Soluble Fas is a marker of peripheral arterial occlusive disease in haemodialysis patients

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Abstract
Background. Peripheral arterial occlusive disease (PAOD) including lower-extremity and cerebrovascular atherosclerosis is a leading cause of morbidity in haemodialysis patients. Recent evidence suggests that the expression of Fas, a molecule implicated in the initiation of apoptosis in various cell types, is increased at sites of atherosclerotic plaques. However, the significance of plasma levels of the soluble form of Fas (sFas) as a marker of peripheral arterial disease has yet to be defined.

Methods. The present report is based on a cross-sectional analysis of baseline data from an ongoing prospective study designed to evaluate the role of sFas as marker of PAOD in end-stage renal disease (ESRD). We evaluated the association between sFas levels and evidence of PAOD in a cohort of 107 chronic haemodialysis patients.

Results. Compared with subjects without evidence of disease (n = 56), subjects with PAOD (n = 51) had significantly higher plasma levels of sFas (30.0 ± 8.9 vs 26.4 ± 9.5 ng/ml; P = 0.04). Using multiple regression, sFas was found to be associated with PAOD independently of classical risk factors for atherosclerosis (hypercholesterolaemia, diabetes, hypertension, and smoking), markers of inflammation (e.g. C-reactive protein, intercellular cell adhesion molecule type 1), and other risk factors (e.g. age, gender). An increase of one quintile in the plasma concentration of sFas was associated with an odds ratio of PAOD of 1.69 (95% CI: 1.09–2.63, P = 0.01). In addition, models that incorporated sFas were significantly better at predicting PAOD than models limited to classical risk factors for atherosclerosis, alone or in combination with CRP levels (P = 0.01).

Conclusions. Increased plasma levels of sFas are associated with established PAOD. These results suggest that sFas may represent a novel and independent marker of atherosclerosis.

Keywords: apoptosis; atherosclerosis; haemodialysis; inflammation; peripheral arterial occlusive disease; sFas

Introduction

Patients with end-stage renal disease (ESRD) are at a substantially increased risk of atherosclerotic vascular disease [1,2]. Both coronary artery disease (CAD) and peripheral arterial occlusive disease (PAOD) are important causes of mortality and morbidity in this population. Lower-extremity vascular disease and cerebrovascular disease are the major manifestations of PAOD and are responsible for disabling strokes and amputations [2,3]. Despite recent advances in our understanding of the uraemic state and improvements in the science and technology of renal replacement therapy, atherosclerotic vascular disease remains a major burden in this population [1,2]. The development of premature atherosclerosis is multifactorial. Several factors have been implicated such as classical cardiovascular risk factors (e.g. hypercholesterolaemia, diabetes, hypertension, and smoking), hyperhomocysteaemia, and various disorders of lipoprotein metabolism. Other contributing factors have also been described recently such as acute or chronic inflammation and increased oxidative stress [4]. However, taken together these factors do not completely account for the excessive burden of atherosclerotic disease experienced by haemodialysis patients. Hence, other risk factors yet to be defined are most likely to be
involved in the accelerated atherosclerosis sustained by these patients.

Disregulation of apoptosis has been implicated as an important factor mediating tissue turnover and plaque development both in humans and in animal models of atherosclerosis [5,6]. Several groups have demonstrated increased apoptotic cell death in vascular smooth-muscle cells and in infiltrating leukocytes at sites of atherosclerotic lesions [7–11]. The Fas/Fas ligand system is a key regulating system responsible for the activation of apoptosis in various cell types, including cellular constituents of the vessel wall [12]. Increased expression of Fas and its ligand (Fas ligand) have been found in human atherosclerotic plaques [8–11].

The soluble form of Fas (sFas) can be measured in human plasma samples [13]. Increased levels of sFas have been reported in the acute phase of myocardial infarction and unstable angina [14]. A positive association between CAD and sFas has also been reported in stable haemodialysis patients [15,16]. However, the importance of circulating levels of sFas as a marker of the peripheral vascular disease is unclear. In the present study, we hypothesized that sFas levels may correlate with evidence of PAOD in ESRD patients and thus could represent a novel marker of atherosclerotic disease in humans.

