66 Contemporary Issues in Diagnostic Hemostasis and Thrombosis

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Chicago, IL 60603
This interactive session will utilize a series of five relevant case studies to explore several contemporary, clinically relevant problems in hemostasis and thrombosis, including anticoagulant monitoring and phrmagenomics, platelet function evaluation, the detection of antiphospholipid and heparin/PF4 antibodies, and the evaluation of patients with suspected hereditary thromboembolic disease. Each case is focused on an important clinical or laboratory problem encountered by pathologists, medical technologists, and hemostasis laboratories. Following a presentation of the basic clinical and laboratory features of each case, session participants will be invited to discuss practical, cost-effective means for further assessment and resolution of the problem and related clinical and laboratory problems. The session will emphasize the integration of modern techniques and discoveries with conventional clot-based assays.

- Interpret important hemostatic laboratory assays and provide recommendations for further patient evaluation or monitoring.
- Recognize technical pitfalls the diagnostic limitations of common hemostatic assays, and develop alternative strategies for laboratory evaluation.
- Understand emerging and expected future developments in hemostatic testing, such as the increasing role of molecular analysis and point of care testing.

FACULTY:

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Blood Banking, Transfusion Medicine
Blood Banking, Transfusion Medicine
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Contemporary Issues in Diagnostic Hemostasis and Thrombosis
Disclosure Statement

I declare that neither my immediate family members nor I have a financial interest in or other relationship with a manufacturer/provider of any commercial product/service presented/discussed in the workshop.

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September 2, 2011

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Case #1

The Case of the Art History Major with Chronic Thrombocytopenia

Clinical History: It was a dark and stormy fall evening in Mason City, MO. Suzanne Cezanne was a 21 year-old art historian and assistant curator at the Art Museum of the Midwest in Mason City, MO. She was working alone in the Edgar Allen Poe exhibit, on loan from the Edgar Allen Poe Museum in Richmond, VA, and scheduled to open the next day. She had worked on the exhibit since early morning and hadn’t had time to eat lunch or dinner. There was great interest in the exhibit, since one of the founders of Mason City, Judge Nathaniel B. Tucker, was a good friend of Poe. As she was adjusting an old smokey, floor-length mirror, the sharp features and piercing eyes of Edgar Allen Poe were suddenly staring at her. She gasped and almost fainted. For a moment thought Poe’s ghost was in the room, but then realized that it was only a reflection from his picture on the wall behind her.

Suzanne knew that Poe had tuberculosis and other medical problems, and she wondered if he also had thrombocytopenia, a problem she had dealt with all of her life. Maybe Dr. Marcus Carrington, Director of the Midwest Hemostasis Institute, could do something for her when she had her first appointment there next week.
Dr. Carrington found that Suzanne had a history of severe, chronic thrombocytopenia first diagnosed at two years of age. Her platelet counts usually varied from 5 x 10^9/L to 20 x 10^9/L. Other than increased bruising, occasional episodes of epistaxis, and heavy menstrual periods, she had not seriously suffered from this problem. Multiple and extensive evaluations for autoimmune, metabolic, and hematologic diseases at other medical centers had been negative negative. These workups had shown normal serum B12 and folate levels, a normal erythrocyte sedimentation rate, negative assays antinuclear antibody and rheumatoid antibodies, a negative direct Coombs test, and normal immunoglobulin levels. Serologic assays for EBV, CMV, HIV, and other infectious diseases were also negative, and a splenic ultrasound at age 19 showed the spleen to be at the upper limits of normal for her age. Bone marrow evaluations at ages 19 and 20 revealed adequate megakaryocytes with normal morphologic features, and cytogenetic analysis of the bone marrow aspirates had showed a normal female karyotype. Since Suzanne's parents perished at an early age in an automobile accident, she grew up in a foster home and did not have any knowledge of her family medical history.

Suzanne had received high-dose steroids, intravenous immunoglobulin, and WinRho injections at different times with no effect on her platelet counts. At the age of 20 years, she was also given four treatments of Rituxan, but her platelet count still remained in the range of 5 x 10^9/L to 20 x 10^9/L. On one occasion when her platelet count was 8 x 10^9/L, she was given several doses of platelets. Her platelet count rose to 48 x 10^9/L one hour later, but it was only 18 x 10^9/L when she returned to the clinic the following week. Suzanne was given a diagnosis of refractory chronic immune thrombocytopenic purpura.

**Physical Examination:** A physical examination by Dr. Carrington revealed an alert, oriented young woman with the following vital signs: BP - 115/79, pulse - 72/min, temperature - 98.1°F, and weight - 57.3 kg. Dermatologic examination showed some bruising at different stages on her upper and lower extremities. The cardiovascular and respiratory examinations were unremarkable. Her abdomen was soft and tender and no organomegaly was found. There were no palpable cervical, supraclavicular, or inguinal lymph nodes. Her sclera were clear and anicteric.

**Laboratory Data:** Suzanne’s blood was drawn for laboratory studies. A comprehensive metabolic panel, including liver and renal function tests, was within normal limits. Blood counts and indices were as follows:

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**Fig. 1.** Suzanne’s CBC data. Red dot represents patient value, reference ranges are indicated by gray bars.
Learning Objectives: (1) Understand the etiology of chronic thrombocytopenia, (2) Understand the classification and clinical features of familial thrombocytopenias, (3) Understand the significance of peripheral blood smear examination and other laboratory assays in the diagnosis of familial thrombocytopenia, (4) Understand the pathogenesis of the MYH-9 related diseases, (5) Understand the treatment options for patients with chronic thrombocytopenia.

Questions:

1. Discuss the etiology of chronic thrombocytopenia. What possible causes of Suzanne’s thrombocytopenia have not been evaluated? In view of her low platelet counts, why have Suzanne’s clinical symptoms been relatively mild?

The general causes of thrombocytopenia include: (1) Artifactual thrombocytopenia due to anticoagulant-dependent platelet agglutinins or other causes, (2) Immune or non-immune destruction of platelets in the circulation, (3) Sequestration of platelets by the spleen, and (4) Failure of platelet production by the bone marrow.

Artifactual thrombocytopenia should be excluded in the evaluation of every new patient with thrombocytopenia. This can easily be done by reviewing the peripheral blood smear for evidence of platelet clumping and satellitism around neutrophils. Other factors that may produce a falsely low platelet count when automated particle counters are used include paraproteins, prior exposure of blood to dialysis membranes, giant platelets, and lipemia. The history of chronic thrombocytopenia beginning at an early age suggests a possible hereditary etiology, although chronic immune thrombocytopenia cannot be excluded. Although Suzanne’s family members are not available for evaluation, repeated evaluation has no revealed evidence of an autoimmune, infectious, or other reason for an acquired thrombocytopenia. Suzanne's relatively mild bleeding symptoms in the presence of severe thrombocytopenia suggests intact thrombopoiesis and the continuing production of young, physiologically active platelets. This phenomenon is often seen in patients with immune thrombocytopenia.

Dr. Carrington performs a bone marrow examination and peripheral blood smear (film) examination. Images from the peripheral blood smear are shown below.
2. Does the peripheral blood smear provide any clues about the etiology of Suzanne’s thrombocytopenia? How would you classify her disease from the peripheral blood smear findings?

Peripheral blood smear examination confirms the presence of severe thrombocytopenia. Most of the platelets are large and show normal granulation. The elevated mean platelet volume (MVP) of 10.3 fL (normal range 6.3 - 9.1) provides additional evidence to support this conclusion. Careful examination of the peripheral blood smear also provides another important clue to the etiology of Suzanne’s disease process; many of the neutrophils contain pale, bluish-purple Dohle-like cytoplasmic inclusions, usually located at the periphery of the cell.

There are many congenital platelet diseases (Table I). In general, these diseases and very rare, and each is characterized by a different constellation of clinical and laboratory findings. The platelet size, presence or absence of thrombocytopenia, platelet size, and other hematologic and non-hematologic clinical features are important diagnostic considerations.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Platelet Count</th>
<th>Platelet Size</th>
<th>Major Clinical Features</th>
<th>Inheritance</th>
<th>Genetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bernard-Soulier syndrome</td>
<td>Low, Variable (Homozygous – severe thrombocytopenia, giant platelets, defective ristocetin-induced platelet aggregation. Heterozygous – Mild macrothrombocytopenia, normal ristocetin-induced platelet aggregation)</td>
<td>Very large</td>
<td>AR, GP Ibβ (22q11), or GPIX (3q21) mutations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congenital amegakaryocytic thrombocytopenia</td>
<td>Severe thrombocytopenia</td>
<td>Normal</td>
<td>AR, c-mpl (1p34) gene mutations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>δγ Storage pool disorder</td>
<td>Low, variable (Combined alpha and dense granule deficiency)</td>
<td>Normal ?</td>
<td>AD, Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dense granule deficiency (Idiopathic δ-storage pool disease)</td>
<td>Normal</td>
<td>Normal ?</td>
<td>AR, AD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dense granule deficiency with pigment abnormalities</td>
<td>Normal</td>
<td>Normal ?</td>
<td>AR, HPS gene mutations [(HPS1, ADTB3A, HPS3, HPS4) or CHS1 gene]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disorder</td>
<td>Platelet count</td>
<td>Platelet size</td>
<td>Morphology/Aggregation</td>
<td>Mutation/Associated gene</td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------------</td>
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</tr>
<tr>
<td>Familial thrombocytopenia with predisposition to acute myelogenous leukemia (AML)</td>
<td>Low, variable</td>
<td>Normal</td>
<td>Defective aggregation, dense granule deficiency, predisposition to myelodysplasia and AML</td>
<td>AD, Transcription factor CBFA2 (AML1)(21q22) mutations</td>
<td></td>
</tr>
<tr>
<td>Glanzmann thrombasthenia</td>
<td>Normal</td>
<td>Normal</td>
<td>Absent or severely decreased aggregation with all agonists except ristocetin due to dysfunctional or deficient GPIIbIIIa</td>
<td>AR, α1β3 or β3 mutations</td>
<td></td>
</tr>
<tr>
<td>Gray platelet syndrome</td>
<td>Low, variable</td>
<td>Large</td>
<td>Gray platelets (severe α-granule protein deficiency) and neutrophils (Romanovsky stains). Dense granule deficiency in dominant form</td>
<td>AR, AR, Unknown</td>
<td></td>
</tr>
<tr>
<td>Macrothrombocytopenia with cytoskeletal abnormalities</td>
<td>Low, variable</td>
<td>Large</td>
<td>Morphologically abnormal platelets with large membrane complexes, disarranged actin and tubulin, no shape change during aggregation</td>
<td>AD?, Unknown</td>
<td></td>
</tr>
<tr>
<td>Montreal platelet syndrome</td>
<td>Low, variable</td>
<td>Giant</td>
<td>Spontaneous platelet aggregation and decreased platelet calpain activity</td>
<td>AD, Unknown</td>
<td></td>
</tr>
<tr>
<td>MYH9 related diseases</td>
<td>Low, variable</td>
<td>Large</td>
<td>Leukocyte inclusions ± nephritis, deafness, cataracts</td>
<td>AD, MYH9 (22q12-13) gene mutations</td>
<td></td>
</tr>
</tbody>
</table>

In Suzanne’s case the findings of thrombocytopenia with large platelets and Dohle-like inclusions in the neutrophils suggests one of the nonmuscle myosin heavy chain IIA syndromes, which include May-Hegglin anomaly, Fechtner syndrome, Sebastian syndrome, Epstein syndrome. In 2003, these diseases were reported to represent variable expressions of a single disease due to mutations in the MYH9 gene encoding for the non-muscle myosin heavy chain IIA (NMMHC-IIA). The terms “MYH9 gene-related autosomal thrombocytopenia” and “MYH9-related macrothrombocytopenia” is now used for this disease. Suzanne does not have a history of renal disease or deafness, but may be too young to show the development of cataracts. Pending further studies of renal function and hearing, the May-Hegglin anomaly appears most likely (Table II).

The May-Hegglin anomaly was initially described by German physician Richard May (1909) in a family with large platelets. In 1945, Swiss physician Robert Hegglin, identified Dohle-like inclusions in a family with dominantly-inherited macrothrombocytopenia, and the triad of thrombocytopenia, giant platelets, and Dohle-like inclusions subsequently became known as the May-Hegglin anomaly (MHA). The Epstein platelet syndrome was subsequently identified in 1972, the Fechtner syndrome in 1985, and the Sebastian platelet syndrome (SPS) in 1990. In 1990 the genetic abnormality in patients with all of these disease was linked to a region on the short arm of chromosome 22, later identified as the MYH9 gene.
The myosin superfamily comprises a large class of ATP-dependent mechanoenzymes involved in muscle contraction and a wide variety of other essential cell motility processes. Of the 18 myosin classes, class II muscle myosins are the most numerous and best understood, since they are a major component of skeletal, cardiac, and smooth muscle. However, structurally similar myosin II molecules termed non-muscle myosin II (NMII) are also present in muscle, as well as all other eukaryotic cells. NMII is essential for all activities involving cell movement and/or changes in cell shape, including cell-cell and cell-matrix adhesion, cell migration, mitosis, differentiation, phagocytosis, cytokinesis, etc. NMII consists of two heavy and two light chains molecules that structurally resemble a “two-headed tadpole.” The heavy chain region of the complete molecule consists of two globular heads (heavy chain motor domains) containing actin-binding regions and ATPase enzyme domains. The motor domains are linked by short neck regions to a long alpha-helical coiled-coil rod tail domain, which ends in a short non-helical region. Two smaller light chain molecules wrap around the neck region and have regulatory functions. There are three different myosin heavy chain isomers (NMHC II-A, NMHC II-B, and NMHC II-C) that occur in different proportions in tissues. In muscle tissue, the head domains bind to actin molecules, while the antiparallel coiled-coil tail domains of the tail regions interact to form a bipolar complex. Muscle contraction occurs through the ATPase enzyme activity of the head domain, which initiates a conformational change in the molecule, sliding the actin molecules, and their attached actin strands, in an anti-parallel direction.

