Levels of inflammatory markers and the development of the post-thrombotic syndrome

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Summary
The post-thrombotic syndrome (PTS) occurs frequently after deep venous thrombosis (DVT) despite appropriate anticoagulant therapy. A close relationship between inflammation and thrombosis exists. While the inflammatory process at the time of DVT appears to improve thrombus resolution, it may promote destruction of venous valves, valvular reflux and subsequent development of PTS. We prospectively evaluated the association between levels of four cytokines (IL-6, IL-8, IL-10 and MCP-1), two adhesion molecules (ICAM-1 and VCAM-1) and the development of PTS in a well-defined cohort of patients with DVT. The study population consisted of 387 patients with objectively diagnosed symptomatic DVT who were followed for two years to determine the incidence of PTS. At the end of follow-up, plasma samples frozen at the four-month visit in 307 study patients were thawed and analyzed for the above inflammatory markers using the Luminex beads technology. Mean levels of IL-6 were significantly higher in patients with PTS compared to patients without PTS (7.35 pg/ml ± 14.26 [SD] vs. 4.60 pg/ml ± 4.90; p=0.03). Logistic regression analyses showed significant associations between PTS and levels above vs. below the median of IL-6 [odds ratio (OR) 1.66; 95% confidence interval (CI) 1.05, 2.62 (p=0.03)] and ICAM-1 [OR 1.63; 95% CI 1.03, 2.58 (p=0.04)]. None of the other markers showed any association with PTS. Our study suggests the presence of significant associations between markers of inflammation such as IL-6 and ICAM-1 and the development of PTS. Further work is needed to evaluate this relationship and to analyse other candidate markers that could be implicated etiologically in the association between DVT and PTS. If confirmed, this could lead to identification of new therapeutic targets for preventing PTS after DVT.

Keywords
Post-thrombotic syndrome, deep venous thrombosis, inflammation, interleukins, adhesion molecules

Introduction
Deep venous thrombosis (DVT) is a common vascular condition that is associated with significant morbidity and mortality. Despite appropriate anticoagulant therapy, the post-thrombotic syndrome (PTS) occurs in 20%-50% of patients after DVT (1–3). PTS is characterized by chronic leg pain, edema and skin changes, which can lead to painful venous leg ulcers that are difficult to treat. As a result, PTS constitutes a high burden to society in terms of costs and impact on quality of life (4–6).

Little is known about PTS and the factors that contribute to its development after DVT. It is believed that interactions among several factors at the level of the affected vein lead to PTS. Such factors include the thrombus itself, inflammatory mediators secreted by blood cells and/or the venous endothelium and the process of fibrin degradation and vein recanalization, all of which might contribute to damage of the vessel wall and valves, leading to valvular incompetence. Reflux, particularly at the level of the popliteal vein, appears to be associated with the clinical manifestations of PTS in combination with residual vein thrombosis (7–9).

There is increasing evidence in the literature supporting a close relationship between inflammation and arterial thrombosis: molecular and cellular components of the clotting cascade seem to trigger and propagate inflammatory processes that can lead to further activation of the clotting cascade and to disease aggravation (10). The major trigger of the inflammatory response is thrombin which affects endothelial cells, smooth
In light of the above evidence, we evaluated whether levels of inflammatory cytokines (IL-6, IL-8, IL-10 and MCP-1) and adhesion molecules (ICAM-1 and VCAM-1) are associated with the development of PTS or venous reflux in a well-defined population of patients with DVT.

### Methods

The present study was a biomarker sub study to the Venous Thrombosis Outcomes (VETO) Study, a multicenter prospective cohort study of patients with acute DVT who were followed for two years to assess clinical, quality of life and economic outcomes (1, 6, 18). Only those procedures that are relevant to the present study are detailed below.

### Study population and procedures

The study population was recruited from among consecutive patients with acute symptomatic DVT at seven hospital centers in Quebec, Canada between April 2001 and July 2002 and one hospital center in Ontario, Canada between November 2001 and September 2004. Patients were eligible to participate if they had objectively diagnosed DVT of the lower limb within the preceding seven days and were excluded from the study if they were incapable of reading or understanding English or French, lived too far from a participating center, had an expected life-span of less than three months, or were unable or refused to provide consent.

