Review

INFLAMMATION AND ENDOTHELIAL DYSFUNCTION IN THE INITIATION AND PROPAGATION OF CARDIOVASCULAR DISEASE IN PATIENTS WITH CHRONIC KIDNEY DISEASE

ABSTRACT

Background: There are many different theories on atherosclerosis pathophysiology. The dominant one is endothelial function disorder resulting from the existence of risk factors such as dyslipidemia, diabetes, smoking, high blood pressure, and hyperhomocysteinemia bacterial and viral infections. The inflammation is an important parameter for CKD appearance and evolution, too. In this review we will summarise the most recent evidence that inflammation and endothelial dysfunction are implicated in the enhanced cardiovascular risk experienced by individuals with CKD, we will not discuss the role of dialysis or transplantation in the propagation of cardiovascular risk.

Literature review: Electronic medical databases were searched using as key-words the terms: “atherosclerosis”, “hemodialysis patient”, “end stage renal disease”, “Chronic Inflammation”, “Endothelial Dysfunction”. The search was conducted in English language. All studies referred to the correlation of the key terms were included and highlight the Inflammation and Endothelial Dysfunction in the Initiation and Propagation of Cardiovascular Disease in patients with Chronic Kidney Disease.

Conclusions: The presence of enhanced CV risk in patients with CKD is well known but the mechanisms by which it occurs are less clear. The endothelium is a complex, multi-functional organ with a variety of vascular homeostatic functions and ED has been shown to result in the initiation and propagation of atherosclerosis. Causes of ED are numerous but inflammation and oxidative stress are clearly highly implicated; albuminuria (even at levels thought to be well below previous definitions of abnormal) may contribute to the inflammatory process, as might dyslipidaemia (though a combination of traditional and no-traditional pathways).

Key-words: atherosclerosis, hemodialysis patient, end stage renal disease, chronic inflammation, endothelial dysfunction.

Introduction

Cardiovascular disease (CVD) results from the formation of occlusive atherosclerotic plaques in the circulation, while it was previously thought that such plaques were inert collections of lipids and fibrous tissue it is now accepted that the atherosclerotic process is one in which inflammation is implicated [1]. It has also been observed that individuals with Chronic Kidney Disease (CKD) are at increased risk of cardiovascular events and while shared traditional risk factors such as diabetes and hypertension explain only in part the increased
cardiovascular risk. It has been proposed that the inflammatory response, through the
initiation and propagation of endothelial dysfunction, may be a cause and consequence of
both CVD and CKD. In this review we will summarise the most recent evidence that
inflammation and endothelial dysfunction are implicated in the enhanced cardiovascular risk
experienced by individuals with CKD. However, we will not discuss the role of dialysis or
transplantation in the propagation of cardiovascular risk.

**Inflammation as a promoter of endothelial dysfunction**

The endothelium is a single layer of cells that separates the vessel wall from the blood
stream. It is now accepted that far from being an inert barrier the endothelium has a number
of vital homeostatic roles. In response to potential damage the endothelium is frequently
activated as a protective response, the result of this activation is reduced vessel dilation and
increased adhesion of leukocytes and platelets. Prolonged activation of the endothelium can
be described as endothelial dysfunction (ED), as the normal homeostatic functions of the
endothelium are disrupted [2].

The relationship between ED and inflammation is complex. In the ‘response to injury’
hypothesis, inflammation is one of several factors which can contribute to ED, and in turn ED
results in atherosclerosis and inflammation. As a result inflammation is an important factor
that not only initiates ED but also propagates and is a consequence of ED [3-5]. In a number
of animal models signs of inflammation are seen in tandem with lipid accumulation in the
vessel wall, leukocytes have been found to localise in early plaques and while the healthy
endothelium does not usually support the binding of these cells, experimental models have
shown that they can be encouraged to do so in the context of an atherogenic diet [6]. When
leukocytes have bound to the endothelium they have then been shown to invade the intima
via a number of chemoattractant molecules, such as Monocyte Chemoattractant protein-1
(MCP-1). The leucocytes are then able to participate in and perpetuate a local inflammatory
response [7-9]. Because of its location measurement of endothelial function was traditionally
complex and involved invasive methodology, however recent advances have lead to a
number of different methods of assessing endothelial function, these have been described in a
recent detailed review by Lekakis [10] and are summarised in Table 1. They include, flow
mediated dilatation (FMD), pulse wave analysis, arterial tonometry and a variety of
biomarkers.

