Anca associated vasculitis
Boomsma, Maarten Michiel

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2001

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 25-10-2018
Plasma levels of soluble endothelial cell protein C receptor
in patients with Wegener's granulomatosis

Maarten M. Boomsma 1, Deborah J. Stearns-Kurosawa 4, Coen A. Stegeman 2,
Elena Raschi 5, Pier L. Meroni 5, Shinichiro Kurosawa 4, Jan Willem Cohen Tervaert 1,3.

Department of Internal Medicine, Division of 1 Clinical Immunology and 2 Nephrology,
University Hospital Groningen, 3 Clinical Immunology, University Hospital Maastricht, The
Netherlands, 4 Free Radical Biology & Aging Research, Oklahoma Medical Research
Foundation, Oklahoma City, USA, 5 Allergy & Clinical Immunology Unit, IRCCS istituto
Auxologico Italiano, University of Milan, Milan, Italy.

Submitted for publication
Abstract

Endothelial cell injury plays an important role in the pathogenesis of Wegener's granulomatosis (WG). Elevated soluble thrombomodulin (sTM) levels are an accepted marker of endothelial damage. The physiological significance of plasma endothelial protein C receptor (sEPCR) levels is not known. To assess the relevance of this plasma protein in WG, sEPCR levels were measured in sera obtained from WG patients and related to disease activity, sTM levels, and other known markers of disease activity. Hundred and twenty-nine sera (16 at presentation, 8 at remission, 42 prior to a relapse, 21 at relapse, and 42 after a relapse) from 31 WG patients were tested. Disease activity (Birmingham Vasculitis Activity Score) was assessed in patients with active disease. During active disease 8 (22%) and 17 (46%) out of 37 patients had elevated levels of sEPCR and sTM, respectively (NS). sEPCR (r = 0.39; P = 0.02) and sTM (r = 0.53; P < 0.01) levels correlated with disease activity. Analysis of longitudinal sera revealed a significant increase in sEPCR (P = 0.01) and sTM (P = 0.04) levels at the moment of a relapse. Corrected for renal function, the increase in sEPCR remained significant (P= 0.04), whereas sTM did not (NS). Elevated sTM levels were related to renal involvement and higher age when compared to those with normal sTM levels. Elevated sEPCR levels was related to higher age. Levels of sEPCR and sTM correlated (r = 0.32; P < 0.001). Plasma levels of sEPCR correlate with disease activity in patients with WG and an increase in this marker can be observed prior to a relapse.

Introduction

Factors reflecting endothelial cell damage or response to injury are of considerable interest in patients with Wegener's granulomatosis (WG), since they may be potential markers of vasculitic disease activity due to the central role of vascular endothelial cells in the pathology of this disease.

Thrombomodulin (TM) is an endothelial cell transmembrane cofactor for thrombin-mediated protein C activation (Esmon et al., 1981; Dittman et al., 1990). Immunohistochemistry studies have shown that membrane-bound TM is expressed on both large vessels and in the microcirculation (Drake et al., 1993; Laszik et al., 1997). While TM expression is not restricted to endothelium (Matsuyama, et al., 2000; Boffa et al., 1987), the surface area of endothelium exposed to blood is much greater than that for other cell types, with greatest exposure in capillary beds. Membrane-bound TM exposed to elastase from activated neutrophils is degraded (Abe et al., 1994; Furuno et al., 1997) and the plasma level of soluble TM antigen (sTM) is an accepted marker of endothelial cell damage (Ishii et al. 1991; Takano et al. 1992). Elevated levels of sTM in relation to disease activity have been measured in autoimmune disorders such as systemic lupus erythematosis (SLE), Churg-Strauss syndrome, and WG (Ohdama et al., 1994; Schmitt et al., 1998; Boehme et al., 1996; Boehme et al., 2000).
The endothelial cell protein C receptor (EPCR) is a recently described member of the protein C anticoagulant pathway (Fukudome et al., 1994). EPCR accelerates formation of activated protein C (Stearns-Kurosawa et al., 1996), a potent anticoagulant and anti-inflammatory agent (Esmon et al., 1997). Immunohistochemistry studies have shown that membrane-bound EPCR is expressed exclusively on endothelial cells, with greater expression corresponding to increasing vessel diameter (Laszik et al., 1997; Fukudome et al., 1998). A soluble form of EPCR (sEPCR) exists in plasma and retains its ability to bind both protein C zymogen and activated protein C (Kurosawa et al., 1997). Recently, it was demonstrated that sEPCR binds to activated neutrophils, partly via proteinase 3 (PR3) (Kurosawa et al., 2000). PR3 is a neutrophil granule protein expressed on the membrane of activated neutrophils (Csernok et al., 1994; Muller Kobold et al. 1998; Campbell et al., 2000) and is the primary target antigen of anti-neutrophil cytoplasmic antibodies (PR3-ANCA) in WG (Labbaye et al., 1991).