At study entry, demographic and clinical characteristics of the 387 participating patients were recorded. Patients subsequently attended five follow-up study visits at one, four, eight, 12 and 24 months. At each visit, trained study personnel assessed subjects’ legs using the Villalta scale, a validated clinical PTS measure that grades the severity, from 0 (absent) to 3 (severe), of each of five patient-rated symptoms (pain, cramps, heaviness, pruritus and paresthesia) and six clinician-rated clinical signs (edema, redness, skin induration, hyperpigmentation, venous ecchymosis and pain during calf compression) (19, 20). Study personnel who rated the clinical signs were blind to patients’ responses to the symptoms component of the scale and did not have access to scores obtained on previous visits. Patients were classified as having developed the PTS if the Villalta score for the same leg as the index DVT was ≥5 at the final follow-up visit. Patients were classified as having developed the PTS if the index DVT was ≥5 on at least two visits starting at the four-month visit or later, or was ≥5 at the final follow-up visit. Among patients with PTS, a Villalta score of 5–9 at all assessments was considered to represent mild PTS, a score of 10–14 on at least one assessment was considered to represent moderate PTS and a score of ≥15 or a venous ulcer was considered to represent severe PTS.

At the 12-month visit, 223 patients who were participants in a reflux substudy underwent evaluation for venous reflux. Reflux was assessed and quantified in a standardized fashion by venous ultrasound using the Valsalva maneuver and manual compression. Sudden cessation of the venous Doppler signal was interpreted as venous valve incompetence (i.e. reflux) (21). As per study procedures, a 10 ml sample of blood was drawn at the four-month visit to obtain DNA for analysis of inherited thrombophilia (results reported elsewhere [11]) and to obtain plasma for future analyses. Plasma samples were preserved at –80°C. In the present study, we analyzed reserved plasma samples for IL-6, IL-8, IL-10, MCP-1, ICAM-1, and VCAM-1.

### Table 1: Patients characteristics* (n=307).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age-years; mean (SD)</td>
<td>55.95 years ± 14.59 (SD)</td>
</tr>
<tr>
<td>Male; n (%)</td>
<td>149/307 (48.5%)</td>
</tr>
<tr>
<td>Proximal DVT; n (%)</td>
<td>178/307 (58.0%)</td>
</tr>
<tr>
<td>Cardiovascular comorbidity; n (%)</td>
<td>25/307 (8.1%)</td>
</tr>
<tr>
<td>Active cancer; n (%)</td>
<td>30/307 (9.8%)</td>
</tr>
<tr>
<td>Venous reflux at 12 months; n (%)</td>
<td>103/223 (46.2%)</td>
</tr>
<tr>
<td>PTS status; n (%)</td>
<td></td>
</tr>
<tr>
<td>No PTS</td>
<td>141 (45.9%)</td>
</tr>
<tr>
<td>Unclassifiable</td>
<td>2 (0.7%)</td>
</tr>
<tr>
<td>PTS severity; n (%)</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>81/141 (57.5%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>38/141 (26.9%)</td>
</tr>
<tr>
<td>Severe</td>
<td>22/141 (15.6%)</td>
</tr>
</tbody>
</table>

* Shown for the 307 of 387 VETO patients who provided blood samples. # reflux testing performed in 223 study patients. PTS= Post-thrombotic syndrome. PTS severity classified as defined in Methods. Proximal DVT= DVT affecting popliteal or more proximal deep veins. Cardiovascular comorbidity refers to angina or myocardial infarction. Active cancer defined as cancer that was diagnosed within last 6 months, or is undergoing treatment, is metastatic or terminal.