### Table 1: Methods of measuring endothelial function in the clinic

<table>
<thead>
<tr>
<th>Technique</th>
<th>Description</th>
<th>Shortcomings</th>
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<tbody>
<tr>
<td>Direct coronary measurement</td>
<td>Coronary angiography with pharmacological stimuli to assess vasodilation [11]</td>
<td>Invasive</td>
</tr>
<tr>
<td>MRI/PET coronary imaging</td>
<td>Allows quantification of myocardial function and microvascular function [12]</td>
<td>Expensive</td>
</tr>
<tr>
<td>Venous occlusion plethysmography</td>
<td>Measurement of muscular blood flow by assessment of tissue volume change induced by the inflation of a cuff proximally [13]</td>
<td>Invasive</td>
</tr>
<tr>
<td>Flow mediated dilation</td>
<td>Vessels are imaged after induced hyperaemia and diameter measured before and after removal of hyperaemia [14]</td>
<td>Significant inter and intra operator variability</td>
</tr>
</tbody>
</table>
Pulse wave analysis
Non-invasive arterial waveform imaging to measure the augmentation index (the difference between the 1st and 2nd systolic peak)
Little data available yet on relationship with treatment and clinical outcomes

Peripheral artery tonometry
After induced hyperaemia the digital pulse wave amplitude is measured
Yet to be validated in large cohorts

Chronic Kidney Disease and endothelial dysfunction
It is well described that individuals with CKD are at enhanced cardiovascular risk [16]. While a number of traditional cardiovascular risk factors may co-exist in individuals with CKD (for example diabetes, hypertension and hyperlipidaemia) it is clear that not all the cardiovascular risk experienced by these individuals can be attributed to these risk factors [17,18]. An explanation for this is that there are non-traditional or novel risk factors, for the development of CVD in CKD that have not previously been considered. These proposed novel risk factors include albuminuria, anaemia, inflammation, abnormal calcium/phosphate metabolism, oxidative stress, malnutrition and ED (which may be the consequence as well as the cause of a number of the other novel risk factors). We will now focus on several significant putative risk factors for ED in CKD; albuminuria, decreased nitric oxide activation, dyslipidaemia and oxidative stress.

Albuminuria and endothelial dysfunction in CKD
Albuminuria has been associated with cardiovascular diseases in both diabetic and non-diabetic patients; the association has been shown to be independent of traditional risk factors such as smoking and hypertension [19-21]. This association has been seen when (microalbuminuria is present) and the pathophysiological basis of the association is not clearly understood. It has been suggested that rather than being a causative association, albuminuria reflects generalised ED, itself a cardiovascular risk factor [22]. As the renal endothelium influences the glomerular capillary barrier it is plausible that renal ED may be involved in the development of albuminuria [23]. To explore the associations a number of studies have been conducted. Stehouwer and others [23] hypothesised that ED and chronic inflammation explained the association between microalbuminuria and mortality; to address this they followed 328 type 2 diabetics for a mean of 9 years (using von Willebrand factor (vWF), soluble E selectin and soluble vascular cell adhesion to assess ED, and C-reactive protein and fibrinogen as markers of inflammation). They found that individuals with markers of both ED and inflammation were at increased risk of death, the presence of such markers were also strongly associated with the development of, and increases in, urinary albumin excretion during follow up; traditional risk factors were also associated with increases in markers of both ED and inflammation. As a result of their findings they concluded that traditional risk factors may contribute to ED and inflammation and microalbuminuria and consequently increase cardiovascular risk.

Another study to explore the relationship between ED and albuminuria involved 94 diabetic subjects, again with vWF being used as a measure of ED; patients were divided into groups dependent upon their baseline urinary albumin excretion and were followed up for 9 up to 53 months. Outcomes related to urinary albumin excretion, cardiovascular event rates and death were collected. The results demonstrated that there was a relationship between increased urinary albumin excretion, cardiovascular events and ED in patients with type 2 diabetes, ED was strongly related to the development of microalbuminuria and the occurrence of cardiovascular events.

UNDER PEER REVIEW
These studies suggest that albuminuria and ED are intimately related and that the relationship is complex, ED being both a potential initiator and propagator of albuminuria, but in addition that albuminuria also serves as a marker of ED.