Elevation of sEPCR levels may result either from vascular injury or through a regulated proteolytic release of sEPCR (Gu et al., 1999). Regulated release of sEPCR, possibly through thrombin stimulation of the endothelium and subsequent metalloproteinase activity (Xu et al., 2000), may modulate inflammation through interaction with activated neutrophils. Consistent with this is an in vitro study showing PR3-ANCA-induced increases in tissue factor expression (de Bandt et al., 1997), the physiological initiator of the coagulation cascade that results in thrombin production. This concept is further supported by observations that significantly elevated levels of circulating sEPCR are observed in patients with SLE and sepsis (Kurosawa et al., 1998), conditions associated with systemic inflammation of the vasculature. However, the clinical relevance of sEPCR in patients with WG is still unknown.

The aim of the present study was to determine whether sEPCR levels are elevated and/or correlate with disease activity in patients with WG. Secondly, we compared this marker with other known markers of endothelial damage and/or disease activity such as sTM, ANCA, anti-endothelial cell antibodies (AECA), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR).

**Patients and methods**

**Patients**

Patients with WG who had been positive for PR3-ANCA (Cohen Tervaert et al., 1990) during an active phase of the disease were eligible for this study. The diagnosis of WG was based on the presence of classical symptoms and histological findings and all patients fulfilled the American College of Rheumatology 1990 criteria for WG (Leavitt et al., 1990) and met the definition for WG of the 1992 Chapel Hill Consensus Conference (Jennette et al., 1994). Patients are followed and treated according to a previously described protocol (Cohen Tervaert et al., 1989; Boomsma et al., 2000) at the vasculitis outpatient clinic, University Hospital Groningen. Patients were evaluated for signs and symptoms of active vasculitis or
Infections at least every 3 months. Patients were treated with prednisolone and cyclophosphamide with or without plasma exchange according to our standard protocol (Franssen et al., 1998; Boomsma et al., 2000). At each visit disease activity was scored using the Birmingham vasculitis activity score (BVAS) (Luqmani et al., 1994). Complete remission was defined as the complete absence of symptoms or signs attributable to active vasculitis (BVAS = 0). A relapse was defined as described previously (Cohen Tervaert et al., 1990; Stegeman et al., 1994). The moment of a clinical relapse was defined as the time at which immunosuppressive treatment was started or intensified. The study was carried out in accordance with the 1997 Declaration of Helsinki of the World Medical Association (JAMA, 1997).

At each visit, blood was collected by venipuncture into EDTA solution, transported on ice, and centrifuged for 10 minutes at 172 g. Plasma was transferred to a fresh tube and re-centrifuged for 10 minutes at 1655 g to remove platelets. Plasma was stored at -20°C until further use. Samples from consecutive patients with WG who were diagnosed in our hospital between October 1992 and March 1997, were collected. When available, samples of these patients were studied at the time a complete remission (i.e. without immunosuppressive treatment). Furthermore, samples from consecutive patients with WG who had a relapse between August 1996 and March 1998 were studied. From these patients, also samples obtained 3 and 6 months prior to, and samples obtained 3 and 6 months after relapse were studied.

