Restoring glutathione as a therapeutic strategy in chronic kidney disease

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Glutathione: antioxidant and cell function regulator

The oxidation–reduction (redox) state of the pool of cellular thiols plays a central role in antioxidant defence and in the regulation of a large number of signal transduction pathways and metabolic functions [1]. The tripeptide glutathione (GSH), i.e. L-γ-glutamyl-L-cysteinyl-glycine (MW 307), represents the major low-molecular-mass thiol compound participating in cellular redox reactions and thio-ether formation. Under oxidative stress, GSH is oxidized to glutathione disulphide (GSSG) and further to other products such as sulphonates. Glutathione-cysteinyl disulphides can also be formed on proteins and such bound glutathione makes up a considerable amount of the cellular glutathione pool.

In January 2004, nearly 60 000 entries could be found under the term ‘glutathione’ in the Medline database, reflecting the importance of this biomolecule. Current knowledge concerning the regulation of mammalian glutathione synthesis is given in Griffith [2]. Briefly, GSH is synthesized from L-glutamate, L-cysteine and glycine in two consecutive steps, catalyzed, respectively, by γ-glutamyl-cysteine synthase and glutathione synthase. The redox reactions are catalyzed by GSH peroxidases (GSH-Px) and GSSG reductases (GSSG-Rd), whereas a major class of enzymes involved in thioether formation is given by the glutathione transferases (GST). Interestingly, GSH-Px activity has been extended to new functions, such as, for instance, peroxynitrite reduction, protection against apoptosis and sperm maturation, and at present glutathione is considered as the most important antioxidant *in vivo* [reviewed in 3].

Besides its antioxidant activity, glutathione has many physiological functions including detoxification of xenobiotics, modulation of redox-regulated signal transduction, storage and transport of cysteine, regulation of cell proliferation, synthesis of deoxyribonucleotide, regulation of immune response, and regulation of leukotriene and prostaglandin metabolism. GSH is able to increase the activation of cytotoxic T cells *in vivo*. The normal functioning of T

lymphocytes is dependent upon cellular supplies of cysteine. The cells acquire the amino acid largely by uptake of GSH by macrophages and lymphocytes. Impaired immune responses are associated with a reduction in the glutathione concentration of immune tissue [4].

Oxidative stress in chronic renal insufficiency

Compelling evidence has shown that oxidative stress, resulting from an imbalance between prooxidant and antioxidant systems in favour of the former, largely contributes to immune system dysregulation and complications observed in end-stage renal disease (ESRD) patients treated with haemodialysis, including β2-microglobulin amyloid arthropathy and accelerated atherosclerosis which are responsible for the high rate of morbidity and mortality in these patients [5–9]. Interestingly, oxidative stress markers have been found to be related to carotid intima media thickness as a reflection of the atherosclerosis process [10] and administration of the antioxidant N-acetyl-cyteine has been found to significantly reduce the incidence of cardiovascular events in these patients [11]. However, several lines of evidence have indicated that chronic renal insufficiency *per se* also induces a state of oxidative stress [12–16] which can be detected long before initiation of maintenance haemodialysis therapy and worsens regularly with renal failure progression [12,13].

On the pro-oxidant side, the salient observations can be summarized as follows: (i) blood interaction with extracorporeal dialysis circuits triggers the activation of circulating phagocytes (polymorphonuclear neutrophils and monocytes) mainly via both complement activation cascade products generated at the contact of bioincompatible dialysis membranes and endotoxin fragments issued from non-ultrapure dialysate; (ii) stimulation of the phagocyte NADPH-oxidase-dependent respiratory burst leads to the univalent reduction of molecular oxygen in the superoxide anion, which following the action of superoxide dismutase, gives rise to hydrogen peroxide and the cascade of reactive oxygen species (ROS); (iii) myeloperoxidase issued from the neutrophil degranulation process provokes the formation of long-lived chlorinated oxidants by catalyzing the reaction between hydrogen peroxide and chloride; and (iv) pro-inflammatory cytokines released by activated monocytes also contribute to enhanced ROS release and deleterious effects.