More than 40 MYH9 mutations have been identified, but most patients with clinical manifestations have mutations in the motor domain of the head region, particularly point mutations at Arg902. The pathophysiology of the clinical manifestations resulting from MYH9 mutations is far from being understood, but it is thought that NMII may have a regulatory role in megakaryocyte development, especially inhibiting the formation of the proplatelet or the migration of megakaryocytes to the vascular space. The mutation may permit the premature release of large functionally compromised platelets with defective ability to undergo shape change. Glomerular podocyte formation is compromised in patients with the renal manifestations of an MYH9 mutation, presumably leading to nephritis. The mechanism of the cataract formation is unknown.

MYH9-related diseases are classified as rare (incidence ~ 1:50,000), but it is believed that the disease may have a much higher incidence, with most cases misdiagnosed as chronic immune thrombocytopenia or another disease. In part, this is because the severity and phenotypic features of these diseases are extremely variable, even within members of the same family. The platelet count is usually between 30 x 10^9/L and 100,000 x 10^9/L and giant platelets are always present. Severe or fatal bleeding episodes are rare, but easy bruising, epistaxis, and other manifestations of mild/moderate platelet function diseases is usually found. Menorrhagia and accompanying iron deficiency are frequent in women with the disease, and female patients may present with these findings. The severity of the renal impairment and the age of the onset of the cataracts and high-tone hearing loss is variable, with possible delayed presentation of the latter two symptoms until the 50s.

3. What additional laboratory studies should be obtained?

MYH9 mutation analysis has recently became commercially available and is indicated in the evaluation of patients with suspected MYH9-related disorders. The diagnostic evaluation of these patients should also include an audiogram, renal function assessment (BUN, creatinine and creatinine clearance, and urinalysis), and ophthalmologic screening for cataracts.

4. What other forms of therapy should be attempted?

Patients with inherited thrombocytopenia have normal platelet survival and will respond to platelet transfusion for major surgery, trauma, or bleeding episodes. In these circumstances, a platelet count >100 x 10^9/L or greater is attempted. Patients with inherited thrombocytopenia do not have immune thrombocytopenia, and will not respond to standard ITP therapies. However, Suzanne should be advised to avoid aspirin, aspirin-containing products, and other prescription drugs, “over-the-counter” drugs, and herbs that interfere with platelet function. Wearing a MedicAlert bracelet is also recommended.
Final Diagnosis: Inherited macrothrombocytopenia, MYH9-related (May-Hegglin anomaly).

References:


The Case of the Middle-Aged Ornithologist with Thrombocytopenia and Expressive Aphasia

**Clinical History:** Vivian Mouette paddled the canoe silently through the Dark Cypress Swamp in the early dawn light, high-powered binoculars at the ready. The 55 year-old leader of the Birdwatchers' Field Club of Mason City was in search of an elusive prey. She had caught a glimpse of an ivory-billed woodpecker, long thought to be extinct, while on a visit to Bayou DeView in Arkansas two years ago. Since then, she was obsessed with trying to find one in the swamps near Mason City, MO. Quietly rounding a bend in the stream, Vivian couldn’t believe what she was seeing. Sitting on the limb of a dead cypress tree was a large black and white woodpecker with a bright red crest and a long, pointed, ivory-colored bill. She quickly snapped several pictures of the creature with the binocular’s built-in 15MP camera before it flew away. As she took her cell phone from her backpack to inform her friend Gladys of the amazing discovery, she noticed that the fingers of her right hand were numb. Gladys answered her call after several rings, but Vivian was unable to speak and couldn’t move her tongue. Her daughter later found her in her home, sitting in a kitchen chair and moaning. She was still unable to speak but was apparently able to understand language. On arrival at the ED of a local hospital, Vivian had a platelet count of $13 \times 10^9/L$ and was given two units of platelets. She was transferred to the Midwest Hemostasis Center with the diagnosis of acute onset aphasia and right-sided hemiparesis with thrombocytopenia.
Vivian was fully functional prior to this event, and had no prior history of strokes or TIA's. However, her past medical history did include Berger's disease, hypothyroidism, COPD, hyperlipidemia, rheumatoid arthritis. She was taking a number of drugs for the treatment of these problems, including prednisone, Simvastatin, Darvocet, Plaquinil, Pentoxyfillin, Clofibenzprine, Zocor, Levothyroxine, Trental, Flexeril, Tricor, Ticlodipine, folic acid, methotrexate, hydrochlorothiazide, and an Atrovent inhaler. She was not allergic to any medications and did not use alcohol or illicit drugs. She had smoked two to three packs of cigarettes per day for greater than 20 years. Vivian had a family history of myocardial infarctions and cerebrovascular accidents on the maternal side of her family.

**Physical Examination:** On admission to the Midwest Hemostasis Center, Vivian had the following vital signs: blood pressure - 146/76 mmHg, heart rate 67/min, temperature - 97.2°F, and respirations -16/min. She was alert, awake, and oriented times four but aphasic with receptive capabilities intact. Her pupils were equally reactive to light and accommodation, and extraocular movements were intact. However, her tongue had a decreased range of motion and was deviated to right. Decreased range of motion of tongue. A cardiovascular examination showed a regular rate and rhythm with no murmur, gallop or rub. There was no carotid bruit. A respiratory examination revealed expiratory wheezing over the posterior field and decreased breath sounds over anterior chest. The abdomen was soft, nondistended, and nontender. There was no hepatosplenomegaly to palpation. Ecchymosis noted over left lower quadrant and there were petechia over anterior chest and lower extremities bilaterally and right and left shoulder bruising. A neurologic examination showed a possible mandibular or cranial nerve V sensory loss with decreased mandible sensation on the left and right and a possible cranial nerve XII lesion as the tongue protruded, deviated to right, and had a decreased range of motion. Vivian also had expressive aphasia with markedly decreased right upper extremity and shoulder strength and 1/5 strength in the right lower extremity, 4/5 in left upper extremity, and 5/5 in left lower extremity. Touch sensation was uniformly decreased on the right. Reflexes were 3+ in right upper and lower extremities. There was a negative Babinski and Vivian was able to sit up with assistance. A head CT revealed an ischemic infarct in the left lentiform nucleus and left basal ganglia with no evidence of hemorrhage.

**Laboratory Data:** Essential laboratory results were as follows: WBC – 7.3 x10^9/L (3.6-9x10^9/L), Hgb – 11.0 g/dL (N 12.9 – 15.5g/dL), Hct – 34% (N 39–47%), RBC – 3.36x10^6/L (N 4.18 – 5.22x10^6/L), MCV – 90.5 fl (N 84.0–100 fl), RDW – 15.8% (N 12–16%), platelets – 23x10^9/L (N 140–440x10^9/L), PT – 9.7 sec (N 13.5–16.5 sec), aPTT – 34.0 sec (N 32 – 48 sec), fibrinogen – 380 mg/dL (N 1500 – 3500 mg/dL), LDH – 481IU/L (N 105 – 250 IU), total bilirubin – 0.7 mg/dL (0.2 – 1.3 mg/dL), conjugated bilirubin – 0 mg/dL (N 0.0 – 0.3 mg/dL), reticulocyte count – 4.1% (N 1-2%), D-dimer 1.14 mcg/mL (normal < 0.41), haptoglobin < 20 mg/dL (normal 50-150), BUN 15, and creatinine 1.09 with glucose of 90. AST 20, ALT 17, alkaline phosphatase 117. Urinalysis revealed small leukocytes with large blood, trace ketones, 1 + bacteria, 0-5 WBC’s and 50-100 RBCs. EKG with normal sinus rhythm, normal axis, isolated Q wave in leads III and IV, R wave progression.

**Learning Objectives:** (1) Understand the etiology of thrombocytopenia, (2) Understand how to examine the peripheral blood smear, and the importance of peripheral blood smear examination in patients with thrombocytopenia, (3) Understand the pathogenesis and clinical presentation of the thrombotic microangiopathies, (4) Understand the biosynthesis and biological functions of von Willebrand factor, (5) Understand the technical aspects, limitations, and clinical interpretation of laboratory assays for thrombotic microangiopathies.
Questions:

1. What is the most likely diagnosis for Vivian?

The findings of severe thrombocytopenia associated with anemia and neurologic abnormalities suggests that Vivian has thrombotic thrombocytopenic purpura (TTP, Moschcowitz syndrome).

TTP is a thrombotic disease first reported by Moschcowitz in 1924 in a 16 year-old girl with anemia, petechiae, and microscopic hematuria, was found to have disseminated microvascular hyaline platelet thrombi at autopsy. The hallmark of TTP is systemic intravascular platelet aggregation, leading to microvascular thrombosis, microangiopathic hemolytic anemia, thrombocytopenia, and organ dysfunction. A classic pentad of microangiopathic hemolytic anemia, thrombocytopenia, CNS involvement, renal involvement and fever is found in some patients but the triad of microangiopathic hemolytic anemia, thrombocytopenia, and neurologic symptomatology is much more common. The differentiation of TTP and HUS from other diseases, including infection, antiphospholipid syndrome, malignant hypertension, and pregnancy-associated DIC syndromes, and other diseases can be difficult, and an atypical clinical presentation is not uncommon.

2. What is the most likely pathogenesis of Vivian’s illness?

Vivian was receiving the antiplatelet drug ticlodipine, which probably triggered the development of TTP.

The pathogenesis of TTP was unknown prior to the 1980’s. The finding of large von Willebrand factor (vWF) multimers in the plasma, first reported in 1982 by Moake, was subsequently followed in 1998 by the discovery of a deficiency or functional abnormality of the enzyme von Willebrand factor (VWF)-cleaving metalloprotease (VWFPCP, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13, ADAMTS-13).

vWF is synthesized in endothelial cells and megakaryocytes as a large precursor molecule, pre-pro-vWF. Proteolytic cleavage of the signal peptide results in 280 kDa pro-vWF monomers that link by disulfide bonds to form large multimers and homodimers with molecular masses into millions of daltons. Ultralarge, proto-vWF (UL-vWF) molecules, formed when the pro peptide is cleaved, are stored in the Weibel-Palade bodies of endothelial cells and the alpha granules of platelets. UL-vWF is secreted into the plasma and mostly circulates in this form. However, encountering conditions of shear force, the molecule undergoes an unfolding conformational rearrangement, and binds to receptors on the endothelial cell, which expose the previously inaccessible Tyr1605-Met1606 peptide bond and permits cleavage by ADAMTS-13 into smaller subunits of two to 20 molecules. The smaller cleaved vWF molecules binds platelets less effectively. ADAMTS-13 is coded by a gene on chromosome 9 and is a member of the zinc-containing ADAMTS metalloproteinase family that share common structural features.

Most cases of TTP are idiopathic, but rare cases of congenital TTP have been described. In addition, acquired, secondary TTP has been described in patients with many diseases and receiving some drugs. An increased incidence of TTP has been described in patients with pregnancy, cancer (especially adenocarcinoma of the breast, GI tract, and prostate), hematopoietic stem cell transplantation, autoimmune disease, vasculitis, and infection (HIV, Streptococcus pneumonia, cytomegalovirus). Drugs associated with TTP include the thienopyridines (ticlopidine, clopidogrel), quinine, immunosuppressive drugs (mitomycin-C, cyclosporine, tacrolimus, deoxycoformycin, a-interferon, gemcitabine) and chemotherapeutic drugs (mitomycin-C, gemcitabine, cisplatin, tamoxifen, bleomycin, cytosine arabinoside, and daunomycin). Idiopathic TTP is of autoimmune origin and associated with specific anti-ADAMTS-13 antibodies that inhibit ADAMTS-13 activity, leading to increased UL-vWF plasma levels and the formation of microthrombi, with subsequent microangiopathic hemolytic anemia and thrombocytopenia. The hereditary form of TTP (Schulman-Upshaw syndrome), and most cases of chronic relapsing TTP, are
due to ADAMTS-13 gene mutations that lead to decreased enzyme activity.\textsuperscript{19, 21} The etiology of the secondary, disease or drug-associated cases of TTP are more controversial, and associated with both microvascular endothelial cell damage and inhibitory anti-ADAMTS-13 antibodies.\textsuperscript{44}

Ticlopidine-associated TTP was first reported in a European dialysis center in 1991, and in the United States in 1998.\textsuperscript{32, 44} Subsequent studies revealed an incidence of 1 in 1600 to 1 in 5000. It usually presents 2 to 12 weeks after the initiation of drug therapy, and is accompanied by severe thrombocytopenia, marked serum LDH elevations, mild renal insufficiency, and ADAMTS-13 inhibitors. Ticlopidine-associated TTP has a good response to plasma exchange but a high incidence of relapse after reexposure to a thienopyridine drug. Clopidogrel-associated TTP was reported in the United States soon after clopidogrel was FDA-approved in 1998, and reportedly has a much lower incidence, of about 1 in 12 million, approximately three times the overall incidence of TTP in the population.\textsuperscript{1, 44} The renal insufficiency in clopidogrel-associated TTP is usually severe, but the thrombocytopenia is mild, and the etiology is microvascular endothelial cell damage, rather than anti-ADAMTS-13 antibodies. Clopidogrel-associated TTP responds slowly to plasma exchange, are relapses are infrequent. The comparative features of TTP induced by clopidogrel and thienopyridine are shown in Table III.