**Laboratory parameters and kidney dysfunction**

At each visit standard laboratory evaluation included determination of the ESR, serum creatinine level, microscopic analysis of the urine sediment, and a 24 hour urine collection for protein determination. In addition, CRP levels were measured by nephelometry (Behring, Marburg, Germany) (Fink et al., 1989), and ANCA levels by IIF and by ELISA (Boomsma et al., 2000).

**Detection of soluble EPCR**

An ELISA for detection of sEPCR antigen in plasma was performed as previously described (Kurosawa et al., 1998). Briefly, microtiter plates (Maxisorp; NUNC NS, Roskilde, Denmark) were coated with 50 µl of 2 µg/ml anti-EPCR 1494 monoclonal antibody (Stearns-Kurosawa et al., 1996) overnight at 4°C. The wells were blocked with assay buffer containing 0.1% (wt/vol) gelatin for at least 1 hour at room temperature. The plates were washed and 50 µl of 1:50 diluted plasma samples were added in duplicate wells and incubated for 1 hour. After washing, 50 µl of 4 µg/ml biotinylated anti-EPCR 1495 MoAb (Stearns-Kurosawa et al., 1996) was added and incubated for 1 hour, followed by detection with streptavidin-alkaline
phosphatase and Blue Phos substrate (KPL, Gaithersburg, MD). The optical density was measured at 650 nm. sEPCR antigen levels were calculated by reference to a standard curve determined with recombinant soluble EPCR (Fukudome et al., 1996) on the same plate. Values of 289 ng/ml (mean + 2 SD of 19 normal controls) or more were considered to be above normal. Since little is known about the clearance of sEPCR, both adjusted (divided by the patient’s serum creatinine value) and unadjusted sEPCR levels are given.

Detection of soluble TM

An ELISA for detection of sTM in plasma was performed essentially as described previously (Laszik et al., 1994; Kurosawa et al., 1998). ELISA plates were coated with 50 µl of 5 µg/ml anti-TM 1029 MoAb overnight at 4°C and subsequently blocked with 200 µl of Tris-HCl, pH 7.5 (TBS) containing 0.1% (wt/vol) gelatin for 1 hour at room temperature. After washing, plates were incubated with 50 µl of 1:20 diluted plasma samples in duplicate for 1 hour at 37°C. After washing, 50 µl of 1.0 µg/ml biotinylated anti-TM 1009 MoAb was added and incubated for 1 hour at 37°C. Bound antibody was detected with 50 µl of 0.1µg/ml streptavidin-alkaline phosphatase (GIBCO BRL, Grand Island, New York) and substrate amplification with an ELISA amplification system (GIBCO BRL). The optical density was measured at 490 nm. For each plate, a standard curve was included using recombinant soluble TM (Laszik et al. 1994). Values of 26.9 ng/ml (mean + 2 SD of 19 normal controls.) or more were considered to be above normal. sTM is cleared from the circulation by the kidneys and marked kidney dysfunction is known to result in accumulation of plasma sTM (Karmochkine et al., 1992; Rustom et al., 1998). Therefore, both adjusted (divided by the patient’s serum creatinine value) and unadjusted sTM levels are given.

Detection of AECA

Human umbilical vein endothelial cells (HUVEC) were isolated from normal term umbilical cord vein by collagenase perfusion and cultured as previously reported (Del Papa et al., 1996). In all the experiments, endothelial cells from at least three different donors were used. AECA were detected by a cell solid-phase ELISA as previously described (Del Papa et al., 1996).

The results of the tested plasma samples were expressed as a percentage of a positive serum arbitrarily chosen as 100% of endothelial binding activity. Values of 27% for IgG-AECA and 11% for IgM-AECA (mean + 3 SD of 35 normal controls) or more were considered positive.
Statistical analysis

To determine differences in levels of serological markers between disease and control groups, the Fischer’s exact test for categorical quantities, and the Mann-Whitney test (unpaired observations) and Wilcoxon test (paired observations) were used when appropriate for continuous quantities. Univariate associations of serological factors with disease activity were evaluated using Spearman’s correlation coefficients for continuous quantities. A Friedman test was used to evaluate difference in the serial samples. A two-sided p-value of < 0.05 was used to indicate statistical significance.

