Genetic And Other Factors Promoting DVT: How Can They Be Detected And Treated
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A-Investigating Biomarkers to Diagnose DVT

A1- Investigating P-selectin, D-dimer, and Wells Score

Venous thromboembolism (VTE) is a substantial health problem in the United States, with an estimated incidence of up to 2 to 3 per 1000 people annually. The morbidity and mortality of undiagnosed VTE remains high, as nearly 60% of undiagnosed deep venous thrombosis (DVT) eventually leads to pulmonary embolism (PE), causing up to 300,000 deaths per year. Therefore, it is important to establish the diagnosis and initiate anticoagulation therapy, when appropriate, in a timely manner. However, the diagnosis of DVT based on clinical presentation alone is unreliable, and this fact has led to the development of clinical scoring systems such as the Well’s score that incorporate signs, symptoms, and risk factors to categorize patients into levels of risk.

Along with clinical scoring systems, biomarkers for thrombosis have also been suggested to help establish the diagnosis of DVT. Currently, the most prominent biomarker used is plasma D-dimer. D-dimer is a product of fibrin degradation and it is a sensitive biomarker that, when low or absent can be used to rule out the diagnosis of DVT in patients with a low Wells’s score. However, this biomarker is not specific for DVT, as it is frequently elevated in the setting of nonspecific inflammation such as cancer, pregnancy, surgery, and trauma. Because of the low specificity of D-dimer, elevated levels cannot be used to “rule in” the diagnosis of DVT, and in such instances an imaging study is needed for definitive diagnosis. The current standard for DVT diagnosis is duplex ultrasound, with or without color, utilizing compression. However, this test is not always available (smaller medical centers, outpatient settings, and on nights and weekends). In these circumstances, a chemical or laboratory diagnosis to “rule in” the diagnosis of DVT without having to rely on duplex ultrasound would be helpful. We have been studying the role of platelet and leukocyte activation, inflammation and thrombosis related to DVT in our laboratory, and we have identified soluble P selectin (sP-sel), soluble E selectin (sE-sel) and microparticles (MP) as potential novel biomarkers to make the diagnosis of DVT. Of these, sP-sel has shown the greatest potential to not only “rule out”, but also establish and “rule in” the diagnosis. Previously we demonstrated that soluble P-selectin (sP-sel) in combination with the Well’s score, establishes the diagnosis of LE-DVT with specificity of 96% and positive predictive value (PPV) of 100%. In order to validate our previous results, we applied the model to a separate but similar patient cohort.

Between April 2009 and March 2012, all patients presenting for a duplex ultrasound exam for concern of DVT were screened. Demographics, clinical data, D-dimer, sPsel, C-reactive protein (CRP), ADAMTS-13, and von Willebrand factor (VWF) levels were prospectively collected in 279 patients (234 LE-DVT, 45 UE-DVT). Continuous and categorical variables were compared to between patients with and without DVT. The diagnostic sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) was then calculated using cut points from our previous work to “rule out” or “rule in” DVT.
Among 234 patients evaluated for LE-DVT, 112 (48%) patients had a confirmed LE-DVT with significant differences in all biomarkers. When Wells score ≥2, sP-sel could rule in LE-DVT with a specificity of 97.5% and a PPV of 91%, which was more accurate than Wells score ≥2 and D-dimer (specificity 65%, PPV 69%) – the current clinical diagnostic paradigm. When Well’s score was <2, D-dimer was superior to sP-sel for excluding the diagnosis of LE-DVT (sensitivity 98%, NPV 95% vs. sensitivity 91%, NPV 79%). The use of additional biomarkers did not increase accuracy. Based on this data, had imaging not been available, 67/234 (29%) of patient could have “ruled out” or “ruled in” LE-DVT for the diagnosis of DVT. The use of sP-sel in UE-DVT was non-diagnostic, likely related to the relatively small thrombi in the UP compared to the LE.

We thus demonstrated that when Wells score ≥2, sP-sel is an excellent biomarker to “rule in” LE-DVT, while D-dimer and a Wells score <2 was most sensitive to “rule out” the diagnosis of LE-DVT. Plasma DNA is elevated in patients with DVT and correlates with biomarkers of DVT.

**B- Investigating New Molecules in the Context of Venous Thrombosis**

**B1- Investigating Extracellular Traps as potential biomarkers for DVT**

Leukocytes release DNA to form extracellular traps (ETs), which have recently been linked to experimental DVT In baboons and mice, extracellular DNA co-localized with von Willebrand factor (VWF) in the thrombus and DNA appeared in circulation at the time of thrombus formation. 