With regard to antioxidant deficiency, it is well established that: (i) the uraemic milieu *per se*induces a deficiency in enzymatic system constituents and cofactors (Zn^{2+}, Se^{2+}, Mg^{2+}); and (ii) this is further aggravated by the dialysis procedure which provokes a loss of major non-enzymatic antioxidant molecules such as vitamin C. The presence of increased plasma levels of oxidatively damaged lipid products (malonedialdehyde, thiobarbituric reactive substances, isoprostanes), DNA and proteins [advanced oxidation protein products (AOPP), chlorodityrosine] reflects the oxidative stress resulting from dialysis-induced oxidant burden and insufficient availability of antioxidants. Ideally, the circulating levels of these markers can serve to assess the quality of the haemodialysis procedure and allow the evaluation to follow the efficacy of antioxidant therapeutic strategies. However, recent findings that plasma levels of some oxidative stress markers are already elevated at an early stage of chronic kidney disease and regularly increase with its progression [13] in parallel with signs of neutrophil priming, support the hypothesis that uraemic toxicity *per se*contributes to the genesis

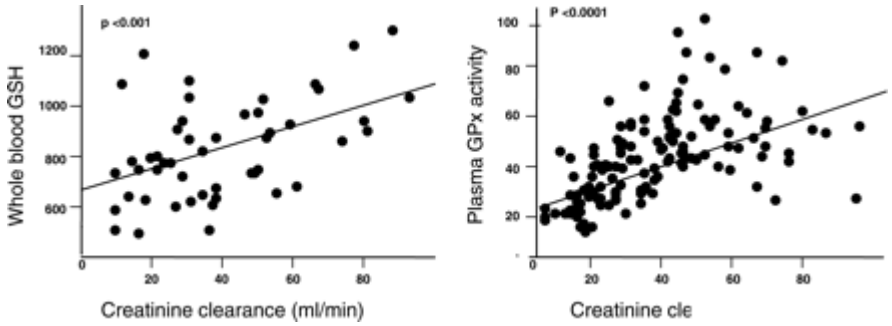
of oxidative stress [17], and that antioxidant therapy may also be recommended long before the start of renal replacement therapy.

Importance of the glutathione system in chronic renal insufficiency-associated oxidative stress

Despite some discrepant findings due to differences in dialyser biocompatibility, duration of haemodialysis, type of blood specimens tested (whole blood, plasma, erythrocytes or white blood cells) and, last but not least, measurement precision for glutathione system components, there is a general consensus that: (i) the activities of GSH-Px and GSSG-Rd are severely impaired in dialysis patients [12,15,18,19], both in erythrocytes and in plasma; (ii) glutathione concentration is dramatically decreased when measured in whole blood [18], whereas in erythrocytes it may either be found decreased [18,20], unchanged [21,22] or even increased [23] with extremely low levels in neutrophils; (iii) the glutathione redox GSSG/GSH ratio probably is a more informative marker both in dialyzed and not yet dialyzed patients [14,15], even though the generally observed marked deficit of GSH appears more relevant to disease.

Our previous study [12] performed in a large cohort of 233 uraemic patients including 185 undialyzed patients with mild to severe chronic renal failure, and 48 patients treated with peritoneal dialysis or haemodialysis, allows the general conclusion that the uraemic state is associated with low circulating levels of GSH and a decreased activity of GSH-Px, which both worsen with the progression of renal failure (Figure 1), and that these deficiencies are aggravated in dialysis patients. Figure 2 depicts the results of a recent compilation of published reports in which GSH and/or GSH-Px were measured in uraemic patients as well as in control subjects and could thus be expressed as percent variation in patients compared with controls. Because of space limitations, we cannot provide the totality of the references used for this review.

Fig. 1.



