

# TTP, APLA, and HIT: Decoding the Alphabet of Immune-Mediated Thrombotic Diseases

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For the hematologist, there are few diseases harder to understand or to explain to a medical student or patient than thrombotic thrombocytopenic purpura (TTP), anti-phospholipid antibodies (APLA), or heparin-induced thrombocytopenia with or without thrombosis (HIT/T). Questions arise: Why do some patients with TTP have low levels of ADAMTS13 and some don't? Is there any way to predict which patients will relapse with their TTP and when? Why do some people with antiphospholipid antibodies clot and others don't? Why do some people clot if they have no platelets, and which patients with HIT/T are going to go on to have thromboses — and when? These were some of the questions addressed during yesterday's Scientific Committee on Clinical Laboratory Hematology (offered again today at 7:30 a.m.).

First, Dr. Don Siegel from the University of Pennsylvania detailed very elegant studies on the genetic restriction of antibodies directed against ADAMTS13 from patients with acquired TTP. Using phage display libraries, more than 130 unique anti-ADAMTS13 antibodies have been analyzed and, remarkably, ~80 percent of antibody heavy chains show genetic restriction to the *VH1-69* immunoglobulin (Ig) germline gene, suggesting that the humoral response in patients with TTP is genetically restricted. Additionally, immunization of rabbits with human ADAMTS13-inhibiting monoclonal antibodies (mAbs) generated anti-idiotypic antibodies that blocked antibody-mediated enzyme inhibition. These data suggest novel therapeutic approaches to TTP, such as selective deletion of B cells utilizing the *VH1-69* heavy-chain gene or using anti-idiotypic antibodies to block inhibition of ADAMTS13.

Next, Dr. Tom Ortel from Duke University Medical Center reviewed the new laboratory criteria for the diagnosis of APLA. In 2006, consensus guidelines for the diagnosis of the antiphospholipid syndrome (APS) were updated, and laboratory criteria now include lupus anticoagulants, anticardiolipin IgG and IgM antibodies, and/or anti- $\beta_2$ -glycoprotein I ( $\beta_2$ GPI) IgG and IgM antibodies, demonstrated on two (or more) occasions at least 12 weeks apart. Antibodies to  $\beta_2$ GPI are reported to be responsible for the major thrombotic complications in patients with APLA. Additionally, Dr. Ortel described other diagnostic assays based on potential prothrombotic mechanisms of antiphospholipid antibodies, such as inhibition of the anticoagulant activity of annexin A5. Lastly, he described use of a gene expression strategy to identify unique gene expression signatures to distinguish patients with APS and venous thrombosis from patients with venous thromboembolism without evidence for antiphospholipid antibodies.

Dr. Michael Reilly from Thomas Jefferson University in the final presentation described the paradigm in laboratory testing for HIT — namely that the ELISA-based assays for detecting antibodies against heparin-PF4 are too sensitive and are positive in many patients without clinical features of HIT, whereas the functional assays for heparin-dependent platelet activation lack sensitivity and are difficult to perform. HIT thus remains a clinical diagnosis. Additionally, it is clear that we need a better assay to distinguish the subset of patients with HIT who are at high risk to go on to develop thrombotic complications. Dr. Reilly presented data from his group describing their cloning of human antibodies from primary human B cells from patients diagnosed with HIT. These cloned human antibodies, used in conjunction with their murine model of HIT, will hopefully allow characterization of pathogenic human HIT antibodies.