![Graphs comparing levels of soluble endothelial protein C receptor (sEPCR), sEPCR corrected for renal function, soluble thrombomodulin (sTM), and sTM corrected for renal function between presentation and remission in 8 patients.](image)

**Figure 1** Soluble endothelial protein C receptor (sEPCR) (A), sEPCR corrected for renal function (B), soluble thrombomodulin (sTM) (C), and sTM corrected for renal function (D) levels derived from paired sera taken at the moment of presentation of disease and disease remission (e.g. without immunosuppressives), respectively, in 8 patients.

Results

Two different cohorts were studied: 1) 16 patients with newly diagnosed WG and from 8 of these patients, a sample at complete remission was available (median [range] interval presentation-remission: 22 months [16-37]), 2) 21 patients with sequential samples collected at 6 and 3 months prior to relapse, at the moment of relapse, and 3 and 6 months after relapse. Furthermore, the composite cohort of 37 samples from patients with active disease (16 samples at presentation from the first cohort and 21 samples at relapse from the second cohort) were analyzed to study the possible relation of the different markers with disease activity.
**sEPCR in Wegener’s granulomatosis**

**Samples at presentation and at remission**

**Clinical characteristics:** The clinical characteristics of the 16 patients of whom a sample at the time of presentation was obtained are listed in Table 1. The median (range) BVAS score was 14 (3–21).

**Soluble EPCR:** At presentation of disease, elevated sEPCR levels were present in 3 out of 16 patients (19%). Remarkably, levels of sEPCR at presentation tended to be lower as compared to levels at remission (P = 0.11) (Table 2; Figure 1a). This difference became significant when sEPCR levels were corrected for renal function (P = 0.04) (Figure 1b). At the moment of remission, elevated sEPCR levels were present in 4 out of 8 patients (50%).

**Table 1** Clinical characteristics of patients with Wegener’s granulomatosis at presentation and at relapse of disease (Median [Range])

<table>
<thead>
<tr>
<th></th>
<th>Patient at time of presentation</th>
<th>Patient at time of relapse</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n.</strong></td>
<td>16</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td><strong>Sex, Male / Female</strong></td>
<td>11 / 5</td>
<td>14 / 7</td>
<td>N.S.</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td>57 (25 - 79)</td>
<td>53 (26 - 77)</td>
<td>N.S.</td>
</tr>
<tr>
<td><strong>BVAS score</strong></td>
<td>14 (3 - 21)</td>
<td>8 (2 - 15)</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Organ involvement a</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic</td>
<td>81%</td>
<td>76%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Cutaneous</td>
<td>31%</td>
<td>24%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Mucous</td>
<td>56%</td>
<td>33%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Ear, nose and throat</td>
<td>88%</td>
<td>67%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Chest</td>
<td>50%</td>
<td>43%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Cardiac</td>
<td>6%</td>
<td>0%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Abdominal</td>
<td>6%</td>
<td>5%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Renal</td>
<td>69%</td>
<td>67%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Nervous system</td>
<td>44%</td>
<td>19%</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

*a Systemic = e.g. fever, arthralgia, myalgia, malaise; Mucous = genital / mouth ulcers or red eyes / proptosis; Renal = positive biopsy and/or decreased creatinine clearance in combination with proteinuria and erythrocyturia; N.S. = not significant.

