Journal of Vascular Surgery: Venous and Lymphatic Disorders
March 2019

Author Disclosures: K. Lobastov: Nothing to disclose; **G. Dementieva:** Nothing to disclose; **N. Soshitova:** Nothing to disclose; **E. Sautina:** Nothing to disclose; **V. Barinov:** Nothing to disclose; **L. Laberko:** Nothing to disclose; **G. Rodoman:** Nothing to disclose.

E-Selectin Inhibition: A New Way to Treat Proximal Deep Venous Thrombosis

Daniel Myers,[1] Patrick Lester,[1] Raymond Adili,[1] Angela Hawley,[1] Laura Durham,[1] Veronica Dunivant,[1] Garrett Reynolds,[1] Suman Sood,[1] William Fogler,[2] John Magnani,[2] Mike Holinstat,[1] **Thomas Wakefield**[1]. [1]University of Michigan Medical School; [2]GlycoMimetics, Inc

Objective: There is a close inter-relationship between thrombosis and inflammation. In previous studies, we have shown the importance of P-selectin in thrombogenesis and thrombus resolution in many preclinical animal models. The role of E-selectin has been explored in rodent models and in a small pilot study of clinical calf vein deep venous thrombosis. The purpose of this study was to determine the role of E-selectin in thrombosis in a primate model of proximal iliac vein thrombosis, a model close to the human condition.

Methods: Iliac vein thrombosis was induced with a well-characterized primate model. Through a transplant incision, the hypogastric vein and iliac vein branches were ligated. Thrombus was induced by balloon occlusion of the proximal and distal iliac vein for 6 hours. The balloons were then deflated, and the primates recovered. Starting on postocclusion day 2, animals were treated with the E-selectin inhibitor GMI-1271, 25 mg/kg subcutaneously once daily (n = 4). Nontreated control (CTR) animals received no treatment (n = 5). All animals were evaluated by magnetic resonance venography (MRV), hematology (complete blood count), coagulation tests (bleeding time, partial thromboplastin time, activated partial thromboplastin time, fibrinogen, and thromboelastography) at baseline, day 2, day 7, day14, and day 21 with euthanasia. In addition, platelet function and CD44 expression on leukocytes were determined.

Results: E-selectin inhibition by GMI-1271 significantly increased vein recanalization by MRV vs CTR animals on day 14 ($P < .05$) and day 21 ($P < .0001$; Fig, *A*). GMI-1271 significantly decreased vein wall inflammation by MRV with gadolinium vein wall enhancement vs CTR also on day 14 ($P < .0001$) and day 21 ($P < .0001$; Fig, *B*). The thromboelastographic measure of clot strength showed significant decreases in animals treated with GMI-1271 vs CTRs at day 2 ($P < .05$) and day 7 ($P < .05$). Importantly, no significant differences in hematology or coagulation test results were noted between all groups, suggesting that E-selectin inhibition carries no bleeding potential. GMI-1271 did not affect platelet function or aggregation or CD44 expression on leukocytes. In addition, no episodes of bleeding were noted in either group.

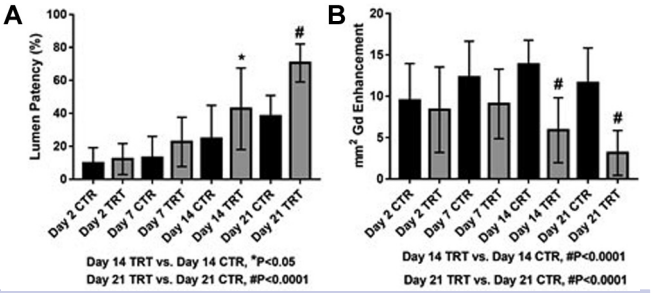

Fig. A, Percentage of vein recanalization. **B,** Vein wall inflammation. *CTR,* control; *TRT,* treated.

Conclusions: The study suggests that E-selectin modulates venous thrombus progression and that its inhibition will increase thrombus recanalization and decrease vein wall inflammation, without affecting coagulation. The use of an E-selectin inhibitor such as GMI-1271 could potentially change how we treat deep venous thrombosis.