The present report is based on the analysis of baseline data from an ongoing prospective cohort study of ESRD patients. Data collected at entry into the study were analysed to evaluate the association between sFas levels and prevalence of PAOD. Adjustment for potential confounders was provided using logistic-regression analysis. Factors considered as potential confounders included classical risk factors of PAOD, and other factors such as age, gender, aspirin intake, and dialysis adequacy. Markers of inflammation (C-reactive protein (CRP) and adhesion molecules) were also considered as potential confounders. Interleukin-2 (IL-2) was also included as a marker of T-cell activation as activated T cells are an important component of atherosclerotic plaques and also secrete high levels of sFas.

Materials and methods

A prospective, open cohort study involving stable ESRD patients on chronic haemodialysis at the Hôpital du Sacré-Cœur de Montréal was initiated in 1999 to evaluate the relative value of sFas as a predictor of atherosclerosis. For the present analysis, we used data collected at study entry on 107 patients enrolled during the first year. The scope of the present analysis is limited to PAOD. The study is ongoing and all living participants are still followed-up in the study. The patients included had to be older than 35 years of age, on chronic haemodialysis for 3 months or more, and without any acute significant illness within 3 months of enrollment. Patients were excluded if they did not agree or could not donate the volume of blood required for the performance of the study and if they had severe unrelated medical conditions with expected survival less than 3 months (e.g. cancer). A total of 20 patients did not meet the inclusion/exclusion criteria and were excluded from the investigation, leaving 107 subjects in the study. The local ethics committee approved the present protocol. All patients signed an informed consent form before entering the study.

Data and diagnostic criteria

Blood samples were collected at the beginning of the study from all participants in tubes containing EDTA and stored at −80°C until the time of analysis. Clinical data were also collected at entry from medical records. When incomplete, the information was obtained directly from participants or their physicians. Data were collected on the following classical risk factors for atherosclerosis: hypercholesterolaemia, diabetes mellitus, hypertension, and smoking. Hypertension was defined as a blood pressure greater than 140/90 mmHg on three or more readings taken before dialysis treatments at least 1 week apart or if the patient was taking antihypertensive medications. Hypercholesterolaemia was defined as the presence of fasting total plasma cholesterol greater than 6.2 mmol/l or if the patient was taking lipid-lowering agents. Subjects were classified as smokers (current smokers or former smokers) or non-smokers. Diabetes mellitus was diagnosed on clinical records or if the fasting glucose concentration was greater than 6.9 mmol/l on two or more tests or if the patient was using insulin or oral hypoglycaemic medications.

PAOD was defined by the presence of either lower-extremity vascular disease or cerebrovascular disease. Peripheral vascular disease was diagnosed if a patient had intermittent claudication, typical rest pain, ischaemic ulceration or gangrene, abdominal aortic aneurysm (detected by ultrasonography or CT scan), or percutaneous or surgical revascularization. Vascular disease involving the arteriovenous fistula was excluded. Cerebrovascular disease was diagnosed if a patient had experienced episode(s) of transient ischaemic attack or ischaemic stroke, or had undergone carotid endarterectomy. Thus, the evaluation of PAOD was based on symptoms/signs, non-invasive radiologic evaluations and surgical procedures. The evaluation was not based on angiography results because such results were not available for all patients and because angiography was found too invasive to be performed systematically on all patients.

Other data included age, gender, race, and body-mass index (BMI: the weight in kilograms divided by the square of the height in meters). Information on aspirin intake was also obtained. Dialysis-related information included diagnosis of renal failure, duration of dialysis, membrane type, and Kt/V, a standard measure of urea removal.

