Inflammatory proteins as predictors of cardiovascular disease in patients with end-stage renal disease

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Abstract
Patients with end-stage renal disease (ESRD) are at high risk from potentially devastating cardiovascular sequelae due to the unique clustering of risk factors in these patients. Inflammation is believed to play a key role in the pathogenesis of these cardiovascular lesions. Both pro- and anti-inflammatory cytokines produced from monocytes, and also from adipocytes, have been studied in this regard. Pro-inflammatory cytokines, although cytoprotective acutely, correlate with increased risk of cardiovascular disease (CVD) in chronic situations. Conversely, elevated levels of anti-inflammatory mediators are associated with increased patient survival times. Statistical modelling, calculation of relative risk and cost considerations indicate that determination of serum C-reactive protein levels may be a useful predictor of CVD in ESRD patients. Adipocytes are a rich source of many of the same cytokines produced by monocytes, including interleukin-6, tumour necrosis factor-α, as well as adipocyte-specific proteins, leptin and adiponectin (ADPN). ADPN, which is produced in much greater quantities than leptin, is inversely related to body mass index and to insulin resistance, suggesting a possible role in type 2 diabetes. Additionally, ADPN has been shown to modulate the endothelial inflammatory response in vitro. Plasma ADPN levels are an inverse predictor of cardiovascular outcomes among patients with ESRD. Furthermore, ADPN is related to several metabolic risk factors in a manner consistent with the hypothesis that this protein acts as a protective factor for the cardiovascular system.

Keywords: adipocytes; cardiovascular disease; cytokines; end-stage renal disease; inflammation; predictors; risk; cardiovascular; uraemia

Introduction
Haemodialysis (HD) patients are at increased risk from cardiovascular disease (CVD) compared with the general population. The potentially life-threatening cardiovascular sequelae of uraemia are manifest as vascular changes, such as tortuosity, dilation and calcification of arteries, and cardiac lesions, including coronary artery thrombosis and calcification of heart valves. The clustering of several risk factors in end-stage renal disease (ESRD) patients places them at unique risk. Risk factors can be grouped as follows: (i) traditional risk factors, e.g., age, sex, hypertension, hypercholesterolaemia, smoking and diabetes; (ii) factors unique to ESRD patients, such as anaemia and elevated calcium–phosphorus product (Ca × P); and (iii) 'emerging' risk factors, including inflammation, hyperhomocysteinaemia and the accumulation of endogenous inhibitors of nitric oxide synthase, such as asymmetric dimethylarginine (ADMA). Although most of these factors confer an independent risk of cardiovascular damage, it is believed that their combined actions are integrated in the progression of atherosclerosis, with inflammation playing a central role.

The most widely accepted explanation for the pathogenesis of the initial stages of atherosclerosis in ESRD patients is the Ross theory [1]. According to this theory, any insult or risk factor affecting the vascular endothelium will induce endothelial activation, characterized by the expression of E-selectin and adhesion molecules, including intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Monocytes and macrophages are attracted to the altered endothelium and subsequently infiltrate the vessel wall to initiate the inflammatory process. These monocytes produce both pro- and anti-inflammatory cytokines. The pro-inflammatory cytokines interleukin-1β (IL-1β), IL-6 and tumour necrosis...
factor-$\alpha$ (TNF-$\alpha$) induce hepatic synthesis of the acute-phase reactant, C-reactive protein (CRP). CRP is not only a cardiovascular risk marker but is also a direct risk factor itself. Another acute-phase reactant, fibrinogen, is produced when an IL-6-sensitive sequence in the fibrinogen gene promoter is activated. It is the production of fibrinogen that links inflammation to clot formation. Anti-inflammatory cytokines produced by monocytes include IL-10, IL-4 and transforming growth factor-$\beta$ (TGF-$\beta$). Adipocytes represent an additional, though less widely recognized, source of both pro- and anti-inflammatory cytokines. Here, we examine the protective and detrimental effects of pro-inflammatory cytokines in acute and chronic situations, respectively, and the protective effect of anti-inflammatory cytokines in relation to the cardiovascular system. The usefulness of cytokines in predicting CVD in ESRD patients is also discussed.

### Protective effects of pro-inflammatory cytokines in acute situations

The role of pro-inflammatory cytokines as immunological mediators is just one facet of their more global function as intercellular messengers co-ordinating multiple, complex cellular responses. For example, in the cardiovascular system, pro-inflammatory cytokines are upregulated in conditions of pressure overload, ischaemia, hypertrophy and congestive heart failure.