**Nitric Oxide and ED in CKD**

Oxidative stress is the imbalance between the production of reactive oxygen species and their clearance, such imbalance results in free radical and peroxide production which result in cellular damage [25]. In combination with these reactive oxygen species and free radicals nitric oxide (NO) contributes to the atherosclerotic process involving the endothelium, with reduced NO bioavailability being associated with increased cardiovascular risk [24-27]. It is not clear by what mechanism this effect takes places, it is possible that reduced NO production (via decreased NO synthase (NOS), itself a consequence of the NOS inhibitor asymmetric Dimethylarginine (ADMA), decreased availability of the NOS substrate L-arginine or increased concentration of oxygen radical species that inactivate NO may explain the reduced bioavailability of NO [28-32].

Wever and others [33] hypothesised that NO production was reduced in individuals with CKD, in a group of 33 patients (7 of whom had CKD, 7 had familial hypercholesterolaemia, 14 healthy controls and 5 healthy smokers) they measured whole body NO production by giving an infusion of $^{15}$N$_2$-arginine and then measured isotopic plasma enrichment of $^{15}$N-citrulline. They found that whole body NO production was significantly lower in patients with CKD than healthy controls, it was also lower in those with familial hypercholesterolaemia than healthy controls though this did not reach significance.

The formation of advanced glycation end products (AGEs) in response to oxidative stress have been implicated as inhibitors of NOS, as AGEs are known to accumulate in CKD. Linden et al hypothesised that AGE excess in CKD resulted in ED via reduced NOS [34,35]. In a cross-sectional study of patients with various stages of CKD and a group of matched healthy controls AGEs were measured in serum and Laser Doppler was used to measure microcirculatory blood flow in hyperaemia. They found that individuals with CKD had increased circulating AGEs and decreased endothelial reactivity; they also found that AGE-rich sera from individuals with CKD inhibited NOS expression; from these findings they concluded that AGEs are influential in the pathogenesis of CVD in patients with CKD. In animal work Vaziri and others [36] tested the hypothesis that CKD results in oxidative stress via NO inactivation which could be ameliorated by anti-oxidant treatment. By performing either sham nephrectomy or nephrectomy on male rats and feeding them either an anti-oxidant rich diet or a normal diet and then measuring a variety of markers of NOS activity. They found that CKD (in the rats who had undergone 5/6 nephrectomy) was associated with decreased tissue NO production and reduced NOS proteins in the renal and cardiac tissues, the anti-oxidant therapy resulted in improved tissue NO production in the CKD subjects. From these findings it is suggested that CKD might result in oxidative stress which in turn results in NO inactivation and that anti-oxidant therapy might increase NO availability, however these findings have not been reproduced in human.

**Dyslipidaemia and oxidative stress and ED in CKD**

Dyslipidaemia is a traditional risk factor for CVD but is increasingly considered as also being a non-traditional risk factor that results in ED, it has been reported that ED was independently related to dyslipidaemia in type 2 diabetics [37].The size of lipid particles and their susceptibility to oxidation has been proposed as a mechanism for ED, in a study of patients with type 2 diabetes and a control group, the diabetic patients had a greater concentration of smaller, dense particles and the rate of oxidation was also greater [38]. When endothelial
function was measured using a brachial artery vasodilation method; these changes were associated with ED.

In a study to investigate the role of chronic versus acute hyperlipidaemia in ED de Man and others gave a group of patients with dyslipidaemia treatment with high dose Atorvastatin and a group with normolipidaemia a high dose infusion of artificial triglycerides and then measured endothelial function using forearm blood flow response [39]. They found that in the patients with chronic dyslipidaemia had evidence of ED that was normalised after 6 weeks of high dose statin treatment; artificially induced dyslipidaemia did not alter the endothelial function of the control subjects, from these findings the authors concluded that only chronic dyslipidaemia results in ED.

Other studies to investigate the potential benefits of the treatment of dyslipidaemia in the setting of ED have been carried out; in a study of Fenofibtrate versus Atorvastatin it was found that both drugs improved endothelial function, with no significant difference between them, the beneficial effects were independent of lipid lowering [40]. Another study by Hamasaki and others [41] considered the effect of cholesterol lowering therapy on vascular re-modelling and endothelial function in patients with normal or mildly diseased coronary arteries. They found that patients who had successful treatment of dyslipidaemia had an increase in the vessel lumen area which was related to both reduction in atherosclerotic plaque size but also to increase in vessel lumen related to vascular re-modelling, the authors suggested that this re-modelling may arise as a result of improved endothelial function.

