and incidence of DVT in critically ill patients was similar to rates in orthopedic surgery. Clinicians should maintain a high index of suspicion for DVT in critical illness.

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A HIGH INCIDENCE OF PATIENTS WITH MTHFR (METHYLENETETRAHYDROFOLATE REDUCTASE) GENE MUTATIONS NOTED IN AN APPALACHIAN POPULATION SEEN FOR THROMBOPHILIA AT THE WEST VIRGINIA UNIVERSITY HOSPITALS, 2006-2012

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Background We noted a higher than expected incidence of MTHFR gene mutations C677T or A1298C in Appalachian patients; a review of 72 patients was presented at American Society of Hematology meeting in 2009. To further evaluate the finding, we looked at 1378 patients, seen at our hospital for thrombophilia work up from 2006-2012 and this is an update to our previous report. Studies have suggested an increased risk of thromboembolism in patients with hyperhomocysteinemia. C677T mutation in the MTHFR gene has been thought responsible for hyperhomocysteinemia. However, the association of MTHFR gene mutations C677T and A1298C and thromboembolism, remains controversial. Methods We reviewed records of 1378 patients for risk factors for acquired or inherited thrombophilia. These patients include both inpatients and outpatients, seen at our institution from 2006-2012 for thrombophilia evaluation and had MTHFR testing done. All available risk factors and laboratory results were reviewed. Results 1378 patients had testing done for MTHFR from 2006-2012. Of 1378 patients 1072(77.7%) were females and 306(22.2%) were males. Median age was 35 years (range18-86). 1161 of the 1378(84%) patients had one or more positive mutations, with alleles C677TT (11.3%-high risk), C677T (25.7%), A1298CC (9%) and A1298C (20.5%). Further 268(19.4%) patients were compound heterozygote and had C677T-A1298C high risk mutation. Thus, 30.7% of all patients tested had a "high risk: MTHFR gene mutation. Coagulation profile of 1378 patients is depicted in Figure 1. Conclusion: Inherited risk factors in patients with established thromboembolism in our patient population is tabulated above. The factor II and factor V mutation incidences of 4.7% and 8.2%, as well the number of abnormalities in Protein C, protein S and anticardiolipin antibody appear similar to data reported in the literature for Caucasian populations. However, Rodrigues et al reported C677TT and C677T prevalence of 14-19% and 36-47% respectively in 1277 normal persons and A1298CC and A1298 C prevalence of 7-11% and 28-35% respectively(Am J Clin Nutr 2006;83:701). In our Appalachian population, the overall incidence of MTHFR mutations previously reported by our group in a small sample size (72) to be 88% still stands true in a larger sample size(1378) per our report but the distribution of the high risk mutations probably is not as high as reported earlier by our group. The incidence of MTHFR high risk mutation in Appalachian population is in accordance with previous population based studies.

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COMPLEXES OF PLATELET FACTOR 4 AND HEPARIN ELICIT TOLL-LIKE RECEPTOR 4-MEDIATED CYTOKINE RELEASE

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Background: Platelet factor 4 (PF4) is a multifunctional protein which serves varied aspects of host defense, including hemostasis, inflammation, innate immunity, and wound repair. PF4 is released from platelets activated by signals indicating microbial infection or tissue or vascular damage. This cationic protein binds to negatively charged extracellular matrix components, microbial membranes or DNA/RNA adducts and forms multimolecular complexes with anionic polymers. Specific complexes formed between PF4 and the anticoagulant, heparin (H), elicit generation of PF4:H antibodies, which are responsible for the life and/or limb threatening syndrome known as heparin-induced thrombocytopenia (HIT). The mechanism of this atypical immune response is poorly understood. Objective: This study was designed to determine whether PF4:H complexes might present a pathogen- or damage-associated molecular pattern recognized by a toll-like receptor (TLR), which would help explain the initiation of an immune response. Methods: PF4 (10 µg/mL; MW 29,000; Haematologic Technologies Inc., Essex Junction, VT) was mixed with 0, 5, 0.5, 0.05 or 0.025 U/mL heparin (Hospira, Inc., Lake Forest, IL) to form complexes of know PF4 to H ratio (PHR)(PHR calculations based on heparin of 140 U/mg: MW 13,000). PF4 alone or in PHR complexes of 1:8, 1.25:1, 12.5:1 or 25:1 were incubated overnight at 37oC, 5% CO2 with acid-citrate-dextrose anticoagulated human whole blood from multiple donors, before plasmas were collected and assayed by ELISA for interleukin-8 (IL-8) (Affimetrix/eBioscience, Inc. San Diego, CA). Incubations with graded concentrations of lipopolysaccharide (LPS) (E.coli O111:B4, Sigma, St. Louis, MO) served as positive control for TLR-mediated IL-8 release. In some experiments, whole blood was incubated with antibody to TLR4 or isotype controls before exposure to PF4:H or LPS. Results: LPS (0 – 1000 ng/mL) caused dose-dependent IL-8 release which was blocked by anti-TLR4 antibody. PF4:H complexes also caused TLR4-mediated IL-8 release that varied depending on PHR; IL-8 release was least when heparin was either absent or in excess (PHR 1:8) and greatest at PHR of 1.25 or 12.5:1. TLR-mediated responses varied widely among donors, in threshold LPS concentration and dose response range, and in the level of IL-8 response to PF4:H complexes. Responsiveness to LPS and to PF4:H complexes was proportional in some but not all experiments. Conclusions: Complexes of PF4 and H, in molar ratios know to be cross-reactive with HIT antibodies, function as a TLR4 agonist and cause IL8 release in cultured whole blood. Toll receptor activation indicates that PF4 bound to heparin can mimic a pathogen- or damage-associated molecular pattern, and supports the suggestion that the HIT immune response represents a misdirected host defense mechanism. Further studies will determine what, if any, other cytokines are released in response to specific PF4:H complexes, and evaluate which of the TLR4-bearing vascular cells are involved in this response. In addition to furthering understanding of the pathogenesis of HIT, this study demonstrates the novel use of the whole blood cytokine release assay assessment of TLR activation as an in vitro method to study the potential immunogenicity of heparin or alternative anticoagulant drugs.