### Table III

<table>
<thead>
<tr>
<th>Feature</th>
<th>Ticlopidine-Associated TTP</th>
<th>Clopidogrel-Associated TTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathophysiology</td>
<td>Anti-ADAMTS-13 antibody Microvascular endothelial damage</td>
<td>Microvascular endothelial damage</td>
</tr>
<tr>
<td>ADAMTS-13 deficiency</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>ADAMTS-13 inhibitors</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Time of onset after drug exposure</td>
<td>2 - 12 weeks</td>
<td>Within 2 weeks</td>
</tr>
<tr>
<td>Renal Disease</td>
<td>Absent to mild</td>
<td>Severe</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>Severe</td>
<td>Mild</td>
</tr>
<tr>
<td>Survival S/P plasma exchange</td>
<td>&gt;90%, rapid response (days)</td>
<td>70%, very slow response (weeks)</td>
</tr>
<tr>
<td>Spontaneous relapse</td>
<td>Occasional</td>
<td>Infrequent</td>
</tr>
<tr>
<td>Relapse with exposure to the other thienopyridine</td>
<td>High</td>
<td>Low</td>
</tr>
</tbody>
</table>

3. What additional laboratory tests should be obtained to confirm the diagnosis?

Laboratory evaluation of patients with TTP usually reveals severe thrombocytopenia (20-50 x 10^9/L) with moderate anemia, and a variable white blood count.\textsuperscript{5, 33} Peripheral smear examination must be performed to confirm the diagnosis, evaluate red blood cell morphology, and rule out leukemia and other diseases. Fragmented red cells are usually frequent, but they may not be abundant during early disease stages. Unfortunately, there are no definitive guidelines regarding the number of schistocytes to exclude TTP from other diseases. The hemolytic anemia also results in elevations of serum lactate dehydrogenase (LDH), decreased haptoglobin levels, and moderate hyperbilirubinemia (2.5-4 mg/dL). A Coomb’s test should be obtained to exclude an autoimmune hemolytic anemia. D-dimers and fibrinogen degradation.
products (FDPs) are elevated from the formation and breakdown of thrombi. The prothrombin time and aPTT are usually within normal limits, while fibrinogen is increased. The BUN and creatinine are elevated proportional to the severity of the renal injury. SLE and other autoimmune disease should be excluded, and serologic studies for HIV should be obtained. Bone marrow evaluation to exclude other causes of thrombocytopenia may be indicated, depending on the clinical picture and other laboratory findings. An image of Vivian’s peripheral blood is shown in Fig. 1.

![Peripheral blood smear, Wright-Giemsa stain, 1000x.](image)

Fig. 1. Peripheral blood smear, Wright-Giemsa stain, 1000x.

The modern era in TTP began with the discovery of the association with ADAMTS-13 and the development of assays for ADAMTS-13 activity and anti-ADAMTS-13 autoantibodies. Although the diagnosis, treatment, and monitoring of TTP is still based on clinical symptomatology supplemented by the basic laboratory tests discussed above, ADAMTS-13-related assays are widely available through reference laboratories, and are increasingly being performed by individual hospital laboratories. These assays are categorized in Table II, and include sequencing of the ADAMTS-13 gene, assays for ADAMTS-13 antigen and enzyme activity, and assays for the detection of anti-ADAMTS-13 neutralizing and non-neutralizing antibodies.

**ADAMTS-13 Antigen Assays**

Immunoassays for plasma ADAMTS-13 use monoclonal or polyclonal antibodies specific for normal or mutated portions of the the molecule. These assays are not clinically beneficial at the present time.
ADAMTS-13 Activity

Current methods for the detection of ADAMTS-13 activity depend on the *in vitro* cleavage of a vWF substrate (purified, plasma-derived or recombinant multimeric vWF or synthetic vWF peptides that incorporate the ADAMTS-13 cleavage site) by ADAMTS-13 activity in patient plasma.\textsuperscript{11, 16, 17, 20} The resulting vWF cleavage products are detected and quantified by direct or indirect methods, and the ADAMTS-13 activity is determined. The direct assays include electrophoresis (SDS agarose or SDS-PAGE) with Western blotting, and Fluorescence Resonance Energy Transfer [FRET], while indirect assay detection methods use collagen binding, ristocetin-induced aggregation, or ELISA. Of these assays, the FRET assays have achieved the most interest and are presently commercially available in kit form through three different manufacturers.

Förster resonance energy transfer, resonance energy transfer, electronic energy transfer) is an interaction between two colored molecules (chromophores) based on their proximity. This phenomenon was originally described by the German scientist Theodor Förster in 1946, and is now a fundamental technique to study molecular interactions in the biosciences. The principle of Förster resonance energy transfer is that a donor chromophore in close proximity to an acceptor chromophore (< 10nm), transfers energy by a non-radiative mechanism termed dipole-dipole coupling. Since fluorescence emissions can be easily and accurately detected, the donor and acceptor chromophores are usually fluorescent molecules, and the term fluorescence resonance energy transfer (FRETS) is used. Since the emission spectrum of the acceptor is predominant when the chromophore molecules are in close proximity, but donor excitation occurs when they separated, an event such as molecular movement or a chemical reaction can be detected. The FRETS assays for ADAMTS-13 activity, as originally described by Kokame and co-workers utilizes a modified A2 domain vWF fragment of vWF in which two amino acid residues (Q1599 and N1610) that span the ADAMTS-13 cleavage site are replaced by fluorescent molecules [A2pr(Nma) and A2pr(Dnp)]. When the vWF fragment is intact the Nma and Dnp groups are in close proximity, so that when Nma is excited at 340 nm, energy is transferred to the quencher, Dnp. However, if the molecules are separated by substrate cleavage, energy transfer does not occur, and Nma emits detectable fluorescent light at 440 nm.

Anti-ADAMTS-13 Antibodies

Anti-ADAMTS-13 autoantibodies are the usual cause of acquired TTP and may be of the neutralizing or non-neutralizing type. Neutralizing antibodies directly bind to the ADAMTS-13 molecule and directly inhibit enzyme activity. They can be detected by ELISA assays utilizing immobilized recombinant ADAMTS-13, or by patient plasma titration with normal plasma, followed by measurements of ADAMTS-13 activity. Non-neutralizing antibodies accelerate the clearance of ADAMTS-13 or its binding to the endothelial cell surface without directly inhibiting enzyme activity. Non-neutralizing antibodies are detected by ELISA.

Genetic Analysis of the ADAMTS-13 Gene

PCR amplification and conventional sequence analysis is used to characterize the ADAMTS-13 gene on chromosome 9q34 in patients with suspected congenital TTP. At this time, approximately 80 mutations and 20 non-synonymous single nucleotide polymorphisms (SNPs) have been described. The mutations are predominately missense mutations, but have also included nonsense mutations, deletions, insertions, and splice site mutations.\textsuperscript{19}

4. What is the best treatment plan for Vivian?

The mortality of untreated TTP from multiple organ damage is as high as 90%, so aggressive therapy is usually undertaken to avoid death and ischemic consequences such as stroke, myocardial infarction, transient ischemic attacks (TIAs), bleeding, and other problems. Daily high-volume plasma exchange for about a week with fresh frozen plasma is the treatment of choice, usually with corticosteroid therapy.\textsuperscript{5, 23, 31} More recently, anti-CD20 monoclonal antibody (Rituximab) has been successfully used in patients un-
responsive to conventional treatment. The current mortality rate is reduced to about 10% with appropriate treatment, although disease relapse is seen in about 15% of patients.

Vivian underwent placement of a left external jugular vein catheter followed by daily plasmapheresis with FFP replacement. The platelet count gradually increased, and reached 150,000 x 10^9/L by day 10. Plasmapheresis was then discontinued. Anticoagulation was started for DVT prophylaxis with 40 units Lovenox subcutaneously every day. However, an ultrasound of the head revealed a large clot around the left IJ internal jugular where the catheter was placed. After surgery to remove the line, a full dose of Lovenox (110 units subcutaneously daily) was began. Vivian slowly recovered was discharged to rehabilitation at a physical and occupational therapy facility. She continued to do well and returned home after approximately 8 weeks. However, one week after returning home Vivian developed easy bruising and returned to the emergency room after passing a melanotic stool. She has found to have thrombocytopenia, with a relapse of her TTP. In spite of specific medication instructions on discharge, Vivian began taking all of her “usual” medications that were in her medicine cabinet, including Ticlopidine. The patient's daughter discovered the medication and disposed of it before the patient was discharged home for the second time.

Final Diagnosis: Thrombotic thrombocytopenic purpura (TTP), Ticlopidine-associated

References


The Case of the Italian Baker with Multiple Myocardial Infarctions and Stent Thrombosis

Clinical History: In Mason City, MO, many folks begin their day with a trip to Guido's Pasticceria and Caffe' for an espresso or cappuccino with some homemade pignolata, ciarduna, bomboloni con la crema, or a slice of Torta della Nonna. Founded in the 1950’s by Guido Santamarin and his wife Maria, immigrants from Treviso, Italy, the business is now ran by Guido’s son, Antonio and his wife Lisa. Antonio, now 47, practically grew up in the bakery, often sleeping on bags of flour while his father baked bread in their brick oven for early morning delivery. Their hard work has resulted in an genuine old world Italian cafe, with Italian marble floors, stained glass windows and a stamped copper ceiling. Mason city citizens daily line up around the block to get into the pasticceria.

One Friday morning, Antonio arrived at the bakery even earlier than usual to begin preparing for the busy weekend pastry sales. He was filling a large tray of cannoli shells when he developed severe substernal chest pressure that radiated around his left side to his back and neck. The pain was associated with lightheadedness, tingling in his right hand, left arm pain, shortness of breath, and palpitations. He stopped filling the shells and took 2 nitroglycerine tablets with some relief. He immediately called the Mason City EMS and received aspirin, an additional nitroglycerin tab, and a lopressor en route to Mason City Medical Center. In the ED he was started on a heparin drip, and received nitropaste, 10mg of IV metoprolol, and 20U insulin for a blood glucose > 500 mg/dL prior to being transferred to the Cardiac ICU.
By the time cardiologist Dr. Renee Hartz was consulted, Antonio only had minimal residual back pain, and no chest pain, shortness of breath, or nausea/vomiting. However, upon reviewing Antonio’s records, Dr. Hartz found an alarming past medical history, with hypertension, diabetes mellitus, hypercholesterolemia, coronary artery disease, multiple (5) myocardial infarctions, peripheral vascular disease, congestive heart failure (EF of 40%), chronic kidney disease (stage III), moderate obesity, and nonsustained ventricular tachycardia (NSVT) status-post placement of an automatic implanted cardiac defibrillator (AICD). He had previously undergone multiple balloon angioplasty and stenting procedures for his coronary artery disease, and had recently undergone a percutaneous transluminal coronary angioplasty with placement of a drug-eluting stent placed for instant thrombosis. In addition to multivitamins, Antonio’s medications include aspirin (325 mg, 1 tab, PO, daily), metoprolol (100 mg, 1 tab, PO, every 12 hours), nitroglycerin (0.4 mg, 1 tab, SL, every 5 minutes, PRN: for chest pain), clopidogrel (75 mg, 1 tab, PO, daily), isosorbide dinitrate (30 mg, 1 tab, PO, every 8 hours, 90 tab), spironolactone (25 mg, 1 tab, PO, bid, 60 tab), lisinopril (40 mg, 1 tab, PO, daily), insulin aspart (100 u/ml subcutaneous solution, 6 Units, SQ, ac tid & hs), hydralazine (50 mg, 1 tab, PO, q 8 hr), clonidine (0.3 mg, 1 tab, PO, q 8 hr), insulin glargine (82 Units, SQ, bedtime), famotidine (20 mg, 1 tab, PO, bedtime), omega-3 polyunsaturated fatty acids (2,000 mg, PO, bid), chlorthalidone (25 mg, 1 tab, PO, bid), and pravastatin (40 mg, 1 tab, PO, bedtime).

Physical Examination: A physical examination revealed: blood pressure - 150/80 mm Hg, heart rate - 80 bpm, and respirations - 16/min. Antonio’s lungs were clear to auscultation and percussion, and he had a regular cardiac rhythm with no murmurs or regurgitation.

Laboratory Data: An ECG was non-ischemic but revealed left ventricular hypertrophy with strain. Troponin I levels were elevated, at 1.0 ng/mL. Dr. Hartz makes a diagnosis of a non-ST elevation myocardial infarction (non-STEMI MI) but is concerned about recurrent stent restenosis or plaque rupture leading to anginal symptoms. She knows that Antonio is very compliant about taking his medications, and also wonders about resistance to clopidogrel and aspirin. Finding no record of a VerifyNow assay in Antonio’s records, she orders P2Y12 and aspirin resistance assays from the coagulation laboratory. The results are as follows:

### Table IV
**Antonio’s Accumetric VerifyNow Assay Results**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Result</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2Y12 % Inhibitor</td>
<td>15%</td>
<td>-</td>
</tr>
<tr>
<td>P2Y12 Assay</td>
<td>252 PRU</td>
<td>-</td>
</tr>
<tr>
<td>Baseline</td>
<td>295 PRU</td>
<td>194-418 PRU</td>
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</table>

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Result</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin Assay</td>
<td>422 ARU</td>
<td>620-672 ARU</td>
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</table>

Contemporary Issues in Diagnostic Hemostasis and Thrombosis
ASCP Annual Meeting, October 2011
2. Discuss the pathophysiology of arterial thrombosis and the management of atherothrombotic disease, including the use of antiplatelet drugs.