... muscle cells of the vascular wall and platelets. By activating pro tease-activated receptors (PARs), in particular PAR1 and PAR2, thrombin induces endothelial cell expression of vascular cell adhesion molecules (VCAMs), intracellular adhesion molecules (ICAMs), P-selectin and E-selectin. Also, endothelial cells secrete chemokines such as monocyte chemotactic protein-1 (MCP-1), platelet-derived growth factor, interleukin (IL)-6, IL-8 and IL-10 (anti-inflammatory). Furthermore, activated platelets express the CD40 ligand (CD40L), which further activates endothelial cells, smooth muscle cells and macrophages, which possess the CD40 receptor for CD40L. As a consequence, the activated cells secrete IL-1, IL-6, IL-8, ICAM-1 and VCAM-1 and E-selectins. In a recent review on endothelial-dependent leukocyte recruitment to the vascular wall, Rao et al. (11) reported that the mere disruption of blood flow leads to a change in the expression of adhesion molecules and the production of chemokines. Studies have shown that IL-8, MCP-1 and vascular endothelial growth factor (VEGF) are involved in the process of thrombus resolution in experimental models of thrombosis (12–15). In DVT, this process may lead to the destruction of the venous valves, venous reflux and thus increase the risk of PTS. In a recent review, Poredos and Jezovnik (16) provide additional evidence on the role of inflammation in venous thrombotic events and also discuss the close link between arterial and venous thrombosis based on the fact that thrombotic events in either site of the circulation share several risk factors including age and body mass index. This association was also explored in a 2008 review by Ageno and Dentali (17).

In light of the above evidence, we evaluated whether levels of inflammatory cytokines (IL-6, IL-8, IL-10 and MCP-1) and adhesion molecules (ICAM-1 and VCAM-1) are associated with the development of PTS or venous reflux in a well-defined population of patients with DVT.
in order to evaluate the role of these markers as potential predictors of PTS.

**Determination of the levels of markers of inflammation**

We used the Luminex micro-beads array system to simultaneously measure circulating levels of four cytokines, IL-6, IL-8, IL-10, and MCP-1 and two adhesion molecules, ICAM-1 and VCAM-1. These markers were selected based on a systematic review of the literature (22). The assays were carried out as per the manufacturer’s instructions (Luminex Corp., Austin, TX, USA) using commercially available kits (Bio-Plex Cytokine Assay, Bio-Rad Laboratories Inc., Hercules, CA, USA), one for the cytokines and one for the adhesion molecules of interest. In brief, the microspheres were incubated with standards, controls, and samples (50 µl) in a 96-well microtiter plate for 1 hour at room temperature. After incubation, the plate was washed to remove any excess reagent and the detection antibodies were added in the form of a mixture containing each of the antibodies for the proteins of interest. After a 30-minute (min) incubation period at room temperature, streptavidin-phycoerythrin (Streptavidin-PE) was added and the plate was incubated for an additional 10 min. After a final wash step, the beads were resuspended in buffer and read on the Luminex 100 instrument using the Bio-Plex Manager Software to determine the concentration of the markers of interest. For levels that were below the detection limit, the value was reported as the limit of detection of the marker being analyzed divided by the square root of 2.

**Statistical analysis**

Statistical analyses were performed using SPSS 15.0 statistical software (SPSS Inc. Chicago, IL, USA) and Intercooled Stata 8.2 statistical software (StataCorp, College Station, TX, USA). Levels of markers of inflammation were described by means and standard deviations and additionally by medians and inter-quartile ranges (75th-percentiles minus 25th-percentiles) because levels were not normally distributed. Two-group comparisons of mean inflammatory marker levels by PTS status were examined using t-tests for independent samples, with the assumption of equal or unequal variances as appropriate. Non-parametric tests of the equality of distributions of marker levels across categories of patient characteristics were conducted using a Kruskall-Wallis test, which is equivalent to a Mann-Whitney U test for a two-group comparison. If an overall significant difference was found for a Kruskall-Wallis test of three groups or more (p-values<0.05), statistical significance levels for multiple pairwise comparisons were determined using a Bonferroni correction to the p-value equal to 0.05/3 = 0.017 for three groups and 0.05/6 = 0.008 for four groups.

Logistic regression was used to model PTS status (yes/no) as an outcome in relation to inflammatory markers and patient characteristics as covariates, and estimates of odds ratios (OR) are provided with 95% confidence intervals (CI) and p-values. Indicator (1/0) variables for high levels of inflammatory markers (using median levels among patients without PTS as cut-offs) were created for regression models to explore whether indicators performed better than continuous level variables in identifying high-risk groups for PTS. Multivariable models for PTS were selected based on a backward elimination procedure with inflammatory markers and all considered patient characteristics. Possible confounding in univariate models of PTS with each inflammatory marker was assessed by adding pre-specified patient characteristics one at a time and checking for a change in the inflammatory marker odds ratio greater than or equal to 20%. P-values < 0.05 were considered statistically significant, unless otherwise specified for multiple pairwise comparisons.