**Soluble TM:** At presentation of disease, elevated sTM levels were present in 8 out of 16 patients (50%). Levels of sTM in patients with WG at presentation of disease were higher than at remission (difference not significant, P = 0.08) (Table 2; Figure 1c). When sTM levels were corrected for renal function (Figure 1d), levels did not appear to be different (P = 0.31). At the moment of remission, elevated sTM levels were present in 1 out of 8 patients (13%).
Other markers: Conventional markers of inflammation and PR3-ANCA antibodies closely followed clinical disease activity. At presentation of disease, CRP levels ($P < 0.01$), ESR ($P = 0.03$) and PR3-ANCA as measured by either IIF or ELISA ($P = 0.02$) were significantly higher compared with their values during remission (Table 2). At presentation of disease, either IgG-AECA, IgM-AECA, or both antibodies were present in 10 out of 16 patients (63%). IgG-AECA ($P = 0.46$) and IgM-AECA ($P = 0.38$) levels at presentation did not significantly differ from levels at remission (Table 2). At the time of remission, either IgG-AECA, IgM-AECA or both antibodies were present in 6 out of 8 patients (75%).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Serological disease activity parameters in patients with Wegener's granulomatosis (Median [Range])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients at time of presentation</td>
<td>Patients in remission</td>
</tr>
<tr>
<td>$n.$</td>
<td>16</td>
</tr>
<tr>
<td>sEPCR (ng/ml)</td>
<td>235 (113 - 373)</td>
</tr>
<tr>
<td>sTM (ng/ml)</td>
<td>28 (13 - 148)</td>
</tr>
<tr>
<td>ANCA (ELISA)</td>
<td>822 (62 - 6597)</td>
</tr>
<tr>
<td>ANCA (IIF)</td>
<td>320 (80 - &gt;640)</td>
</tr>
<tr>
<td>IgG-AECA (%)</td>
<td>27 (11 - 73)</td>
</tr>
<tr>
<td>IgM-AECA (%)</td>
<td>14 (0 - 31)</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>101 (12 - 315)</td>
</tr>
<tr>
<td>ESR (mm/hour)</td>
<td>83 (42 - 122)</td>
</tr>
</tbody>
</table>

sEPCR = soluble endothelial protein C receptor; sTM = soluble thrombomodulin; ANCA = antineutrophil cytoplasmic antibodies; ELISA = enzyme linked immunosorbent assay; IIF = indirect immunofluorescence; AECA = antiendothelial cell antibodies; CRP = C-reactive protein; ESR = erythrocyte sediment rate.

Sequential samples at relapse

Clinical characteristics: The clinical characteristics of the 21 patients of whom samples prior to, at the time of, and following relapse were obtained are listed in Table 1. The median (range) BVAS score at the moment of relapse was 8 (2-15), which is significantly lower ($P = 0.05$) than BVAS scores at the time of presentation of the 16 patients from the first cohort.

Soluble EPCR: sEPCR levels rose prior to relapse and levels dropped to baseline (-6 months) levels after treatment ($P = 0.01$) (Figure 2a). Correction of sEPCR levels for renal function did not affect the results ($P = 0.04$) (Figure 2b). In 5 out of 21 patients (24%) levels of sEPCR were above the normal range at the time of relapse.

Soluble TM: sTM levels rose prior to relapse, and levels dropped below baseline (-6 months) levels after treatment ($P = 0.04$) (Figure 2c). When sTM levels were corrected for renal function, these changes of sTM levels were no longer significant ($P = 0.23$) (Figure 2d). In 9 out of 21 patients (43%) levels of sTM were above the normal range at the time of relapse.
**Other markers:** CRP levels (P < 0.001) and ESR (P < 0.01) rose at relapse, and levels dropped to baseline (-6 months) levels after treatment (Figure 3a-b). PR3-ANCA levels as detected by both IIF and ELISA rose prior to relapse, and levels dropped below baseline (-6 months) levels after treatment (P < 0.0001) (Figure 3c-d). In all patients PR3-ANCA as detected by either IIF or ELISA were present at relapse. No significant change was noted in IgG-AECA and IgM-AECA levels during relapse (Figure 3e-f). At the moment of relapse, 10 out of 21 patients (48%) had either IgG-AECA, IgM-AECA or both antibodies present.