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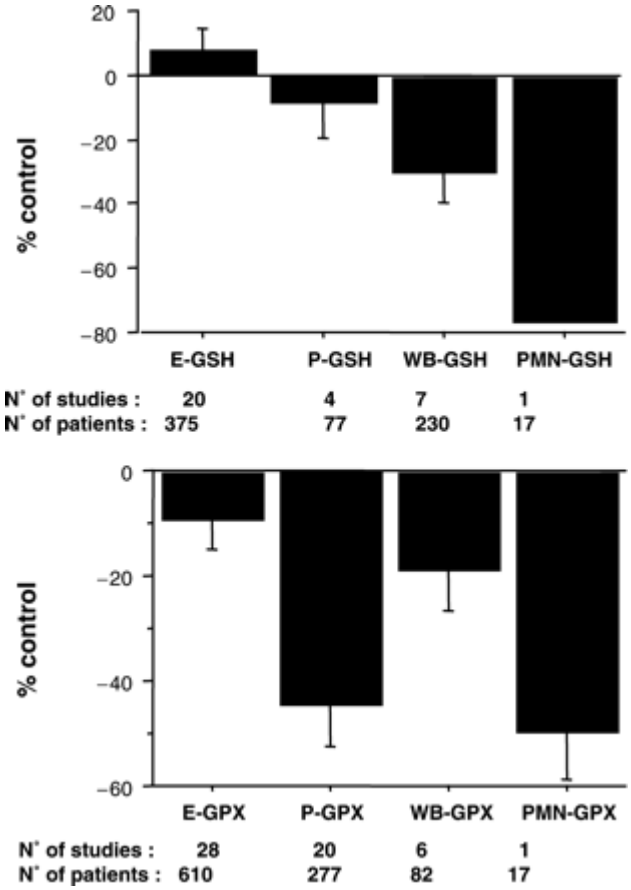
Regression analysis between progression chronic renal insufficiency (measured by creatinine clearance) and whole blood glutathione (GSH) (left panel) and plasma glutathione peroxidase (GSH-Px) (right panel) concentrations (figure adapted from ref. 12).

Fig. 2.



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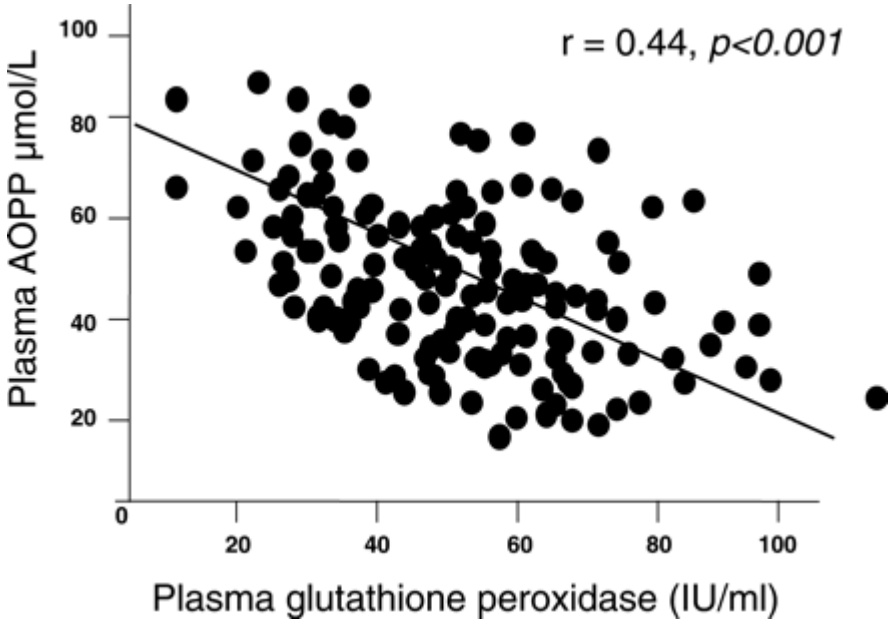
Glutathione (GSH) and glutathione peroxidase (GSH-Px) concentrations in erythrocytes (E), plasma (P), whole blood (WB) and polymorphonuclear neutrophils (PMN) in uraemic patients. Data issued from a total of 51 papers of the literature are expressed as % variation of controls (mean ± SD).

Interestingly, we also found that plasma AOPP levels in uraemic patients were inversely related to GSH-Px activities (Figure 3), corroborating the validity of AOPP as a relevant oxidative stress marker.

Fig. 3.



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Relationship between plasma AOPP and GSH-Px plasma levels in 153 chronic renal failure patients (BDL and VWS, unpublished data).