Procedures

Plasma samples from all patients were assayed for sFas, CRP, soluble intercellular cell adhesion molecule type 1 (sICAM-1), soluble vascular cell adhesion molecule type 1 (sVCAM-1), and interleukin 2 (IL-2). Plasma levels of sFas, sICAM-1, sVCAM-1, and IL-2 were measured using commercially available ELISA kits (Biosource International, CA, USA for sFas and IL-2; Chemicon, Mississauga, Ontario, Canada for sICAM-1 and sVCAM-1). The ELISA method was similar for all plasma markers. Briefly, monoclonal antibodies specific for a plasma marker are adsorbed onto microwells. Samples from patients are pipetted into these wells and the plasma marker present then binds to the
antibodies. A second, streptavidine–peroxidase-conjugated monoclonal antibody is added and binds to the plasma marker captured by the first antibody. A substrate solution is added, which is acted upon by the bound enzyme to produce colour. The intensity of this coloured product is directly proportional to the concentration of plasma marker present in the sample. The absorbance is measured at 450 nm for sFas, sICAM-1, sVCAM-1, and at 405 nm for IL-2. The reported intra-assay coefficients of variability are 4.9, 4.1, 3.1, and 4.7 for sFas, sICAM-1, sVCAM-1, and IL-2 respectively. The inter-assay coefficients of variability are 7.9, 7.7, 5.2, and 6.9 for sFas, sICAM-1, sVCAM-1, and IL-2 respectively. CRP was measured using a nephelometric-based procedure (BNA 100 by Dade Behring). All samples were analysed in duplicate. When the difference between duplicates was greater than 15%, the assay was repeated. Samples were analysed in a similar and blind fashion for all the tests in order to reduce systematic bias.

Statistical analysis

Means and proportions were calculated for subjects with confirmed PAOD and those without evidence of disease for the following variables: demographics, BMI, prevalence of classical risk factors for atherosclerosis, plasma levels of selected markers (sFas, CRP, sICAM-1, sVCAM-1, and IL-2), prevalence of aspirin intake, duration of dialysis, and Kt/V. The significance of differences in means between the two groups was assessed using Student’s t-test or Wilcoxon Rank-Sum test for non normal data, and the differences in proportions was tested with the use of the $\chi^2$ statistic or Fischer exact test.

The association between prevalence of PAOD and sFas was assessed using several separate analyses. First, difference in mean sFas plasma levels between subjects with confirmed PAOD and those without evidence of disease was evaluated using Student’s t-test. Secondly, each cohort was divided into quintiles according to the distribution of sFas levels. Logistic-regression analysis was used to adjust for potential confounders and compute risk estimates (odds ratio) of PAOD according to increasing quintiles of sFas levels. Variables included in the analysis were: plasma levels of selected markers (sFas, CRP, sICAM-1, sVCAM-1, and IL-2) expressed in quintiles, classical risk factors for atherosclerosis (hypercholesterolaemia, diabetes mellitus, hypertension, and smoking) age, gender and any of the remaining variables (aspirin intake, duration of dialysis, Kt/V) that were statistically associated with the presence of PAOD on univariate analysis. The logistic-regression analysis included all 107 patients and did not compare one specific quintile versus another. It took into account all five quintiles (and thus all 107 patients) at the same time to evaluate the association between increasing sFas levels from quintile 1 to quintile 5 and PAOD. Finally, we used the likelihood-ratio test to evaluate whether models that incorporated sFas were significantly better at identifying patients with PAOD than models limited to classical risk factors for atherosclerosis alone or models limited to both classical risk factors and CRP levels. As the relative contribution of sFas, CRP and other risk factors may differ between lower-extremity and cerebrovascular atherosclerosis, we also conducted the above analyses with the corresponding subgroups. Separate analyses were conducted for subjects with or without confirmed lower-extremity vascular disease and for subjects with or without confirmed cerebrovascular disease.

Results

The baseline characteristics of patients with confirmed PAOD and those without are shown in Table 1. Fifty-one subjects (47.7%) had evidence of PAOD whereas 56 subjects (52.3%) were free of disease. Among the 51 subjects with PAOD, 14 (27.4%) had evidence of cerebrovascular disease alone, 19 (37.3%) had evidence of lower-extremity vascular disease alone, and 18 (35.3%) had evidence of both. Race, BMI, prevalence of hypertension, duration of dialysis, and Kt/V were similar in both groups. As expected, patients with PAOD were older and had a higher prevalence of several classical risk factors for atherosclerosis (male gender, diabetes mellitus, hypercholesterolaemia, and smoking status) than subjects without evidence of disease. The proportion of subjects using aspirin was also significantly higher among subjects with evidence of PAOD (Table 1). Diagnosis of renal failure was the following: glomerulonephritis in 26.2%, diabetes mellitus in 19.6%, hypertension in 12.1%, polycystic kidney disease in 4.7%, tubulo-interstitial disease in 3.7%, obstructive uropathy in 3.7%, and other:unknown in 30.0%. There was no significant difference in renal diagnosis between subjects with and without PAOD. Mean ESRD duration was 60.8 ± 56.4 months. All subjects were dialysed thrice weekly on cellulosyntetic (hemophan) membrane or synthetic (polycrylonitrile) membrane with bicarbonate dialysis solution and standard water treatment.