**Results**

**Patient characteristics**

Of the 387 participants in the VETO study, blood samples were available for 307 (some patients refused to provide blood due to unwillingness to provide DNA samples to test for inherited thrombophilia). Patient characteristics are summarized in Table 1. Mean age was 56 years ± 15 (standard deviation [SD]), 49% were male, 8% had concomitant cardiovascular comorbidity (angina or myocardial infarction) and 10% had active cancer. The initial DVT affected the proximal deep venous system in 58% of patients. Among 223 patients with reflux data at one year, 46.2% had ipsilateral reflux. All patients received initial heparin treatment for a mean of 7.4 days (95% CI, 6.8 to 8.0 days) (2/3 received low-molecular-weight heparin and 1/3 unfractionated heparin) followed by warfarin for a mean duration of 34.1 weeks (95% CI 30.0 to 37.3 weeks). Decisions to prescribe compres-

Table 2: Levels of markers of inflammation in all patients and by PTS status (mean ± SD)*.

<table>
<thead>
<tr>
<th>Marker</th>
<th>All patients n=307</th>
<th>PTS n=141</th>
<th>No PTS n=164</th>
<th>P-value (PTS vs. no PTS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>5.85 ± 10.38</td>
<td>7.35 ± 14.26</td>
<td>4.60 ± 4.90</td>
<td>0.03</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>4.27 ± 10.87</td>
<td>4.47 ± 12.12</td>
<td>4.14 ± 9.77</td>
<td>0.79</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>3.71 ± 17.79</td>
<td>4.53 ± 25.13</td>
<td>3.04 ± 7.10</td>
<td>0.47</td>
</tr>
<tr>
<td>MCP-1 (pg/ml)</td>
<td>49.81 ± 58.23</td>
<td>49.43 ± 40.46</td>
<td>50.41 ± 70.37</td>
<td>0.88</td>
</tr>
<tr>
<td>ICAM-1 (ng/ml)</td>
<td>176.61 ± 74.61</td>
<td>179.83 ± 56.83</td>
<td>173.40 ± 87.41</td>
<td>0.46</td>
</tr>
<tr>
<td>VCAM-1 (ng/ml)</td>
<td>373.56 ± 112.90</td>
<td>377.00 ± 113.16</td>
<td>370.67 ± 113.48</td>
<td>0.63</td>
</tr>
</tbody>
</table>

IL= Interleukin. MCP= Monocyte chemotactic protein. ICAM-1= Intracellular adhesion molecule-1. VCAM-1= Vascular cellular adhesion molecule-1. P-values were calculated using an independent t-test assuming equal or unequal variances as appropriate. * Blood samples were available for 307 patients; of those, 293 were obtained at 4 months and 14 at 8 months.
Figure 1: Values for some data points are shown as well as the 25th-percentile, median, and 75th-percentile levels (boxed values) for the different markers. For boxplot figure of IL-10 levels, PTS=1, data label 298.43 pg/ml was omitted from the graph for better graph representation.
sion stockings were left to treating physicians rather than by protocol. Overall, 52% of patients reported using compression stockings at some time during study follow-up.

Of the 307 participants, 141 (45.9%) developed PTS during the two-year follow-up period and 164 did not. The risk of developing PTS was not influenced by type of heparin or use of stockings (1). Among patients with PTS, 57% had mild PTS, 27% had moderate PTS and 16% had severe PTS. Because of incomplete follow-up, two patients were unclassifiable with respect to PTS status and were not considered further in the markers of inflammation analyses.

### Markers of inflammation

Fourteen of 307 patients who provided blood samples at eight months instead of four months were included in all analyses because mean and median levels of markers of inflammation did not differ by time of blood draw.