From an evolutionary perspective, the cytokine response has been very highly conserved, indicating that it confers a survival advantage. This protective function is demonstrated in the control of oxidative stress, where cytokines induce the synthesis of superoxide dismutase, a crucial system for scavenging free radicals. Cytokines also induce anti-apoptotic genes encoding proteins of the Bcl-2 family. The protective effect of pro-inflammatory cytokines in acute conditions has been demonstrated experimentally. In a murine model of cardiac ischaemia, myocardial infarction following coronary artery occlusion was far more extensive in TNF receptor knockout mice than in wild-type controls [2].

### Detrimental effects of pro-inflammatory cytokines in chronic situations

Sustained elevations of pro-inflammatory cytokines have deleterious effects on the cardiovascular system. The potential for TNF-$\alpha$ to induce cardiac hypertrophy has been demonstrated by observing the effect of exposure of adult cardiac myocytes to TNF-$\alpha$ in vitro [3]. Cardiac myocyte proliferation, measured by phenylalanine incorporation, was increased when TNF-$\alpha$ was added to the medium. Further, this proliferation was blocked by addition of anti-TNF-$\alpha$ antibodies (Figure 1). The effect of TNF-$\alpha$ on cardiac remodelling in vivo has been elegantly investigated using transgenic mice with cardiac-restricted overexpression of TNF-$\alpha$ [4]. Compared with age-matched, wild-type controls, transgenic mice showed an enlarged and dilated left ventricle. Other potentially cardiotoxic cytokines have been identified in a community-based sample where elderly patients, without prior myocardial infarction, were observed over 7 years [5]. The results of this study showed that patients with elevated initial levels of IL-6

![Fig. 1. Effect of TNF-$\alpha$ on rates of general protein synthesis in adherent myocytes. PHE = phenylalanine. Reproduced, with permission, from Yokoyama et al. [3].](image)
Using cytokine levels to predict CVD in ESRD patients

The previous observations suggest that determination of serum cytokine levels might be useful for predicting survival in HD patients. A study examining the relationship between cytokines and mortality was published in 1998 [6]. This study was based on a cohort of 233 patients, recruited over 3 years, from 10 haemodialysis (HD) centres in Washington and Bethesda, USA. The independent relative risk associated with the increase of several pro-inflammatory cytokines was calculated. To allow comparison between cytokines, each was expressed as the increase in plasma concentration in terms of standard deviation (SD). The risk associated with a 1 SD increase in plasma IL-1β was ~1.7, a risk slightly higher than that of IL-6 and TNF-α. The anti-inflammatory cytokine IL-4 was also studied. The relative risk reduction associated with a 1 SD increase in the plasma concentration of IL-4 was ~0.7, which coincides with a risk reduction of ~30%. The predictive power of IL-6 for mortality has been confirmed independently [7]. In this study, a significant difference in mortality rate was observed when patients were divided into quartiles according to IL-6 concentration. The 5 year survival was 100% in the first quartile, i.e. in patients with relatively low levels of IL-6. This figure fell to 40% in patients with the highest IL-6 levels.

The inflammatory marker on which most attention has been focused in ESRD patients is CRP. A direct relationship between CRP level and the severity of carotid atherosclerosis, as assessed by the number of atherosclerotic plaques, was demonstrated in 1998 [8]. This was followed by the first demonstration of the predictive value of CRP for both overall death and cardiovascular death in HD patients [9]. Zimmermann found that survival rates were ~95% in patients with low levels of CRP but decreased to 65% in patients in the highest CRP quartile. The finding that CRP is a strong predictor of survival has been confirmed in a study of 663 European patients [10] (Figure 3).

Investigation of the acute-phase reactant fibrinogen has shown that over half (56%) of a group of HD patients had elevated levels of plasma fibrinogen (>350 mg/dl) [11]. Moreover, fibrinogen was ~200 mg/dl higher in uraemic patients compared with healthy subjects. Elevated fibrinogen is potentially harmful not only because it induces clot formation, but also because, at higher concentrations, denser clots are formed, limiting the access of fibrinolytic enzymes. It has been calculated that, taking into account other risk factors such as age, previous cardiovascular events, diabetes, CRP and homocysteine levels, a 200 mg/dl elevation in fibrinogen levels (i.e. the difference between the average normal value and the average value found in HD patients) translates to an independent increased risk of 50%.

Conversely, although less extensively studied, a protective effect for anti-inflammatory cytokines has been demonstrated. Quantitative production of IL-10 is subject to genetic variation on the basis of polymorph-
isms in the promoter of the IL-10 gene. In a prospective study of 300 HD patients, the allele associated with low production of IL-10, −1082A*, was predictive for a higher cardiovascular morbidity [12]. Similar results were demonstrated in a separate study looking at TGF-β, where levels of this cytokine were significantly reduced in HD patients, in particular in those with severe CVD [13].

