A NEW PLAYER IN CAROTID ARTERY DISEASE IN DIALYSIS PATIENTS: THE GENETIC POLYMORPHISM OF THE ENDOTOXIN RECEPTOR CD 14

Attilio Losito, Renal Unit, Policlinico Monteluce, 06123 Perugia, Italy

Introduction

Cardiovascular risk in patients with end-stage renal disease (ESRD) on dialytic treatment is much higher than in the general population [1]. Early research pointed to accelerated atherosclerosis as responsible for the cardiovascular morbidity and mortality in long-term maintenance hemodialysis [2]. Although the so-called conventional risk factors such as hypertension, dyslipidemia, diabetes mellitus, and smoking operate also in dialysis patients, they do not fully account for the magnitude of the atherosclerotic process in this particular population. Therefore, recently a great deal of research has been addressed to risk factors that are particularly present in ESRD or in dialytic treatment. Among others, inflammation is now regarded as one of the primary and most important mechanisms in atherogenesis [3]. In hemodialysis patients an activated acute phase response, revealed by elevated C-reactive protein, has been shown to be associated with high cardiovascular death rate [4]. Proinflammatory cytokines, such IL-6, have been frequently found to be elevated in hemodialysis patients [5]. The association of high levels of IL-6 with carotid atherosclerosis has been considered a confirmation of the role of inflammation in the atherosclerotic process in dialysis [6]. This cytokine seems to be at the center of a vicious circle that comprises inflammation, malnutrition, and cardiovascular disease [7]. Studies on genetic polymorphisms of IL-6 in hemodialysis patients also support its role in cardiovascular disease of ESRD [8]. Chronic hemodialysis should be regarded as a complex state where procedure-related and disease factors coexist. In this setting other proinflammatory substances with potential atherogenic properties may play a role.

Endotoxin and sCD14

The hemodialytic procedure is associated with an exchange of substances through membranes of different degrees of permeability. Years ago passage of bacterial endotoxin, from clinically used
dialysate to the blood, through dialytic membranes of different types, was demonstrated [9]. In the general population raised concentrations of endotoxin are found in association with heart failure suggesting that endotoxin may trigger immune activation during edematous episodes [10]. Other research have shown that circulating endotoxin provokes severe endothelial dysfunction and may be an important atherogenic factor [11]. Strong support to this hypothesis has been provided by a large epidemiological study on the general population. In the study, plasma endotoxin levels were measured and carotid disease was assessed simultaneously. The results showed that subjects with high levels of circulating endotoxin had a high risk of carotid atherosclerosis [12]. The atherosclerotic risk was higher in smoker and ex-smokers.

CD14 is a pattern recognition receptor, on the membranes of monocytes and macrophages, for bacteria produced endotoxin. A soluble form of CD14 (sCD14) is present in serum, whose role is the control of the bioactivity of circulating endotoxin: high levels of sCD14 enhance the blood clearance of endotoxin [13]. CD14, after binding with bacterial lipopolysaccharides evokes the endothelial activation and the secretion of proinflammatory cytokines, followed by a complex cascade of events that eventually leads to the atherosclerotic lesion. The final effect of this mechanism is on the vulnerability of the atherosclerotic plaque. The sCD14 is therefore at the crossroads between infection and inflammation [14]. The levels of circulating sCD14 are regulated by a genetic-environmental interaction. A recently discovered polymorphism (-59C/T) in the gene of CD14 has been shown to control the levels of circulating sCD14 in interaction with infections and bacterial contamination [15]. A recent report has in fact described the association between this polymorphism and coronary artery disease (CAD) patients chronically infected with C. pneumoniae [16]. Subsequent research on the association of this polymorphism and cardiovascular disease has produced variable and sometimes conflicting results. In some cases an association of this polymorphism with cardiovascular disease was found, while other studies did not confirm this association [17, 18]. Unfortunately most of the reported studies are of the cross-sectional type and the indices of disease examined differ substantially. Another explanation of these controversial results may lie with the genetic-environmental interaction. In fact, identifying genetic variants as a single cause of cardiovascular disease would be an oversimplification. Instead, the contribution of these polymorphisms should also be considered in the context of nongenetic risk factors (e.g. smoking, diabetes, hyperlipidemia) and the examined patients populations. In the general population an interesting relationship has been found between smoking habits, carotid atherosclerosis, and the CC genotype of this polymorphism [19]. Since endotoxemia is a mediator of inflammation, and smokers have elevated plasma levels of endotoxin, carriers of the CC genotype may be at risk of atherosclerosis because the reduced clearance of endotoxin associated with the C allele of the -159C/T polymorphism. It has also been suggested that this polymorphism is a marker for smoking dependence with all its consequences [20]. Therefore the possible role of this polymorphism is to be searched not in the general population, but in subsets of patients exposed at a genetic-environmental interaction. Patients chronically treated with hemodialysis may be an example.

**Polymorphism of sCD14, Hemodialysis, and Carotid Disease**

Carotid disease is found frequently in ESRD and has been associated with a poor outcome. Risk factors for carotid atherosclerosis have been related to complications of ESRD, mostly linked to chronic inflammation or to abnormal levels of toxic mediators [21, 22]. A genetic enhancement
of the toxic effect of angiotensin II associated with ESRD has been also suggested [23]. The population of dialysis patients with chronic exposure to exogenous endotoxin represents a model in which to verify the genetic-environmental interaction and its effect on the atherosclerotic process. In patients with ESRD who are undergoing chronic hemodialysis, increased serum levels of sCD14 have been found, possibly as a response to chronic exposure to trace amounts of endotoxin [24]. The rise of sCD14 levels were observed during acute or chronic infections and are the effect of the combination between immune response and a functional genetic trait. Carriers of the CC genotype of the -159C/T polymorphism not only produce lower levels of sCD14 as immune response compared to TT carriers but also present lower baseline serum levels [25]. This finding confirms the functional nature of this genetic polymorphism. The theoretical consequence is that carriers of CC genotype have slower clearance of bacterial endotoxin, and therefore are more exposed to the toxic effects of this substance. Indirect evidence that endotoxin may be a cause of carotid disease in ESRD, comes from the finding of an association between smoking and atherosclerosis in dialysis patients [26]. A putative link between smoking, endotoxemia, and atherosclerosis is the CD14 receptor, and in turn its polymorphism [19]. The association of this polymorphism with carotid atherosclerosis and cardiovascular mortality has been investigated in a cohort of ESRD patients. The multivariate analysis showed that age, smoking, and the -159C/T polymorphism were independent predictors of carotid disease [27]. Carriers of the CC genotype had a relative risk of 8.5 compared to the TT (95% CI = 1.554 – 83.950). In the longitudinal part of the study the association of cardiovascular mortality with different risk factors was assessed. The Cox analysis produced a model with the following predictors: age, serum albumin, hypertension, and CD14 polymorphism. All these selected variables are known risk factors for cardiovascular disease in ESRD, the new one is the CD14 polymorphism. The results of this study confirm that performing genetic association studies in the search of a single genetic cause of cardiovascular disease may be an impossible task [28]. The analysis of genetic-environment interaction with functional polymorphisms in selected populations may instead be more meaningful. It may allow insights into the pathogenetic putative mechanism. This type of study, in selected populations, may also contribute to design specifically aimed at therapeutic interventions.

References