Soluble CD40 ligand is predictive of combined cardiovascular morbidity and mortality in patients on haemodialysis at a relatively short-term follow-up

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Abstract

Background. We tested the hypothesis that soluble CD40 ligand (sCD40L), a biomarker of proatherogenic inflammation, may be predictive of cardiovascular (CV) events in a subgroup of patients from the RISCAVID study, an observational and prospective study in patients on haemodialysis (HD).

Methods. Plasma sCD40L levels were assessed at the time of the enrollment in 300 HD patients (mean age: 65 ± 15 years), recruited in five different centres. During a follow-up of 24 months, overall mortality, CV mortality and CV major nonfatal events (acute myocardial infarction, congestive heart failure and stroke) were registered. Cox proportional hazards regression assessed adjusted differences in CV morbidity and mortality risk.

Results. Stratifying patients according to plasma sCD40L levels in those with levels lower or equal to (sCD40L\textsubscript{≤}) and greater than (sCD40L\textsubscript{>}) the median value of 7.6 ng/mL, no significant difference was observed at baseline between the two groups in age, gender, blood pressure values and previous CV events. At 24-month follow-up, a significant (P < 0.01) lower incidence of the combined end point of CV morbidity and mortality was observed in the sCD40L\textsubscript{≤} group (29%) as compared to the sCD40L\textsubscript{>} group (36%). In the multivariate Cox proportional hazards regression model, the presence of sCD40L above the median value is associated with a significant increase in the risk of CV morbidity and mortality (hazard ratio: 1.61, 95% confidence interval 1.03–3.11).

Conclusions. These observational results support the prognostic value of sCD40L in end-stage renal disease, thus providing a useful tool to better stratify CV prognosis in these patients.

Keywords: atherosclerosis; cardiovascular disease; ESRD; inflammation

Introduction

Chronic kidney disease is associated with substantially increased risk for cardiovascular (CV) disease morbidity and mortality independent of traditional CV risk factor [1–3].
Patients on haemodialysis (HD) are characterized by an annual CV mortality ~10%, 10- to 20-fold greater than in the general population [4]. It has been recently proposed that enhanced vascular inflammation might represent the pathophysiological background underlying the increased susceptibility of patients in HD to develop CV events [5–7].

In this regard, it is now generally accepted that CD40–CD40 ligand interaction is a main determinant of the proatherogenic phenotype [8, 9]. Originally identified in B and T lymphocytes as being involved in T-cell-dependent B-cell activation and differentiation, the CD40–CD40 ligand system has been implicated in the pathophysiology of several chronic inflammatory diseases including risk factor-related vascular damage [8]. CD40, a 50-kDa integral membrane protein of the tumour necrosis factor receptor family, and its cognate agonist CD40 ligand, also known as CD154, a transmembrane 39-kDa protein structurally related to tumour necrosis factor-alpha, are co-expressed by several cells of the vasculature, including endothelial cells, smooth muscle cells and macrophages [10]. CD40 ligand also occurs in a soluble form (sCD40L) that is considered to possess a full biological activity [11]. Increased sCD40L levels have been described in obesity [12], hypercholesterolaemia [13], diabetes [14, 15] and unstable angina [16]. Furthermore, it has been recently reported that circulating sCD40L has a strong independent prognostic value among apparently healthy individuals [17] and patients with acute coronary syndromes [18] and represents an independent predictor of restenosis after percutaneous transluminal angioplasty [19]. Thus, the clinical association between sCD40L and CV events suggests that sCD40L function spans the time interval from early atherogenesis to late thrombotic complications.

According to this, Hocher et al. [20] recently demonstrated during a follow-up period of 52 months that sCD40L is an independent predictor of atherothrombotic events in patients on HD. Herein, we expand on this topic demonstrating that this prognostic value of sCD40L is evident also at 24-month follow-up, further confirming the strong link between sCD40L and clinical outcomes in patients in HD.