Glutathione repletion in dialysis patients

Cysteine supply

Evidence for a beneficial effect of glutathione repletion in dialysis patients is still fragmentary and often indirect. The first possible interpretation of the reason of low blood GSH levels has been the hypothesis of an impaired GSH synthesis, although plasma levels of cysteine are unexpectedly elevated in comparison with healthy subjects. On the other hand, it has been established that cysteine is the main compound and limiting product in GSH synthesis. The administration to peritoneal dialysis patients of L-2-oxothiazolidine-4-carboxylic acid, a cysteine prodrug which has been investigated in a blinded, placebo-controlled study [24], resulted in a significant rise in whole-blood glutathione levels at days 7 and 14 compared with baseline, in contrast to the placebo group. Glutathione was also significantly increased if normalized by haematocrit or haemoglobin to correct for anaemia status and returned to baseline at follow-up. More recently, in a pilot study conducted in six haemodialysis patients, a significant increase in predialysis whole blood GSH was observed following the IV infusion of 1 g of *N*-acetylcysteine during 12 dialysis sessions, followed by 4 weeks of wash-out and 2 g of *N*-acetylcysteine during 12 dialysis sessions [25].

The effect of parentally administered reduced glutathione [26] on anaemia status has been examined in 20 chronic haemodialysis patients. It is known that GSH infused to healthy subjects is catabolized fast to the three constituent amino acids. When reduced glutathione (1.2 g) or placebo were given to haemodialysis patients in a randomized double-blind fashion for 120 days, there was an increase in reduced glutathione levels in both RBC and plasma and an increase in haemoglobin, with a concomitant decrease in plasma oxidized glutathione and reticulocyte count in the GSH-treated

alone group, but not in the placebo-treated group. The most marked effect was observed on the 120th day of therapy. After the interruption of GSH treatment, the above parameters returned to pre-treatment values. Similar findings have been reported in another trial employing similar doses of reduced GSH [27]. After the first 3 months of treatment, anaemia improved significantly in 17 patients (60%), as long as they were undergoing therapy, but dropped rapidly to pre-treatment values when GSH was discontinued. Taken together these studies support the hypothesis that GSH deficiency is due to insufficient supply and/or biotransformation of cysteine.

During continuous ambulatory peritoneal dialysis the peritoneal immune cells, mainly macrophages, are highly compromised by multiple factors, including oxidative stress, and this results in a loss of functional activity and an imbalance in the thiol-disulphide status. The treatment of peritoneal macrophages with flavonoids alone or in combination with N-acetylcysteine as a cysteine donor resulted in a shortened and more efficient time course of thiol normalization as well as in a further increase in phagocytosis [28]. Besides the stabilization of cellular thiol status, this treatment also significantly improved phagocytosis and the degree of macrophage maturation and led to significant changes in IL-6 and IL-1ra synthesis, supporting the important role of thiol restoration in the regulation of pro-inflammatory functions.

The successful treatment with *N*-acetylcysteine of two cases of haemodialysis-associated pseudoporphyria [29] shows another positive effect of thiol administration. The cutaneous lesions rapidly healed in both patients after the start of oral *N*-acetylcysteine treatment. Furthermore, the transient discontinuation of this medication in one of the two patients led to a recurrence of cutaneous blistering. Such data support the hypothesis that ROS may contribute to oxidation-dependent skin damage in haemodialysis patients.

Restoring the thiol pool—a worthwhile therapeutic strategy?

Given the widespread role of the thiol pool in key cellular functions and the pathogenesis of various disease states, a clinical benefit can be expected following restoration of thiol levels [30]. In haemodialysis patients, a therapeutic advantage may be foreseen by reducing the stimulated activity of the immune system, expressed as an activation of leukocytes and lymphocytes and, notably, an increase in circulating oxidative stress markers as well as an imbalance between inflammatory cytokines and their inhibitors [31]. Consequently, a benefit in terms of oxidative-stress-related complications of ESRD patients can be expected. Many pieces of information are already available, demonstrating that the administration of antioxidants contributes to an improvement of anaemia despite either no change or even a decrease in erythropoietin needs. Recently, Usberti *et al.* [32] reported that a combination of a vitamin E-bonded dialysis membrane and glutathione infusion achieved better control of anaemia than a vitamin E-bounded membrane alone, with a significant saving of erythropoietin. Finally, recent pilot studies and therapeutic trials in haemodialysis patients are also in support of a reduction of cardiovascular complications, following the administration of, respectively, vitamin E [33] and oral *N*-acetylcysteine [11], which both may act through the thiol restoration pathway.