Using univariate analysis, we evaluated the differences in mean plasma levels of sFas and markers of inflammation (CRP, sICAM-1, sVCAM-1, and IL-2) in patients with confirmed PAOD and those without. Plasma levels of sFas were significantly higher ($P = 0.04$) among subjects with evidence of PAOD than among those without (Figure 1). Plasma levels of CRP were also significantly higher among subjects with evidence of PAOD than among those without (Table 2). There were no statistically significant differences between the two groups in the plasma levels of sICAM-1, sVCAM-1, and IL-2 (Table 2).

We used logistic regression to evaluate whether sFas and PAOD were associated independently of potential confounders. The analysis provided simultaneous adjustment for plasma levels of selected markers (CRP, sICAM-1, sVCAM-1, and IL-2) in addition to classical risk factors for atherosclerosis (hypercholesterolaemia, diabetes mellitus, hypertension, and smoking) and other confounders (age, gender, and aspirin intake). In addition, Kt/V was included in this analysis to take into account the effect of dialysis adequacy and clearance. Table 3 shows the results of the
logistic-regression analysis. An increase of one quintile in the plasma concentration of sFas was associated with an odds ratio of PAOD of 1.69 (95% CI: 1.09–2.63, \( P < 0.01 \)). Thus, increasing levels of sFas were associated with a significant increase in the risk of PAOD. In addition to sFas, CRP levels were found to be independent predictors of PAOD (OR \( s = 1.55 \), 95% CI: 1.01–2.39, \( P = 0.04 \)). Male gender, diabetes, and hypercholesterolaemia were also independently associated with PAOD (Table 3). The level of sICAM-1, sVCAM-1, and IL-2 were not found to be independently associated with PAOD. Removing aspirin intake from the model did not change significantly the results shown in Table 3.

Table 1. Characteristics of subjects with or without PAOD

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients without PAOD ((n = 56))</th>
<th>Patients with PAOD ((n = 51))</th>
<th>( P ) value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>65.6 ± 12.8</td>
<td>70.7 ± 10.7</td>
<td>0.03</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>44.6</td>
<td>68.6</td>
<td>0.01</td>
</tr>
<tr>
<td>White race (%)</td>
<td>89.3</td>
<td>86.3</td>
<td>0.89</td>
</tr>
<tr>
<td>BMI</td>
<td>25.9 ± 5.8</td>
<td>24.9 ± 5.3</td>
<td>0.36</td>
</tr>
<tr>
<td>Risk factors (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>17.9</td>
<td>41.2</td>
<td>0.0008</td>
</tr>
<tr>
<td>Hypertension</td>
<td>83.9</td>
<td>94.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>14.3</td>
<td>39.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Smoking</td>
<td>28.6</td>
<td>52.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Aspirin intake (%)</td>
<td>14.3</td>
<td>35.3</td>
<td>0.01</td>
</tr>
<tr>
<td>Dialysis duration (years)</td>
<td>4.7 ± 4.5</td>
<td>5.5 ± 5.0</td>
<td>0.41</td>
</tr>
<tr>
<td>Kt/V</td>
<td>1.3 ± 0.3</td>
<td>1.3 ± 0.2</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table 2. Levels of plasma markers in subjects with or without PAOD

<table>
<thead>
<tr>
<th>Marker</th>
<th>Patients without PAOD ((n = 56))</th>
<th>Patients with PAOD ((n = 51))</th>
<th>Normal range*</th>
<th>( P ) value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/l)</td>
<td>17.4 ± 23.3</td>
<td>23.7 ± 27.8</td>
<td>&lt; 5.0</td>
<td>0.03</td>
</tr>
<tr>
<td>sICAM-1 (ng/ml)</td>
<td>435.0 ± 110.6</td>
<td>431.4 ± 126.4</td>
<td>&lt; 400</td>
<td>0.87</td>
</tr>
<tr>
<td>sVCAM-1 (ng/ml)</td>
<td>7198.3 ± 8302.1</td>
<td>7833 ± 9458.4</td>
<td>&lt; 1700</td>
<td>0.71</td>
</tr>
<tr>
<td>IL-2 (pg/ml)</td>
<td>1.18 ± 0.19</td>
<td>1.16 ± 0.19</td>
<td>&lt; 1.15</td>
<td>0.64</td>
</tr>
</tbody>
</table>

*Expected values for normal individuals.

\( P \) value for the comparison of patients with and without PAOD.

Fig. 1. Plasma levels of sFas in subjects with or without PAOD. Mean plasma levels 30.0 ± 8.9 vs 26.4 ± 9.5 ng/ml; \( P = 0.04 \). Expected values for normal individuals less than 7.0 ng/ml.
Patients on haemodialysis, the amputation rate is close to 200 per 1000 patient years for patients with diabetes and the death rate from cerebrovascular disease is close to 25 per 1000 patient years for patients older than 65 years [2].

In the present study, we found that sFas is independently associated with PAOD. In other words, differences in age, gender or differences in prevalence of diabetes, hypercholesterolaemia, smoking, or aspirin intake cannot explain the association between sFas and PAOD. sFas was found to be associated with PAOD independently of the effect of all major risk factors for atherosclerosis. After adjustment for all of the above confounders and risk factors, an increase of one quintile in the plasma concentration of sFas was associated with an odds ratio of PAOD of 1.69 (95% CI: 1.09–2.63, \( P = 0.01 \)). Adjustment for covariates was partly responsible for the large observed effect when compared with the univariate results. In addition, measurement of sFas increased the predictive value of multivariate models based on classical risk factors for atherosclerosis, alone or in combination with the inflammatory marker CRP.

Increasing levels of sFas were associated with significant increases in the risk of both lower-extremity and cerebrovascular atherosclerosis. An increase of one quintile in the plasma concentration of sFas was associated with an odds ratio of lower-extremity and cerebrovascular atherosclerosis of 1.68 (95% CI: 1.07–2.62, \( P = 0.02 \)) and 1.62 (95% CI: 1.07–2.46, \( P = 0.02 \)), respectively. This finding is not unexpected. Atherosclerosis is a generalized disease, and most patients who have vascular disease in one organ system often exhibit evidence of vascular disease elsewhere. Close to 60% of individuals with lower-extremity atherosclerosis are reported to have some evidence of cerebrovascular atherosclerosis [17]. The risk factors are believed to be similar for both lower-extremity and cerebrovascular atherosclerosis, but their relative influence may differ. For lower-extremity atherosclerosis, smoking is the most significant risk factor, whereas the major risk factor for cerebrovascular disease is hypertension [17]. In our study, after adjustment for these risk factors, sFas was found to represent an additional and independent risk factor for both lower-extremity and cerebrovascular atherosclerosis.

Other evidence of the generalized nature of atherosclerosis in ESRD patients is the prevalence of CAD, which is reported in at least 40–60% of patients with peripheral vascular disease [17]. Increased levels of sFas were also found in previous reports to represent an independent risk factor for CAD [15,16].

Fas is a 45 kDa transmembrane protein that initiates an apoptotic signal when bound to its natural ligand, Fas-L [12]. The sFas form represents an alternatively spliced product of the Fas gene [13]. Increased expression of Fas has been identified at sites of atherosclerotic lesions both in animal models and humans [9–11]. Also, increased levels of sFas have been reported in the acute phase of myocardial infarction and unstable angina [14]. Our results suggest that the association between sFas and atherosclerosis is

### Table 3. Adjusted odds ratio of PAOD associated with an increase of one quintile in the plasma levels of sFas and other markers