Materials and methods

Setting

A prospective observational study (RISCAVID, ‘Rischio Cardiovascolare nei pazienti afferenti all’Area Vasta In Dialisi’) was started with the aim to investigate the link between chronic inflammation and morbidity in a large and homogenous HD population of the north-west Tuscany, including 757 prevalent HD patients on 1235 062 inhabitants and 15 dialysis facilities. Five centres agreed to participate in the present study aiming to investigate the possible predictive role of circulating sCD40L levels on CV morbidity and mortality rates in 300 HD patients during a follow-up of 24 months.

The protocol was in conformity to the ethical guidelines of our institutions, and informed consent was obtained from each participant. All the evaluations were performed during a midweek non-dialysis day.

Patients

Three hundred patients with end-stage renal disease (ESRD) (54% males) who had been on regular HD for at least 3 months and with a dialytic vintage of 46 months (range: 3–377 months) were enrolled in the study. All patients were anuric (24-h diuresis < 300 mL) and were on thrice weekly HD. No further selection criteria were adopted to enroll study participants. The patients’ dry weight was targeted to achieve a normotensive oedema-free state. Two hundred and forty-seven patients had arteriovenous fistula, 23 had a subcutaneous polytetrafluoroethylene graft and 30 a semipermanent tunnelling access.

Chronic renal failure was caused by primary and secondary glomerulonephritis (24%), congenital or hereditary kidney disease (12%), diabetic nephropathy (14%), chronic pyelonephritis (9%), vascular nephropathies (17%) or interstitial nephritis (3%), and the remainder had renal failure of uncertain etiology (21%).

Atherosclerotic disease was ascertained according to the following criteria: serial 12-lead electrocardiogram evidence or Q-wave infarction and appropriate myocardial enzyme elevations; coronary revascularization including coronary artery bypass surgery or percutaneous transluminal coronary angioplasty; typical history of angina with abnormal coronarography; neurological examination findings consistent with new onset focal neurological deficits, with or without computed tomography or magnetic resonance imaging evidence of cerebral infarction; symptomatic extracranial artery stenosis resulting in carotid endarterectomy; abdominal aortic or lower extremity arterial disease: abdominal aortic repair; lower extremity revascularization via bypass surgery or angioplasty; lower extremity amputation; new onset of intermittent claudication confirmed by Doppler or angiography findings. Two physicians independent of the study were responsible for the clinical ascertainment. This analysis was performed without knowledge of baseline characteristics.

Diabetes was defined by the use of insulin or oral hypoglycaemic agents. Furthermore, data regarding smoking status, body mass index, blood pressure, use of antihypertensive medications, calcium, phosphate, serum intact parathormone levels, albumin, total serum cholesterol, haemoglobin, use of erythropoetin, Kt/V and type of membranes used were recorded.

**HD modalities**

Standard low or high-flux bicarbonate HD was performed using either synthetic low-flux (Fresenius Medical Care, Bad Homburg, Germany) or high-flux polysulfone membranes (from 1.4 to 1.6 m², Fresenius Medical Care), polyamide (Gambro), cellulose-modified membranes and polymeric-thacrylate. Haemodiafiltration (HDF) was performed using high-flux polysulfone (from 1.8 to 2.0 m², Fresenius Medical Care) or polyamide using commercially available sterile bicarbonate bags (10–15 L/session) or performed as acetate-free biofiltration. On-line HDF was performed by the on-line production of ultrapure bicarbonate-buffered dialysate (22–25 L/session). In all centres, analysis of the dialysis system was customary and revealed absence of bacteria (<100 colony forming Units/mm) or bacteriological contaminant products (endotoxin levels < 0.025 endotoxin units).

**Follow-up**

The occurrence of morbid or fatal events was recorded at every 6-month interval or a total of 24 months of follow-up by five physicians who periodically visited each centre. During the first visit, a questionnaire addressed ESRD history, medical and psychosocial history, dialysis prescription, laboratory data and prescribed medications at the time of study enrollment of all prevalent patients. In the following visits, overall and CV mortality, major non-fatal CV events (myocardial infarction, congestive heart failure, stroke and sudden death), dialysis prescription and medication were recorded.

