS since this 2004 study.

Another product of lipid peroxidation, 4-hydroxynonenal (4-HNE) appears to be particularly important for the regulation of vascular redox state in humans. 4-Hydroxynonenal is produced from the reaction of OH° with lipid structures, and is highly reactive with proteins, giving rise to a wide range of protein adducts. Recent evidence suggests that 4-HNE produced in the vascular wall may exert paracrine effects on the neighbouring perivascular adipose tissue, leading to the activation of peroxisome proliferator-activated receptor-γ signalling in this fat depot. As a result, perivascular fat releases the antioxidant adipokine, adiponectin, which exerts a paracrine effect back onto the vascular wall, reducing NADPH oxidase activity, and improving eNOS coupling. 4-Hydroxynonenal thus restores the balance between NO and O₂⁻ in the vascular endothelium. This cascade also underlines the complexities of regulation of vascular redox state in humans, involving multiple intravascular feed-back loops in addition to communication signals with other tissues, which host either pro- or antioxidant mechanisms depending on the underlying diseases state. It also highlights that the oxidation products (used also as clinical biomarkers) may not always be ‘simple by-products’ of oxidation with no biological effects, but might play an active role in the regulation of vascular redox state, e.g. as rescue signals released from the vascular wall.

**Protein oxidation**

The direct, mostly reversible, functional effects of oxidative post-translational modifications on many cellular proteins suggest they could be strong candidates for assessment of cellular redox haemostasis. Tyrosine nitration, protein carbonylation, and S-glutathionylation are the modifications that have been thus far investigated, both of specific proteins, as well as their total levels. The nitration of protein tyrosines is an important consequence of increased ROS. The in vivo reaction occurs through two predominant pathways peroxynitrite and haem peroxidase-dependent nitration with steric effects resulting in altered protein function. Many proteins including fibrinogen, plasmin, Apo A-I in the plasma, Apo B, Mn-superoxide dismutase (SOD) in the vessel wall, and creatine kinase (isoenzyme MM) as well as sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA) in the myocardium undergo nitration, with important functional effects.

Both free circulating 3-nitrotyrosine (3-NO₂-Tyr), which possibly reflects the turnover of nitrated proteins with the modified amino acid not recycled for de novo protein synthesis, and total protein 3-NO₂-Tyr measured by hydrolyzing the protein fraction of the biological sample to its constituent amino acids, have been examined as biomarkers. The gold standard technique for both approaches is tandem mass spectrometry coupled to gas chromatography or HPLC. However, semi-quantitative strategies such as ELISA using an antibody against 3-NO₂-Tyr are more user-friendly and widely applied. Although not used in clinical practice, the 3-NO₂-Tyr has achieved a number of intermediate milestones, including demonstration of the levels as independent predictors of cardiovascular risk, and modulation by statin therapy in a small clinical study. Protein carbonyls can form by the oxidation of a few amino acid side chains via the addition of aldehydes such as those generated from lipid peroxidation. Carbonyl compounds are widely used markers of severe protein oxidation, with several assays developed for quantification. The chemical stability of protein carbonyls makes them suitable targets for laboratory measurement and for their storage. As a marker of oxidative damage to proteins, carbonyls have been shown to accumulate during aging, ischaemia/reperfusion, diabetes, and obesity.

Protein S-glutathionylation, the formation of a mixed disulphide bond between the reactive cysteine residue and the abundant glutathione is an excellent candidate for oxidative signalling due to its stability and reversibility. By conferring a 305 Da negatively charged adduct, it exerts steric effects on proteins similar to phosphorylation. S-Glutathionylation of critical cysteines plays a particularly important role in the cell membrane, mediating redox regulation of eNOS, the ryanodine receptor, SERCA, and the Na⁺–K⁺ pump, to name a few. In contrast to these, S-glutathionylation can also occur in non-critical cysteines without functional or regulatory effects. Thus measuring ‘total S-glutathionylated proteins’ in serum, in a manner similar to that applied to protein nitrosylation, faces problems of both not representing S-glutathionylation at target tissues, as well as accounting for the subpopulation of ‘silent’ S-glutathionylated proteins. We have recently presented data for S-glutathionylation of the Na⁺–K⁺ pump in erythrocytes, which closely parallels that in the myocardium in both animals and patients with heart failure, suggesting its biological validity as a circulatory marker in heart failure.

**DNA oxidation**

Reactive oxygen species mediate damage to all components of the DNA molecule, the purine and pyrimidine bases, as well as the deoxyribose backbone. One of the most abundant products of cellular DNA damage, 8-hydroxy-2′-deoxyguanosine (8-OhdG) can be detected by HPLC, and has been used as a redox biomarker, particularly in cancer research. Although application to cardiovascular disease has been relatively infrequent, levels of 8-OHdG have been found to be elevated in patients with CAD and may also be useful for risk stratification in patients with subclinical cardiovascular disease, as shown for carotid atherosclerosis in a small study of haemodialysis patients.

**Advanced glycation end products**

Advanced glycation end products are a class of molecules resulting from modifications of proteins or lipids that become non-enzymatically glycated and oxidized after contact with aldose sugars. They form in vivo in hyperglycaemic environments and during the ageing process, and mediate vascular disease in diabetes. Because of their severe instability, most of the AGES are difficult to correctly analyse, and are not practical for measurement as biomarkers in cardiovascular disease.
Antioxidant defence molecules and by-products of reactive oxygen species generation

Activity of antioxidant enzymes can be quantified in blood, including catalase, glutathione peroxidase (GPX-1), and SOD. However, interpretation of the serum levels is complex. Multiple factors may lead to low antioxidant capacity, thus predispose to disease progression. However, conversely, although high levels of antioxidant enzyme activity may be protective, they may also reflect transcriptional upregulation of protective mechanisms in response to chronically high levels of ROS. Hence, although a moderate sized prospective study has demonstrated that erythrocyte GPX-1 activity was inversely associated with cardiovascular events in patients with suspected CAD, 65 this has not been reproduced, and it is unlikely that such markers will become clinically useful.

Xanthine oxidoreductase, the enzyme that catalyses the conversion of hypoxanthine to uric acid in the final steps of purine degradation, also produces ROS as part of this process. 66 Xanthine oxidase and XOR activity are upregulated in myocardial ischaemia/reperfusion injury and in heart failure. 66 Although uric acid possesses some limited antioxidant properties in hydrophilic environments, XOR-derived ROS generation in proportion to uric acid production has led to uric acid emerging as a useful redox biomarker in cardiovascular disease. 67 Indeed, this relationship of uric acid with redox state may contribute to its ability to predict mortality in ischaemic heart disease as observed in a large cross-sectional study of the general population. 68 The association with mortality has also been seen in a retrospective cohort analysis of a moderate sized cohort with diabetes, 69 and in a smaller study of patients with chronic heart failure. 70 Indeed, in chronic heart failure, uric acid predicted prognosis better than established indicators such as age, renal function, Na , and exercise capacity. 70 Uric acid has the advantage of wide availability in most clinical biochemical laboratories and substantial evidence supporting its clinical validity. 66 However, uric acid levels are altered by renal function and diuretic use, thus reducing the specificity for redox signalling.

Future directions

With improved knowledge of oxidative signalling and focus on measuring functionally significant oxidative modifications, redox biomarkers have the potential to live up to the historical expectations and provide significant contribution to diagnosis, prevention, and treatment of cardiovascular disease. In addition to development of pathophysiologically relevant redox biomarkers, taking a ‘multi-marker’ approach is also likely to improve their overall performance. In this regard, integrating redox biomarkers with specific markers of disease process has a great potential, e.g. identifying vulnerable plaque and subsequent cardiovascular events in combination with atherosclerosis identified on CT coronary angiography.

Biomarkers, in general, are increasingly used to select subjects in whom preventative treatments might be beneficial to halt a specific pathophysiological process. This is also the case for redox biomarkers, for instance to identify subjects with elevated ROS levels and depleted antioxidant capacity, who are likely to benefit from ‘specific antioxidant’ therapies. 35,71 This notion is supported by the fact that none of the antioxidant trials have selectively enrolled subjects in whom increased ROS levels and the response to antioxidants has been demonstrated by redox biomarkers. It is plausible that this approach may overcome the disappointment of the non-specific antioxidants. 7 Moreover, established pharmacotherapies, such as b-blockers and inhibitors of the renin–angiotensin pathway in heart failure exert their efficacy, at least in part, by specific receptor-coupled antioxidant effects. Measurement of oxidative biomarkers as an indicator of response to b-blockers or angiotensin-converting enzyme inhibitors, may allow for targeted approaches, particularly in those patients who do not tolerate combined therapy. 4

As new oxidative biomarkers emerge, their relevance to the underlying cardiovascular disease process could also make them candidates as surrogate endpoints in clinical trials. Such surrogate markers may also be used to facilitate drug development. Indeed, validated biomarkers are recognized by the FDA to suggest efficacy in Phase III trials. Despite this, potential pitfalls including non-disease-specific effects of the drugs, or their effects on disease processes independent of the redox biomarkers necessitate demonstration of benefits based on clinical endpoints in phase IV studies. 72 Thus, biomarkers of signalling by ROS have the potential to fill an important gap in the assessment of biological processes in cardiovascular clinical studies.

Summary and conclusion

Despite the biological plausibility of redox biomarkers as important adjuncts in diagnostic and prognostic armamentarium, their validation for clinical application has been slow and none have yet reached clinical use. Appreciation of the complexities of redox signalling, such as the compartmentalized signal transduction, and careful selection of markers that represent functionally significant oxidative modifications, may assist in guiding future efforts for development of novel biomarkers. In our opinion, receptor-coupled membrane protein oxidative modifications that occur in a similar fashion in circulating cells in parallel to cardiac myocytes and endothelial cells present a great potential. However, such biomarkers are in early phases of development and validation. A major challenge that must be addressed in large clinical trials is to demonstrate the incremental value of redox markers in addition to the established sophisticated models of cardiovascular risk prediction. Identification of new biomarkers that reflect the pivotal cellular redox mechanisms involved in cardiovascular disease will enable rational patient selection and therapeutic response monitoring to evaluate a new generation of redox therapies.

Supplementary material

Supplementary Material is available at European Heart Journal online.

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