Thus, DNA, if found in human DNA, could also be a marker for DVT (and VTE).

For the ETs, a subgroup of patients were analyze consisting of 47 patients who were symptomatic for DVT and confirmed by compression duplex ultrasound, 28 patients who presented with swelling and leg pain but had a negative compression duplex ultrasound, and 19 controls of healthy non-pregnant volunteers without signs or symptoms of active or previous DVT. Exclusion criteria included age less than 18 years, unwillingness to consent, current pregnancy, on an anticoagulant, or diagnosed with isolated calf vein thrombosis. Additionally, the Wells score for a patient’s risk of DVT was assessed in every case.

Regarding ETs, we used circulating plasma DNA as a surrogate marker. Our results demonstrated that circulating DNA was significantly elevated in patients positive for DVT by ultrasound, as opposed to both patients negative for DVT by ultrasound (57.7±6.3 vs. 17.9±3.5ng/mL, P<.01) and controls (57.7±6.3 vs. 23.9±2.1ng/mL, P<.01). There was a strong positive correlation between levels of circulating plasma DNA and CRP (P<.01), D-dimer (P<.01), VWF (P<.01), Wells score (P<.01) and myeloperoxidase (MPO) (P<.01), along with a strong negative correlation between plasma DNA and ADAMTS13 (P<.01) and the ADAMTS13/VWF ratio.

The logistic regression model showed a strong association between plasma DNA and the presence of DVT (ROC curve was determined to be 0.814). In conclusion, a strong correlation between circulating DNA and MPO suggests that neutrophils may be a source of plasma DNA in patients with DVT.
B2. Investigating Galectin-3 binding protein and Galactin-3 in VT

Galactin-3 binding protein (Gal-3BP) was detected in procoagulant microparticles from mice and patients with venous thrombosis (VT) in prior studies from our laboratory. Subsequently, we demonstrated that both the inhibition of Gal3-BP in wild type (WT) mice and the absence of Gal-3 in Gal-3 knock-out mice decreased VT. However, the precise role of Gal-3BP and Gal-3 in the pathophysiology of thrombosis remains unknown. We hypothesized that Gal-3BP and Gal-3 are critical to thrombus venous formation. In order to investigate, we performed studies in the mouse and in man.

**Mice:** Using our inferior vena cava (IVC) ligation model of venous thrombosis in WT mice, circulating microparticle, platelet, and red blood cell samples from thrombosed (VT) and non-thrombosed (non-VT) mice were analyzed for Gal-3BP and Gal-3 by western blot. The expression of Gal-3BP and Gal-3 in the IVC of mice with venous thrombosis and mice without venous thrombosis was evaluated by RT-PCR. Additionally, Gal-3BP and Gal-3 was qualitatively evaluated in thrombi and the surrounding vein wall by western blot. Finally, circulating Gal-3BP and Gal-3 was measured in mouse plasma using ELISA. **Patients:** Microparticles and plasma samples from patients who were positive (n=80) or negative (n=79) for venous thrombosis by compression duplex ultrasound were tested for Gal-3BP and Gal-3 using ELISA.

**Mice:** Gal-3BP protein levels were not significantly different in mice with or without venous thrombosis in vein wall, thrombi, circulating microparticle, platelet, or red blood cell samples. Gal-3 monomers and multimers were more abundant in the circulating microparticles, platelets, and red blood cells of mice with venous thrombosis compared to non-thrombosed mice. In the IVC, Gal-3 gene expression was significantly increased in mice with venous thrombosis compared to mice without venous thrombosis (p<0.01), and Gal-3 protein levels were likewise increased. There were no significant differences in Gal-3BP gene expression between mice with and without venous thrombosis. Gal-3BP and Gal-3 were both found in 2-day old murine thrombi. Circulating Gal-3BP was elevated in plasma and significantly elevated in microparticles (p<0.05) from mice with venous thrombosis, while circulating Gal-3 was significantly elevated in both plasma and microparticles (p<0.05) from these same mice with venous thrombosis.

**Patients:** Gal-3BP was significantly elevated in microparticles (p<0.05) and in plasma (p<0.05) from patients positive for DVT by compression ultrasound, compared to patients negative for DVT by ultrasound but with leg pain.

We thus demonstrated that Gal-3BP and Gal-3 play a role in venous thrombosis in two species, mice and humans. To the best of our knowledge, this is the first study to shed light upon the role of Gal-3BP and Gal-3 in venous thrombosis. Gal-3BP and Gal-3 are promising potential targets for therapeutic interventions and potential biomarkers for venous thrombosis.
References: