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DOI:
10.1111/j.1365-2362.2011.02607.x

Citation for published version (Harvard):

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An observational study of endothelial function in early arthritis

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ABSTRACT

Background Endothelial dysfunction is present in established rheumatoid arthritis, but it is not clear at what stage of the disease this abnormality develops. We set out to determine whether endothelial damage/dysfunction is present in a group of patients with early arthritis (EA) (new onset inflammatory arthritis, EA).

Materials and methods Eighteen patients with EA, 48 healthy controls and 25 disease controls were recruited. Plasma was obtained for endothelial (von Willebrand factor (vWF) and soluble E-selectin) and angiogenesis markers (vascular endothelial growth factor and its receptor sFlt-1), adhesion molecules (intercellular adhesion molecule 1 and vascular cell adhesion molecule 1) and circulating endothelial cells (CECs, as a marker of endothelial damage). Microvascular endothelial function was assessed using laser Doppler perfusion imaging and macrovascular function using flow-mediated dilatation of the brachial artery.

Results von Willebrand factor and CECs (both \( P < 0.05 \)) were significantly elevated in EA suggesting endothelial dysfunction and damage but were unrelated to classical laboratory markers of inflammation C-reactive protein, erythrocyte sedimentation rate or IL6. No other biomarkers was elevated in EA. Microvascular and macrovascular abnormalities were confined to endothelium-independent (smooth muscle cell) responses.

Conclusions Endothelial damage/dysfunction is present early in the course of inflammatory arthritis but is not directly related to inflammation markers.

Keywords Circulating endothelial cells, early arthritis, endothelium, vascular endothelial growth factor, vascular function, von Willebrand factor.


Introduction

Patients with established rheumatoid arthritis (RA) have abnormal endothelial (and thus vascular) function, which may be related to inflammation. Evidence of this comes from many sources and includes raised levels of von Willebrand factor (vWF) and soluble E-selectin, and although there are also reports of raised levels of soluble forms of adhesion molecules intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 (i.e. sICAM and sVCAM), these are, despite being influenced by inflammation, not specific for the endothelium [1–4]. More recent work implies a role for angiogenic vascular endothelial growth factor (VEGF) and its receptor (soluble Flt-1) in RA, which may be related to angiogenesis in the synovium [5–7]. Other methods for assessing vascular function include large artery (e.g. brachial) flow-mediated dilatation (FMD), changes in microvascular blood flow (e.g. of the forearm) in response to agonists such as acetylcholine [8–11], and increased numbers of circulating endothelial cells (CEC) which are presumed to be driven from the intima by the disease process [12]. All these changes support the concept that RA is a vascular disease, a view now widely recognised [13].

In addition to established RA, ‘newly diagnosed’ RA (as defined by not more than 18 months of symptoms) has also been associated with abnormalities of endothelial function [14]. However, there are few data on the state of the endothelium in the very early stages of arthritis, i.e. within the first few weeks after onset of symptoms, and thus before it can be correctly defined as ‘rheumatoid’. This is pertinent as it may be that early inflammatory changes play a part in the development or establishment of semi-permanent vascular dysfunction as is present in classical RA. Indeed, anti-inflammatory treatment in established disease can ameliorate many of the adverse changes described previously [10,15–17].
We therefore set out to test the hypothesis that patients presenting with a new onset of inflammatory arthritis (before any more refined diagnosis had been made) have abnormalities of endothelial function. We tested our hypothesis in a simple case–control study of subjects with early arthritis (EA) compared with healthy (i.e. negative) controls and a group of hypertensive diabetes as ‘disease’ (i.e. positive) controls.