Figure 2 Box plots indicating the overall range (error bars), 25-75% range (boxes), and median value (horizontal lines) of levels of soluble endothelial protein C receptor (sEPCR) (A), sEPCR corrected for renal function (B), soluble thrombomodulin (sTM) (C), and sTM corrected for renal function (D), at 6 months, and 3 months prior to relapse, at the time of relapse, and 3 months, and 6 months after relapse, in 21 Wegener’s granulomatosis patients studied longitudinally.
Correlation of markers with disease activity

The correlation between the different markers and BVAS-scores of 37 patients with active disease (16 at presentation and 21 at relapse) was calculated and is listed in Table 3. Spearman’s correlation of the sEPCR (\(r = 0.39; P = 0.02\)), sTM (\(r = 0.53; P < 0.01\)), and CRP (\(r = 0.59; P < 0.001\)) levels revealed a strong association with the BVAS score. When sEPCR (\(r = -0.14; P = 0.39\)) and sTM (\(r = 0.21; P = 0.20\)) levels were corrected for renal function, these correlation were no longer significant.

Figure 3 Box plots indicating the overall range (error bars), 25-75% range (boxes), and median value (horizontal lines) of levels of C-reactive protein (CRP) (A), erythrocyte sedimentation rate (ESR) (B), antineutrophil cytoplasmic antibody (ANCA) as detected by indirect immunofluorescence (IIF) (C), and ANCA as detected by enzyme-linked immunosorbent assay (D), IgG-anti-endothelial antibodies (AECA) (E), and IgM-AECA (F), at 6 months, and 3 months prior to relapse, at the time of relapse, and 3 months, and 6 months after relapse, in 21 Wegener’s granulomatosis patients studied longitudinally.
### Table 3
Spearman's correlation between serological disease activity parameters and disease activity in patients with active Wegener's granulomatosis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Disease activity (BVAS score)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sEPCR (ng/ml)</td>
<td>0.39</td>
<td>0.02</td>
</tr>
<tr>
<td>sTM (ng/ml)</td>
<td>0.51</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ANCA (ELISA)</td>
<td>0.27</td>
<td>0.11</td>
</tr>
<tr>
<td>ANCA (IIF)</td>
<td>0.31</td>
<td>0.06</td>
</tr>
<tr>
<td>IgG-AECA (%)</td>
<td>0.05</td>
<td>0.76</td>
</tr>
<tr>
<td>IgM-AECA (%)</td>
<td>-0.02</td>
<td>0.91</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ESR (mm/hour)</td>
<td>0.29</td>
<td>0.11</td>
</tr>
</tbody>
</table>

sEPCR = soluble endothelial protein C receptor; sTM = soluble thrombomodulin; BVAS = Birmingham vasculitis activity score; ANCA = anti-neutrophil cytoplasmic antibodies; ELISA = enzyme linked immunosorbent assay; IIF = indirect immunofluorescence; AECA = anti-endothelial cell antibodies; CRP = C-reactive protein; ESR = erythrocyte sediment rate.

**Figure 4** Levels of soluble endothelial protein C receptor (sEPCR) and soluble thrombomodulin (sTM) in 37 Wegener's granulomatosis patients studied at active disease (16 samples at presentation, 21 samples at relapse). Dotted lines indicate the upper limit of the normal range.

In Figure 4, sEPCR and sTM levels are displayed from 37 samples from patients with active disease (21 samples at relapse, 16 samples at presentation). There was a significant correlation between sEPCR and sTM levels (r = 0.40; P = 0.01). Elevated sEPCR levels were correlated with higher age, while elevated sTM levels were associated with higher creatinine levels, renal involvement, BVAS scores, and age (Table 4).
Table 4 Soluble TM and EPCR in patients with active Wegener's granulomatosis (Median [Range])