Table 2 shows mean levels of markers of inflammation for all patients and according to PTS status. Patients with PTS had higher mean levels of IL-6 than patients without PTS (7.35 pg/ml ± 14.26 vs. 4.60 pg/ml ± 4.90; p=0.03). Median levels of markers of inflammation are shown in Figure 1 and Table 3. There was a trend toward higher median levels of IL-6 and ICAM-1 in patients with PTS vs. without PTS (p=0.07 and 0.06, respectively), and there was a suggestion of a pattern of higher median levels of some of the markers of inflammation with increasing PTS severity (Table 3). Comparison of values for the 90th percentiles (cut-offs defined by the No PTS group) did not show significant differences between the groups (data not shown). Levels of markers did not vary by type of heparin used or extent of index DVT (data not shown).

Univariate logistic regression analyses of markers of inflammation as predictors of PTS (Table 4) showed that IL-6 levels (continuous) and indicators of elevated IL-6 and ICAM-1 levels (using median levels in patients with no PTS as a cut-off) were predictive of PTS [ORs of 1.04 (p=0.046), 1.66 (p=0.030) and 1.63 (p=0.035), respectively]. In multivariate analyses, addition of prespecified patient characteristics (gender, age, body mass index [BMI], extent of index DVT [proximal vs. distal], recurrent DVT, cancer or cardiovascular comorbidities) to the models did not change the ORs for IL-6 and ICAM-1 by more than 10%, however, age and BMI appeared to be potential confounders. ORs for IL-6 became 1.55 (95% CI 0.97–2.47; p=0.064) and 1.36 (95% CI 0.84–2.21; p=0.207) when BMI and age, respectively, were added to models, while ORs for ICAM-1 became 1.74 (95% CI 1.10–2.99; p=0.046) and 1.33 (95% CI 0.82–2.16; p=0.242) when BMI and age, respectively, were added to the models.

The percentage of patients who had ipsilateral valvular reflux did not differ according to PTS status (Table 1). Levels of markers of inflammation did not differ according to the presence or absence of reflux (data not shown). In bivariate analyses, there were no associations between markers of inflammation and PTS stratified by presence of absence of reflux (data not shown), except that among patients without valvular reflux, median levels of IL-6 were significantly higher in patients with PTS than in those without PTS (3.86 vs. 2.23 pg/ml; p=0.011); this finding was similar to results for the population as a whole.

### Discussion

The results of our prospective study of patients with acute DVT suggest the presence of associations between markers of inflammation such as IL-6 and ICAM-1 and the development of PTS during two years follow-up. Mean and median IL-6 levels and median ICAM-1 levels were higher in patients with PTS compared to patients without PTS. Although statistical significance was somewhat attenuated by the addition of age and BMI to the predictor models, the magnitude of the associations between IL-6, ICAM-1 and PTS remained robust.

In a prospective study, Roumen-Klappe et al. (23) reported that elevated levels of IL-6 and C-reactive protein were associated with elevated venous resistance, a surrogate marker of PTS, after DVT. Similarly, our results also demonstrated a potential

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<table>
<thead>
<tr>
<th>Marker</th>
<th>IL-6 pg/ml</th>
<th>IL-8 pg/ml</th>
<th>IL-10 pg/ml</th>
<th>MCP-1 pg/ml</th>
<th>ICAM-1 ng/ml</th>
<th>VCAM-1 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (n=164)</td>
<td>3.04 (1.56–5.65)</td>
<td>2.10 (0.35–4.07)</td>
<td>1.52 (0.85–2.89)</td>
<td>38.76 (29.92–51.59)</td>
<td>157.62 (135.14–192.53)</td>
<td>357.12 (296.88–432.69)</td>
</tr>
<tr>
<td>Yes (n=141)</td>
<td>3.86 (1.8–6.68)</td>
<td>1.77 (0.35–4.97)</td>
<td>1.63 (0.94–2.69)</td>
<td>42.44 (30.52–54.36)</td>
<td>169.01 (141.03–206.4)</td>
<td>358.41 (303.38–424.92)</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td>0.0656</td>
<td>0.7863</td>
<td>0.8419</td>
<td>0.5435</td>
<td>0.0382</td>
<td>0.7462</td>
</tr>
</tbody>
</table>

**Table 3: Median levels (interquartile ranges) of markers of inflammation by PTS status (n=305*).**
role for IL-6. Conversely, we did not detect associations between IL-8 and IL-10 levels and PTS. Although IL-10 has been shown in previous studies to limit the inflammatory response (24), IL-10 levels were not found to be protective for PTS in our study patients. Although an animal study by Ali et al. (25) showed that MCP-1 treatment of thrombi induced in the vena cava of rats enhanced thrombus resolution through stimulation of recanalisation, we did not detect significant differences between MCP-1 levels in patients with PTS compared to those who did not develop PTS.