**Patients and methods**

**Subjects**

Eighteen patients who had been referred to an early arthritis clinic, 48 healthy controls (HC) and 25 type 2 diabetic hypertensive patients [the disease control group (DC)] were recruited. The EA group suffered symptoms for a minimum of 1 month, median of 2 months [inter-quartile range (IQR) 1–30 months] and were recruited from a dedicated EA clinic in a UK teaching hospital, with referrals directly from primary care physicians. The median (IQR) rheumatoid factor titre was 0 (0–160) units with two-thirds of patients being classified as sero-negative. The tender joint score was 5.5 (2–7.5), and the swollen joint score was 4.5 (2–6.5). Anti-CCP antibodies were present in 16.6% of the patients. Subsequently, in two-thirds of the patients, the EA developed into RA. In the remaining one-third, the final diagnoses were equally distributed between reactive arthritis (resolving), unclassified arthritis (persistent), unclassified arthritis (resolving) and sarcoid-related arthritis. The HC group was recruited from hospital staff, and friends or relatives of the EA group or the DC group [11,12]. The HC self-reported as being in good health and denied any past medical history of note. The disease (positive) control group was diabetic hypertensives recruited from a cardiovascular risk factor clinic. Median (IQR) disease duration was 7 (5–15 years) years. All subjects were free of overt cardiovascular, renal, hepatic and neoplastic disease and were not taking antibiotics or oral anticoagulants. Clinical and demographic data are presented in Table 1. Local ethical committee approval was obtained, as was informed consent from each participant.

**Assessment of endothelial function**

All subjects answered questions relating to general health, medications, duration of arthritis symptoms (for the EA group), and had baseline measurement of blood pressure and routine biochemistry and haematology. All subjects then had venous

### Table 1 Background data for EA patients, DCs and HCs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EA (n = 18)</th>
<th>DC (n = 25)</th>
<th>HC (n = 48)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58 ± 16</td>
<td>67 ± 8.9*</td>
<td>54 ± 10</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>7/11</td>
<td>19/6*</td>
<td>17/34</td>
<td>0.001</td>
</tr>
<tr>
<td>Creatinine (µM)</td>
<td>86 ± 19</td>
<td>101 ± 24*</td>
<td>76 ± 16</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total cholesterol (mM)</td>
<td>51 ± 1.1</td>
<td>43 ± 0.8*</td>
<td>57 ± 1.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HDL (mM)</td>
<td>15 ± 0.35</td>
<td>1.4 ± 0.5*</td>
<td>1.8 ± 0.5</td>
<td>0.005</td>
</tr>
<tr>
<td>Triglycerides (mM)</td>
<td>1.59 ± 0.48</td>
<td>1.98 ± 0.97</td>
<td>1.67 ± 0.87</td>
<td>0.235</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>12.9 (12.3–13.5)*</td>
<td>14.0 (12.5–14.5)</td>
<td>14 (13.3–14.65)</td>
<td>0.025</td>
</tr>
<tr>
<td>WCC (×10^9/L)</td>
<td>8.1 (6.5–10.1)*</td>
<td>6.6 (5.6–7.3)</td>
<td>6.0 (5.0–7.2)</td>
<td>0.002</td>
</tr>
<tr>
<td>Ptt (×10^9/L)</td>
<td>336 (271–461)*</td>
<td>246 (208–269)*</td>
<td>262 (240–332)</td>
<td>0.003</td>
</tr>
<tr>
<td>Active smoker</td>
<td>4/18 (22%)</td>
<td>2/25 (8.0%)</td>
<td>4/48 (8.33%)</td>
<td>0.241</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>135 ± 26</td>
<td>150 ± 17*</td>
<td>135 ± 22</td>
<td>0.013</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>81 ± 13</td>
<td>80 ± 11</td>
<td>83 ± 11</td>
<td>0.655</td>
</tr>
<tr>
<td>BP lowering drugs</td>
<td>3/18 (16.7%)</td>
<td>23/25 (92%)*</td>
<td>3/48 (6.25%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Statin</td>
<td>1/18 (5.6%)</td>
<td>17/23 (73.9%)*</td>
<td>1/48 (2.1%)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

M, male; F, female; Cholesterol, serum total cholesterol; HDL, serum high-density lipoprotein cholesterol; Hb, haemoglobin concentration; WCC, total white cell count; Ptt, platelet count; SBP, systolic blood pressure; DBP, diastolic blood pressure; Rx for BP, number (percentage) of subjects taking medication to control blood pressure; Statin, number (percentage) or subjects taking a regular Statin; IQR, inter quartile range; SD, standard deviation; EA, early arthritis patients; HC, healthy controls; DC, disease controls (diabetic hypertensives).

Data are mean ± SD for normally distributed data and median (IQR) for non-normally distributed data.

P value is difference comparing EA, HC and DC. *P < 0.05 compared with HC, post hoc test.
blood taken for later batch analysis of plasma and serum markers of endothelial function (vWF and soluble E-selectin, sE-sel), angiogenesis (VEGF and the VEGF receptor sFlt-1) and adhesion molecules (intercellular adhesion molecule 1, ICAM-1 and vascular cell adhesion molecule 1, VCAM-1) by commercial ELISA (R&D Systems, Abingdon, UK; Dako-Cytomation, Ely, UK). Inflammatory markers C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were measured by the routine Hospital Pathology service, while interleukin 6 (IL-6) was measured by ELISA (R&D Systems) (Table 2). Inter/intra-assay coefficient of variations and lower limits of detection were < 10% and < 5%, respectively.

Physiological vascular function was assessed in large and small arteries. Briefly, microvascular endothelial function was assessed using laser Doppler perfusion imaging using the Perimed system (Bury St Edmunds, Suffolk, UK) with iontophoresis of ACh for endothelium-specific vasodilatation and sodium nitroprusside (SNP) for the endothelium-independent response [10,11]. The technology relies on differences between transmitted and returned wavelengths that reflect changes in blood flow in a unit area of subcutaneous tissue. Aliquots of 1% ACh and 0.1% SNP were prepared in sterile filtered distilled water (Sigma-Aldrich, Poole, Dorset, UK).

For assessment, the patient sat comfortably in a constant-temperature room for 20 min before the start of measurements. A hairless area of forearm skin in the nondominant arm was selected, and the iontophoresis chamber was placed over this skin. A second electrode was placed over the volar aspect of the ipsilateral wrist. The chamber was filled with SNP, then covered with a glass slip and clipped in place ensuring that no air bubbles were visible below the glass cover slip. The cathode was attached to this and the anode to the other (wrist) electrode. Iontophoresis was not commenced until two complete baseline readings had been taken, following which the iontophoresis driver delivered 0.1 mA over 2 min (total charge delivered 12 mC) as a continuous current. The chamber used for SNP was then removed and a fresh iontophoresis chamber placed approximately 4 cm further away from the site of the initial assessment (so that the iontophoresis electrode did not lie within the path of the current from the previous recording).

The same procedure was used for ACh except that the electrode polarity was reversed and the current used was 0.2 mA (total charge delivered 24 mC). The dedicated software produces a table of values for each time point that is used to determine the dilation peak response (in terms of absolute perfusion units, and also % change from baseline), over the 10-min experiment. Inter- and intra-assay variation was < 10%.

Flow-mediated dilatation has previously been described in detail [18]. High-resolution ultrasound was used to assess changes in the diameter of the brachial artery.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EA</th>
<th>DC</th>
<th>HC</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inflammatory markers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>22.5 (0–34.5)*</td>
<td>0 (0–5)</td>
<td>0 (0–0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>34 (11–56)**</td>
<td>13 (4–24)*</td>
<td>5.5 (3–29)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>59 (25–95)*</td>
<td>11.5 (2.5–33)</td>
<td>10 (2.5–51.5)</td>
<td>0.626</td>
</tr>
<tr>
<td><strong>Endothelial markers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vWF (IU/dL)</td>
<td>123 (11)*</td>
<td>124 (18)*</td>
<td>109 (17)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>sE-sel (ng/mL)</td>
<td>70 (42–79)</td>
<td>72.5 (50–90)</td>
<td>57.5 (35–75)</td>
<td>0.117</td>
</tr>
<tr>
<td><strong>Angiogenic markers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF (pg/mL)</td>
<td>49 (34–140)</td>
<td>37 (28–92)</td>
<td>39 (25–120)</td>
<td>0.155</td>
</tr>
<tr>
<td>sFlt-1 (ng/mL)</td>
<td>5.4 (0–12.8)</td>
<td>0 (0–18.3)</td>
<td>2.0 (0–23.5)</td>
<td>0.221</td>
</tr>
<tr>
<td><strong>Adhesion molecules</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICAM-1 (ng/mL)</td>
<td>173 (157–213)</td>
<td>207 (173–267)*</td>
<td>179 (147–217)</td>
<td>0.006</td>
</tr>
<tr>
<td>VCAM-1 (ng/mL)</td>
<td>330 (200–462)</td>
<td>565 (422–625)*</td>
<td>365 (272–476)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IL-6, interleukin 6; vWF, von Willebrand factor; sE-sel, soluble E-selectin; VEGF, vascular endothelial growth factor; sFlt-1, VEGF receptor sFlt-1; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; EA, early arthritis; DC, disease controls (diabetic hypertensives); HC, healthy controls; IQR, inter quartile range.