Atherosclerosis (arteriosclerotic vascular disease, ASVD, hardening of the arteries) is the most common vascular disease in the world. It is a chronic disease affecting the large and medium-sized arteries, that requires decades to develop. The pathogenesis is extremely complex, but a chronic inflammatory response in the wall of the artery causes the accumulation of fatty substances, especially cholesterol, macrophages, calcium, and other substances, to form a firm structure along the intraluminal wall of the vessel termed a plaque. The formation of atherosclerotic plaques is accentuated by low-density lipoproteins and countered by high density lipoproteins (HDL). Stable plaques rich in smooth muscle narrow the lumen and decrease the elasticity of the artery, but generally do not produce clinical symptomatology. However, plaques rich in macrophages and foam cells (unstable plaque, vulnerable plaque) may have a weak, narrow layer of tissue (i.e., fibrous cap) separating them from the lumen. These unstable plaques can rupture, expose thrombogenic material to the circulation, initiate activation of the platelets and the coagulation system, and promote the development of a blood clot (thrombus). The sudden intraluminal occlusion of an artery can rapidly lead to cardiac ischemia/necrosis, with the clinical consequences of angina pectoris, myocardial infarction, stroke, or peripheral vascular disease, depending on the anatomic location of the lesion. The thrombi can also detach and move down the bloodstream to smaller arteries anywhere in the body (thromboembolic disease). The risk of developing atherosclerosis is determined by genetic factors in combination with multiple acquired risk factors, including body weight, lifestyle, diet, hypertension, tobacco smoking, other diseases (diabetes, dyslipoproteinemia), stress, medications, etc.

Diet and behavioral modification is initially used to prevent or alleviate atherosclerosis, but a combination of dietary modification and drugs (i.e., statins, niacin, intestinal cholesterol absorption-inhibiting substances, Omega-3 oils) are used for patients with more advanced disease to promote a more favorable lipoprotein ratio. Patients with severe acute or life-threatening disease may require angioplasty with stent placement to expand the narrowed arteries, or bypass surgery to open alternate sources of blood flow around the diseased vessel. Since anticoagulants are not effective in arterial disease, therapy with antiplatelet drugs are used as a prophylactic measure in patients at risk for thrombotic cerebrovascular or cardiovascular disease, or secondarily in patients status-post treatment by medical and surgical means. The antiplatelet drugs currently in use are briefly reviewed below.

2. Describe the pharmacological effects, medical indications, and side effects of antiplatelet drugs, including aspirin, clopidogrel, ticlopidine, prasugrel, and dipyridamole.

Antiplatelet drugs interact with different pathways of platelet metabolism to prevent platelet activation. Common targets of current antiplatelet drugs include cyclooxygenase 1 (COX-1)(aspirin), P2Y12 receptor (ticlopidine, clopidogrel, prasugrel), phosphodiesterase (Cilostazol), glycoprotein 1Ib/IIIa receptor (abciximab, eptifibatide, tirofiban), and the thromboxane receptor (Terutroban). Another antiplatelet drug (Dipyridamole) acts through multiple mechanisms to inhibit adenosine reuptake. The major properties of these drugs is summarized in Table V. These drugs comprise a multibillion dollar industry, and may new antiplatelet drugs are in development or undergoing clinical trials.

Aspirin
Salicylates are among the oldest and most versatile human drugs. Salicylates are heterocyclic compounds present in several common plants, including willow, birch, beech, and popular. Due to its potent analgesic, antipyretic, and antiinflammatory effects, salicylic acid has been used as a human pharmacological agents for at least 2400 years. Acetylsalicylic acid, in which a carboxyl group is substituted for the hydroxy moiety of salicylic acid (o-hydroxybenzoic acid), was originally synthesized by French chemist Charles Frederic Gerhardt in the mid-1850’s and termed “salicylic-acetic anhydride.” In 1897, chemists at the German firm Bayer AG resynthesized acetylsalicylic acid (2-acetoxybenzoic acid) from salicin and found that it had similar anal-

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The compound was named aspirin “acetyl” and the old German name for salicylic acid “spirsäure.” The identity of the Bayer chemist responsible for the discovery is a matter of controversy. However, there is considerable evidence that Jewish chemist Arthur Eichengrün actually made the discovery, but that records of the discovery were expunged by the Nazi regime, and credit was given to another chemist Felix Hoffman. There is a controversy whether Felix Hoffmann, but the later claimed that he was the lead investigator and records of his contribution were expunged under the Nazi regime. Aspirin quickly became one of the world’s most widely used drugs, with nearly 40,000 tons consumed yearly.

<table>
<thead>
<tr>
<th>Class</th>
<th>Drug</th>
<th>Mechanism</th>
<th>Route</th>
<th>Side Effects</th>
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<tbody>
<tr>
<td>Cyclooxygenase Inhibitors</td>
<td>Aspirin</td>
<td>Irreversible acetylation of cyclooxygenase 1</td>
<td>Oral</td>
<td>Bleeding</td>
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<td></td>
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<td></td>
<td></td>
<td>GI toxicity</td>
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<tr>
<td>ADP Receptor Inhibitors</td>
<td>Clopidogrel (Plavix)</td>
<td>Irreversible inhibition of P2Y12 receptor</td>
<td>Oral</td>
<td>Bleeding</td>
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<td>Rash, Neutropenia</td>
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<tr>
<td></td>
<td>Ticagrelor (Brilinta)</td>
<td>Reversible, allosteric P2Y12 inhibitor</td>
<td>Oral</td>
<td>Dyspnea</td>
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<td>Bleeding, Rash</td>
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<tr>
<td></td>
<td>Prasugrel (Effient)</td>
<td>Irreversible inhibition of P2Y12 receptor</td>
<td>Oral</td>
<td>Bleeding</td>
</tr>
<tr>
<td></td>
<td>Ticlopidine (Ticlid)</td>
<td>Irreversible inhibition of P2Y12 receptor</td>
<td>Oral</td>
<td>Bleeding</td>
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<tr>
<td>GPIib/IIIa Inhibitors</td>
<td>Abciximab (ReoPro)</td>
<td>Integrin αIIbβ3 antagonist</td>
<td>IV</td>
<td>Bleeding</td>
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<td>Thrombocytopenia</td>
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<td>Eptifibatide (Integrilin)</td>
<td>Integrin αIIbβ3 antagonist</td>
<td>IV</td>
<td>Bleeding</td>
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<td>Thrombocytopenia</td>
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<tr>
<td></td>
<td>Tirofiban (Aggrastat)</td>
<td>Integrin αIIbβ3 antagonist</td>
<td>IV</td>
<td>Bleeding</td>
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<td></td>
<td></td>
<td></td>
<td>Thrombocytopenia</td>
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<tr>
<td>ADP Reuptake Inhibitors</td>
<td>Dipyridamole (Persantine)</td>
<td>Inhibition of ADP uptake; inhibition of phosphodiesterase</td>
<td>Oral</td>
<td>Headache</td>
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<td></td>
<td>Dizziness, Hypotension, Flushing</td>
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<td>GI toxicity</td>
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<tr>
<td>Phosphodiesterase Inhibitors</td>
<td>Cilostazol (Pletal)</td>
<td>Inhibition of phosphodiesterase</td>
<td>Oral</td>
<td>Bleeding</td>
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<td>Diarrhea, Palpitations, Dizziness</td>
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<td>Rash, Pancytopenia</td>
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In the United States, at least 26 million individuals ingest aspirin daily to prevent heart disease and stroke. The antithrombotic effects of aspirin were not recognized until the mid-20th century, when Gibson, and later Craven reported its beneficial effect in patients with myocardial infarction, strokes, and other vascular diseases.\(^{15, 16}\)

The pharmacologic effects of aspirin remained unexplained until 1971, when British pharmacologist John Vane reported that it interferes with the synthesis of prostaglandins and thromboxane.\(^{12, 17}\) Prostaglandins are fatty acid derivatives that, together with the thromboxanes and prostacyclins, comprise the prostanoids. These compounds exert a wide variety of important physiologic effects by acting as autocrine or paracrine signaling agents. At the cellular level, the enzyme phospholipase A\(_2\) generates arachidonic acid from membrane phospholipids, which serves an intermediary for additional chemical reactions. Leukotrienes are generated from arachidonic acid (AA) through the actions of the enzymes 12-lipoxygenase and 5-lipoxygenase, while prostaglandin \(H_2\) (PGH\(_2\)) synthase (cyclooxygenase, COX, PHS) and other synthases. COX is an unusual enzyme with two active sites. A cyclooxygenase site converts arachidonic acid into prostaglandin \(G_2\) (PG\(_G_2\)), while a heme site with peroxidase activity converts the PG\(_G_2\) into prostaglandin \(H_2\) (PGH\(_2\)). PGH\(_2\) is further modified by other synthases into additional prostanoids, including PGD\(_2\), PGE\(_2\), PGF\(_{2\alpha}\), PGI\(_2\) (prostacyclin), and thromboxane \(A_2\) (TXA\(_2\)).

Three isoenzymes of COX have been discovered. COX-1 is a constitutive enzyme expressed in nearly all mammalian cells, while COX-2 is inducible by inflammatory stimuli, and expressed by microvascular endothelial cells, macrophages, and other cells at the sites of injury or inflammation, where it generates prostaglandin inflammatory mediators. COX-3 (COX-1b, COX-1v) is a splice variant of COX-1. Aspirin irreversibly acetylates serine 529 of COX-1 and serine 516 of COX-2.\(^{18, 19}\) The acetylated enzymes cannot convert arachidonic acid to PGH\(_2\), and additional prostanoids cannot be generated. The inhibition of COX-1 is responsible for the antithrombotic effects of aspirin, while the inhibition of COX-2 explains its antiinflammatory role. The major role of platelet COX-1 is the generation of thromboxane \(A_2\), a potent mediator of vasoconstriction and platelet aggregation. However, since COX-1 is not involved in other mechanisms of platelet activation through physical forces or other platelet membrane receptors, aspirin is a relatively weak inhibitor of platelet function.

Aspirin is rapidly absorbed in the stomach and upper gastrointestinal tract, appears in the circulation within 20 minutes of ingestion, and reaches a peak plasma level within 30-40 minutes. Aspirin-induced platelet inhibition largely occurs in the portal circulation, can be detected within one hour, and persists for the entire life of the platelet (8-10 days). However, in view of the continuing production and release of platelets, platelet COX function recovers by approximately 10% per day after a single dose of aspirin, and the bleeding time normalizes in 3 to 4 days. The appropriate dose of aspirin has been the subject of extensive research studies. A relative low daily dose of aspirin (i.e., 81 mg) has been generally accepted as optimal for the general population, since it completely inhibits TXA\(_2\)-mediated vasoconstriction and platelet aggregation while leaving the endothelial cell prostacyclin generation unaffected. However, in patients with acute vascular injury, higher-dose aspirin (i.e., 325 mg) may also prevent the deleterious effect of collagen-induced platelet aggregation. Daily ASA doses greater than 325 mg are associated with significant gastric toxicity.

**Clopidogrel**

Clopidogrel bisulfate, marketed under the trade name Plavix by Bristol-Myers Squibb and Sanofi-Aventis, is an oral antiplatelet agent in the thienopyridine class of drugs. Since 2007, it has been the second most widely prescribed drug worldwide, reaching US sales of approximately $4.2 billion in 2009. Clopidogrel was discovered by Jean-Pierre Maffrand, Director of Discovery Research at the pharmaceutical company Sanofi-Aventis. Plavix is a prescription medication available in tablets that supply the molar equivalent of 75 or 300 mg of clopidogrel. The medical applications of clopidogrel, alone or in combination with aspirin and other antithrombotic drugs, include: (1) the prevention of vascular ischemic events in patients with symptomatic atherosclerosis, (2) treatment of acute coronary syndrome without ST-segment elevation (NSTEMI), and (3) treatment of ST elevation MI (STEMI). It is also used for the prevention of thrombosis after the placement of intracoronary stents, and in patients with a history of aspirin-induced gastric ulcers who require antiplatelet drug therapy.

Clopidogrel is a prodrug that is metabolized by a hepatic CYP450 cytochrome isoenzymes (CYP3A4, CYP3A5, CYP2C19) to produce an active thiol metabolite that irreversibly inhibits ADP binding by forming a disulfide.
bond between two groups in the extracellular domain of the platelet low affinity ADP receptor P2Y12. This inhibits ADP-mediated activation of the GPIIb/IIIa complex. Platelet function inhibition is present as soon as two hours after clopidogrel ingestion and reaches a steady state of 40% to 60% inhibition between Day 3 and Day 7. Approximately 5 days is required for platelet function to return to baseline following discontinuation of clopidogrel administration. Hemorrhage is the major adverse effect of clopidogrel, with an 2% incidence of gastrointestinal hemorrhage annually. Other serious, but rare adverse effects include neutropenia (0.05% incidence) and TTP (0.004% incidence). Clopidogrel also interacts with many other drugs, especially those metabolized by CYP450 enzymes. Approximately 2% to 14% of the population have CYP2C19 polymorphisms that prevent the conversion of clopidogrel base into its active metabolite. As of May, 2009, the FDA has required a black box warning to the drug label recommending CYP2C19 genotyping to identify poor clopidogrel metabolizers.

**Ticagrelor**
Ticagrelor is a new platelet ADP inhibitor approved by the FDA on July 12, 2011, and marketed under the trade name of Brilinta by AstraZeneca (Wilmington, Del.) in the US. Ticagrelor is a reversible P2Y12 antagonist that binds to a different site than clopidogrel. In contrast to clopidogrel, it also does not require hepatic activation. Ticagrelor is rapidly absorbed from the gastrointestinal tract, reaches peak plasma concentration in about 1.5 hours, and has a plasma half-life of approximately 7 hours. It is metabolized by hepatic CYP450 cytochrome enzymes and excreted mainly in the bile and feces. The major side effect is dyspnea, but as will all platelet agonists, there is a risk of bleeding. Reportedly, ticagrelor has a lower mortality rate than clopidogrel. The use of Ticagrelor is contraindicated in patients with active bleeding or a history of intracranial bleeds, reducted liver function, or the concomitant use of other drugs that interfere with CYP3A4.

**Ticlopidine**
Ticlopidine (ticlid) is a thienopyridine ADP receptor inhibitor discovered by Dr. Jean-Pierre Maffrand that is similar to clopidogrel in structural and pharmacological properties. However, because of a relatively higher incidence of neutropenia, aplastic anemia, TTP, and gastric hemorrhage, it is not widely used.

**Prasugrel**
Prasugrel is a third member of the thienopyridine class of platelet ADP receptor inhibitors. It was developed by the Japanese pharmaceutical company Daiichi Sankyo Co. and is marketed in the United States by Eli Lilly and Company under the trade name Effient. It was approved by the FDA in July, 2009 for patients with thrombotic cardiovascular diseases planned for management by percutaneous coronary intervention (PCI). Prasugrel is a more potent and clinically effective platelet inhibitor than clopidogrel, but is also associated with a higher risk of adverse bleeding. However, prasugrel is metabolized by the CYP3A4 and CYP2B6 isoforms of CYP450, and genetic polymorphism has not been demonstrated.

**Dipyridamole**
Dipyridamole (Permole, Persantine) is a vasodilator first used in the early 1960s for patients with chest pain. It was reported to have antiplatelet activity in 1968. Dipyridamole exerts multiple effects that decrease platelet function. It inhibits the uptake of adenosine into platelets and endothelial cells and inhibits several enzymes, including thromboxane synthetase, adenosine deaminase, and cAMP/cGMP phosphodiesterases. These actions lead to decreased availability of TXA2 and increased local concentrations of adenosine which inhibit platelet aggregation to PAF, collagen, and ADP. Dipyridamole is highly bound to plasma proteins and metabolized in the liver. Dipyridamole is used in combination with warfarin for postoperative thromboembolic prophylaxis in patients receiving prosthetic heart valves. A combination of modified release dipyridamole (Aggrenox) and aspirin is also used for the prevention of stroke and transient ischemic attacks.

**Abciximab**
Abciximab (RePro, Eli Lily) consists of the Fab fragments of a monoclonal anti-glycoprotein IIb/IIIa antagonist. The drug has a short plasma half-life but induces an inhibition of platelet function that persists for at least 48 hours. Abciximab is primarily used as an antithrombotic agent in patients undergoing percutaneous
transluminal coronary angioplasty (PTCA) with or without stent placement. Severe thrombocytopenia is a rare but serious complication with this agent.

Tirofiban
Tirofiban (Aggrastat, Medicure Pharma) is a synthetic, rapid acting, non-peptide glycoprotein IIb/IIIa antagonist with a short half-life modeled from an anticoagulant present in the venom of the saw-scaled viper (Echis carinatus). It is used in combination with heparin and aspirin in the management of patient with unstable angina or non-ST-wave myocardial infarction planed for percutaneous transluminal coronary angioplasty (PTCA).

Eptifibatide
Eptifibatide (Integrilin, Millennium Pharmaceuticals) is a rapid acting glycoprotein IIb/IIIa antagonist used in combination with heparin and aspirin or clopidogrel in the management of patients with unstable angina or non-ST-segment-elevation (e.g., non-Q-wave) myocardial infarction. Structurally, it is an unusual cyclic haptapeptide modeled from a compound present in the venom of the southeastern pygmy rattlesnake (Sistrurus miliarius barbouri). Bleeding and hypotension are serious side effects of eptifibatide.

3. Define “aspirin resistance” and “clopidogrel resistance” and discuss the significance of these phenomenon in the care of patients with thrombotic disease.

Clinically, “resistance,” “nonresponsiveness”, or “failure” to aspirin and/or clopidogrel refers to the development of a thrombotic vascular event prescribed an adequate dose of the drug(s). This may occur due to poor patient compliance or suboptimal drug absorption or other pharmacokinetic factors. Genetic variability can also affect the magnitude of response in individual patients. In the laboratory, the time of specimen collection and the method of platelet function quantitation can cause variability in the results. Unfortunately, the reported incidence of these phenomenon has varied widely in different studies, depending on the patient population and the means of determination. Until precise criteria for these phenomenon are established, “nonresponsiveness” is preferred over other terminology.

Clinically, thrombosis occurs in approximately 13% of patients receiving aspirin therapy (Antithrombotic Trialist Collaboration. Collaborative meta-analysis of randomized trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. However, attempts to quantify the effects of aspirin by laboratory measurement of platelet function have resulted in extremely variable findings, with a reported frequency of aspirin nonresponsiveness varying from 5% to 60%. Proposed explanations for the mechanism of aspirin resistance have included poor patient compliance, suboptimal aspirin absorption, increased platelet receptor activity, increased platelet cyclooxygenase-2 and isoprostane activity, and genetic polymorphisms of cyclooxygenase-1 or platelet glycoprotein IIla.

The reported prevalence of nonresponsiveness to clopidogrel has varied from 5% to 44%, primarily related to the loading dose (300 mg vs 600 mg) and the dosing protocol. Patient noncompliance, individual variability in the platelet ADP receptor, polymorphisms in the platelet ADP receptor, and polymorphisms in the cytochrome P450 drugs that metabolize clopidogrel, especially CYP2C19.

4. Discuss laboratory methods for the determination of patient responsiveness to aspirin and clopidogrel, including light transmission aggregometry, Plateletworks, PFA-100, and the Accumetrics Verify-Now assay.

Light transmission aggregometry (turbidimetric aggregometry) measures the in vitro response of platelets to various chemical agents (i.e., aggregating agents, platelet agonists) that induce platelet functional responses. In the clinical laboratory, platelet aggregometry is utilized for the diagnosis of inherited and acquired platelet disorders, the assay of von Willebrand factor activity (ristocetin cofactor assay), the diagnosis of heparin-induced thrombocytopenia, and monitoring patients receiving antiplatelet drugs.
let aggregometers are modified spectrophotometers that measure light transmission through platelet-rich plasma (PRP). Although the turbidity of fresh PRP limits light transmission, transmission progressively increases as platelet aggregation causes the formation of larger and larger particles. More recent innovations of platelet aggregometry include whole blood aggregometers and lumiaggregometers. Whole blood aggregometers require less patient blood and provide faster turn-around time than optical aggregometers. Lumiaggregometers simultaneously measure platelet aggregation and ATP secretion to provide a more accurate diagnosis of platelet function defects. The platelet agonists routinely used in the clinical laboratory to differentiate various platelet function defects include adenosine diphosphate (ADP), epinephrine, collagen, ristocetin, and arachidonic acid. Other agonists, such as thrombin, vasopressin, serotonin, thromboxane A2 (TXA2), platelet activating factor, and other agents are used by research and specialized clinical laboratories. Conventional platelet aggregation is a complex laboratory assay that is particularly sensitive to the assay conditions, as well as drugs and other substances in the blood. Because of these influences, platelet aggregometry is an advanced, manually intense, costly assay restricted to specific clinical circumstances. A variety of commercial instruments and reagents for platelet aggregometry are available from Chrono-Log Corporation (Havertown, PA), Bio/Data Corporation (Horsham, PA), and Helena Laboratories (Beaumont, TX).

Plateletworks (Helena Laboratories, Beaumont, Texas) is a rapid in vitro point of care platelet aggregation screening technique based on impedance platelet counting and specifically developed for cardiopulmonary bypass and cardiac catheterization settings. The technique uses whole blood to measure the change in the platelet count due to platelet aggregation. Whole blood is drawn and separate 1 mL aliquots are placed into tubes containing EDTA (baseline), an ADP agonist, a collagen agonist, and an arachidonic acid agonist. The tubes are well mixed by inversion and the platelet count is measured in each tube using a hematology analyzer. The percent aggregation is calculated in each tube by the following formula:

\[
\text{% Aggregation} = \frac{\text{Baseline Platelet Count} - \text{Agonist Platelet Count}}{\text{Baseline Platelet Count}} \times 100
\]

The Plateletworks assay has been used to monitor the reversal of platelet inhibition with clopidogrel or NSAIDS in elective cardiac surgery patients, monitoring the efficacy of therapy with platelet GpIIb-IIIa antagonists in patients undergoing percutaneous coronary intervention or receiving medical therapy for non-ST elevation acute coronary syndromes, and predicting post-operative bleeding and blood product utilization in patients undergoing cardiac surgery with cardiopulmonary bypass.

The PFA-100 (Siemens Healthcare Diagnostics, Deerfield, IL) is a rapid, automated laboratory instrument that is sensitive to quantitative and qualitative abnormalities of platelets and von Willebrand factor (vWF). In the PFA-100, citrated whole blood is aspirated from a reservoir under constant vacuum conditions through a microscopic 150 µm aperture. This aperture is cut into a biologically active nitrocellulose membrane in a disposable cartridge device coated with a combination of platelet agonists. These agonists are either collagen (fibrillar Type I equine tendon) and epinephrine (C/Epi) or collagen and adenosine-5’- diphosphate (C/ADP). The blood is forced through the aperture at a high shear rate that roughly corresponds to the flow conditions present in small arteries. As the blood is forced through the aperture, platelets undergo adherence, activation and aggregation on the membrane surrounding the aperture and progressively form a plug that finally occludes the aperture. The closure time (CT) is the time required for the complete occlusion to occur. The PFA-100 is more rapid and less expensive than the bleeding time for the evaluation of platelet function. Since there is a good correlation between the bleeding time and the PFA-100 in certain patient populations, there is a trend to replace the bleeding time with the PFA-100 for a first-line screening test for platelet dysfunction in patients undergoing preoperative evaluation. Other clinical applications of the PFA-100 include the following:
Screening for impaired primary hemostasis, including von Willebrand disease and inherited platelet dysfunction

Verification of desmopressin acetate (DDAVP) therapy efficacy in pre-surgical patients

Evaluate platelet dysfunction due to antiplatelet drugs

Evaluate patients with high-risk pregnancy, menorrhagia, and other diseases for bleeding dysfunction

There are several cavets in the clinical utilization of the PFA-100. Strict adherence to specimen requirements, specimen transportation, and specimen processing is required, since the PFA-100 is affected by critical pre-analytical variables such as hematocrit or platelet count, blood collection technique, and transportation through pneumatic tube systems. Since the PFA-100 has been reported as insensitive to some patients with platelet function defects, clinical correlation is critical, with follow-up with a different screening technique in cases of high clinical suspicion. The PFA-100 is insensitive to alterations in the quantity or quality of fibrinogen and therefore has not been shown to be useful in evaluating patients for the presence of dysfibrinogenemia or hypofibrinogenemia. It is not sensitive to defects or deficiencies in the classic coagulation factors and appears to have little if any significant utility in assessing Hemophilia A and B.

The VerifyNow System (Accumetrics, San Diego, CA), formerly the Ultegra Accumetrics RPFA, is a point-of-care platelet function analyzer specifically designed and marketed for the monitoring of selected antiplatelet drugs, including aspirin, P2Y12 inhibitors (clopidogrel, prasugrel, ticlopidine), and GPIIb/IIIa antagonists. The VerifyNow System is comprised of: (1) a small analyzer that uses turbidimetric optical detection to measure the agglutination of fibrinogen-coated microparticles in anticoagulated whole blood, and (2) disposable, single-use assay cartridges that contain fibrinogen-coated beads, a platelet agonist, and other necessary reagents. The patient collection tube (whole blood in 2mL Greiner partial fill Vacuette with 3.2% sodium citrate) is placed into the analyzer where an appropriate aliquot is automatically dispensed from the into the assay cartridge without operator intervention. The results are available in several minutes. In the assay, platelets with unblocked receptors are activated and cause microparticle agglutination with a change in optical light transmission.

The Accumetrics Verifynow test for aspirin resistance uses an instrument which measures the increase in light transmittance when platelets aggregate in response to arachidonic acid. The results of the test are expressed in Aspirin Reaction Units (ARU), which reflect the rate and extent of platelet aggregation. The expected therapeutic range for platelet function in patients receiving aspirin is 350-549 ARU. Patients on aspirin with greater than 550 ARU are considered aspirin resistant. After aspirin discontinuation, an ARU of less than 550 indicates residual responsiveness.

The VerifyNow P2Y12 Plavix Response Assay quantitates platelet inhibition after P2Y12 receptor blockade, including the thienopyridine class of drugs (i.e., clopidogrel, prasugrel and ticagrelor). The P2Y12 assay can be useful for detecting thienopyridine resistance, monitoring thienopyridine dosage effect, and ensuring patient compliance. Patients with adequate platelet inhibition have an increased risk of bleeding, and discontinuation of thienopyridine administration is recommended five days prior to surgery. The results of the P2Y12-mediated platelet aggregation are reported in P2Y12 Reactivity Units (PRU). Baseline platelet function (BASE) is measured in a separate channel and reported as BASE PRU Units. The percent change from baseline is calculated from the BASE and PRU results and reported as percent P2Y12 Inhibition (%).