The possibility that repletion of thiols will not prevent the increased oxygen radical production associated with uraemia, but may actually mitigate its effect by providing adequate scavenging capacity would support the development of therapeutic trials associating, at least in one therapeutic arm, vitamin E and/or other, as yet unavailable reactive oxygen scavengers. If such therapeutic protocols of thiol restoration are developed in uraemic patients, either alone or combined with another antioxidant, both a clinical endpoint, such as the occurrence of cardiovascular events, and levels of oxidative stress and inflammatory markers, such as CRP, fibrinogen and AOPP, could be used to check their efficacy.

Conclusion

The reality of oxidative stress, defined as a rupture between pro-oxidant and anti-oxidant systems, has been established in chronic renal insufficiency patients. To date, it is considered a major player in uraemia-associated morbidity and mortality. Therapeutic approaches aimed at an upregulation of intracellular thiol concentration, which controls key molecular mechanisms of cell life, have already led to the demonstration of promising beneficial effects in terms of cardiovascular complications and anaemia correction. They deserve to be further validated in both predialysis and dialysis patients.

References

1 Sies H. Glutathione and its role in cellular functions. *Free Radic Biol Med* 1999; 29: 916–921
Google Scholar

2 Griffith OW. Biologic and pharmacologic regulation of mammalian glutathione synthesis. *Free Radic Biol Med* 1999; 27: 922–935
Google Scholar

3 Brigelius-Flohé R. Tissue-specific functions of individual glutathione peroxidases. *Free Radic Biol Med* 1999; 27: 951–963
Google Scholar

4 Droge W, Eck HP, Gmunder H, Mihm S. Modulation of lymphocyte functions and immune responses by cysteine and cysteine derivatives. *Am J Med* 1991; 91: 140S–144S
Google Scholar

5 Canaud B, Cristol J, Morena M *et al.* Imbalance of oxidants and antioxidants in haemodialysis patients. *Blood Purif* 1999; 17: 99–106
Google Scholar

6 Galli F, Canestrari F, Buoncristiani U. Biological effects of oxidant stress in haemodialysis: the possible roles of vitamin E. *Blood Purif* 1999; 17: 79–94
Google Scholar

7 Handelman GJ. Evaluation of oxidant stress in dialysis patients. *Blood Purif* 2000; 18: 343–349
Google Scholar

8 Himmelfarb J, Stenvinkel P, Ikizler TA, Hakim RM. The elephant in uremia: oxidant stress as a unifying concept of cardiovascular disease in uremia. *Kidney Int* 2002; 62: 1524–1538
Google Scholar

9 Locatelli F, Canaud B, Eckardt KU *et al.* Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. *Nephrol Dial Transplant* 2003; 18: 1272–1280
Google Scholar

10 Drüeke T, Witko-Sarsat V, Massy Z *et al.* Iron therapy, advanced oxidation protein products, and carotid artery intima-media thickness in end-stage renal disease. *Circulation* 2002; 106: 2212–2217
Google Scholar

11 Tepel M, van der Giet M, Statz M *et al.* The antioxidant acetylcysteine reduces cardiovascular events in patients with end-stage renal failure: a randomized, controlled trial. *Circulation* 2003; 107: 992–995
Google Scholar

12 Ceballos-Picot I, Witko-Sarsat V, Merad-Boudia M *et al.* Glutathione antioxidant system as a marker of oxidative stress in chronic renal failure. *Free Radic Biol Med* 1996; 21: 845–853
Google Scholar

13 Witko-Sarsat V, Friedlander M, Nguyen Khoa T *et al.* Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. *J Immunol* 1998; 161: 2524–2532
Google Scholar

14 Annuk M, Fellstrom B, Akerblom O *et al.* Oxidative stress markers in pre-uremic patients. *Clin Nephrol* 2001; 56: 308–314
Google Scholar