Data are median with IQR or mean with standard deviation.
P value is difference comparing EA, HC and DC. *P < 0.05 compared with HC, **P < 0.01 post hoc test.
were taken after the patient had rested in a supine position for 20 min in a quiet room. High-quality ultrasound scans of the brachial artery were obtained using a 10-MHz vascular ultrasound probe. A longitudinal section of the brachial artery was identified 5 cm above the antecubital fossa. Vessel diameter was measured on a frozen image of the vessel using electronic callipers in 2D imaging at the upstroke of the R wave on the ECG trace. Five measurements of vessel diameter over a 1-cm segment of the artery were taken, and the mean value was calculated. After a baseline scan, a standard pneumatic sphygmomanometer cuff was placed at the level of the mid forearm and inflated to 250 mm Hg for 4½ min, and then rapidly deflated. Sixty seconds after cuff release, the second reading was taken in an identical way to the first; care was taken to use the same segment of artery for this scan.

The patient then relaxed in the same position for 20 min for the vessel changes to be reversed. The scans were then repeated in a similar way before and after the administration of 400 μg sublingual glyceryl trinitrate (GTN) (delivered as a two spray puffs from a metered dose delivery device) to assess the endothelium-independent response. The results were expressed as a percentage change in vessel diameter from the baseline reading in each case. Images were saved to a disk so that the images could be blindly re-interpreted to assess inter- and intra-observer variability. Inter- and intra-assay variation was < 10%.

Data analysis
We took vWF to be our test statistic, hypothesising an increase in a standard deviation (SD, e.g. 15 IU/dL) in mean levels in both the DC and EA groups compared with the HCs – i.e. that endothelial perturbation is of approximately the same magnitude in EA (e.g. mean 115 SD 15) as it is known to be in the DC group, as defined by peak response to ACh or % change in blood flow following both endothelium-dependent and independent stimuli, compared with HC. Patients in the EA group had lower haemoglobin, higher white blood cell count and higher platelet count than the two other groups. The majority of EA patients (61%) were taking nonsteroidal anti-inflammatory drugs, with a median duration of use of 2 weeks. One patient had been started on methotrexate (for 4 weeks) and one patient on oral steroids (for 2 weeks).

Results
Clinical and demographic data are provided in Table 1. DCs were older than the two other groups, had a higher creatinine level and included more men. Total cholesterol was lower, almost certainly because of the use of statins, while blood pressure was higher and subjects were more likely to be taking anti-hypertensives. Patients with EA had lower haemoglobin, higher white blood cell count and higher platelet count than the two other groups. The majority of EA patients (61%) were taking nonsteroidal anti-inflammatory drugs, with a median duration of use of 2 weeks. One patient had been started on methotrexate (for 4 weeks) and one patient on oral steroids (for 2 weeks).

Markers of inflammation, endothelial function, angiogenesis and adhesion molecules
As expected, patients with EA had significant elevations of the inflammatory markers (CRP, ESR and IL6) compared with HC and DC. There was a significant increase in endothelial marker vWF in EA compared with controls (to a similar level as that seen in DC) (Table 2). This result justifies our sample size calculation. None of the other measured parameters (of angiogenesis and adhesion molecules) were abnormal in the patients with EA. The adhesion molecules sICAM-1 and sVCAM-1 were both significantly elevated in DC compared with HC and EA.