Adhesion molecules have also been reported to play a major role in inflammation. VCAM-1 is a ligand expressed by activated endothelial cells and monocytes whose function is to recruit leukocytes to sites of inflammation. Soluble fractions of VCAMs are secreted into the blood circulation when their expression is upregulated. Elevated serum concentrations of VCAMs have been reported in patients with atherosclerosis (26, 27), hypertriglyceridemia (28), diabetes (29) and acute DVT (30, 31). ICAMs are also secreted mainly after stimulation of endothelial cells, vascular smooth muscle cells, macrophages and thrombocytes (32, 33). Although expected to be elevated in conditions where inflammatory processes are triggered, a study by Bucek et al. (34) showed that serum ICAM-1 levels were not different in patients with DVT compared to controls. In a recent publication by Smith (35), elevated circulating levels of adhesion molecules such as VCAM, ICAM and ELAM were found in patients with chronic venous disease compared to controls. In the present report, we showed that ICAM-1 levels appear to be elevated in DVT patients who developed PTS compared to patient who did not develop PTS.

As described in the introduction, inflammatory processes appear to be involved in the pathophysiology of venous disease, including thrombosis. Elevated levels of various inflammatory mediators have been found in patients with DVT, chronic venous diseases and PTS (e.g. CRP, IL-6). Although the major role of these mediators appears to relate to thrombus resolution (29, 36–40), how might they be implicated in the development of PTS? Phillips and Sarkar (41) suggest that increased fibrosis of the vein wall after DVT appears to be the main cause of valve damage, reflux, varicose veins and ulcers and is thought to be caused by inflammatory cells and mediators. Complex molecular mechanisms involving a large number of activated metalloproteinases (MMPs) have been hypothesized to explain the scarring process and subsequent fibrosis development (41). These MMPs, including MMP-1, MMP-2, MMP-3, MMP-8 and MMP-9 are expressed and released by leukocytes and when activated, are believed to result in extracellular matrix degradation and collagen fiber digestion, thus impeding the healing process and leading to vein wall weakening, varicosities and chronic venous ulcers.

The strengths of our study include recruitment of a well defined population of patients with objectively diagnosed DVT who were prospectively followed for two years for the development of PTS, and that we used a new, efficient technique that is known to be reliable, reproducible and sensitive compared with ELISA techniques to measure markers of inflammation (42, 43). However, there are some limitations to our study. First, as this was a sub-study to the main VETO Study, we were limited as to the timepoint at which blood samples were collected from the study patients, i.e. at four months post-DVT instead of at baseline. Hence, we were not able to assess if levels of markers at the time of acute DVT influence the subsequent development of PTS. Of interest, a recent Spanish study (44) showed that elevated levels of IL-6 at the time of DVT diagnosis predicted higher risks of death and PTS during the subsequent 12 months. Nevertheless, we did detect associations between markers of inflammation measured at four months and PTS, suggesting that the four-month timepoint may be relevant to identify DVT patients with a sustained inflammatory response and a higher risk of developing PTS. In the course of an ongoing clinical trial, we plan to examine associations between markers of inflammation drawn at three different time points after DVT diagnosis and PTS (45). Second, the integrity of the plasma samples could be questioned as samples were collected between 2001 and 2004. Although all samples were frozen at −80°C until the time of analysis, several factors might affect the levels measured such as sample processing (presence of hemolyzed red blood cells), the number of freeze-thaw cycles, and/or other proteins or chemicals that might act as contaminants. To avoid this, samples were collected and shipped regularly to the coordinating center for storage until analysis was performed, all samples were stored under the same conditions in a dedicated study freezer and, in the event that a haemolyzed sample was obtained, a second sample was collected to replace the haemolyzed one. Further, to avoid inter-sample variability for the Luminex technique, all samples were treated and analyzed in the same way by the same technician and calibration of the apparatus was done before each assay using manufacturer standards in order to avoid any possible inter-assay variability. Also, the levels of markers of inflammation found in our study are within the ranges of those previously published in

