Nephrol Dial Transplant (2003) 18: 1272–1280
DOI: 10.1093/ndt/gfg074

Nephrology Dialysis Transplantation

Consensus Paper

Oxidative stress in end-stage renal disease: an emerging threat to patient outcome

Francesco Locatelli[1], Bernard Canaud[2], Kai-Uwe Eckardt[3], Peter Stenvinkel[4], Christoph Wanner[5] and Carmine Zoccali[6]

[1]Department of Nephrology and Dialysis, Azienda Ospedale di Lecco, Ospedale A. Manzoni, Lecco, Italy, [2]Service de Nephrologie, Hôpital Lapeyronie, Montpellier, France, [3]Department of Nephrology and Medical Intensive Care, Charité, Campus Virchow Klinikum, Berlin, Germany, [4]Division of Renal Medicine, Department of Clinical Science, Karolinska Institute, Huddinge University Hospital, Stockholm, Sweden, [5]Division of Nephrology, University Hospital Würzburg, Germany and [6]CNR Centre of Clinical Physiology, Reggio Calabria, Italy

Abstract

Introduction. Patients affected by end-stage renal disease (ESRD) experience an excess of morbidity and mortality due to cardiovascular disease (CVD), which cannot be fully explained by the classical CVD risk factors. Among emerging CVD risk factors, oxidative stress is currently being given emphasis.

Methods. We achieved a consensus on key points relating to oxidative stress in ESRD patients.

Results. ESRD patients are subjected to enhanced oxidative stress, as a result of reduced anti-oxidant systems (vitamin C and selenium deficiency, reduced intracellular levels of vitamin E, reduced activity of the glutathione system) and increased pro-oxidant activity (advanced age, high frequency of diabetes, chronic inflammatory state, uraemic syndrome, bio-incompatibility of dialysis membranes and solutions). Oxidative stress and inflammation are deeply inter-related, as different oxidant free radicals are generated by phagocytic cells in response to inflammatory stimuli: both are related to endothelial dysfunction, as the endothelium is a source and a target of oxidants and participates in the inflammatory response. There is growing evidence, from experimental and clinical studies, that oxidative stress may be implicated in the pathogenesis of atherosclerosis and other complications of ESRD, namely dialysis-related amyloidosis, malnutrition and anaemia. Given that free radicals have very short half-lives (seconds), the clinical assessment of oxidative stress is based on the measurement of different stable oxidized compounds (such as lipid peroxidation products, advanced glycation and oxidation lipid and protein products, nucleic acid oxidation derivatives) or antibodies directed against oxidized epitopes (such as anti-oxidized low-density lipoprotein antibodies). At the same time, both enzymatic anti-oxidants (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic anti-oxidants (glutathione, vitamin C, vitamin E, negative inflammatory proteins) can be evaluated. However, many laboratory methods assessing various oxidative stress components still have to be standardized. Moreover, it is still uncertain whether it is better measuring plasma and/or intracellular concentrations or activities of these components. The possibility of improving patient outcome by therapeutic interventions aimed at reducing oxidative stress, e.g. by vitamin C or vitamin E supplementation, currently is to the fore, but results so far have remained inconclusive.

Conclusions. It is important to consider oxidative stress as a potentially important source of patient morbidity and mortality, although this knowledge is not yet immediately applicable in the clinical arena. Further well-designed, randomized controlled clinical trials with anti-oxidants (e.g. vitamin E, vitamin C, *N*-acetyl cysteine, L-arginine) are required to establish evidence-based recommendations for clinical practice.

Keywords: anti-oxidant therapy; atherosclerosis; dialysis complications; endothelial dysfunction; inflammation; oxidative stress

Introduction

Cardiovascular disease (CVD) is the major cause of death in patients affected by end-stage renal disease (ESRD) [1,2]. According to two of the largest ESRD registries, the US Renal Data System (USRDS) and the European Registry of patients on renal replacement

Correspondence and offprint requests to: Professor Francesco Locatelli, Department of Nephrology and Dialysis, Ospedale A. Manzoni, Via Dell'Eremo 11, 23900 Lecco, Italy.
Email: nefrologia@ospedale.lecco.it

therapy (ERA–EDTA), the estimated risk for cardiac events such as myocardial infarction is 3.5–50 times higher among patients on renal replacement therapy (RRT) than in the general population [3,4]. The classical risk factors for CVD (such as old age, hypertension, diabetes, smoking, dyslipidaemia, left ventricular hypertrophy, heart failure and physical inactivity) are over-represented in patients on RRT. This is partly because of the epidemiology of the ESRD population, which now consists mainly of old patients affected by diabetes and vascular disease [5] and who often have survived previous CVD events [6]. In part, chronic renal insufficiency (CRI) *per se* is associated with both classical CVD risk factors and uraemia-specific risk factors, such as chronic volume expansion, anaemia, disturbances of calcium–phosphate metabolism, hyperhomocysteinaemia and a microinflammatory state associated with increased oxidant stress. These non-traditional CVD risk factors are being given emphasis not only because they could explain the high incidence of CVD events in the ESRD population in comparison with the general population [7], but also because they may represent new targets for therapeutic intervention. In this respect, oxidative stress certainly is a fascinating emerging issue. ESRD patients are characterized by an imbalance between pro-oxidant and anti-oxidant factors, and increased oxidant stress has been associated with typical complications of ESRD such as atherosclerosis and β_2-microglobulin amyloidosis [8]. Although oxidative stress is not readily detected, therapies aimed at reducing it (for instance by anti-oxidant administration) have been investigated recently. However, the results so far have been contradictory and require further well-designed, randomized controlled trials.

Issues relating to oxidative stress in ESRD will be presented and discussed in this report.

Oxidative stress: what it is and how to measure it

Oxidative stress is defined as the tissue damage resulting from an imbalance between an excessive generation of oxidant compounds and insufficient anti-oxidant defence mechanisms [9].