Microvascular and macrovascular endothelial function, and circulating endothelial cells data
Microvascular endothelium-dependent function in the EA group, as defined by peak response to ACh or % change in response, was not significantly different to the HCs (Table 3). Endothelium-independent responses were abnormal, with percentage change of smooth muscle response to SNP from baseline significantly impaired compared with HC. In the DC, there were markedly significant differences in percentage increase in blood flow following both endothelium-dependent and independent stimuli, compared with HC. Patients in the EA group has preserved FMD compared with the HCs. There was a significant impairment of the endothelium-independent response to GTN. For the DC group, highly significant differences between HC and DC were observed in both endothelium-dependent and endothelium-independent brachial artery responses. CECs were higher in EA compared with both other groups (P < 0.025).

Correlations
A crude exploration of potential correlations failed to find any correlation coefficient (r) of > 0.65.
Circulating endothelial cells (CECs) and Microvascular function

They also observed modest positive correlations of sFlt-1 levels with ESR (r = 0.17, P = 0.006) and CRP (r = 0.14, P = 0.02). Interestingly, only VEGF levels measured at baseline correlated with a disease activity score measured 1 year later, and VEGF levels were higher in patients with radiographic progression. We clearly lack the power for these analyses.

We report that endothelial damage/dysfunction is present very early in inflammatory arthritis, despite the short duration of symptoms in this group. This is perhaps somewhat surprising, given that the median duration of symptoms in this group was only 2 months. On a background of cardiovascular disease, endothelial dysfunction is thought to arise from chronic endothelial activation [28] and endothelial damage to be the consequence of endothelial dysfunction. However, acute elevations in CECs have been recorded following myocardial infarction [29], confirming that acute severe endothelial insults may result in rapid shedding of CECs. Thus, the new onset of inflammatory arthritis in the EA group seems to be sufficient to cause abnormalities of endothelial function and also to result in endothelial damage, even over a time course of only a few weeks. These changes are summarised in Table 4.

The lack of significant and strong correlations between inflammation and endothelial markers suggest that inflammation is not necessarily directly responsible for the endothelial abnormalities. Possible explanations for this include other as yet unidentified endothelial insults in EA, those unlinked to inflammation being responsible for the endothelial insult, a time lag between inflammation and its effect on the

### Table 3 Microvascular and macrovascular endothelial function, and CECs in EA, DCs and HCs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EA</th>
<th>DC</th>
<th>HC</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microvascular function</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACh peak (perfusion units)</td>
<td>1.73 (1.46–1.96)</td>
<td>1.55 (1.36–1.82)*</td>
<td>1.766 (2.188–3.884)</td>
<td>0.020</td>
</tr>
<tr>
<td>ACh % change (%)</td>
<td>286 (215–344)</td>
<td>175 (137–231)*</td>
<td>326 (258–448)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SNP peak (perfusion units)</td>
<td>1.46 (1.17–1.88)</td>
<td>1.39 (1.09–1.53)</td>
<td>1.47 (1.22–1.84)</td>
<td>0.160</td>
</tr>
<tr>
<td>SNP % change (%)</td>
<td>271 (203–328)*</td>
<td>187 (141–220)*</td>
<td>362 (244–485)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Macrovascular function</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMD %</td>
<td>4.6 (1.89–6.37)</td>
<td>1.56 (–0.39–2.67)*</td>
<td>3.68 (2.37–5.97)</td>
<td>0.005</td>
</tr>
<tr>
<td>GTN %</td>
<td>12.5 (6.1–17.5)*</td>
<td>9.8 (6.9–13.0)</td>
<td>17.8 (12.8–22.8)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

CECs (cells/mL) 7 (3.5–14.5)* 4 (2.0–8.5) 4 (1.0–8.0) < 0.01

GTN, glyceryl trinitrate; FMD, flow-mediated dilatation; EA, early arthritis; HC, healthy controls; DC, disease controls (diabetic hypertensives). ACh peak, maximum skin perfusion following iontophoresis of acetylcholine, measured in perfusion units; ACh % change, percentage change from baseline blood flow following iontophoresis of acetylcholine; SNP peak, maximum skin perfusion following iontophoresis of sodium nitroprusside, measured in perfusion units; SNP % change, percentage change from baseline blood flow following iontophoresis of SNP.