The target level for patients receiving chronic antiplatelet therapy is a percent P2Y12 inhibition ≥ 50%. Patients with suboptimal platelet inhibition show a PRU > 235 U and/or percent P2Y12 Inhibition <20%. These patients may have an increased risk of cardiac events, such as stent thrombosis and recurrent cardiac events. Baseline PRU values below 193 U usually indicate an interfering substance (i.e., recent glycoprotein IIb/IIIa inhibitor use) and are not likely to be valid.
The results of the VerifyNow assay should be interpreted in light of all the clinical and laboratory data available on the patient. Strict adherence to the collection protocol and timing of analysis is essential for meaningful results, and the possibility of improper sample collection or handling should be considered for patients with unexpected P2Y12 assay results. The assay has not been studied in patients with significant thrombocytopenia (PLT <100 x 10^9/L) and is contraindicated in patients with von Willebrand factor deficiency and inherited platelet disorders, such as Glanzmann thrombasthenia and Bernard-Soulier syndrome.

5. Does Antonio have aspirin resistance, clopidogrel resistance, or both?

Antonio appears to be resistant to clopidogrel, since his P2Y12 inhibition is <20% (15%) and his PRU is > 235 U (252 U). The results of the assay appear valid, since the baseline is > 193 U (295 U).

References


The Case of the Architect with Post-operative Thrombocytopenia

Clinical History: Nearly early every home and business in Mason City, MO has a plaque somewhere inside with the inscription “Built by Biggs.” Situated on the outskirts of Mason City, the offices of the Biggs Construction Company were surrounded by an army of red and yellow trucks and construction equipment. 65-year-old Luther Bigg’s philosophy of building was “fast and frugal,” and his well-organized construction crews could build anything within budget nearly overnight. Luther continually bounced from administrative meetings to on-site supervision, as he tried to be personally on hand for the important phases of every construction project. However, the hard work and long hours were taking their toll on Luther. One morning, while working on the new Mason City Art Center, he had severe chest pain and was rushed to Mason City Medical Center, where he underwent three-vessel coronary artery bypass surgery the same afternoon. Luther’s postoperative course was complicated by transient atrial fibrillation and volume overload, causing him to remain ventilator-dependent. Intravenous heparin was administered to prevent the possibility of thrombosis. Luther’s platelet count was 200x10⁹/L preoperatively and 182x10⁹/L on postoperative day 2 but fell to 70 x 10⁹/L on the seventh postoperative day. Fortunately, there were no clinical signs of thrombosis, which was confirmed by arterial and venous Doppler ultrasonography of all four extremities. The on-call vascular surgery resident speculated about the possibility of disseminated intravascular coagulation (DIC) as the cause of the thrombocytopenia and decided to order blood cultures, since Luther had a slight fever. That evening, the chief resident was quite outraged, and immediately gave other orders. What did he order?
Questions

1. What is the most likely etiology of the thrombocytopenia in Mr. Biggs?

Until proven otherwise, thrombocytopenia occurring in a patient receiving heparin in any form should be considered heparin-induced thrombocytopenia (HIT). In these patients, all available clinical and laboratory data should be reviewed to exclude other causes of acute thrombocytopenia, especially spurious thrombocytopenia, sepsis with disseminated intravascular coagulation (DIC) and drug-induced thrombocytopenia (DIT) caused by other medications the patient is receiving. Other than heparin, common causes of DIT include antibiotics (vancomycin, beta-lactam antibiotics), quinine, antiepileptic drugs (phenytoin, carbamazepine, valproic acid), glycoprotein IIb/IIIa inhibitors, etc. Overall, more than 300 drugs have been associated with thrombocytopenia.

Heparin can induce thrombocytopenia by two different mechanisms. A transient, non-immune mediated decrease in platelets occurs in about 30% of patients after the initiation of heparin therapy, and was formerly referred to as HIT type I, non-immune mediated HIT, or heparin-associated thrombocytopenia. This form of thrombocytopenia, caused by a direct interaction of heparin with the platelet surface, is usually mild (platelet count > 100 x 10^9/L), occurs within the first few days after heparin administration, and resolves within several days, even without heparin discontinuation. Type I HIT is not associated with an increased risk of thrombosis.

In contrast to HIT Type I, Type II HIT (hereafter referred to as HIT) is a dramatic and potentially catastrophic complication. This uncommon complication of heparin therapy occurs in approximately 1-3% of heparinized patients, but is significantly higher in cardiovascular patients, who are often repeatedly exposed to heparin. Thrombocytopenia can occur at any time, but typically begins 5 to 10 days after initial heparin exposure, or within hours of reexposure to heparin. Although more common in patients receiving unfractionated heparin than low molecular weight heparin (LMWH), HIT can occur with heparin exposure of any type, including subcutaneous heparin injections or heparin flushes through intravenous lines or catheters. Unlike other types of DIT, HIT is associated with a low risk of bleeding, but a substantial risk of arterial or venous thrombosis. This thrombosis is unique in that it is typically a "white thrombus" composed predominantly of platelets and little fibrin. The overall occurrence of thrombosis is approximately 20-50%, with the highest incidence in women and post-surgical patients. In medical inpatients, a high unfractionated heparin dose, exposure to heparin for more than five days were the most significant risk factors for the development of HIT. The thrombosis is usually of venous origin but arterial thrombosis can occur, particularly in patients with arteriosclerosis. There is a high incidence of morbidity and mortality in HIT patients with thrombosis, largely from acute myocardial infarction, pulmonary embolism, and peripheral arterial thrombosis. Other adverse consequences can result for microvascular thrombosis and include skin necrosis, adrenal hemorrhagic necrosis, and anaphylactoid reactions. The term heparin-induced thrombocytopenia and thrombosis (HITT) has been used for patients with thrombocytopenia and thrombosis. The financial impact of developing HIT are considerable, and largely a consequence of medication costs, prolonged in-hospital stay, diagnostic and therapeutic interventions, laboratory tests, blood transfusions. In one study, the projected annual financial impact was estimated at $700,000 to $1,000,000 for an institution with an incidence of 50 HIT cases per year.

The etiology of HIT has been extensively studied in the laboratory, although many details have yet to be resolved. Heparin binds to platelet factor 4 (PF4, cytokine CXCL4) to form an antibody-inducing heparin/PF4 complex. PF4 is a 70 amino acid protein produced by megakaryocytes and released from the alpha-granules of activated platelets. The normal physiologic role of PF4 as an inflammatory mediator is not completely understood, but it has high binding affinity for negatively-charged glycosaminoglycans, including heparin, and may also bind to thrombomodulin to enhance protein C activation. Recently, the molecular structure of the heparin/PF4 complex has been reported to mimic repetitive viral epitopes that are thought to trigger a rapid T-cell independent immune response with little anamnestic response. Regardless of their mechanism of formation, anti-PF4/heparin antibodies in HIT are primarily of the IgG class and interact with the heparin/PF4 complex to form large macromolecular immune complexes with high affinity for the FCyRIIA (CD32) receptors on platelets and monocytes. The activated platelets release additional PF4 that further exacerbate the platelet activation and lead to thrombin generation and thrombin generation ("thrombin storm"). Anti-heparin/PF4 antibodies have re-
ently been found to inhibit the protein C regulatory system, which also potentiates the prothrombotic state.\textsuperscript{15} Other investigators have reported a role for heparin contaminants in activating the contact factor system or activated monocytes in promoting thrombus formation.\textsuperscript{16-18} The antibody-coated platelets are eventually removed by the reticuloendothelial system, leading to thrombocytopenia. The pathogenesis of the thrombosis is multifactorial and less well defined. The major pathogenic mechanisms include the generation of procoagulant, platelet-derived microparticles and other procoagulants from activated platelets, thrombin generation resulting from heparin neutralization by PF4 released by activated platelets, and vascular damage resulting from the interaction of the anti-heparin/PF4 to heparan sulfate and other heparinoid-like structures on the endothelial cell.

The clinical diagnosis of HIT is complicated by the variability in the platelet count, the time course of the thrombocytopenia, and the existence of other diseases and medication effects in the target patient population.\textsuperscript{19} Since the platelet count can remain in the normal range (i.e., >150,000 x 10\(^9\)/L) in approximately 10% of patients with HIT, a relative decrease in the baseline or post-operative platelet count of >50% is used as a diagnostic marker of the disease, rather than an absolute platelet count. Most patients with HIT have platelet counts in the range of 50,000 to 60,000 x 10\(^9\)/L, and a few have severe thrombocytopenia of <30 x 10\(^9\)/L. “Typical-onset” HIT develops 5-14 days after heparin exposure, while “rapid-onset” HIT occurs within 24 hours in patient previously exposed to heparin, usually within the past 30 days. Rare patients with “delayed-onset” thrombocytopenia develop HIT more than 10 days following heparin exposure. Because of the variability in the temporal onset of HIT, the American College of Chest Physicians (ACCP) recommends the procurement of a base-line platelet count prior to heparin administration, 24 hours after beginning therapy, and at regular intervals until the discontinuation of heparin therapy. Routine platelet count monitoring is recommended at least every other day in high-risk patients and every two to three days in moderate risk patients. Routine platelet count monitoring is usually not recommended for patients receiving LMWH.

Laboratory assays for HIT that are sensitive, specific, and rapidly available do not exist at this time. In order to facilitate rapid clinical decision-making, Warkentin and Heddle developed a clinical scoring system for HIT (4 T) based on four available clinical criteria, including the platelet count, time course for the development of thrombocytopenia, the presence or absence of thrombosis, and the presence or absence of other causes of thrombocytopenia.\textsuperscript{20-25} Algorithms combing the 4 T scoring system with laboratory criteria were subsequently developed, including the HIT Expert Probability Score.\textsuperscript{26, 27} The 4 T clinical scoring system is detailed in Table VI.

### Table VI

4 T’s Clinical Scoring System for HIT*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count</td>
<td>&lt;30% fall or nadir &lt;10 x 10(^9)/L</td>
<td>30-50% fall or nadir 10-19 x 10(^9)/L</td>
<td>&gt;50% fall and nadir &gt;20 x 10(^9)/L</td>
</tr>
<tr>
<td>Timing of platelet count decrease</td>
<td>Fall &lt; 4 days without recent exposure</td>
<td>Uncertain onset days 5-10 (missing platelet counts), or onset ≤1 day in case of heparin exposure 30-100 days ago</td>
<td>Clear onset days 5-10; or onset ≤1 day in case of heparin exposure &lt;30 days ago</td>
</tr>
<tr>
<td>Thrombosis or other sequelae</td>
<td>None</td>
<td>Progressive or recurrent thrombosis; non-necrotizing (erythematous) skin lesions; suspected thrombosis (not proven)</td>
<td>New thrombosis (confirmed); skin necrosis; acute systemic reaction after intravenous heparin bolus injection</td>
</tr>
<tr>
<td>Other cause of thrombocytopenia</td>
<td>Definite</td>
<td>Possible</td>
<td>None apparent</td>
</tr>
</tbody>
</table>

Scoring - ≤3 points: Low probability for HIT; 4-5 points: Medium probability; 6-8 points: High probability

*Warkentin and Heddle, 2003
2. What immediate action should be undertaken?

The following actions must be undertaken in any patient suspected of having HIT, type II:

- All forms of heparin must be immediately discontinued.
- Anticoagulation with a direct thrombin inhibitor (DTI) must be started and continued until the platelet count increases to >100 x 10^9/L.
- The patient must be carefully monitored for the development of thromboembolic disease.
- Anti-vitamin K (i.e., warfarin) therapy is initiated when the platelet count exceeds 100 x 10^9/L with a period of dual therapy “bridging” until a therapeutic INR is achieved.”

Treatment with a DTI is critical to decrease the risk of thrombosis after the discontinuation of heparin therapy. If DTI therapy is not begun, the risk of thrombosis is approximately 50% after twenty days. The first DTI, hirudin, was originally isolated from the saliva of the medicinal leech (Hirudo medicinalis) in the 1800’s, but it was not widely used after the discovery of heparin in the early 1900’s. Worldwide, five DTI’s are presently used in patient care. Four DTI’s are FDA-approved, and three are used for the treatment of HIT (Table II). DTI’s are classified as univalent or bivalent depending on their interaction with thrombin. Bivalent DTI’s (Lepirudin, Bivalirudin, and Desirudin) bind to both the active (catalytic) site and fibrinogen-binding, regulatory exosite (exosite 1) on the thrombin molecule, while univalent DTI’s (Argatroban, Dabigatran) bind selectively to the active site. Another univalent DTI, Ximelagatran (Exanta, Exarta) was withdrawn from the market after reports of hepatotoxicity. However, fondaparinux (Arixtra, GlaxoSmithKline, Brentford, Middlesex, United Kingdom), a synthetic pentasaccharide Factor Xa inhibitor, and other drugs are under evaluation.

Lepirudin (Refludan, Bayer Corporation, West Haven, CT) is a desulfated recombinant form of hirudin approved by the FDA in 1998 for the treatment of HIT. Lepirudin inhibits both free and bound and does not interact with HIT antibodies. However, it is largely excreted by the kidneys, so care must be used in renal patients with HIT. Bivalirudin (Angiomax, Angiox, The Medicines Company, Parsippany, NJ) is a short, synthetic compound comprised of the C-terminal and N-terminal portions of the active peptide regions of the hirudin molecule. It has a rapid onset and short half-life, inhibits both circulating and clot-bound thrombin, and inhibits thrombin-mediated platelet activation. Since lepirudin is largely eliminated by proteolytic enzymes, it can be used in patients with renal or hepatic dysfunction. It is used, in combination with aspirin and GPIIb/IIIa platelet inhibitors for undergoing percutaneous transluminal coronary angioplasty (PTCA) or percutaneous coronary intervention (PCI), and in patients at risk of HIT undergoing PCI. Desirudin (Revasc, Rhone-Poulenc-Rorer) is a synthetic, essentially irreversible recombinant form of hirudin licensed in Europe for the prevention of DVT in patients undergoing elective hip and knee replacement surgery.