15 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
Google Scholar

16 Tarng DC, Wen Chen T, Huang TP *et al.* Increased oxidative damage to peripheral blood leukocyte DNA in chronic peritoneal dialysis patients. *J Am Soc Nephrol* 2002; 13: 1321–1330
Google Scholar

17 Vanholder R, Argiles A, Baurmeister U *et al.* Uremic toxicity: present state of the art. *Int J Artif Organs* 2001; 24: 695–725
Google Scholar

18 Ross EA, Koo LC, Moberly JB. Low whole blood and erythrocyte levels of glutathione in hemodialysis and peritoneal dialysis. *Am J Kidney Dis* 1997; 30: 489–494
Google Scholar

19 Schettler V, Wieland E, Methe H *et al.* Oxidative stress during dialysis: effect on free radical scavenging enzyme (FRSE) activities and glutathione (GSH) concentration in granulocytes. *Nephrol Dial Transplant* 1998; 13: 2588–2593
Google Scholar

20 Galli F, Rovidati S, Benedetti S *et al.* Overexpression of erythrocyte glutathione S-transferase in uremia and dialysis. *Clin Chem* 1999; 45: 1781–1788
Google Scholar

21 Daschner M, Lenhartz H, Botticher D *et al.* Influence of dialysis on plasma lipid peroxidation products and antioxidant levels. *Kidney Int* 1996; 50: 1268–1272
Google Scholar

22 Suliman ME, Divino Filho JC, Barany P *et al.* Effects of high-dose folic acid and pyridoxine on plasma and erythrocyte sulfur amino acids in hemodialysis patients. *J Am Soc Nephrol* 1999; 10: 1287–1296
Google Scholar

23 Cristol JP, Bosc JY, Badiou S *et al.* Erythropoietin and oxidative stress in haemodialysis: beneficial effects of vitamin E supplementation. *Nephrol Dial Transplant* 1997; 12: 2312–2317

Google Scholar

24 Moberly JB, Logan J, Borum PR *et al.* Elevation of whole-blood glutathione in peritoneal dialysis patients by L-2-oxothiazolidine-4-carboxylate, a cysteine prodrug (Procysteine). *J Am Soc Nephrol* 1998; 9: 1093–1099

Google Scholar

25 Martino C, Gianello P, Manfredini P *et al.* Antioxidant activity of N-acetylcysteine in patients undergoing haemodialysis. In *Free Radicals, Nitric oxide and Inflammation: Molecular, Biochemical and Clinical Aspects* . Antalya, Turkey, 2001.

Google Scholar

26 Costagliola C, Romano L, Scibelli G *et al.* Anemia and chronic renal failure: a therapeutical approach by reduced glutathione parenteral administration. *Nephron* 1992; 61: 404–408

Google Scholar

27 Usberti M, Lima G, Arisi M *et al.*: Effect of exogenous reduced glutathione on the survival of red blood cells in hemodialyzed patients. *J Nephrol* 1997; 10: 261–265

Google Scholar

28 Tager M, Dietzmann J, Thiel U *et al.* Restoration of the cellular thiol status of peritoneal macrophages from CAPD patients by the flavonoids silibinin and silymarin. *Free Radic Res* 2001; 34: 137–151

Google Scholar

29 Vadoud-Seyedi J, De Dobbeleer G, Simonart T. Treatment of haemodialysis-associated pseudoporphyria with *N*-acetylcysteine: report of two cases. *Brit J Dermatol* 2000; 142: 580–581

Google Scholar

30 Santangelo F. Intracellular thiol level concentration modulating inflammatory response: influence of the regulation of cell function through cysteine prdrug approach. *Curr Med Chem* 2003; 10: 2599–2610

Google Scholar

31 Descamps-Latscha B, Drüeke T, Witko-Sarsat W. Dialysis-induced oxidative stress: biological aspects, clinical consequences and therapy. *Semin Dial* 2001; 14: 193–199

Google Scholar

32 Usberti M, Gerardi G, Micheli A *et al.* Effects of a vitamin E-bonded membrane and of glutathione on anemia and erythropoietin requirements in hemodialysis patients. *J Nephrol* 2002; 15: 558–564

Google Scholar

33 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

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