Data are median (inter quartile range).

P value is difference comparing EA, HC and DC. * P < 0.05 compared with HC, post hoc test.

### Discussion

In a simple cross-sectional study, we found evidence of damage/dysfunction (i.e. raised vWF and CECs) of the endothelium in EA which parallels similar changes in established RA [1,2,12]. This observation is novel and extends previous studies as we are unaware of other data on CECs in EA. Although the degree of increase in vWF was comparable to that seen in the DCs, the latter failed to demonstrate increased numbers of CECs. This was unexpected as raised CECs have been described in diabetes but not in uncomplicated hypertension [21,22]. Raised soluble adhesion molecules in the DCs were described in diabetes but not in uncomplicated hypertension [23,24], it is notable that we failed to find raised levels in EA. Although there are ample evidence of active inflammation in the EA group (raised CRP, ESR, IL6), notably these markers failed to correlate with the increased vWF.

Indeed, increased vWF need not always correlate with inflammation markers and thus its position as an acute-phase reactant remains unclear [25,26]. Finally, lack of abnormalities in levels of markers of angiogenesis (VEGF, sFLT-1), or abnormal micro- or macrovascular function in EA, unlike in established RA, implies these changes have a long-standing and complex aetiology. Indeed, with considerably greater power (n = 310 patients with EA), Clavel et al. [27] reported that at baseline and after 1 year, VEGF levels correlated with clinical and biological parameters of inflammation. They also observed modest positive correlations of sFlt-1 levels with ESR (r = 0.17, P = 0.006) and CRP (r = 0.14, P = 0.02). Interestingly, only VEGF levels measured at baseline correlated with a disease activity score measured 1 year later, and VEGF levels were higher in patients with radiographic progression. We clearly lack the power for these analyses.

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The lack of significant and strong correlations between inflammation and endothelial markers suggest that inflammation is not necessarily directly responsible for the endothelial abnormalities. Possible explanations for this include other as yet unidentified endothelial insults in EA, those unlinked to inflammation being responsible for the endothelial insult, a time lag between inflammation and its effect on the
endothelium masking the correlation, or possibly a complex interaction with multiple factors contributing to endothelial damage and dysfunction.

The lack of abnormalities of endothelium-dependent function in the forearm skin microcirculation and brachial artery in EA contrasts with the finding of abnormal vWF levels in this group, and also the observation for endothelial damage in the form of CECs, as has been found in patients with RA [12]. As for that group, the explanation may lie in the fact that the two physiological tests measure endothelial function in distinct sites (forearm skin for laser Doppler derived microvascular function, and the brachial artery for macrovascular function), whereas vWF and CECs are systemic manifestations of an abnormal endothelium. vWF and CECs may be released from any site and circulate until sampled by phlebotomy and therefore may reflect a process going on in the diseased joints of patients with arthritis. In contrast, endothelium-dependent microvascular and macrovascular function tests were abnormal in the EA group. It is therefore likely that short-duration inflammatory arthritis is associated with localised endothelial dysfunction and damage, as well as wider abnormalities of vascular function (both small and large vessels) that are not endothelium dependent.

We recognise several limitations, such as those of our cross-sectional design (with no intervention or longitudinal data) and of the small sample size. However, despite the latter, we found clear laboratory evidence of inflammation in EA. We are also unable to explore processes that may provide clear mechanistic insight at a tissue level into the endothelial abnormalities seen, and without further data we cannot explore any associations or causality. Indeed, patients in the EA group have preserved FMD, and without further data, we cannot explore any associations or causality. Furthermore, the plasma markers may reflect systemic changes rather than tissue-specific changes, and the vascular flow data provide information on a large artery and on skin microvessels, not on the most likely site where the disease may be more active, i.e. the synovium.

We conclude that endothelial damage/dysfunction is present early in the course of inflammatory arthritis and is not directly related to inflammatory markers. These changes may reflect the different pathophysiological processes evident in EA, which may have implications for disease evolution and/or progression.

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Received 29 December 2010; accepted 17 September 2011

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