Generation of oxidative compounds is physiologically relevant as an important step in inflammation and tissue repair processes. Therefore, it represents part of the defence mechanisms against invading microorganisms and malignant cells, as well as of tissue healing and remodelling. On the other hand, an improper, or maladaptive, activation of oxidative processes may be chronically present in pathological situations, such as uraemia, contributing to cell and tissue injury [10].

Sources of oxidative stress

The mitochondrial respiratory chain represents the most powerful cellular source of oxidants in the body. Mitochondrial oxidants may exert deleterious effects and are thought to contribute to cellular senescence, as well as neurodegenerative diseases. However, to date, there is no method available to determine their potential contribution to cellular pathology.

The phagocyte oxidant generation system is based on the inducible production of reactive oxygen species (ROS) via univalent reduction of molecular oxygen (O_2): following exposure to appropriate stimuli, both polymorphonuclear neutrophils (PMNs) and monocyte-macrophages activate and increase their O_2 consumption (the so-called respiratory burst). The NADPH-oxidase enzyme system, which is bound to cellular membranes, reduces O_2 to superoxide anion (O_2^-), which is highly unstable and, as soon as it is formed, is converted into hydrogen peroxide (H_2O_2). Both O_2^- and H_2O_2 are precursors for the production of more powerful oxidants. O_2^- interacts with nitric oxide (NO) to form highly reactive nitrogen species (nitrosative stress), while H_2O_2 reacts with intracellular iron to form hydroxyl radicals (OH^-), that are heavily implicated in cell membrane lipid degradation, protein aggregation and DNA damage. Moreover, H_2O_2 is the substrate for myeloperoxidase (MPO) to produce the chlorinated oxidants. In the presence of Cl^-, MPO converts H_2O_2 into hypochlorous acid (OCl^-), a powerful compound capable of oxidizing a number of molecules, such as lipids, proteoglycans and other membranous or intracellular constituents, particularly the thiol groups of membrane proteins (chlorinative stress). In addition, it may react with endogenous amines (R-NH_2) to produce chloramines (RNH-Cl).

The ROS are released together with pro-inflammatory cytokines, which in turn amplify oxidant generation [8].

Oxidative stress markers

Oxidants are highly reactive compounds with a half-life of only seconds. Therefore, their *in vivo* determination is generally not feasible. In contrast, lipids, proteins, carbohydrates and nucleic acids, after being modified by oxy-radicals, have lifetimes ranging from hours to weeks, which makes them ideal markers of oxidant stress (Table 1) [11].

Table 1. Markers of oxidative stress and anti-oxidants

Markers of oxidative stress	Anti-oxidants
Lipid peroxidation	Enzymatic
Acrolein	Superoxide dismutase
Malonyldialdehyde	Catalase
4-Hydroxynonenal	Glutathione peroxidase
Thiobarbituric acid-reactive substances	Non-enzymatic
F_2-isoprostanes	Glutathione
Advanced lipid oxidation products	Vitamin E
Oxidized LDL antibodies	Vitamin C
Protein oxidation	Ferritin
Advanced oxidation protein products	Transferrin
Carbohydrate oxidation	Albumin
Advanced glycosylation end-products	etc.
Nucleic acid oxidation	
8-Hydroxy-2′-deoxyguanosine	

During lipid peroxidation, unstable hydroperoxides, resulting from peroxyl radical-dependent chain reactions among unsaturated fatty acyl moieties, break down to smaller and more stable products, e.g. aldehydes, such as acrolein, malonyldialdehyde (MDA), 4-hydroxynonenal (HNE) or thiobarbituric acid-reactive substances (TBARS). F_2-isoprostanes are primarily products of arachidonic acid oxidation and may serve as stable markers of free-radical attack of the cell membrane phospholipids *in vivo* [10]. Furthermore, increased levels of advanced lipid oxidation end-products (ALEs) and the presence of specific antibodies directed against oxidized low-density lipoproteins (LDLs) may represent useful markers of enhanced oxidative stress [12].

Proteins are elective targets of oxidant-mediated injury, leading to cross-linking and aggregation products that may be resistant to proteolysis. However, it has long been difficult to find markers of protein oxidation. Searching for oxidatively modified proteins in uraemic patients, Witko-Sarsat *et al.* [13] were able to characterize the so-called advanced oxidation protein products (AOPPs). This name was used in analogy with advanced glycation end-products (AGEs), with which AOPPs share several homologies. AOPPs, closely related to markers of monocyte activation, may serve as mediators of inflammation and correlate positively with dityrosine and AGE pentosidine plasma concentrations, which indicate oxidant-mediated damage. The existence of a strong relationship between AOPPs and AGEs led to the concept of carbonyl stress, where oxidation acts together with glycation in the formation of AGEs [12].

Finally, oxidative compounds may interact with nucleic acids and contribute to mutagenesis and oncogenesis. Oxidative damage of leukocyte nucleic acids has recently been shown in ESRD. Determination of the 8-hydroxy-2′-deoxyguanosine (8-OHdG) content by high performance liquid chromatography (HPLC) was used to evaluate leukocyte DNA damage [14]. 8-OHdG levels were found to be elevated in CRI, with the highest levels being observed in ESRD.

Anti-oxidant defence systems

To prevent the harmful effects of ROS, anti-oxidant systems, both enzymatic and non-enzymatic, are naturally present and counteract free radicals (Table 1).

Superoxide dismutase (SOD) accelerates the dismutation rate of O_2^- to H_2O_2 as the first line of enzymatic anti-oxidant defence. Catalase reduces H_2O_2 to water. Selenium-containing glutathione peroxidase (GSH-Px) reduces all organic lipid peroxides and requires GSH as a hydrogen donor [12].