**Laboratory measurements**

Blood sampling was performed after an overnight fast between 7:00 a.m. and 9:00 a.m. always during a midweek non-dialysis day. After 20–30 min of quiet resting in semirecumbent position, samples were taken into chilled EDTA vacutainers, placed immediately on ice and centrifuged within 30 min at –4°C, and the plasma was stored at –80°C before assay. Serum lipids, albumin, calcium, phosphate and haemoglobin measurements were made using standard methods in the routine clinical laboratory. Plasma sCD40L levels were measured by immunoenzymatic methods (R&D systems, Minneapolis, MN). Serum albumin and C-reactive protein (CRP) were centrally determined in the Immunopathology Laboratory of the Internal Medicine Department of the University of Pisa. Serum albumin was measured with a nephelometric technique (Dade Behring GmbH, Marburg, Germany) with an intra- and inter-assay variability of 4.3 and 4.4%, respectively. CRP was measured by a high-sensitivity-modified laser nephelometry technique (Berhing Diagnostics GmbH, Barburg, Germany). The CRP assay was standardized according to the WHO First
International Reference Standard and had a sensitivity of 0.1 mcg/mL, with a standard reference range of between 0.1 and 0.4 mg/L.

**Statistical analysis**

Fisher’s exact test and Mann–Whitney test were used to compare proportions and means, respectively. The cumulative probability of survival from the entry in the study to the events was estimated by the product-limit (Kaplan–Meier) method. The log-rank test was used to compare the homogeneity of survival functions across strata defined by binary transformation of prognostic variables. In the regression analysis, the explanatory variables were recorded to binary variables.

Cox’s proportional hazard model was also used to compute multivariate-adjusted relative risk estimates and 95% confidence intervals (CIs) including the most important confounders. Results are expressed as mean and standard deviation and median (range, as appropriate). All test were considered significant with $P < 0.05$.

**Results**

All patients enrolled in the study were followed prospectively for 24 months. Clinical characteristics of the study population are reported in Table 1. Patients were relatively old, non obese, with a wide range of HD duration. Extracorporeal substitutive treatment, haematological and calcium/phosphorus balance were adequately controlled (Table 1).

The median plasma sCD40L concentration was 7.6 (interquartile range 3.9–12.0) ng/mL (Figure 1). Patients were stratified according to plasma sCD40L levels in those with levels below or equal to ($\leq$) and upon (>) the median value of 7.6 ng/mL. At baseline, no significant differences were observed in age, gender distribution, BMI, HD modalities or vintage or received single-pool extracorporeal substitutive treatment (Table 1). Underlying renal diseases and concomitant pharmacological treatment were similar in the two groups. Phosphate levels and calcium/phosphate product were higher in sCD40L$>$ than sCD40L$\leq$ patients. The two groups did not differ for the incidence of previous CV events or risk factors such as smoking history, diabetes mellitus, hypertension and dyslipidaemia (Table 1).

A total of 79 (26.5%) participants died within the 24-month follow-up (35 patients of CV disease and 44 of other causes (infections, malignances hyperkalaemia and gastrointestinal bleeding).
At 24-month follow-up, 69 patients experienced a nonfatal CV event. A significantly (P < 0.01) higher incidence of the combined end point of CV morbidity and mortality was observed in the sCD40L+ group as compared to sCD40L− group (Table 2, Figure 2). The calculated relative difference in the combined end point was 28%.

CV events and total mortality did not significantly differ between the two groups (Table 2). In the multivariate Cox proportional hazards regression model, including all possible demographic, inflammatory, HD-related confounders and comorbidity, with the exclusion of hypertension and calcium × phosphate product, which were related to sCD40L levels (P = 0.04 and P = 0.01, respectively), the presence of previous CV disease, diabetes mellitus and sCD40L above the median value (hazard ratio: 1.61, 95% CI 1.03–3.11, Table 3) was associated with a significant increase in the risk of the combined end point of CV morbidity and mortality but not of the separate end points or total mortality.