<table>
<thead>
<tr>
<th></th>
<th>Elevated sTM</th>
<th>Normal sTM</th>
<th>P-value</th>
<th>Elevated sEPCR</th>
<th>Normal sEPCR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n.</td>
<td>17</td>
<td>20</td>
<td></td>
<td>8</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Sex, Male / Female</td>
<td>14 / 3</td>
<td>11 / 9</td>
<td>0.09</td>
<td>6 / 2</td>
<td>19 / 10</td>
<td>1.00</td>
</tr>
<tr>
<td>Renal</td>
<td>88%</td>
<td>50%</td>
<td>0.017</td>
<td>88%</td>
<td>62%</td>
<td>0.23</td>
</tr>
<tr>
<td>BVAS</td>
<td>13 [6-21]</td>
<td>7 [2-21]</td>
<td>&lt;0.01</td>
<td>13 [6-18]</td>
<td>9 [2-21]</td>
<td>0.20</td>
</tr>
<tr>
<td>Age, y</td>
<td>59 [26-79]</td>
<td>50 [24-76]</td>
<td>0.05</td>
<td>62 [50-71]</td>
<td>52 [24-79]</td>
<td>0.04</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>81 [12-307]</td>
<td>52 [53-315]</td>
<td>0.30</td>
<td>47 [53-191]</td>
<td>67 [6-315]</td>
<td>0.57</td>
</tr>
<tr>
<td>Leukocytes (x10⁹/l)</td>
<td>7.9 [4.4-24.7]</td>
<td>7.2 [4.0-17.4]</td>
<td>0.57</td>
<td>7.2 [4.4-12.1]</td>
<td>7.9 [4.0-24.7]</td>
<td>0.45</td>
</tr>
<tr>
<td>IgG-AECA (%)</td>
<td>19 [0-90]</td>
<td>24 [4-51]</td>
<td>0.99</td>
<td>18 [9-71]</td>
<td>25 [0-90]</td>
<td>0.92</td>
</tr>
<tr>
<td>IgM-AECA (%)</td>
<td>4 [0-36]</td>
<td>8 [0-26]</td>
<td>0.61</td>
<td>3 [0-26]</td>
<td>10 [0-36]</td>
<td>0.06</td>
</tr>
<tr>
<td>ANCA (ELISA)</td>
<td>726 [54-6597]</td>
<td>411 [10-14370]</td>
<td>0.32</td>
<td>638 [26-6201]</td>
<td>424 [10-14370]</td>
<td>0.87</td>
</tr>
<tr>
<td>ANCA (IIF)</td>
<td>640 [80-640]</td>
<td>320 [40-640]</td>
<td>0.17</td>
<td>640 [320-640]</td>
<td>640 [40-640]</td>
<td>0.57</td>
</tr>
<tr>
<td>Atherosclerotic events, Yes/No</td>
<td>4 / 13</td>
<td>5 / 15</td>
<td>1.00</td>
<td>3 / 5</td>
<td>6 / 23</td>
<td>0.37</td>
</tr>
</tbody>
</table>

sEPCR = soluble endothelial protein C receptor; sTM = soluble thrombomodulin; BVAS = Birmingham Vasculitis Activity Score; CRP = C-reactive protein; AECA = anti-endothelial cell antibodies; ANCA = antineutrophil cytoplasmic antibodies; ELISA = enzyme linked immunosorbent assay; IIF = indirect immunofluorescence; Atherosclerotic events = e.g. angina, myocardial infarction, stroke etc.

**Discussion**

In this study, we measured levels of soluble EPCR in plasma samples from patients with WG and related those levels to clinical disease activity. Consistent with previous findings that sTM, which is an established marker of endothelial cell damage (Ishii et al., 1991; Takano et al., 1992), was found to correlate with disease activity in WG (Boehme et al., 1996), we also found that sEPCR was related to disease activity in WG patients. However, only a minority (22%) of patients with active WG had elevated sEPCR levels compared with healthy controls, whereas sTM levels were increased in 46% of these patients. The reason for this is not known, but there are several possible interpretations. Since WG patients with active disease express PR3 on their neutrophils (Muller Kobald et al., 1998) and PR3 supports sEPCR binding to activated neutrophils (Kurosawa et al., 2000), plasma sEPCR levels might be underestimated in patients with active disease due to binding of sEPCR to activated circulating neutrophils. It is also possible that the plasma sTM and sEPCR levels are reflecting different mechanisms of release from their endothelial-bound parents. sTM arises from elastase proteolytic degradation and endothelial cell damage. In contrast, thrombin interaction with its receptor on endothelial cells induces subsequent metalloproteolytic activity to give rise to sEPCR. Therefore, sEPCR levels may be reporting a response to injury, rather than cellular damage per se. This
consideration may give insight into the apparent paradox that sEPCR levels correlated with WG disease activity, despite the fact that membrane-bound EPCR is expressed primarily on larger vessels and WG is primarily a disease of small to medium-sized vessels by clinical criteria. In patients with WG, levels of sEPCR correlated not only with disease activity scores, but also with sTM levels. This contrasts with an earlier study in which there was no correlation observed between sEPCR and sTM levels in patients with sepsis or SLE (Kurosawa et al., 1998). The simplest explanation is that this results from differences in the sites of vascular injury between WG, SLE and sepsis. We also found that patients with active disease and elevated sEPCR levels tended to be older than patients with active disease but normal sEPCR levels. Thus, there may be multiple, chronic pathologic processes contributing to sEPCR levels, such as atherosclerotic damage of the larger vessels. This is, however, purely speculative and additional studies in different patient populations remain necessary.