The most active non-enzymatic antioxidant is represented by GSH itself, which is a scavenger for H_2O_2, OH^- and chlorinated oxidants. Vitamin E protects the cell membrane from lipid peroxidation by forming a low-reactivity tocopheroxyl radical. Vitamin C directly scavenges O_2^- and OH^-. Inflammation proteins such as ferritin, transferrin and even albumin exert a non-enzymatic anti-oxidant effect by sequestrating transition metal ions [12].

A reliable measurement of the global anti-oxidant capacity in CRI patients would be useful but, in contrast to other diseases, the determination of the total peroxyl radical-trapping antioxidant potential (TRAP) (by measuring the ability of a biological fluid to resist 2,2′azobis [2-amidinopropane] hypochlorite-induced linoleic acid peroxidation) failed to act as a relevant parameter for evaluating plasma and LDL anti-oxidant capacity in ESRD [15].

Therefore, the estimation of the anti-oxidant status of CRI patients comprises the measurement of the different compounds of the anti-oxidative system in plasma and cells. Despite the lack of standards and conflicting results, the determination of plasma levels of vitamin C and GSH-Px, and the erythrocyte content of SOD, GSH, GSH-Px and vitamin E has been applied successfully, revealing anti-oxidant deficiency in CRI.

Oxidative stress and anti-oxidants in ESRD and beyond: reality and promises

In CRI patients, the balance between pro- and anti-oxidant capacity is shifted towards an increased oxidative stress. Several deficiencies in different components of the anti-oxidant defence mechanisms have been demonstrated, including reduced levels of vitamin C (primarily due to dietary restriction of fresh fruits and vegetables to avoid hyperkalaemia, and loss of the vitamin during dialysis), reduced intracellular levels of vitamin E, reduced selenium concentrations and deficiency in the GSH scavenging system [8,12]. At the same time, pro-oxidant activity is increased. Factors contributing to increased pro-oxidant activity include characteristics of the patient population suffering from renal disease, such as advanced age and diabetes, uraemia *per se* (through mechanisms as yet poorly defined), chronic inflammation and factors associated with RRT. Haemodialysis (HD) may induce repetitive bouts of oxidative stress, primarily through membrane bio-incompatibility and endotoxin challenge [16,17]. While alterations in pro- and anti-oxidant capacity start during the early stages of CRI, they are most pronounced in patients on dialysis. Overall, however, there is some controversy as to whether the onset of regular dialysis improves or worsens oxidative stress.

Because of the imbalance between pro- and anti-oxidant systems, indicators of oxidative stress are increased in patients with CRI. During HD, a direct increase in blood levels of ROS has been demonstrated using whole blood chemiluminescence [16]. Neutrophils of uraemic patients show overproduction of ROS in response to activating stimuli. Blood levels of several lipid and protein oxidation products are increased in patients with renal failure. Oxidative stress also promotes the formation of AGEs, independently of glucose levels. The recently characterized carbonyl

stress products may also be a consequence of increased oxidative stress. In addition, the degree of haemolysis can indirectly reflect oxidative stress, since oxidation of plasma membrane proteins can reduce membrane elasticity and erythrocyte survival.

Consequences of increased oxidative stress in CRI

Oxidative stress is likely to contribute to patient morbidity and, presumably, mortality. Most importantly, oxidative stress is believed to promote endothelial dysfunction and atherosclerosis and, therefore, cardiovascular complications. Among several qualitative changes in LDL in dialysis patients, increased levels of oxidized LDL and elevated titres of anti-oxidized LDL antibodies can be found. Dialysis-related amyloidosis has also been linked to increased oxidative stress. Malnutrition, a significant adverse risk factor for patient prognosis, is associated with surrogate markers of oxidative stress, although it is unclear whether and in which direction this association may reflect causality. Recent experimental data have also implicated oxidative stress in the pathogenesis of hypertension.

The relationship between malonyldialdehyde levels as an indicator of oxidative stress and the development of atherosclerosis was demonstrated recently in a cross-sectional study in dialysis patients [18]. However, no prospective epidemiological studies are yet available to confirm a link between the extent of oxidative stress and patient outcome.

Use of antioxidants in patients with CRI

Attempts to improve patient prognosis with anti-oxidative therapy have been made in the general population and in patients with renal disease.

In the general population, epidemiological data suggested that the intake of vitamin E is inversely related to the development CVD. Subsequently, large prospective randomized controlled trials have tested whether vitamin E supplementation improves cardiovascular outcomes, the most recent one being the Heart Protection Study [19]. Of these studies, some showed no benefit from vitamin E supplementation. The single study showing a reduced rate of non-fatal myocardial infarction in patients with angiographically proven coronary atherosclerosis [20] has been criticized for imbalances in baseline characteristics between the control and treatment arms.

In patients affected by CRI, oral vitamin E supplementation has been shown to reduce the oxidative susceptibility of LDL [21], prevent the oxidative stress associated with anaemia therapy or improve erythropoietin (Epo) responsiveness, which may be impaired by oxidative stress. The SPACE trial tested the effect of vitamin E (800 IU/day) on a combined cardiovascular endpoint in 196 HD patients with pre-existing CVD, and showed a significant benefit from vitamin E supplementation [22]. Based on these interesting results, a larger trial, sufficiently powered to assess effects on mortality, appears highly desirable.

Specific dialysis techniques have been introduced in an attempt to reduce oxidative stress, such as vitamin E-modified cellulose membranes and haemolipodialysis [23]. Given that blood–membrane interaction can trigger oxidative stress, direct scavenging at the membrane site is an attractive approach. Vitamin E-modified cellulose membranes bear a hydrophilic polymer that strongly binds vitamin E. Haemolipodialysis also employs vitamin E, but in the form of vitamin E-containing liposomes, that interact with blood components at the HD membrane without passage through the membrane itself. In addition, the liposomes can be oxidized themselves, acting as a 'radical sink'. However, so far, only one study has demonstrated a functional benefit from vitamin E-modified dialysis membranes [24], and the more widespread use of both techniques is severely hampered by their cost.

Another aspect of the management of CRI with potential implications on oxidative stress is the treatment of anaemia. Red blood cells contain high levels of anti-oxidants, in particular reduced GSH. A rise in red cell mass may therefore increase the total anti-oxidative capacity. On the other hand, some studies have suggested that the intravenous injection of iron, as well as Epo therapy, may at least transiently enhance the oxidative stress [25]. The hypothesis that anti-oxidant supplementation may improve the anaemic condition of CRI patients, as assessed by lower Epo requirements, is a fascinating issue and needs to be addressed adequately by randomized controlled clinical trials.

Inflammation and oxidative stress in ESRD: is there a link?

As increased oxidative stress and inflammation are both common features of ESRD, it has been speculated that there may be an association between them and endothelial dysfunction, contributing to an increased risk for CVD. Indeed, Memon *et al.* [26] recently demonstrated that LDL isolated from animals treated with bacterial lipopolysaccharide (LPS) was more susceptible to *ex vivo* oxidation with copper than LDL isolated from saline-treated animals. Thus, one could speculate that chronic inflammation causes increased oxidative stress. On the other hand, oxidative stress may also stimulate an inflammatory response. Interestingly, several recent clinical studies suggest that oxidative stress and inflammation may be linked in ESRD patients. First, Nguyen-Khoa *et al.* [27] suggested that the presence of inflammation and the duration of dialysis are the most important determinants of oxidative stress in HD patients. Secondly, an association between F_2-isoprostanes and C-reactive protein (CRP) levels has been reported recently in HD patients [28]. Thirdly, in a study by Mezzano *et al.* [29], in which 64 patients with advanced ESRD were studied, a significant positive correlation was found between acute phase proteins and markers of oxidative

stress. Finally, it has been demonstrated that AOPPs act as mediators of oxidative stress and monocyte respiratory burst, which points to monocytes as both targets and actors in the immune dysregulation associated with ESRD [30].

Activation of PMNs is a well-recognized feature in dialysis patients, which may represent an important pathway for tissue damage and LDL oxidation *in vivo*. Interestingly, increased MPO secretion could also contribute to CVD by promoting endothelial dysfunction as it attenuates nitric oxide (NO)-dependent smooth muscle relaxation [31]. Taken together, activation of PMNs and secretion of MPO may be an important link between inflammation, oxidative stress and endothelial dysfunction in ESRD patients. This hypothesis is supported by a recent study, which has shown that elevated leukocyte count and blood MPO levels are associated with the presence of coronary artery disease in a group of non-renal patients [32]. Moreover, a recent study has shown that a functional variant of the MPO gene is associated with cardiovascular disease in ESRD patients [33].

If oxidative stress and inflammation are important for the pathogenesis of CVD in ESRD patients, one could speculate that anti-oxidative treatment strategies may be beneficial and decrease the prevalence of CVD in this patient group. Thus, since oxidation products may mediate inflammation in ESRD patients, nutrients and/or anti-oxidants, which modulate cytokine activity, are of particular interest. Since inflammation, oxidative stress and atherosclerosis are closely intertwined, further studies are needed to investigate the ability of oral supplementation, or vitamin E-coated dialysers, to reduce the inflammatory process and improve outcome in HD patients. It is of note that a recent study reported that γ-tocopherol and its major metabolite (but not α-tocopherol) may possess significant anti-inflammatory activity [34]. This suggests that the inclusion of both α- and γ-tocopherol in vitamin E supplements may be more effective in preventing human disease [35].

In conclusion, inflammation may be one important cause of increased oxidative stress in ESRD patients. As MPO, generated in response to activation of PMNs, generates spreading oxidants and inactivation of NO, this may link inflammation to increased oxidative stress and endothelial dysfunction in ESRD patients. Thus, further studies are needed to investigate whether anti-oxidant treatment strategies, such as α- and γ-tocopherol supplementation, may decrease the inflammatory response and the unacceptably high mortality rate in ESRD patients.

AGEs and cardiovascular damage in ESRD: hypotheses, experimental evidence and clinical observations

The causes of the high frequency of CVD and the high incidence of cardiovascular mortality in dialysis patients are multi-factorial in origin. Disturbances in carbohydrate and lipid metabolism, the imbalance between oxidants and anti-oxidants and the immuno-inflammatory system are all thought to play a role. More specifically, chronic uraemia is characterized by the accumulation of AGEs, as well as the activation of the acute phase response, for which CRP is the prototype in humans.

AGEs characteristics and serum levels

AGEs are diverse fluorescent and non-fluorescent compounds which are formed in complex, not yet fully understood reactions starting with the non-enzymatic reaction of reducing sugars or other carbonyl compounds with free amino groups of proteins. Only a few physiologically occurring AGEs have been characterized chemically, such as *N*-(carboxymethyl)-lysine (CML), a colourless and non-fluorescent compound. AGEs accumulate during ageing and in the course of many degenerative diseases. It was first noted by Makita and co-workers in 1991 that ESRD patients had dramatically increased serum levels of unspecific AGE-modified peptides [36]. Meanwhile, numerous studies in which levels of total chemically undefined AGEs, AGE-dependent fluorescence and defined AGEs such as CML and pentosidine were measured have confirmed these results. It is worth noting that diabetic ESRD patients actually have lower CML levels than non-diabetics, an as yet unexplained finding [37].

Since it has been postulated that the kidney is responsible for the removal of AGEs, the accumulation of AGEs in CRI has been attributed partly to the progressive loss of renal function. On the other hand, increased *de novo* generation due to enhanced oxidative stress and accumulation of reactive precursors, such as di-carbonyls, appears to be implicated as well [38]. In particular, the important role of the liver in modulating the AGE pool *in vivo* has so far been underestimated.