Selecting pro-inflammatory cytokines for measurement in clinical practice

Several complementary approaches have been used to determine which of the candidate pro-inflammatory cytokines demonstrates the greatest prognostic utility. To address this question, we have used a statistical modelling technique using data from the Cardiovascular Risk Extended Evaluation in Dialysis patients (CREED) database. Those risk factors independently associated with mortality were identified from all potential risk factors affecting HD patients. These factors [age, previous cardiovascular events, asymmetric dimethyl arginine (ADMA) levels, treatment modality and cholesterol level] were used to produce a basic model. Six models were then created using the basic model, by adding one of the following cytokines: IL-6, IL-18, CRP, IL-1β, TNF-α and fibrinogen. When these models were ranked according to their statistical strength in predicting mortality (−2 log likelihood statistics), the model based on IL-6 was found to be the best ($P < 0.01$).

A complementary approach to select an appropriate clinical marker is calculating the risk associated with a standard increase in each cytokine. On comparison, it emerged that the relative risk associated with being in the highest quartile of IL-6 was similar to that of being in the highest quartile of IL-18, or in the highest quartile of serum CRP. Thus, the risk estimate obtained by measuring serum CRP was similar to that obtained by using IL-6. Because the cost of measuring IL-6 is about four times that of measuring CRP, measurement of CRP emerges as a reasonable, cost-effective option in clinical practice.

Cytokine production by adipocytes

Adipose tissue produces a variety of cytokines including IL-6, TNF-α, leptin and the recently discovered protein adiponectin (ADPN). Leptin and ADPN are produced almost exclusively in adipose tissue. ADPN, a protein of the collectin family, has a plasma concentration 1000 times higher than that of leptin, at $\sim 5 \mu g/ml$ in healthy individuals. Interestingly, ADPN is inversely, rather than directly, related to fat mass and to insulin levels, i.e. higher insulin levels and insulin resistance correlate with lower ADPN levels. This observation suggests that low levels of ADPN may herald, or even trigger, type 2 diabetes [14]. In the rhesus monkey Macaca mulatta, an animal that spontaneously develops obesity and type 2 diabetes, longitudinal observations have clearly established that plasma ADPN declines in parallel with insulin sensitivity and remains low once the animal has developed diabetes [15].

ADPN attenuates endothelial inflammatory responses in vitro [16]. In an adhesion assay, low levels of monocytes spontaneously adhered to endothelial cells. This process was amplified by the addition of TNF-α, because this cytokine induces the expression of adhesion molecules by endothelial cells. The addition of ADPN to the medium reversed the effect of TNF-α in a dose-dependent manner. This study...
also showed that, in humans, plasma ADPN concentration is lower in patients with severe coronary artery disease than in healthy individuals, an effect that was equally evident in men and women. These data support the idea that ADPN behaves as an anti-inflammatory cytokine.

We investigated the clinical and biochemical correlates of plasma ADPN levels. Plasma ADPN levels were 2.5 times higher among dialysis patients than among healthy patients [17]. ADPN concentration in dialysis patients was inversely related to body mass index values, plasma leptin levels, insulin levels and insulin sensitivity. Furthermore, plasma ADPN levels were directly related to the level of the protective lipoprotein, high-density lipoprotein cholesterol, but inversely related to the level of serum triglyceride. Although it might be expected that ADPN, an anti-inflammatory protein, would be inversely related to CRP, a recognized marker of inflammation, no such relationship was found. Whether the apparently independent variability of ADPN and CRP in this study indicates true biological independence is not yet known.

The predictive power of ADPN levels with respect to survival rates and cardiovascular events was tested prospectively in a cohort of 227 HD patients [17]. Overall, high ADPN levels were associated with longer survival. At the end of the monitoring period (31±13 months), 70% of patients with high ADPN levels (i.e. ADPN in the highest tertile) remained free of cardiovascular events, while the proportion of event-free patients in the lowest ADPN tertile was only 54%. The difference between the highest and the lowest tertile was ~5 µg/ml (Figure 4). When adjusted for traditional and non-traditional risk factors, this translated to a risk increase of 19% for a decrease in ADPN level of 5 µg/ml. Thus, ADPN has a potentially protective role in HD patients.

**Conclusion**

Inflammation is a fundamental protective response in acute situations. However, sustained inflammatory processes may induce severe cardiovascular damage.

Inflammatory cytokines interact in a complex way in patients with cardiovascular damage and ESRD. Some cytokines demonstrate pro-inflammatory effects, whereas others are anti-inflammatory.

The plasma concentration of cytokines predicts cardiovascular mortality in patients with ESRD. CRP is a reasonably precise and cost-effective risk marker in these patients.

Adipose tissue is an important source of cytokines and the most abundant protein synthesized in fat cells, ADPN, is an inverse predictor of cardiovascular events.

**Conflict of interest statement.** None declared.
References

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