We did find that sTM levels, before correction for renal function, correlated with disease activity. These findings are in agreement with previous studies in which elevated levels of sTM were demonstrated in patients with active WG (Ohdama et al., 1994; Boehme et al., 1996). In our study, patients with active disease and elevated sTM levels had a higher prevalence of renal involvement, higher serum creatinine levels, and higher age as compared to patients with active disease but normal sTM levels. These observations are consistent with the known renal damage associated with WG pathology and previous studies in a variety of clinical populations demonstrating that impaired renal function is associated with elevated levels of sTM (Karmochkine et al., 1992; Hergesell et al., 1996).

The second part of our study addressed the relevance of serial measurements of sEPCR for monitoring disease activity during follow-up of patients with WG. We found that serum levels of sEPCR rose prior to a diagnosed relapse and dropped to baseline levels after treatment. These results remained significant even after correction of sEPCR levels for renal function. Therefore, our results indicate that measurement of serum levels of soluble EPCR may have some potential as a marker of disease activity. sTM levels also rose prior to the moment of a relapse and levels dropped below baseline levels after treatment. However, when sTM levels were corrected for renal function, these changes in sTM levels were no longer significant. Longitudinal data from other clinical studies are not available.

Previous in vitro studies have shown that autoantibodies like PR3-ANCA activate cytokine-primed neutrophils and monocytes leading to the release of proteolytic enzymes and the production of oxygen radicals and pro-inflammatory cytokines (reviewed in Heerenga et al., 1998) and that AECA may play an important role in vascular injury by attracting leucocytes to the inflammatory site (reviewed in Meroni et al., 1999). Interaction of autoantibodies with their target antigens bound to the endothelium may aggravate the inflammatory process. These effects may contribute to vascular injury and by these means induce sTM and sEPCR production. Consistent with this observation, ANCA and AECA are often detected in patients
with active vasculitis and PR3-ANCA levels mirror disease activity (Cohen Tervaert et al., 1989; Boomsma et al., 2000). Although previously it was reported that AECA levels showed a relationship to disease activity in vasculitis similar to that for PR3-ANCA (Chan et al., 1993; Frampton et al., 1990), we could not confirm this in the present study. In conclusion, we found in a minority of patients with active WG have significantly elevated plasma levels of sEPCR. Moreover, the levels of sEPCR correlated with disease activity. Serial measurement of levels of sEPCR may have some potential as a marker of disease activity in WG patients, although more clinical studies are required to establish the utility of sEPCR as a marker of vascular disease processes.

Acknowledgements

This study was supported by a project grant from the University Hospital Groningen (AZG-stimuleringsgelden) and a grant from Ricerca Corrente 2000 IRCCS Istituto Auxologico Italiano. Research support was provided by an Initial Investigator Award (D.S.K.) and a Scientist Development Grant (S.K.) from the American Heart Association, and grants from the National Institutes of Health (NHLBI R01 HL647867 to S.K.). The antibodies and recombinant proteins used in the sTM and sEPCR ELISAs were a kind gift of Dr. Charles Esmon (Howard Hughes Medical Institute, Oklahoma Medical Research Foundation, Oklahoma City, OK). We wish to thank Roelie van Wijk, Marcel van der Leij and Kandice Swindle for technical support.

Literature