AGE fluorescence usually correlates with CML levels, showing common origins of formation. Fluorescence is not specific for individual compounds, and interference with other fluorescent compounds may occur. However, it may comprise unknown AGE compounds with physiological impact. The physiological activity of the well-characterized AGEs, such as CML, pentosidine or imidazolone, is still not proven unequivocally. For example, pentosidine shows a much higher relative degree of accumulation in plasma ($\sim$10-fold as compared with 3.6-fold for CML), and different pathways of AGE formation may exist *in vivo*. However, the absolute concentration of pentosidine in plasma is 100-fold lower than that of CML.

AGEs and atherosclerosis

AGEs can also contribute to the rapidly progressive atherosclerosis that develops in patients with diabetes

and CRI. They promote the influx of mononuclear cells and stimulate cell proliferation [39,40]. Collagen-linked AGEs within the atherosclerotic lesion bind plasma proteins, interact with macrophage receptors to induce cytokine and growth factor release, and quench NO activity [41]. There is also evidence that AGEs modify LDL, making it less able to be cleared by the LDL receptors [42]. AGEs are also identified as inducing endothelial dysfunction. A recent observation suggested that glycoaldehyde generated by the MPO–H_2O_2–chloride system, involving activated phagocytes, might lead to CML production and thereby play an important role in tissue damage at sites of inflammation, as encountered in atherosclerosis. Besides the classical pathway, CML may be formed by glyoxal generated during lipid peroxidation or glucose auto-oxidation. Moreover, co-staining of CML and macrophages [39] was found in atherosclerotic lesions where CRP is also deposited. It has also been proposed that CRP may play a part in the recruitment of monocytes during atherosclerosis [40].

The contradiction

Recently, it was hypothesized that circulating AGEs may promote the inflammatory status seen in HD patients [41] subsequently leading to high concentrations of CRP in serum and to cardiovascular mortality. In stark contrast to this theory, a recent study performed by Schwedler *et al.* [43] indicated that high levels of AGEs, as determined by total serum fluorescent AGEs (AGE-fl) and CML, were linked to better survival in HD patients who were followed over a period of 32 months. A correlation with the acute phase response, as determined by CRP, could not be demonstrated, and the hypothesis that high levels of CML and AGE-fl may activate the acute phase response could not be demonstrated by correlating serum markers. Furthermore, recent data showed that pentosidine was unrelated to intima media thickness and to the number of atherosclerotic plaques [43]. Therefore, serum AGE levels in HD patients may not be indicative of cardiovascular outcome. Whether the benefit of high serum AGEs is an epiphenomenon or reflects better nutritional support requires further studies.

In conclusion, the above findings suggest that the mechanisms proposed for noxious AGE effects based on experiments *in vitro* do not necessarily translate into the *in vivo* setting of uraemic patients. However, it is still possible that damaging cardiovascular effects of AGEs occur locally at the tissue or cellular level, or else that such effects are mediated by other types of AGEs. Furthermore, it is likely that the plasma concentration of different AGE species represents only a small fraction of total body AGE content and that serum levels do not reflect specific changes in the overall AGE pool well—besides reflecting kidney function. Thus, circulating AGEs may not be adequate parameters for the demonstration of AGE effects with respect to atherosclerosis-related outcome. It may be more appropriate to focus on changes in AGE pools and intracellular AGEs.

The endothelium, oxidative stress and cardiovascular damage in ESRD

The endothelium is perhaps one of the major sources of ROS, but is also the major target of these substances. Measuring the haemodynamic response to acetylcholine (Ach) in the forearm is an accepted means, and probably the one used most frequently, to estimate endothelial function in man. When one injects increasing doses of Ach in healthy subjects or in persons affected by coronary heart disease, there is a clear vasodilator dose–response relationship. Studying the vasodilator response to Ach is clinically meaningful because this response predicts the incidence of cardiovascular events. Indeed, ~90% of patients with forearm blood flow (FBF) above the median have been shown to remain event free for 7 years after the haemodynamic study, whilst fewer than a half of the patients with FBF below the median have a favourable course [44].

Vitamin C given intra-arterially at high doses is a potent anti-oxidant and is able to improve the vasodilatation induced by Ach. When patients were divided into those who experienced cardiovascular events on follow-up and those who did not, the vasodilator dose–response relationship to Ach was steep at baseline and remained almost unchanged in patients without cardiovascular events. This phenomenon could be explained by low ROS generation at baseline. In contrast, the basal dose–response relationship was flatter in those who experienced cardiovascular events and the response to vitamin C was greatly enhanced, suggesting a high rate of ROS formation that could be counteracted by vitamin C. Again, survival in patients with a vitamin C response suggestive of low ROS generation was much higher than in those with a response suggestive of high ROS generation [44]. Taken together, these findings indicate a clear link between ROS formation at the endothelial site, endothelial dysfunction and cardiovascular damage.

Among the biochemical mechanisms involved in tissue damage by ROS, nitrosative stress is currently being looked at carefully, as will be discussed in more detail below.

In the endothelium, the very basic regulatory reaction is the generation of NO from L-arginine, which is controlled by NO synthase. Tetrahydrobiopterin is an essential factor for NO synthase function. When tetrahydrobiopterin is oxidized, the synthesis of NO is compromised. Oxidation of tetrahydrobiopterin has relevant consequences because in this particular situation, NO synthase functions as an oxidizing enzyme itself and generates superoxide anion, thus amplifying oxidative stress [45]. Anti-oxidants such as vitamin C and GSH may be useful because they

reconvert tetrahydrobiopterin to the non-oxidized state, a phenomenon accompanied by normalization of NO synthesis.

It is worth noting that O_2^- may also induce tissue damage by reacting with NO itself. This generates peroxynitrite, a highly reactive compound that adds nitrate to tyrosine residues in proteins, thus causing impairment of various enzymatic reactions and tissue damage. Limiting the availability of NO in this particular situation may even be useful.