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**Table 4: Univariate logistic regression analysis of markers of inflammation as predictors of PTS (n=305).**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Univariate Odds Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>1.04 (1.00–1.07)</td>
<td>0.046</td>
</tr>
<tr>
<td>Categorical</td>
<td>1.66 (1.05–2.62)</td>
<td>0.030</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>1.00 (0.98–1.02)</td>
<td>0.789</td>
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<tr>
<td>Categorical</td>
<td>0.763 (0.49–1.20)</td>
<td>0.240</td>
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<tr>
<td>IL-10 (pg/ml)</td>
<td></td>
<td></td>
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<tr>
<td>Continuous</td>
<td>1.00 (0.99–1.02)</td>
<td>0.504</td>
</tr>
<tr>
<td>Categorical</td>
<td>1.05 (0.67–1.65)</td>
<td>0.826</td>
</tr>
<tr>
<td>MCP-1 (pg/ml)</td>
<td></td>
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<tr>
<td>Continuous</td>
<td>1.00 (0.99–1.00)</td>
<td>0.884</td>
</tr>
<tr>
<td>Categorical</td>
<td>1.38 (0.88–2.17)</td>
<td>0.160</td>
</tr>
<tr>
<td>ICAM-1 (ng/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>1.00 (0.99–1.00)</td>
<td>0.462</td>
</tr>
<tr>
<td>Categorical</td>
<td>1.63 (1.03–2.58)</td>
<td>0.035</td>
</tr>
<tr>
<td>VCAM-1 (ng/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>1.00 (0.99–1.00)</td>
<td>0.626</td>
</tr>
<tr>
<td>Categorical</td>
<td>1.03 (0.66–1.62)</td>
<td>0.880</td>
</tr>
</tbody>
</table>

*Categorical defined as above or below median levels of patients without PTS.
What is known about this topic?
- A close relationship between inflammation and thrombosis exists.
- The inflammatory process at the time of deep venous thrombosis (DVT) may promote destruction of venous valves, valvular reflux and subsequent development of post-thrombotic syndrome (PTS), a frequent complication of DVT.

What does this paper add?
- We evaluated whether levels of inflammatory cytokines (IL-6, IL-8, IL-10 and MCP-1) and adhesion molecules (ICAM-1 and VCAM-1) are associated with the development of PTS or venous reflux in a well-defined population of patients with DVT.
- Our study suggests the presence of significant associations between markers of inflammation such as IL-6 and ICAM-1 and the development of PTS.
- Further work is needed to evaluate this relationship and to analyze other candidate markers that could be implicated etiologically in the association between DVT and PTS.
- If confirmed, this could lead to identification of new therapeutic targets for preventing PTS after DVT.

the cardiovascular literature for IL-6, ICAM-1, VCAM-1, and other markers of inflammation (46–50). Third, we observed fairly large variability in some marker levels which could be attributed to the disease state studied (i.e. PTS development stage) as well as comorbid conditions that could affect marker levels and the time of day samples were collected Fourth, it would have been of interest mechanistically to measure the levels of markers of inflammation directly in the affected vein at the level of the thrombus, in addition to measuring them in plasma. However, this would require biopsy of the thrombosed vein which is invasive and painful for patients, and our study objective was to identify conveniently measurable markers that might be predictive of future development of PTS.

Several other markers present in the circulation may also be involved in the occurrence, resolution or recurrence of DVT or PTS; however, elucidation of their specific roles is yet to be accomplished. These include von Willebrand factor, vascular endothelial growth factor, tissue plasminogen activator and platelet activator inhibitor.

In conclusion, our study supports the hypothesis that inflammatory processes contribute to the development of PTS after DVT. In ongoing work, we are further studying the influence of inflammatory markers at various timepoints after acute DVT on the development of PTS and attempting to identify pathways leading to this condition. Confirmation and elucidation of the association between inflammation and PTS could have important therapeutic implications for the prevention and treatment of PTS.

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