Argatroban (Arganova, Argatra, Novastan, GlaxoSmithKline, Brentford, Middlesex, United Kingdom) is small molecule, L-arginine derivative, and reversible thrombin inhibitor that binds to free, fibrin-bound, and clot-associated thrombin. Argatroban was FDA-approved in 2000 for the prevention/treatment of thrombosis in HIT, and in 2002 for the treatment of patients at risk of HIT undergoing percutaneous coronary intervention (PCI). Argatroban undergoes hepatic clearance, and must be dose-adjusted in patients with hepatic dysfunction.

Dabigatran etexilate (Pradaxa, Pradax, Prazaxa, Boehringer Ingelheim, Ingelheim Germany). Dabigatran is a derivative of a benzamidine-based thrombin inhibitor originally discovered in the 1980’s. Dabigatran is orally administered as a prodrug that is converted by hepatic esterases into the active metabolite. Dabigatran was approved by the FDA in 2010 for the for prevention of stroke in patients with non-valvular atrial fibrillation. The active metabolite is excreted by the kidneys, and it is contraindicated in patients with renal dysfunction.

Vitamin K antagonists such as warfarin are contraindicated for the treatment of patients with HIT since they rapidly decrease the production of protein C. Protein C deficiency, in the thrombotic environment of HIT, is a adverse factor that could trigger severe microvascular thrombosis and additional clinical consequences.
### Table VII
Features of Direct Thrombin Inhibitors Used to Treat HIT

<table>
<thead>
<tr>
<th>Drug</th>
<th>Lepirudin</th>
<th>Argatroban</th>
<th>Bivalirudin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-life</td>
<td>1.3 hours</td>
<td>39-51 minutes</td>
<td>25 minutes</td>
</tr>
<tr>
<td>Clearance</td>
<td>Renal</td>
<td>Hepatic</td>
<td>Renal, proteolytic</td>
</tr>
</tbody>
</table>
| Dose            | IV bolus: 0.4 mg/kg  
IV infusion: 0.15 mg/kg/h | IV infusion: 2 µg/kg/min  
IV infusion: 1.75 mg/kg/h | IV bolus: 0.75 mg/kg  
IV infusion: 1.75 mg/kg/h |
| Monitoring      | aPTT, ACT       | aPTT, ACT      | ACT, aPTT, thrombin time |
| FDA approved    | Yes             | Yes            | No              |
| Mechanism of action | Bivalent       | Univalent      | Bivalent        |

3. What additional laboratory assays should be requested? Would a positive laboratory result confirm the diagnosis of this disease entity in Mr. Biggs?

Laboratory assays for the detection of anti-heparin:PF4 complex antibodies should be obtained, although the results have a poor correlation with clinical symptomatology and are usually of little help in the immediate management of the patient with suspected HIT. In this regard, the role of laboratory testing in HIT diagnosis was comprehensively reviewed by Baldwin and collaborators. Overall, they found anti-heparin/PF4 antibodies are discovered in approximately 17% of patients treated with UFH, and 8% of those receiving LMWH. However, only 20% of antibody-positive patients develop thrombocytopenia, and very few of those develop significant thrombosis.

These assays include: (1) antibody assays that directly determine the presence of the antibody in plasma or serum by enzyme immunoassay (EIA) and other techniques, and (2) functional assays, which indirectly determine the presence of the antibody by measuring platelet activation in the presence of heparin. The HIT EIA assays are highly sensitive (~ 99%), commonly available, and relatively easy and rapid to perform. Unfortunately, they are less specific (50-90%), and cannot differentiate pathogenic from non-pathogenic antibodies. The functional assays are much more specific, but technically difficult to perform and less readily available. Consequently, the recommended course of action in most laboratories is to screen for the presence of HIT antibodies by EIA, and to follow-up with a functional assay in positive patients where the diagnosis still remains controversial.

Conventional solid-phase EIA assays for HIT antibodies in patient serum utilize a PF4/polyanion complex (PF4:heparin or PF4:polyvinylsulfonate (PVS)) coated on microtiter trays as a capture antigen. Patient antibodies binding the PF4/polyanion complex are detected with an enzyme-labeled (usually alkaline phosphatase) anti-human globulin detection antibody. The presence of an anti-heparin:PF4 antibody induces a chromogenic reaction with the release of a colored reaction product measured as a change in the optical density (OD). A cutoff (usually 0.4) is specified by each manufacturer to differentiate a positive and negative result, although some authorities believe a higher OD should be used to increase the specificity of the assay and avoid overdiagnosis. Since the antibody avidity determines the OD units and may correlate with the pathogenic significance of the antibody, some laboratories report the quantitative OD unit value. In most systems, “weak” antibodies have an OD value in the range of 0.4 to 1.0, while stronger antibodies produce an OD > 1.0. Unfortunately, some HIT EIA assays can detect the clinically irrelevant IgA and IgM antibodies, as well as the pathogenic IgG forms. These assays may also yield false positive results in the presence of autoimmune-related antibodies, immune complexes, and other non-HIT related antibodies. False negative results can occur in patients with low-avidity, low titer antibodies. The diagnostic specificity of the assay can be
increased with a “confirmatory” step using high concentrations of heparin that disrupt formation of the reaction and/or IgG-specific immunoassays. Commercial HIT EIA assays are available from several companies (Asserachrom-HPIA, Stago, Asniere, France; GTI Diagnostics, Brookfield, WI; Aniara Hyphen BioMed, Mason, OH). Other HIT immunoassays have been reported but are not widely used in the clinical laboratory. These include the fluid-phase EIA and particle gel immunoassay.

Table VIII

<table>
<thead>
<tr>
<th>Assay</th>
<th>Specimen/Principle</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin Release Assay (SRA)</td>
<td>Heat-treated patient serum Donor platelets isolated, labeled with 14C serotonin, washed incubated with patient serum in presence of heparin, 14C measured</td>
<td>High sensitivity High specificity</td>
<td>Uses radioisotopes Technically demanding Not readily available</td>
</tr>
<tr>
<td>Platelet aggregation</td>
<td>Patient platelet-poor plasma Platelet anti-PF4 antibodies induces aggregation of normal platelets in presence of heparin</td>
<td>High specificity</td>
<td>Low sensitivity Technique dependent Technically time-consuming and demanding Not readily available</td>
</tr>
<tr>
<td>EIA</td>
<td>EIA with PF4/heparin-coated microplates and patient serum or plasma</td>
<td>High sensitivity Relative technical simplicity</td>
<td>Low specificity Poor correlation with SRA</td>
</tr>
</tbody>
</table>

The $^{14}$C-serotonin release assay (platelet serotonin release assay, SRA), platelet aggregation test (PAT), and heparin-induced platelet activation (HIPA) test are the most widely used functional assays for the detection of anti-heparin:PF4 antibodies. The SRA, developed by Sheridan et al. in 1986, uses FcγRIIa receptor-phenotyped “normal” platelets from patients reactive to heparin:PF4 antibodies. The platelets are incubated with radioactive serotonin, washed, resuspended in calcium-containing assay buffer, and then incubated with the heat-treated test serum in the presence and absence of low, therapeutic (0.1 to 0.3 U/mL) and high, supratherapeutic (10 to 100 U/mL) concentrations of heparin. Platelet activation is indicated by the release of radioactive serotonin into the incubation supernatant, with a positive result signified by the release of > 20% of the radioactive serotonin to therapeutic levels of heparin, but not to supratherapeutic levels. Supratherapeutic heparin concentrations should decrease serotonin release by altering the binding ratio and decreasing the formation of the IgG/PF4/heparin immune complexes. Platelet activation at both low and high heparin concentrations (“intermediate” result) indicate the presence of preformed immune complexes or non-heparin dependent antibodies. Fc(gamma)RIIa receptor blocking monoclonal antibodies can also be used to confirm the specificity of the analysis. In view of the high specificity of the SRA, it is commonly used to confirm the diagnosis of HIT in patient’s with a positive HIT ELISA assay. Some procedural variations further increase the sensitivity by However, in some patients, non-drug-dependent antiplatelet autoantibodies can induce a false positive result. Due to the expense and technical complexity of the SRA, it is only performed by a few laboratories in the United States. The HIPA is similar to the SRA, but uses the macroscopic agglutination of platelets in a microtiter tray as the end point. Non-radioactive variations of the SRA measure serotonin release by EIA or platelet-derived microparticles by flow cytometry. The source of the control platelets is an important factor, since platelet FcγRIIa receptor polymorphisms at amino acid 131 influence the binding of heparin-dependent antibodies. In this regard, control platelets with histidine-histidine (His-His) or histidine-arginine (His-Arg) at FcγRIIa-
131 were found to greatly increase the sensitivity of the assay relative to platelets with arginine-arginine (Arg-Arg). The platelet FcγRIIa phenotype may also influence the risk of developing HIT. (Brandt, 1995)

The heparin platelet aggregation test (HPAT) uses a commercial platelet aggregometer to quantitatively measure the aggregation of donor platelets (citrated platelet rich-plasma or washed platelets) by patient platelet-poor plasma in the presence of low-dose (0.2-0.5 U/mL) and high-dose (100 U/mL) unfractionated heparin. The formation of antibody/PF4/heparin immune complexes activate the platelet FcγRIIa receptor and cause platelet aggregation. The assay is considered positive with a platelet aggregation response of > 20% with low dose heparin, and <20% aggregation with high-dose heparin. The specificity of the HPAT is higher than that of the EIA, but the sensitivity is lower (50-80%). The assay requires meticulous attention to detail, with the concentration of heparin and the source of the control platelets being the major determinants of the sensitivity and specificity of the reaction. In this regard, it is best to use a mixture of platelets from several HIT antibody responsive donors. A related assay, the heparin-induced platelet aggregation (HIPA) assay uses washed normal platelets, patient serum, and heparin in microtiter trays, with a visual assessment of platelet aggregation. The lag time until platelet aggregation occurs is inversely proportional to the strength of the reaction. The flow cytometric detection of heparin-induced thrombocytopenia has been reported by the measurement of platelet activation using platelet microparticles detection, annexin V binding to phosphatidylserine, and expression of the platelet activation marker P-selectin (CD62p).

4. Who discovered heparin? What is the chemical structure of heparin? How is heparin manufactured?

Dr. William Henry Howell, former dean of the School of Medicine at Johns Hopkins University and chairman of the Department of Physiology reoriented his research studies to the increasingly important field of coagulation in 1916. As part of this effort, Dr. Howell enlisted the help of Jay McLean, a medical student transferring from the University of California, to isolate procoagulant substances from animal tissue. Dr. Howell was initially skeptical when McLean claimed to have isolated a fat-soluble coagulation-inhibiting compound, or “anticoagulant,” from canine hepatic tissue but later repeated McLean’s work and isolated a water-soluble polysaccharide anticoagulant that he named “heparin,” from the ancient Greek word (hepar, liver). In 1920 Howell The structure of heparin was elucidated by Erik Jorpes at Karolinska Institute in 1936, a technique for producing a safe salt solution was identified by Dr. Charles Best at the University of Toronto, and human clinical trials began in Toronto in 1935. The experimental and clinical work of Dr. Gordon Murray, a prominent Toronto heart surgeon, was crucial for the perfection of heparin as a human anticoagulant.

Arterial thrombosis was reported in patients receiving heparin in the late 1950’s and early 1960’s, but an association with thrombocytopenia was never made because platelet counts were not routinely monitored at that time. However, in the early 1970’s a group of vascular surgeons at Duke University finally associated thrombosis with heparin administration, and proved that it was an immune-mediated problem. Diagnostic assays for HIT were developed in the 1980’s, and therapeutic agents other than aspirin and warfarin developed in the 1990’s.

Heparin is a glycosaminoglycan polymer (3 kDa to 30 kDa) of variably-sulfated, highly negatively-charged, repeating disaccharide units, most commonly 2-O-sulfated iduronic acid and 6-O-sulfated glucosamine, IdoA(2S)-GlcNS(6S). Heparin is naturally produced by basophils and mast cells, stored in their secretory granules, and released into the vasculature at the sites of tissue injury. The normal biological function of heparin is unclear; it may act as an anticoagulant, but may also have a role in host defense against bacteria and other foreign substances. Pharmaceutical-grade heparin is isolated from animal tissues, usually bovine lung or porcine intestinal mucosa, through a complex extraction process.
5. What physiologic effects are exerted by heparin?

The anticoagulant effect of heparin is mediated by its interaction with anti-thrombin (AT). The binding of a specific pentasaccharide sequence in the heparin polymer to the AT molecule results in a 1000-fold increase in AT reactivity against thrombin, factor Xa, and other proteases. Although the anti-factor Xa activity of unfractionated heparin requires only the presence of the pentasaccharide heparin sequence, the inactivation of thrombin is size dependent, and requires the binding of thrombin both to the pentasaccharide sequence and a second site proximal to the pentasaccharide sequence. A recent variety of fractionated, low-molecular weight heparins (LMWH) have been produced, as well as a synthetic analogue mirroring the pentasaccharide sequence (fondaparinux). These newer drugs exhibit a more subtle anticoagulant effect than unfractionated heparin, since they target only factor Xa.