Author Disclosures: D. Myers: Nothing to disclose; **P. Lester:** Nothing to disclose; **R. Adili:** Nothing to disclose; **A. Hawley:** Nothing to disclose; **L. Durham:** Nothing to disclose; **V. Dunivant:** Nothing to disclose; **G. Reynolds:** Nothing to disclose; **S. Soo:** Nothing to disclose; **W. Fogler:** salary, stock options GlycoMimetics, Inc; **J. Magnani:** salary, stock options GlycoMimetics, Inc; **M. Holinstat:** Nothing to disclose; **T. Wakefield:** Nothing to disclose.

Association of Inflammatory and Hemostatic Biomarkers with Inflammasomes in Septic Patients at Risk for Development of Coagulopathy

Evi Kalodiki,[1,2] Debra Hoppensteadt,[1] Richard Green,[1] Amanda Walborn,[1] Grace Wegryzn,[1] Jawed Fareed[1]. [1]Thrombosis Hemostasis, Loyola University Medical Centre; [2]Vascular Surgery, Josef Pflug Vascular Laboratory, Ealing Hospital, and Imperial College and West London Vascular and Interventional Centre

Objective: Sepsis is a catastrophic complication of infection that results in systemic inflammatory responses. Inflammasomes initiate the inflammatory cascade that results in the activation of caspase 1, leading to the upregulation of inflammatory cytokines such as interleukins B and 18. The NRLP-3 inflammasome contributes to the innate immune response identification of pattern recognition receptors on pathogens including bacteria and viruses. Whereas the role of inflammasome in the inflammatory response is known, it is not clear how inflammasome contributes to the hemostatic dysregulation observed in sepsis-associated coagulopathy. The purpose of this study was to quantitate inflammasome levels in defined sepsis-associated patients and to determine its potential relevance to various biomarkers of hemostatic dysregulation.

Methods: Plasma samples from 52 adults with sepsis and suspected coagulopathy were analyzed. Samples were collected from intensive care unit patients on day 0, under an Institution Review Board-approved protocol. Samples were stored at −80°C before analysis. Platelet count was determined as part of standard clinical practice. Healthy control samples were purchased from George King Bio-Medical (Overland, Kan). Prothrombin time and international normalized ratio were measured using recombiplastin reagent. Fibrinogen was measured using a clot-based method on ACL ELITE (Instrumentation Laboratory, Bedford, Mass) coagulation analyzer. Cortisol, D-dimer, plasminogen activator inhibitor 1 (PAI-1), NLRP-3 inflammasomes, microparticle-associated tissue factor, fibronectin, and CD40L were measured using commercially available enzyme-linked immunosorbent assays.

Results: In comparing patients with sepsis and suspected disseminated intravascular coagulation (DIC) with the normal plasma samples, there was a significant elevation in NLRP-3 inflammasome levels in the sepsis cohort ($P < .0001$). The Fig shows that the NLRP-3 inflammasome concentration in the sepsis cohort did not correlate with other biomarkers. An elevated level of NLRP-3 inflammasomes was significantly

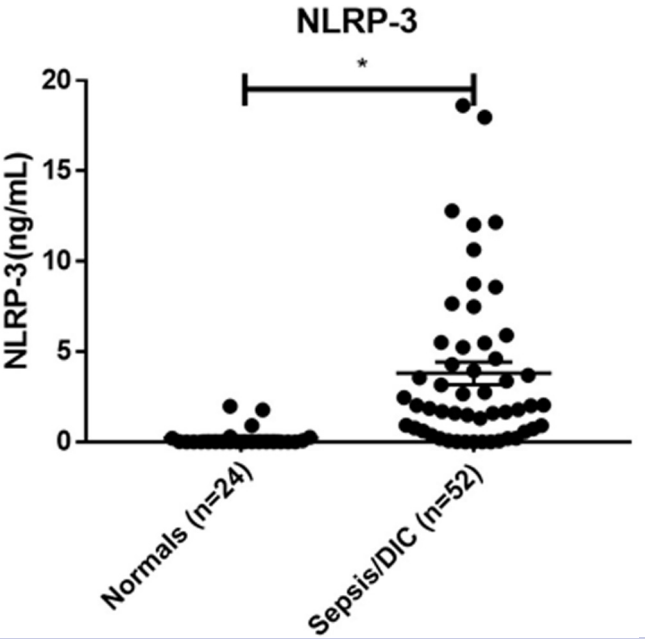

Fig. NLRP-3 inflammasomes in patients with sepsis and suspected disseminated intravascular coagulation (*DIC*) on day 0 (N = 52) compared with normal healthy controls (N = 24).

Table. Inflammatory and hemostatic biomarkers correlated with NLRP-3 inflammasome levels in patients with sepsis and suspected disseminated intravascular coagulation (DIC)

NLRP-3 inflammasome correlation	*P* (Mann-Whitney)	Spearman *r*
CD40L	.5716	−0.08028
PAI-1	.0041	0.3915
MP-TF	.1491	0.2528
Fibrinogen	.7685	0.04314
Fibronectin	.8291	−0.03067
Cortisol	.0758	0.2484
D-Dimer	.3272	0.1495

MP-TF, Microparticle-associated tissue factor; *PAI-1*, plasminogen activator inhibitor 1.

associated with increased levels of PAI-1 ($P < .0004$). No other inflammatory or hemostatic markers were significantly correlated with NLRP-3 inflammasomes. This is depicted in the Table.

Conclusions: The study shows a significant relationship between inflammasomes and PAI-1 levels in patients with sepsis-associated coagulopathy. The positive correlation between NLRP-3 inflammasomes and PAI-1 shows that the activation of inflammasomes may have a role in the upregulation of PAI-1 and the observed hemostatic dysregulation. The strong association between NLRP-3 inflammasome and PAI-1 in baseline samples of patients with sepsis and DIC also suggests that NLRP-3 inflammasome may contribute to the fibrinolytic dysregulation in sepsis and DIC.

Author Disclosures: E. Kalodiki: Nothing to disclose; **D. Hoppensteadt:** Nothing to disclose; **R. Green:** nothing to disclose: **A. Walborn:** Nothing to disclose; **G. Wegryzn:** Nothing to disclose; **J. Fareed:** Nothing to disclose.

Venous Reflux Changes After Physical Exercise

Roman A. Tauraginskii,[1] Fedor Lurie,[2,3] Sergei S. Simakov,[4] Denis A. Borsuk[5]. [1]International Institution of Health Care Research Institute of Clinical Medicine; [2]Jobst Vascular Institute; [3]University of Michigan; [4]Moscow Institute of Physics and Technology; [5]The Clinic of Phlebology and Laser Surgery "Vasculab" Ltd

Objective: It is known that the presence of venous reflux affects the arterial inflow to the lower limb. However, the effect of changes in arterial inflow on the parameters of venous reflux has not been studied. The purpose of this investigation was to assess the influence of changes in arterial inflow during exercise on quantitative parameters of reflux.

Methods: There were 61 lower limbs of patients with primary incompetence of the great saphenous vein (GSV) included in the study. The diameter, cross-sectional area, average velocity, and reflux time (RT) were measured by duplex ultrasound. Reflux volume (RV) flow (Q) and absolute RV (ARV) were calculated. The measurements were performed standing at rest before physical exercise and 60 seconds after physical exercise (30 lifts to tiptoes at a frequency of 1 time per second). A decrease in the absolute volume of reflux (DRV) after exercise was calculated (DRV = ARV [after] − ARV [before] / ARV [before] * 100%). Automatic distal compression-decompression (120 mm Hg) was used as a provoking maneuver. Median and interquartile range were used for describing quantity parameters.

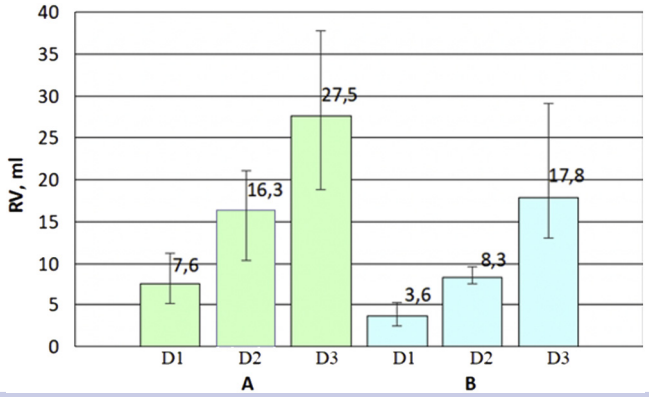

Fig 1. Reflux volume (*RV*) depending on diameter of the great saphenous vein (GSV). **A,** Before physical exercise. **B,** After physical exercise. All groups have statistically significant difference.