Age, CRP, albumin and HD modalities were not predictive of the combined CV end point, while they proved to be significant predictors of total mortality (age, P = 0.001; CRP, P = 0.04; albumin P = 0.03; HDF, P = 0.01).

**Discussion**

sCD40L has been recently proposed as a prognostic factor in patients who have ESRD and are on HD because of its ability to predict nonfatal and fatal atherothrombotic events in a 4-year follow-up study (20). Our data further expand findings from this quoted study demonstrating that the prognostic value of sCD40L is already evident at 24-month follow-up thus reinforcing the strong link between sCD40L and clinical outcomes in patients on HD and suggesting a possible clinical use of this biomarker to better define CV prognosis in these patients. In this regard, the CV risk profile of our study population was similar to that of the population studied by Hocher et al. [20] with the only relevant exception of more prevalent previous CV events in our study population (46% versus 27%). In addition, the rate of fatal CV events during follow-up was quite similar in the two studies (52.6% of the participants died of CV disease within the 52 months versus 26.3% within 24 months). These similarities make the two studies comparable and the results complementary leading together to the first identification of a biomarker potentially suitable in a clinical setting.

The strong impact of sCD40L levels on CV prognosis might have relevant clinical implications. Indeed, the evidence that patients with circulating sCD40L levels exceeding the cutoff value of 7.6 mg/dL, a discriminating value similar to that identified by Hocher et al. [20] but 2- to 3-fold higher than mean plasma concentrations observable in healthy subjects [12], might allow a better identification patients with poor CV prognosis thus needing a tight clinical follow-up. In addition, the evidence that long-term statin treatment is able to reduce increased sCD40L levels in patients with ESRD, at least in those peritoneally dialysed [21], might contribute to explaining the benefits deriving from statin treatment in uraemic patients [22] and might help to better determine the response to a preventive approach.

As far as the biological significance of our findings, it is generally accepted that CD40–CD40L interaction is an initial event in atherothrombosis, leading in turn to the activation of several pro-inflammatory mediators [23]. In fact, CD40L may promote the expression of vessel-remodelling metalloproteinases and induce pro-coagulant activity in vascular cells, and the absence of CD40L affects the stability of arterial thrombi and delays arterial occlusion in vivo [23]. Moreover, ligation of CD40 on endothelial cells up-regulates monocyte chemoattractant protein-1, soluble E-selectin, soluble vascular cell adhesion molecule-1 and soluble intercellular adhesion molecule-1 [24], all

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**Table 2.** Cumulative events at 24-month follow-up in patients on HD according to the level of sCD40L (median value 7.6 ng/mL)*

<table>
<thead>
<tr>
<th>End point (n, %)</th>
<th>Patients with sCD40L &gt; median</th>
<th>Patients with sCD40L ≤ median</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite end point*</td>
<td>54 (36%)</td>
<td>44 (29%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Nonfatal CV events</td>
<td>38 (25%)</td>
<td>31 (20%)</td>
<td>0.21</td>
</tr>
<tr>
<td>CV mortality</td>
<td>21 (14%)</td>
<td>17 (11%)</td>
<td>0.14</td>
</tr>
<tr>
<td>Total mortality</td>
<td>42 (27%)</td>
<td>38 (25%)</td>
<td>0.32</td>
</tr>
</tbody>
</table>

*Combined end point: nonfatal and fatal CV events.

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**Table 3.** Cox proportional hazards analysis of factors predicting the combined end point of CV morbidity and mortality in patients on HD

<table>
<thead>
<tr>
<th>Factor</th>
<th>Hazard ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>1.33 (0.47–2.18)</td>
<td>0.42</td>
</tr>
<tr>
<td>Age &gt;65 years</td>
<td>1.83 (1.10–3.04)</td>
<td>0.004</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.44 (0.71–3.33)</td>
<td>0.19</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>1.48 (0.73–3.04)</td>
<td>0.28</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2.67 (1.43–5.67)</td>
<td>0.001</td>
</tr>
<tr>
<td>Previous CV event</td>
<td>1.94 (1.04–3.81)</td>
<td>0.01</td>
</tr>
<tr>
<td>sCD40L &gt; 7.6 mg/dL</td>
<td>1.61 (1.03–3.11)</td>
<td>0.04</td>
</tr>
<tr>
<td>CRP &gt;4.6 mg/dL</td>
<td>1.17 (0.59–2.34)</td>
<td>0.41</td>
</tr>
<tr>
<td>HDF</td>
<td>0.72 (0.35–1.56)</td>
<td>0.33</td>
</tr>
<tr>
<td>HD duration &gt;48 months</td>
<td>0.91 (0.48–1.75)</td>
<td>0.78</td>
</tr>
<tr>
<td>Kt/V &lt;1.46</td>
<td>1.34 (0.69–2.60)</td>
<td>0.28</td>
</tr>
<tr>
<td>Albumin &lt;3.7 mg/dL</td>
<td>1.13 (0.57–2.25)</td>
<td>0.73</td>
</tr>
</tbody>
</table>

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**Fig. 2.** Kaplan–Meier curve of combined CV morbidity and mortality at 24-month follow-up in patients on HD according to the level of sCD40L (median value 7.6 ng/mL). Continued line: patients with sCD40L > 7.6 ng/mL; dotted line: patients with sCD40L ≤ 7.6 ng/mL.
molecules that have been associated with atherothrombosis by mediating the recruitment and adhesion of monocytes and lymphocytes to the injured vessel wall [25, 26]. In addition, sCD40L exerts a profound inhibitory effect on endothelial migration [19], a process considered critical for the re-endothelization of the injured vessel [26]. Thus, CD40/CD40L has all the biological potential to affect the onset and progression of atherosclerotic disease in patients with ESRD and to explain per se the different incidence of the composite end point in the two groups distinguished according to median sCD40L values. According to this, Campean et al. [27] recently described a higher inflammatory status of coronary lesions as well as the involvement of CD40–CD40 ligand signalling cascade in chronic renal failure, especially in cases of calcified atherosclerosis.

The striking prognostic impact sCD40L on the clinical course in patients on HD raises questions about the origin of this biomarker. Platelets represent the main source of circulating sCD40L in patients with acute coronary syndrome [16] and in hypercholesterolaemia [13]. Accordingly, plasma levels of sCD40L correlate closely with markers of platelet activation in these patient populations [13, 16]. Thus, increased circulating levels of sCD40L might reflect enhanced platelet activation in HD. According to this, it has been demonstrated that circulating activated platelets (P-selectin/CD63-positive platelets) are higher in HD patients than in controls and further increase during HD sessions [28]. Potential causes of such activation include possible stimulation of platelets by proinflammatory cytokines that have been reported to be increased in patients with ESRD [29]. Furthermore, the increased lipid peroxidation that has been found in patients with chronic renal failure might also participate in activating platelets [30]. On the other hand, the lack of any correlation between circulating levels of sCD40L and CRP seems to exclude a role for this platelet-activating inflammatory biomarkers [26] in the enhanced sCD40L signalling observed in our study population. CRP is not associated with the composite CV end point in the present study, while it is one of the significant predictors of total mortality, together with age, albumin and HD modality as already shown in the original RISCAVID population [5].

The present study has some limitations. The population represents a subgroup analysis of a larger observational study [5]. Results are at least in part confirmatory of previous results showing the prognostic role of sCD40L on CV risk in HD patients [20]. Nevertheless, the present study suggests a prognostic value of this marker of vascular inflammation on the composite end point of CV morbidity and mortality in relatively short-term follow-up and beyond traditional risk factors, systemic markers of inflammation and malnutrition.

In conclusion, these observational results, obtained at 2-year follow-up in a population of patients on HD, strongly support the prognostic value of sCD40L, a biomarker of proatherogenic inflammation, in patients with ESRD, thus providing a useful tool to better stratify CV prognosis in HD patients.

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References


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