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CLINICAL RESEARCH STUDIES

Association between inflammation biomarkers, anatomic extent of deep venous thrombosis, and venous symptoms after deep venous thrombosis

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Objective: **Inflammation may play a role in pathogenesis of venous thromboembolism, but the nature of this relationship is not yet understood. The objective of this study was to assess whether inflammation marker levels measured at diagnosis of deep venous thrombosis (DVT) and change in levels during the first month after DVT are associated with anatomic extent of DVT and severity of venous signs and symptoms at baseline and 1 month.**

Methods: **The BioSOX study is a biomarker substudy of the Compression Stockings to Prevent the Post-Thrombotic Syndrome (SOX) trial, a multicenter, randomized controlled trial that included patients with a first, acute, symptomatic, proximal DVT. Blood samples were collected from participants at baseline and 1 month, and C-reactive protein (CRP), intercellular adhesion molecule 1, interleukin (IL)-6, and IL-10 were measured by established assays. Linear regression was used to assess the association between continuous log-transformed baseline biomarker levels and anatomic extent of DVT, classified as iliac or common femoral DVT vs femoral or popliteal DVT (reference). Proportional odds ordinal logistic regression models were used to analyze the association between biomarker level and Villalta score (as a measure of severity of venous signs and symptoms) at baseline and 1 month.**

Results: **Among 717 patients, 60.2% were male, and the mean age was 55.2 years. There was a significant association between more extensive DVT (common femoral or iliac) and levels of CRP and IL-6 at DVT diagnosis. Median (interquartile range) CRP level was 11.6 mg/L (3.84-39.5) in patients with common femoral or iliac DVT vs 6.86 mg/L (3.11-22) in patients with popliteal or femoral DVT, and median IL-6 level was 6.36 pg/mL (1.09-14.37) vs 4.40 pg/mL (2.35-8.27), respectively. These differences were statistically significant in linear regression analyses. In addition, compared with those in the lowest quartile, each higher quartile of baseline CRP concentration was associated with an odds ratio of 2.89 (1.93-4.33) for having a more severe Villalta category at baseline and 1.98 (1.28-3.08) for having a more severe Villalta category 1 month after DVT. Higher baseline levels of IL-6 were associated with Villalta severity category at baseline (odds ratio, 2.40 [1.61-3.59]). Change in biomarker levels during the first month after DVT was not strongly associated with the 1-month Villalta score.**

Conclusions: **Levels of CRP and IL-6 at DVT diagnosis were associated with thrombotic disease burden, as measured by DVT extent, and severity of DVT symptoms and signs. Further studies are required to more fully elucidate the role of inflammation in DVT and its clinical course. (J Vasc Surg: Venous and Lym Dis 2015;3:347-53.)**

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The BioSOX Investigators are listed in the Appendix (online only).

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Venous thromboembolism (VTE) is the third leading cause of vascular death, with a high incidence, especially among older persons, and an overall estimated incidence between 0.7 and 2 per 1000 person-years.[1] Post-thrombotic syndrome (PTS) is the most common chronic complication of deep venous thrombosis (DVT), occurring in 20% to 50% of patients with DVT.[2,3]

The importance of the inflammatory response in venous thrombosis has been shown in in vitro studies[4,5] and in animal models.[6-8] Data regarding this association in humans are accumulating[9-16]; however, it is still not known whether inflammation is causal in the development or clinical course of venous thrombosis or rather is a consequence of venous thrombosis.

The Villalta scale can be used to quantify the severity of venous symptoms and signs after DVT. Baseline Villalta score, 1-month Villalta score, and extensive (iliac or common femoral) DVT are significant predictors of later PTS.[3,17] To improve our ability to predict which DVT patients will develop

PTS, inflammatory biomarkers measured at DVT diagnosis may prove useful if they are significantly associated with these clinical predictors. Identifying such associations could better define, at an early stage, patients who are at high risk for future development of PTS. Demonstrating such an association will also provide additional evidence for the role of inflammation in DVT and provide a basis for future intervention studies. We hypothesized that inflammation marker levels may relate to anatomic extent of acute DVT and severity of acute DVT signs and symptoms.

We aimed to assess whether inflammation marker levels measured at DVT diagnosis and change in levels during the first month after DVT are associated with the anatomic extent of DVT and baseline and 1-month Villalta scores.