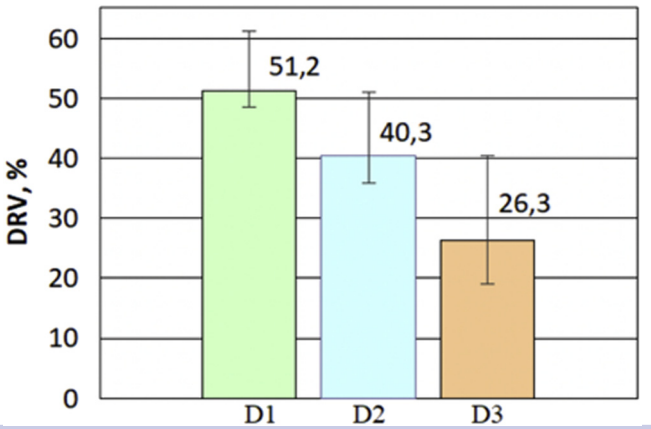

Fig 2. Decrease in reflux volume (*DRV*) changes depending on diameter of the great saphenous vein (GSV). D1 and D3, $P = .0002$. D1 and D2, $P = .099$. D2 and D3, $P = .0581$.

Results: Before exercise, reflux parameters were as follows: RT = 4.85 (3.71-6.00) seconds; Q = 3.89 (2.03-5.81) mL/s; RV = 17.05 (10.32-25.34) mL. After physical exercise, they changed to RT = 2.86 (2.14-3.33) seconds, Q = 3.61 (2.06-6.37) mL/s, and RV = 10.07 (6.08-16.48) mL. The changes in RT and RV were statistically significant ($P < .0001$ and $P = .0007$, respectively; Fig 1).

DRV was statistically significantly different only between groups D1 and D3 ($P = .0002$; Fig 2).

DRV was reversely related to the GSV diameter ($r = −0.56$; $P < .05$) and to the disease severity measured by Venous Clinical Severity Score ($r = −0.41$; $P < .0001$).

Conclusions: The increase in arterial inflow during physical activity leads to the decrease in the volume of reflux, mainly due to decrease in RT. The decrease in the volume of reflux after exercise is inversely proportional to the diameter of the GSV.

Author Disclosures: R. A. Tauraginskii: Nothing to disclose; **F. Lurie:** Nothing to disclose; **S. S. Simakov:** Nothing to disclose; **D. A. Borsuk:** Nothing to disclose.

In Vivo and Ex Vivo Thrombin Generation in Noncomorbid Patients with Suspected Deep Venous Thrombosis

Evi Kalodiki,[1,2] Fredrik Wexels,[3] Ola Dahl,[3] Jeanine Walenga,[3] Walter Jeske,[3] Omer Iqbal,[3] Debra Hoppensteadt,[3] Jawed Fareed[3]. [1]Loyola University Medical Centre; [2]Vascular Surgery, Josef Pflug Vascular Laboratory, Ealing Hospital, and Imperial College and West London Vascular and Interventional Centre; [3]Thrombosis Hemostasis, Loyola University Medical Centre

Objective: Thrombin generation in vivo can be assessed by measuring prothrombin fragment 1+2 (F1+2) and D-dimer. The F1+2 fragment is generated during prothrombin conversion to thrombin and thus reflects thrombin generation. Degradation of cross-linked fibrin produces D-dimer, which reflects both ongoing coagulation and blood clot dissolution. Ex vivo, thrombin generation can be assessed by the thrombin generation assay, in which the endogenous thrombin potential (ETP) reflects the total enzymatic activity of thrombin. The aim was to compare thrombin generation in vivo and ex vivo in patients with suspected deep venous thrombosis (DVT).

Methods: Patients with clinically suspected DVT and without known comorbidities or taking anticoagulants were included. Blood samples were collected before examination with compression ultrasound of the lower extremities. In vivo parameters were analyzed with commercially