Lipid oxidation has been implicated in ED via a mechanism of the release of soluble cell adhesion molecules (CAMs) and vWF. In order to evaluate the relationship between lipid oxidation and the inflammatory state in CKD Bolton et al conducted a cross-sectional study of individuals with CKD and angina and a group of healthy controls [42]. They measured a variety of adhesion molecules, vWF, circulating levels of cytokines and CRP; endothelial function was assessed using a forearm FMD technique. In contrast to other research they found that ED was not related to lipid oxidation and concluded that the role of lipid oxidation in the development of ED had been previously overstated. In fact ED was more severe in patients with CKD than angina and was associated with increased acute phase proteins and cytokines.

Other work has considered the role that inflammation plays in the initiation and propagation of ED, in CKD there is an increased plasma concentration of cytokines and chemokines (resulting from increased production and decreased clearance). Oberg and others [43] measured a number of biomarkers of inflammation and oxidative stress (CRP, IL-6, plasma protein free carbonyl group content and plasma free F2-isoprostane content) in a group of patients with CKD 3-5 and a group of matched healthy controls. They found that there was evidence of oxidative stress and inflammation in patients with CKD but that this did not correlate with degree of renal impairment, there was a correlation between CVD and inflammation in patients with CKD and that there was an inverse correlation with angiotensin blocker and statin use in this group, oxidative stress was present in those individuals with diabetes and dyslipidaemia as has previously been shown.

Strategies for intervention in relation to reduction of inflammation/ED in CKD

Given that inflammation and ED appear to have a significant role in the initiation and propagation of CV disease, strategies to reduce inflammation and ED would be potentially very beneficial. However apart from the management of traditional risk factors (control of hypertension, diabetes, smoking cessation and lipid lowering) there is little evidence for other interventions (though as we have seen there is overlap between traditional and novel risk factors).
factors with many traditional risk factors having their effect via an inflammatory pathway).

Table 2 summarises some potential targets for intervention, however much of the evidence for these targets comes from dialysis populations which are not analogous to CKD populations.

### Table 2: Targets for intervention to reduce inflammation in CKD

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Example</th>
<th>Proposed mechanism of action</th>
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<tbody>
<tr>
<td>Renal Angiotensin Aldosterone blockade</td>
<td>ACEi/ARB</td>
<td>By reducing proteinuria these agents have been shown to improve both cardiovascular and renal outcomes beyond the blood pressure lowering effect [44-47]</td>
</tr>
<tr>
<td>Anti-oxidants</td>
<td>Vitamin E, Acetylcysteine</td>
<td>Vitamin E is a potent anti-oxidant with anti inflammatory properties and has been shown in patients on dialysis to improve cardiovascular outcomes [48] Acetylcysteine is another anti-oxidant that may reduce pro-inflammatory cytokine release [49]</td>
</tr>
<tr>
<td>Treatment of periodontal disease</td>
<td>D Paricalcitol</td>
<td>Periodontal disease is prevalent in patients with CKD and has been postulated as a driver of chronic inflammation and endothelial dysfunction though no large randomised control trials have been conducted in patients with CKD Vitamin D deficiency thought to have haemodynamic and pro-inflammatory effects, paricalcitol has been shown to reduce inflammation and improve cardiovascular end point in patients on dialysis (thought there was no improvement in endothelial function) [50]</td>
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</tbody>
</table>

**Conclusions**

The presence of enhanced CV risk in patients with CKD is well known but the mechanisms by which it occurs are less clear. The endothelium is a complex, multi-functional organ with a variety of vascular homeostatic functions and ED has been shown to result in the initiation and propagation of atherosclerosis. Causes of ED are numerous but inflammation and oxidative stress are clearly highly implicated; albuminuria (even at levels thought to be well below previous definitions of abnormal) may contribute to the inflammatory process, as might dyslipidaemia (though a combination of traditional and no-traditional pathways).

There are a number of limitations to many of the studies described here, many of the measures of endothelial function are putative, invasive or suffer from poor reproducibility, and many biomarkers of inflammation such as cytokines are unstable and difficult to measure in routine clinical practice. While statin use and angiotensin blockade has been shown to improve endothelial function in some studies no randomised controlled trials have been conducted with the specific aim of trying to demonstrate an improvement in endothelial function resulting from one treatment intervention or another. An additional limitation is that many of these interventions have known and well understood effects on traditional risk factors as well as on some non-traditional inflammatory risk factors. Thus documenting would be challenging. The future direction of research in this area is likely to require such studies to take place to result in patient benefit from what is currently a very interesting and promising area.
REFERENCES


44. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, et al. Effects of Losartan on Renal and Cardiovascular Outcomes in Patients with Type 2