<table>
<thead>
<tr>
<th>Markers</th>
<th>Odds ratio (95% CI)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sFas</td>
<td>1.69 (1.09–2.63)</td>
<td>0.01</td>
</tr>
<tr>
<td>CRP</td>
<td>1.55 (1.01–2.39)</td>
<td>0.04</td>
</tr>
<tr>
<td>sCAM-1</td>
<td>0.90 (0.57–1.44)</td>
<td>0.68</td>
</tr>
<tr>
<td>sVCAM-1</td>
<td>1.02 (0.63–1.65)</td>
<td>0.93</td>
</tr>
<tr>
<td>IL-2</td>
<td>1.06 (0.69–1.64)</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Additional risk factors

- **Age (years)**: 1.04 (0.99–1.10), \( P = 0.10 \)
- **Male gender**: 5.04 (1.40–18.20), \( P = 0.01 \)
- **Diabetes mellitus**: 4.21 (1.13–15.68), \( P = 0.03 \)
- **Hypertension**: 4.86 (0.28–84.00), \( P = 0.27 \)
- **Hypercholesterolaemia**: 4.11 (1.05–16.15), \( P = 0.04 \)
- **Smoking**: 1.88 (0.56–6.26), \( P = 0.30 \)
- **Aspirin intake**: 2.34 (0.63–8.63), \( P = 0.20 \)
- **Kt/V**: 6.79 (0.50–92.16), \( P = 0.14 \)

*Odds ratios of PAOD associated with an increase of one quintile in the plasma levels of listed markers. The odds ratios are adjusted for the other plasma markers and for the additional risk factors.

To evaluate whether measurement of sFas added to classical risk factors and markers of inflammation in identifying PAOD patients, likelihood-ratio tests were used to compare the fit of regression models that specifically included or excluded sFas. In these analyses, models that incorporated sFas were significantly better at identifying PAOD patients than models limited to classical risk factors for atherosclerosis alone (\( P = 0.01 \)) or models limited to both classical risk factors and CRP levels (\( P = 0.01 \)).

As the relative contribution of sFas, CRP, and other risk factors may differ between lower-extremity and cerebrovascular atherosclerosis, we also conducted the above analyses for each corresponding subgroup. An increase of one quintile in the plasma concentration of sFas was associated with an odds ratio of lower-extremity vascular disease of 1.68 (95% CI: 1.07–2.62, \( P = 0.02 \)). Similarly, an increase of one quintile in the plasma concentration of sFas was associated with an odds ratio of cerebrovascular disease of 1.62 (95% CI: 1.07–2.46, \( P = 0.02 \)). Thus, increasing levels of sFas were associated with significant increases in the risk of both lower-extremity and cerebrovascular atherosclerosis.

### Discussion

Atherosclerotic vascular disease is a major cause of morbidity and mortality in patients with chronic renal failure undergoing renal replacement therapy [1–3]. Compared with the general population, the risk of PAOD is substantially higher among dialysis patients. Lower-extremity vascular disease and cerebrovascular disease result in considerable morbidity including disabling strokes and amputations [2]. In ESRD patients on haemodialysis, the amputation rate is close to 200 per 1000 patient years for patients with diabetes and the death rate from cerebrovascular disease is close to 25 per 1000 patient years for patients older than 65 years [2].

In the present study, we found that sFas is independently associated with PAOD. In other words, differences in age, gender or differences in prevalence of diabetes, hypercholesterolaemia, smoking, or aspirin intake cannot explain the association between sFas and PAOD. sFas was found to be associated with PAOD independently of the effect of all major risk factors for atherosclerosis. After adjustment for all of the above confounders and risk factors, an increase of one quintile in the plasma concentration of sFas was associated with an odds ratio of PAOD of 1.69 (95% CI: 1.09–2.63, \( P = 0.01 \)). Adjustment for covariates was partly responsible for the large observed effect when compared with the univariate results. In addition, measurement of sFas increased the predictive value of multivariate models based on classical risk factors for atherosclerosis, alone or in combination with the inflammatory marker CRP.