Several endogenous inhibitors of NO synthase may participate in endothelial dysfunction. The most important one is asymmetric dimethyl arginine (ADMA). When the intracellular concentration of this substance in endothelial cells is high, the generation of NO is markedly reduced. To a small extent, ADMA is eliminated by the renal route. The intracellular concentration of ADMA is very much dependent on the activity of the enzyme dimethyl-diaminohydrolase (DDHA), which transforms ADMA into citrulline. The activity of this enzyme is blocked by oxidative stress. Indeed, hypercholesterolaemia, hyperhomocysteinaemia and perhaps smoking are associated with a marked increase in plasma ADMA concentration. The median plasma concentration of ADMA is $\sim$1 μmol/l in healthy subjects [46] and $\sim$2.5 μmol/l in HD patients when sampling is performed during the dialysis interval, i.e. in a situation that can be considered as 'steady state' in these patients [46,47], a 1.5–5 μmol/l difference in comparison with the general population.

Since oxidative stress is high in ESRD, in theory it should be useful to reduce the availability of NO, thereby limiting the production of peroxynitrite and nitrosative stress. Therefore, it is important to investigate how ADMA relates to death and cardiovascular outcomes in ESRD patients. This relationship has been tested in a prospective cohort study, the CREED study, in 225 ESRD patients undergoing HD [47]. First of all, some crude analyses. In this study, patients were divided in two groups, namely survivors and non-survivors. Plasma ADMA concentration was 3 μmol/l in non-survivors and 2 μmol/l in survivors; similarly, it was higher in patients who experienced cardiovascular events than in those who did not. Patients were then stratified into three groups: the first group included those with plasma ADMA levels less than the 50th percentile (the median), the second those with ADMA levels between the 50th and the 75th percentile, and the third those with values above the 75th percentile. All-cause mortality and both fatal and non-fatal cardiovascular events increased dramatically from the first to the third group. Given that crude analyses may be misleading, because they do not take into account the influence of other risk factors, the independent effect of ADMA on survival and cardiovascular events was tested by the multivariate Cox regression model: concentrations of ADMA in plasma ranked as the second factor predicting all-cause mortality (hazard ratio: 1.26) and fatal and non-fatal cardiovascular events (hazard ratio: 1.17). There is another way to extrapolate the implication of this survival analysis. As plasma ADMA is higher by 1.5–5 μmol/l in HD patients than in healthy subjects, this difference can be regarded as a 39% higher risk of death and a 25% higher risk of cardiovascular events in HD patients compared with the general population. Thus, raised ADMA levels in ESRD patients appear to be a maladaptive process rather than a useful compensatory mechanism. This may depend on the fact that NO synthesis in ESRD is very low because of the severity of atherosclerosis (i.e. reduction in endothelial surface area) and low L-arginine availability [48]. The question remains as to whether ADMA is causally involved in the high cardiovascular complications of dialysis patients. To answer this question intervention studies are needed. In this regard, there are several options:

(i) We may intervene by reducing oxidative stress, which is the trigger of high ADMA levels, by anti-oxidant supplementation (e.g. vitamin E supplementation). We may reduce oxidative stress by counteracting inflammatory processes (e.g. by aspirin). Statins represent another intriguing possibility, not only because lipid lowering *per se* is associated with reduced oxidative stress, but also because these drugs may interfere with factors regulating NADPH activity. Finally, angiotensin-converting enzyme (ACE) inhibitors may remove a strong oxidant stimulus by reducing angiotensin-II levels.

(ii) Alternatively, we may try to increase NO synthesis by administering high doses of L-arginine. This latter possibility may be problematic because L-arginine (given as the chloride salt) is an acidifying agent. Moreover, any combination therapy based on the association of statins with anti-oxidants should be evaluated carefully because in a recent trial in patients with coronary heart disease, the association of anti-oxidants with a simvastatin–niacin regimen blunted the favourable effects of this regimen [49].

Final accord

After intensive discussion, the panel reached consensus on the following key points.

The importance of oxidative stress

- Oxidative stress is a double-edged sword: it is naturally occurring in humans as an important part of host defence mechanisms, but excessively activated in many pathological conditions.
- In ESRD patients, oxidative stress appears to play a crucial role in the pathogenesis of atherosclerosis, malnutrition, dialysis-related amyloidosis and anaemia.
- A tentative association exists between increased oxidative stress and inflammation, which are both

common features of ESRD. This may contribute to endothelial dysfunction and an increased risk of coronary heart disease.

The causes of oxidative stress

- Oxidative stress results from an imbalance between anti-oxidant defence mechanisms and excessive generation of oxidants, leading to cell and tissue injury. In CRI patients, there is a deficiency in anti-oxidant systems (vitamin C and selenium deficiency, reduced intracellular vitamin E, reduced activity of GSH system). At the same time, pro-oxidant activity is increased due to advanced age, diabetes, chronic inflammation and bioincompatibility of dialysis membranes and solutions.
- Tissue damage occurs through a number of biochemical mechanisms, all of which have in common the formation of highly reactive intermediate compounds (free radicals) that can oxidize proteins, lipids and nucleic acids.

Determining oxidative stress

- As oxidants have very short half-lives (seconds), they cannot be evaluated reliably in clinical conditions. Therefore, the determination of oxidative stress requires the use of more stable markers.
- These markers include lipid peroxidation products (such as acrolein, malonyldialdehyde, 4-hydroxynonenal, thiobarbituric acid-reactive substances, F_2-isoprostanes), advanced lipid oxidation products, specific anti-oxidized LDL antibodies; oxidatively modified proteins and AOPPs; evaluation of enzymatic anti-oxidant systems (erythrocyte content of SOD and GSH, plasma levels of GSH-Px), non-enzymatic anti-oxidants (plasma levels of vitamin C, erythrocyte content of GSH and vitamin E) and inflammatory proteins (CRP, albumin).

Measuring the consequence of oxidative stress

- The endothelium is a source and also a major target of ROS. Measuring the haemodynamic response to Ach in the forearm is an accepted means of estimating endothelial function *in vivo*.
- In the presence of high generation of ROS, high dose vitamin C given intra-arterially produces a steeper dose–response, that could be taken as an index of ROS generation.

Reducing oxidative stress

- As oxidative stress and inflammation appear to be important in the pathogenesis of CVD in ESRD patients, anti-oxidant treatment strategies could be beneficial.
- Although anti-oxidant therapy with vitamin E and C has not been shown to reduce cardiovascular risk in the general population, the increased level of oxidative stress in CRI patients makes it an attractive approach; a recent study in dialysis patients points in this direction, but further well-designed clinical trials are recommended.
- The reduction in dialysis-induced oxidative mechanisms by biocompatible membranes and ultrapure dialysis fluids is desirable.

Acknowledgements. Francesco Locatelli, as chairman of the Accord programme, wrote the Introduction and oversaw the consistency of the manuscript edition of the Accord workshop. Other sections were by the following participants: Bernard Canaud: Oxidative stress: what it is and how to measure it. Kai-Uwe Eckardt: Oxidative stress and anti-oxidants in ESRD and beyond: reality and promises. Peter Stenvinkel: Inflammation and oxidative stress in end-stage renal disease: is there a link? Christoph Wanner: AGEs and cardiovascular damage in ESRD: hypotheses, experimental evidence and clinical observations. Carmine Zoccali: The endothelium, oxidative stress and cardiovascular damage in ESRD. We would like to thank Thorsten Brandt, Helen Wright and Marco D'Amico for their help in editing the manuscript.

Note added in proof

The article by Tepel *et al.* [50] suggests a beneficial effect of acetylcysteine (600 mg given orally BID) on composite cardiovascular events occurring in haemodialysis patients followed up for a median of 14.5 months. However, no effect was reported on total or cardiovascular mortality.

References

1. Luke RG. Chronic renal failure—a vasculopathic state. *N Engl J Med* 1998; 339: 841–843
2. Locatelli F, Marcelli D, Conte F *et al*. Cardiovascular disease in chronic renal failure: the challenge continues. *Nephrol Dial Transplant* 2000; 15 [Suppl 5]: S69–S80
3. Levey AS, Beto JA, Coronado BE *et al*. Controlling the epidemic of cardiovascular disease in chronic renal disease: what do we know? What do we need to learn? Where do we go from here? *Am J Kidney Dis* 1998; 32: 853–906
4. Brunner FP, Selwood NH on behalf of the EDTA Registry Committee. Profile of patients on RRT in Europe and death rates due to major causes of death groups. *Kidney Int* 1992; 42 [Suppl 38]: S4–S15
5. Locatelli F, Manzoni C, Del Vecchio L, Di Filippo S. Changes in the clinical condition of haemodialysis patients. *J Nephrol* 1999; 12 [Suppl 2]: S82–S91
6. Muntner PM, Coresh J, Klag MJ, Powe NR. Does improved myocardial infarction and stroke survival explain the increase in ESRD incidence? [abstract]. *J Am Soc Nephrol* 1999; 10: 175
7. Zoccali C. Cardiovascular risk in uraemic patients—is it fully explained by classical risk factors? *Nephrol Dial Transplant* 2000; 15: 454–457
8. Descamps-Latscha B, Drüeke T, Witko-Sarsat V. Dialysis-induced oxidative stress: biological aspects, clinical consequences, and therapy. *Semin Dial* 2001; 14: 193–199
9. Sies H. Oxidants and antioxidants. *Exp Physiol* 1997; 82: 291–295
10. Handelman GJ. Evaluation of oxidant stress in dialysis patients. *Blood Purif* 2000; 18: 343–349
11. Pryor WA. Oxy-radicals and related species: their formation, lifetimes and reactions. *Annu Rev Physiol* 1986; 48: 657–667

12. Canaud B, Cristol JP, Morena M, Leray-Moragues H, Bosc JY, Vaussenat F. Imbalance of oxidants and antioxidants in haemodialysis patients. *Blood Purif* 1999; 17: 99–106
13. Witko-Sarsat V, Friedlander M, Capeillere-Blandin C *et al*. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int* 1996; 49: 1304–1313
14. Tarng DC, Huang TP, Wei YH *et al*. 8-Hydroxy-2′-deoxyguanosine of leukocyte DNA as a marker of oxidative stress in chronic hemodialysis patients. *Am J Kidney Dis* 2000; 36: 934–944
15. Khoa T, Massy ZA, Witko-Sarsat V *et al*. Critical evaluation of plasma and LDL oxidant-trapping potential in hemodialysis patients. *Kidney Int* 1999; 56: 747–753
16. Nguyen A, Lethias C, Zingraff J, Herbelin A, Naret C, Descamps-Latscha B. Hemodialysis membrane-induced activation of phagocyte oxidative metabolism detected *in vivo* and *in vitro* within microamounts of whole blood. *Kidney Int* 1985; 28: 158–167
17. Descamps-Latscha B, Goldfarb B, Nguyen AT *et al*. Establishing the relationship between complement activation and stimulation of phagocyte oxidative metabolism in hemodialyzed patients: a randomized prospective study. *Nephron* 1991; 59: 279–285
18. Boaz M, Matas Z, Biro A *et al*. Serum malondialdehyde and prevalent cardiovascular disease in hemodialysis. *Kidney Int* 1999; 56: 1978–1983
19. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20 536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 2002; 360: 20–33
20. Stephens NG, Parsons A, Schofield PM *et al*. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet* 1996; 347: 781–786
21. Islam KN, O'Byrne D, Devaraj S, Palmer B, Grundy SM, Jialal I. Alpha-tocopherol supplementation decreases the oxidative susceptibility of LDL in renal failure patients on dialysis therapy. *Atherosclerosis* 2000; 150: 217–224
22. Boaz M, Smetana S, Weinstein T *et al*. Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomised placebo-controlled trial. *Lancet* 2000; 356: 1213–1218
23. Galli F, Rovidati S, Chiarantini L, Campus G, Canestrari F, Buoncristiani U. Bioreactivity and biocompatibility of a vitamin E-modified multi-layer hemodialysis filter. *Kidney Int* 1998; 54: 580–589
24. Miyazaki H, Matsuoka H, Itabe H *et al*. Hemodialysis impairs endothelial function via oxidative stress. Effects of vitamin E-coated dialyzer. *Circulation* 2000; 101: 1002–1006
25. Sommerburg O, Grune T, Hampl H *et al*. Does long-term treatment of renal anaemia with recombinant erythropoietin influence oxidative stress in haemodialysed patients? *Nephrol Dial Transplant* 1998; 13: 2583–2587
26. Memon RA, Staprans I, Noor M *et al*. Infection and inflammation induce LDL oxidation *in vivo*. *Arterioscler Thromb Vasc Biol* 2000; 20: 1536–1542
27. Nguyen-Khoa T, Massy ZA, De Bandt JP *et al*. Oxidative stress and haemodialysis: role of inflammation and duration of dialysis treatment. *Nephrol Dial Transplant* 2001; 16: 335–340
28. Handelman GJ, Walter MF, Adhikarla R *et al*. Elevated plasma F2-isoprostanes in patients on long-term hemodialysis. *Kidney Int* 2001; 59: 1960–1966
29. Mezzano D, Pais EO, Aranda E *et al*. Inflammation, not homocysteinemia, is related to oxidative stress and hemostatic and endothelial dysfunction in uremia. *Kidney Int* 2001; 60: 1844–1850
30. Witko-Sarsat V, Friedlander M, Khoa TN *et al*. Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. *J Immunol* 1998; 161: 2524–2532
31. Aby-Soud HM, Khassawneh MY, Sohn JT, Murray P, Haxhiu MA, Hazen SL. Peroxidases inhibit nitric oxide (NO)-dependent broncodilation: development of a model describing NO–peroxidase interactions. *Biochemistry* 2001; 40: 11866–11875
32. Zhang R, Brennan M-L, Fu X *et al*. Association between myeloperoxidase levels and risk of coronary artery disease. *J Am Med Assoc* 2001; 286: 2136–2142
33. Pecoitys-Filho R, Stenvinkel P, Marchlewska A *et al*. A functional variant of the myeloperoxidase gene is associated with cardiovascular disease in end-stage renal disease patients. *Kidney Int* in press
34. Jiang Q, Elson-Schwab I, Courtemanche C, Ames BN. Gamma-tocopherol and its major metabolite, in contrast to alpha tocopherol, inhibit cycloxygenase activity in macrophages and epithelial cells. *Proc Natl Acad Sci USA* 2000; 97: 11494–11499
35. Handelman GJ, Machlin LJ, Fitch K, Weiter JJ, Dratz EA. Oral alpha-tocopherol supplements decrease plasma gamma-tocopherol levels in humans. *J Nutr* 1985; 115: 807–813
36. Makita Z, Radoff S, Rayfield EJ *et al*. Advanced glycosylation end products in patients with diabetic nephropathy. *N Engl J Med* 1991; 325: 836–842
37. Weiss MF, Erhard P, Kader-Attia FA *et al*. Mechanisms for the formation of glycoxidation products in end-stage renal disease. *Kidney Int* 2000; 57: 2571–2585
38. Miyata T, Van Ypersele de Strihou C, Kurokowa K, Baynes JW. Alterations in nonenzymatic biochemistry in uremia: origin and significance of 'carbonyl stress' in long-term uremic complications. *Kidney Int* 1999; 55: 389–399
39. Schleicher ED, Wagner E, Nerlich AG. Increased accumulation of the glycoxidation product N(epsilon)-(carboxymethyl)lysine in human tissues in diabetes and aging. *J Clin Invest* 1997; 99: 457–468
40. Torzewski M, Rist C, Mortensen RF *et al*. C-reactive protein in the arterial intima: role of C-reactive protein receptor-dependent monocyte recruitment in atherogenesis. *Arterioscler Thromb Vasc Biol* 2000; 20: 2094–2099
41. Schwedler S, Schinzel R, Vaith P, Wanner C. Inflammation and advanced glycation end products in uremia: simple coexistence, potentiation or causal relationship? *Kidney Int* 2001; 59 [Suppl 78]: S32–S36
42. Zoccali C, Mallamaci F, Asahia K *et al*. Pentosidine, carotid atherosclerosis and alterations in left ventricular geometry in hemodialysis patients. *J Nephrol* 2001; 14: 293–298
43. Schwedler SB, Metzger T, Schinzel R, Wanner C. Advanced glycation end products and mortality in hemodialysis patients. *Kidney Int* 2002; 62: 301–310
44. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* 2001; 104: 2673–2678
45. Laursen JB, Somers M, Kurz S *et al*. Endothelial regulation of vasomotion in apoE-deficient mice: implications for interactions between peroxynitrite and tetrahydrobiopterin. *Circulation* 2001; 103: 1282–1288
46. Kielstein JT, Böger RH, Bode-Böger S *et al*. Asymmetric dimethylarginine plasma concentrations differ in patients with end stage renal disease: relationship to treatment method and atherosclerotic disease. *J Am Soc Nephrol* 1999; 10: 594–600
47. Zoccali C, Bode-Böger S, Mallamaci F *et al*. Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. *Lancet* 2001; 358: 2113–2117
48. Wever R, Boer P, Hijmering M *et al*. Nitric oxide production is reduced in patients with chronic renal failure. *Arterioscler Thromb Vasc Biol* 1999; 19: 1168–1172
49. Brown BG, Zhao XO, Chait A *et al*. Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N Engl J Med* 2001; 345: 1583–1592
50. Tepel M, van der Giet M, Statz M, Jankowski J, Zidek W. The antioxidant acetylcysteine reduces cardiovascular events in patients with end-stage renal failure: a randomized, controlled trial. *Circulation* 2003; 107: 992–995