METHODS

The BioSOX study is a biomarker substudy of the Compression Stockings to Prevent the Post-Thrombotic Syndrome (SOX) trial, a multicenter, randomized placebo-controlled trial of elastic compression stockings for the prevention of PTS. Patients with a first, acute, symptomatic, proximal DVT of the lower limbs were observed for 24 months. Baseline, 1-month, and 6-month blood samples were collected. This paper focuses on the baseline and 1-month samples as our interest was in the acute phase of DVT.

Proximal DVT, defined as DVT in the popliteal or more proximal deep leg veins, had to have been objectively confirmed by ultrasound. Patients were excluded from the SOX trial if they had a contraindication to or inability to apply elastic compression stockings, an expected life span of <6 months, or geographic inaccessibility to follow-up or had received thrombolytic therapy for the initial treatment of DVT.

Serum concentrations of C-reactive protein (CRP), intercellular adhesion molecule 1 (ICAM-1), interleukin (IL)-6, and IL-10 were measured at each time point.

CRP was measured with a BN II nephelometer (Siemens Healthcare, Malvern, Pa). The interassay coefficient of variation (CV) range was 2.8% to 4.8% with a lower detection level of 0.15 mg/L. ICAM-1 was measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minn) with an interassay CV range of 5.6% to 6.0% and a detection range of 2 to 1000 ng/mL. IL-6 was measured by a chemiluminescent enzyme-linked immunosorbent assay (R&D Systems) with an interassay CV range of 12.0% to 15.9% and a detection range of 0.48 to 1500 pg/mL. IL-10 was measured by the multiplex CVD3 panel (Millipore, Billerica, Mass) with an interassay CV range of 3.6% to 10.3% and a lower detection limit of 0.013 pg/mL.

The study was approved by the research ethics boards at all participating centers, and written informed consent was obtained from all patients.

Statistical analysis. Continuous variables were described as medians and interquartile ranges. Linear regression models were used to assess the association between continuous baseline biomarker levels (log transformed where appropriate) and highest anatomic extent of acute DVT, classified a priori as iliac or common femoral DVT (more extensive DVT) vs femoral or popliteal DVT (reference category; less extensive DVT).

To quantify DVT symptoms and signs and their severity at DVT diagnosis (baseline) and 1 month after DVT, we used the Villalta scale, a reproducible clinical 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 ectasia, and pain on calf compression).[18] The presence of leg ulcers was also documented. A higher total Villalta score indicates greater severity of signs and symptoms (score of 5 to 9, mild; 10 to 14, moderate; >14 or leg ulcer, severe), in accordance with PTS severity level when this scale is used for PTS diagnosis at later time points after DVT.

Proportional odds ordinal logistic regression models were used to analyze the association between (1) baseline biomarker level and baseline Villalta score category, (2) baseline biomarker level and 1-month Villalta score category, and (3) change in biomarker level from baseline to 1 month and 1-month Villalta score category.

Percent change from baseline to 1 month was defined as negative, no or minimal change, and positive change from baseline biomarker level. To define no or minimal change in biomarker levels, we used a conservative estimate defined as two times the upper limit of the interassay CV supplied by the laboratory: 4.8% for CRP, 6% for ICAM-1, 15.9% for IL-6, and 10.3% for IL-10. Negative percent change was divided at the median and categorized as strongly decreasing (referent) and decreasing.

Adjustment variables included age, sex, and body mass index; smoking (any vs none at time of baseline assessment); type of DVT (unprovoked vs secondary); cancer-related DVT; history of infectious or inflammatory conditions; stroke or myocardial infarction within 1 month before DVT; and use of antiplatelet agents, nonsteroidal anti-inflammatory drugs, steroids, or statins in the month before DVT. Secondary DVT was defined as post partum or immobilized within the last month or trauma or surgery within the last 3 months. Cancer-related DVT was defined as active malignant disease (diagnosed within the last 6 months or metastatic or ongoing treatment or palliative treatment). In adjusted analyses focused on the percent change in biomarker levels from baseline to 1 month, we also adjusted for baseline quartile of biomarker level. Adjustment for multiple testing was performed with the Bonferroni correction.

All analyses were done with Stata/IC 11.3 (Stata Corp, College Station, Tex).

RESULTS

Among 803 patients enrolled in the SOX trial at 24 Canadian and U.S. centers from 2004 to 2010, 725 agreed to participate in the BioSOX substudy, and 717 provided a baseline sample. Baseline characteristics of study subjects are described in Table I. Mean age was 55.2 years, and 61.2% were male.

Median (interquartile range) CRP level at DVT diagnosis was 11.6 mg/L (3.84-39.5) in patients with common