Increasing levels of sFas were associated with significant increases in the risk of both lower-extremity and cerebrovascular atherosclerosis. An increase of one quintile in the plasma concentration of sFas was associated with an odds ratio of lower-extremity and cerebrovascular atherosclerosis of 1.68 (95% CI: 1.07–2.62, \( P = 0.02 \)) and 1.62 (95% CI: 1.07–2.46, \( P = 0.02 \)), respectively. This finding is not unexpected. Atherosclerosis is a generalized disease, and most patients who have vascular disease in one organ system often exhibit evidence of vascular disease elsewhere. Close to 60% of individuals with lower-extremity atherosclerosis are reported to have some evidence of cerebrovascular atherosclerosis [17]. The risk factors are believed to be similar for both lower-extremity and cerebrovascular atherosclerosis, but their relative influence may differ. For lower-extremity atherosclerosis, smoking is the most significant risk factor, whereas the major risk factor for cerebrovascular disease is hypertension [17]. In our study, after adjustment for these risk factors, sFas was found to represent an additional and independent risk factor for both lower-extremity and cerebrovascular atherosclerosis.

Other evidence of the generalized nature of atherosclerosis in ESRD patients is the prevalence of CAD, which is reported in at least 40–60% of patients with peripheral vascular disease [17]. Increased levels of sFas were also found in previous reports to represent an independent risk factor for CAD [15,16].

Fas is a 45 kDa transmembrane protein that initiates an apoptotic signal when bound to its natural ligand, Fas-L [12]. The sFas form represents an alternatively spliced product of the Fas gene [13]. Increased expression of Fas has been identified at sites of atherosclerotic lesions both in animal models and humans [9–11]. Also, increased levels of sFas have been reported in the acute phase of myocardial infarction and unstable angina [14]. Our results suggest that the association between sFas and atherosclerosis is
not limited to acute CAD. In our cohort of stable ESRD patients, sFas was found to be significantly and independently associated with both lower-extremity and cerebrovascular atherosclerosis.

The inflammatory marker CRP was also found to be a significant marker of PAOD in haemodialysis patients. This is consistent with recently published data that suggest a role for inflammation markers as predictors of vascular disease amongst apparently healthy individuals and/or ESRD patients [18]. As both sFas and CRP were found to be significant and independent predictive factors of PAOD in these patients, our results suggest that pathways responsible for increased levels of sFas and CRP are independent and concur to the expression of PAOD in these patients.

The present results may have potentially important public health implications. Measurement of sFas may improve the ability to predict PAOD in high-risk patients. Our results also open the way to further research aimed at identifying novel targets for preventive and therapeutic interventions that take into account the role of the Fas/Fas ligand system in the development of atherosclerosis.

The present study was not designed to delineate the mechanisms underlying the association between increased sFas levels and PAOD. Nonetheless, some potential mechanisms may be excluded based on the present results. Elevated levels of sFas have been reported in patients with impaired renal function [19,20]. However, differences in sFas clearance between patients cannot account for our results as all patients were on haemodialysis with no or insignificant residual renal function. In addition, all analyses were adjusted for Kt/V, a standard measure of solute removal and clearance during haemodialysis, to take into account differences in dialysis clearance. After adjustment for Kt/V, sFas remained an independent marker of PAOD in ESRD patients. Also, activated T cells could have been hypothesized as a potential source of sFas in patients with PAOD as these cells secrete sFas [13,21] and are an important component of atherosclerotic plaques [4]. In our analysis, IL-2, a marker of T-cell activation, was not associated with either PAOD or sFas levels. Other Fas-expressing cellular constituents of the atherosclerotic vessel wall, such as endothelial cells, macrophages, and vascular smooth-muscle cells may be contemplated as potential sources of sFas. Future studies will be needed to test these hypotheses and delineate further the mechanisms underlying the association between sFas and PAOD.

Finally, the present report was based on a cross-sectional analysis of baseline data from an ongoing prospective study. Data collected at entry in the study were analysed to evaluate the association between prevalent atherosclerotic disease and markers of apoptosis and inflammation. The ongoing prospective phase of the study should delineate further the role of sFas as a predictor of PAOD in ESRD patients.

In conclusion, our results suggest that sFas represents a novel and independent marker of vascular disease in ESRD patients. After adjustment for classical risk factors for atherosclerosis, plasma markers of inflammation and other confounders such as age, gender, BMI, and dialysis adequacy, sFas was found to be significantly and independently associated with PAOD.

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