6. Describe the administration and pharmacokinetics of heparin.

UFH is not absorbed from the gastrointestinal tract because of its high negative charge density and large size. This property, and its short, but variable biologic half-life has precluded its application as an outpatient drug. However, UFH is the most widely used anticoagulant for hospitalized patients, to whom it is administered by intravenous (IV) or subcutaneous (SQ) routes. In the bloodstream, heparin is extensively bound to plasma proteins, von Willebrand factor, macrophages, and endothelial cells. IV heparin exerts a nearly immediate anticoagulant effect, but there is a delay of > 20 minutes after the subcutaneous administration of heparin. Heparin has an unusual non-linear does-response relationship, such that there is a disproportional increase in the half-life and intensity of the anticoagulant effect as the dosage is increased.

Fig 2. A typical heparin dose-response curve. The solid line represents the regression line of aPTT values plotted against heparin levels determined in fresh plasma specimens from at least 60 patients on heparin therapy by the anti-factor Xa assay. In this case, the therapeutic aPTT range corresponding to heparin levels of 0.3 to 0.7 U/mL is approximately 75-125 seconds.
Heparin is cleared from the body by dual mechanisms, including a rapid, saturable phase mediated by the reticuloendothelial system, and a slower, non-saturable, dose-independent phase due to renal clearance.

5. **What is the most common complication of heparin therapy?**

Hemorrhage is the most common complication of postoperative heparin therapy. The risk of hemorrhage is small in anti-coagulated patients without underlying complications, and often presents as hematuria or mild oozing from a mucosal site. However, life-threatening bleeding from the brain, gastrointestinal tract, and other sites can also occur. In general, the more prolonged the aPTT, the greater the likelihood of hemorrhage. Heparin therapy may also aggravate preexisting minor hemorrhage from pathologic lesions, such as colonic carcinoma or gastric ulcer. For this reason, bleeding from the gastrointestinal, respiratory, or urinary tract in patients on heparin should prompt a thorough search for a potential source of the bleeding. The risk of bleeding varies with the dosage and duration of treatment. The overall risk of bleeding is approximately 10% overall, but as high as 20% with high-dose heparin therapy in certain patients, including elderly patients, and those with other illness, renal failure, alcohol abuse, etc.

Patients on long-term heparin therapy are at risk of developing other complications, including osteoporosis and spontaneous vertebral fractures.

6. **How is heparin therapy monitored by the laboratory?**

Traditionally, the aPTT has been used to monitor the therapeutic effect of heparin. This test remains the most readily available and practical method to follow anti-coagulation by heparin. However the relationship between the aPTT and the concentration of heparin varies between labs reflective of differences in reagents, methodologies, sample size, and other factors. The current recommendations are for each lab to standardize the aPTT therapeutic range to an anti-factor Xa assay of 0.3 to 0.7 U/mL or a direct heparin measurement by protamine titration of 0.2 to 0.4 U/mL. The current standardized recommended therapeutic ranges of aPTT are reported with each test result and are regularly updated for each new reagent lot, or other changes in laboratory reagents or instrumentation. One heparin unit (the "Howell Unit") is the quantity of heparin required to keep 1 mL of cat’s blood fluid for 24 hours at 0 °C. This is approximately equivalent to 0.002 mg of pure heparin.

The “Brill-Edwards” protocol is the most widely used technique to measure the heparin-responsiveness of aPTT assay in a clinical laboratory. In the Brill-Edwards protocol, 50-100 plasma samples from different individuals receiving unfractionated heparin are obtained. The heparin concentration of each sample is determined by the chromogenic anti-Factor Xa technique, and the corresponding aPTT of each sample is measured with the laboratories reagents and instrumentation. A heparin response curve is constructed by plotting the aPTT values against the corresponding heparin concentrations (Fig. 2). Regression analysis is used to determine the range of aPTT values corresponding to UFH levels of 0.3-0.7 IU/mL (“heparin therapeutic range”). Theoretically, heparin dosing to maintain aPTT values within the therapeutic range will prevent over- or underdosing.

A “baseline” aPTT should be obtained prior to the administration of heparin. Regular monitoring should begin with the administration of heparin and continue regularly thereafter. Due to the complicated pharmacodynamics of heparin, and the potential for changes in clearance, the aPTT should be monitored daily, even after a therapeutic dose is found. Monitoring becomes a more difficult problem in patients with an abnormal aPTT at baseline, those who require higher than average doses of heparin, and those who are undergoing transition to oral anticoagulation. Citrated blood for measurement of the aPTT should be drawn six hours after a heparin bolus, and the result should be used to adjust the next dose.

Heparin monitoring by measurement of heparin plasma levels using the anti-factor-Xa assay is highly recommended in any patient with a known preexisting condition that may alter the heparin concentration-aPTT relationship or produce heparin resistance. In view of the many disadvantages of the aPTT, there is a growing consensus that the anti-factor Xa assay should be used for monitoring all patients receiving UFH. Disadvantages of the aPTT include the lack of a relationship between the aPTT and the clinical efficacy of heparin,
variation in the responsiveness of commercial aPTT reagents and reagent/instrument combinations to heparin, the nonlinearity of the heparin dose response curve, and the lack of sensitivity of the aPTT to drugs such as LMWH and factor Xa inhibitors.

Treatment options for patients resistant to heparin include increasing the dose of heparin, using LMWH, a direct thrombin inhibitor, fondaparinux, fresh frozen plasma (FFP), and/or AT concentrate.

Final diagnosis: Heparin-induced thrombocytopenia (HIT)

References:


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<th>Reference</th>
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Case #4 - References


Clinical History: Homes are difficult to find in the Mason City, MO region. Moving vans continually enter the area, and most leave empty. Many Mason City families living in a home are deeply indebted to Sandra Branson, owner of the There’s No Place Like Home Real Estate Company. Sandra is comfortably wealthy and has a beautiful home in the exclusive Foxwood Forest Estates subdivision of Mason City. Sandra enjoys the excitement of finding another house for sale, and closing yet another deal, but she also wants to retire and spend more time with her family. Fate intervenes when Sandra notices several large bruises while showing a home. The bruising continues for several weeks. Sandra reluctantly goes to her family physician. He finds that the bruises are unprovoked, present mostly on the extremities, and seem to extravasate under the skin rather than surfacing. Sandra also complains of intermittent headaches and numerous blood blisters in her mouth. Sandra is immediately referred to the Midwest Hemostasis Institute for definitive evaluation.

Dr. Marcus Carrington sees Sandra the next day. He finds that she has a long history of arthritis and a complex, but more recent history of bleeding related problems. Her history began eight years previously for profuse bleeding following a mastoidectomy. On that occasion, the her platelet count was normal, but her bleeding time was markedly increased (>20 minutes), and platelet aggregation studies revealed an absent platelet response to ADP. Other laboratory parameters were unremarkable. Five years previously she was seen for the evaluation of bleeding from the right ear. The PT, aPTT, platelet count, and fibrinogen were normal at that time,
but the bleeding time was again prolonged to >20 minutes. Sandra was advised against the use of alcohol, aspirin, and other drugs that could affect platelet function. She was seen two years later for petechiae and a bleed in the conjunctiva of the lower lid of her right eye. A tourniquet test on the right arm was strongly positive. One month later she developed massive bleeding in the right knee two days after an injection of Cortisone for pain.

**LABORATORY STUDIES:** Initial laboratory studies at the Midwest Hemostasis Institute reveal the following: Hgb - 8 g/dL, Hct - 28%, platelet count 28,000/µL. Peripheral blood smear examination reveals approximately 10% nucleated red blood cells and a rare "blast-like" cell. Serum B₁₂, folate and ferritin levels were within normal limits.

**Questions**

1. **What process could account for Sandra’s history of bleeding problems? What additional laboratory studies should be performed?**

   A bone marrow study was performed. The bone marrow was hypercellular (95% cellular) with 6% myeloblasts. Cytogenetic analysis of the bone marrow revealed 47,XX,+8. Platelet aggregation studies were remarkable for a markedly decreased response to ADP and for a slight prolongation in the lag time for collagen of uncertain cause.

2. **What disease process does Sandra have?**

   A diagnosis of refractory anemia with excess blasts (RAEB) was rendered.

3. **What is the pathophysiologic explanation for the bleeding?**

   This is the unusual case of a patient with a myelodysplastic syndrome (RAEB) that was manifested years earlier as a platelet function abnormality with intermittent, unusual episodes of bleeding.

   The myelodysplastic syndromes (MDS) are a heterogenous group of chronic neoplasms characterized by variable peripheral blood cytopenias resulting from ineffective hematopoiesis.¹⁻⁶ First identified as a distinct disease category in 1976, MDS is now recognized as one of the most common hematologic diseases. They are largely diseases of advanced age, but some cases occur in children and younger individuals. The incidence of MDS is approximately 35,000 to 55,000 cases per year in the United States, with 86% occurring in patients > 60 years of age.⁷, ⁸ Immunosuppression, prior chemotherapy or radiotherapy, and exposure to benzene and other solvents have been identified as risk factors for MDS. The molecular pathogenesis of MDS is not well understood, but is believed to be similar to acute myelogenous leukemia, with at least two somatic gene mutations leading to enhanced proliferation and impaired differentiation of hematologic progenitor cells.², ⁹⁻¹² Chromosomal abnormalities are common in MDS and include trisomy 8 and deletions of chromosomes 5, 7, 13, 20, and the sex chromosomes. Although the precise etiologic mechanism of many of these abnormalities remains to be discovered, the 5q- syndrome, associated with the loss of 5q31-33, has been associated with gene RPS14.¹³, ¹⁴ A variety of genetic mutations, including JAK2, TET2, and NPM1, have recently been identified in patients with MDS, and their significance is under investigation.¹³, ¹⁵⁻²¹

   The clinical symptoms of MDS result from cytopenia, and include variable combinations of anemia, neutropenia, and thrombocytopenia.² Physical examination may reveal evidence of bleeding (petechiae, ecchymoses, hemoptysis, hematuria, melena), anemia (pallor, tachycardia, or congestive heart failure), or infection (fever, cough, dysuria, shock).

   MDS must be differentiated from other causes of anemia, neutropenia, and thrombocytopenia. This requires complete blood counts and peripheral blood smear examination, bone marrow examination with cytogenetic and molecular studies, and the exclusion of other diseases, including congenital diseases, vitamin deficiencies, lupus, hepatitis, HIV, hemolytic anemia, plasma cell neoplasms, etc.).²² The bone marrow is usually hypercellular for the age of the patient, but may be hypocellular. However, the morphologic hallmark of MDS is aberrant morphologic changes in the erythroid, myeloid, and megakaryocytic cell lines.¹⁶, ²³ Dysplastic
granulocytes in the bone marrow or peripheral blood may be hypersegmented or hyposegmented, and show decreased cytoplasmic granules (hypogranularity) or large, pseudo-Chediak Higashi granules. The peripheral blood erythrocytes show anisopoikilocytosis, while erythroid precursors in the bone marrow may show binucleation, internuclear bridging, or aberrant nuclear features (irregularity, budding, strings). Peripheral blood platelets may be large, giant, and/or hypogranular, while megakaryocytes may be hyposegmented or hypersegmented. A Prussian blue stain for iron can reveal abnormal, iron-containing erythroid precursors (ringed sideroblasts). The World Health Organization (WHO) recently issued a revised and updated classification for MDS, summarized in Table IX.

Table IX
WHO (2008) Classification of Myelodysplastic Syndromes

<table>
<thead>
<tr>
<th>Disease</th>
<th>Peripheral Blood Findings</th>
<th>Bone Marrow Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractory cytopenia with unilineage dysplasia (RA, RN, RT)</td>
<td>Cytopenia, blasts ≤1%, monocytes ≤1 X 10⁹/L</td>
<td>Dysplasia (&gt;10%) in one lineage only, &lt;5% blasts, &lt;15% ringed sideroblasts</td>
</tr>
<tr>
<td>Refractory anemia with ringed sideroblasts</td>
<td>Anemia, no blasts, monocytes ≤1 X 10⁹/L</td>
<td>Erythroid dysplasia only, &lt;5% blasts, ≥15% ring sideroblasts</td>
</tr>
<tr>
<td>Refractory cytopenia with multilinage dysplasia</td>
<td>Cytopenia(s), &lt;1% blasts, monocytes ≤1 X 10⁹/L, no Auer rods</td>
<td>Dysplasia in &gt;10% of the cells of ≥2 myeloid lineages, &lt;5% blasts, &lt;15% ringed sideroblasts, no Auer rods</td>
</tr>
<tr>
<td>RAEB-1</td>
<td>Cytopenias, &lt;5% blasts, monocytes ≤1 X 10⁹/L, no Auer rods</td>
<td>Unilineage or multilineage dysplasia, 5%-9% blasts, no Auer rods</td>
</tr>
<tr>
<td>RAEB-2</td>
<td>Cytopenias, 5%-19% blasts, monocytes ≤1,000/µL, Auer rods ±</td>
<td>Unilineage or multilineage dysplasia; 10%-19% blasts; Auer rods ±</td>
</tr>
<tr>
<td>MDS with isolated del(5q)</td>
<td>Anemia, normal to slightly increased platelets, &lt;5% blasts, no Auer rods</td>
<td>Normal to increased megakaryocytes with hypolobated nuclei, &lt;5% blasts, del (5q) as sole cytogenetic abnormality, no Auer rods</td>
</tr>
</tbody>
</table>

An increased bleeding tendency is common in patients with a myelodysplastic syndrome. The bleeding is usually due to thrombocytopenia, but dysplastic megakaryopoiesis with morphologically and functionally aberrant platelets, thrombotic thrombocytopenic purpura, acquired factor VIII inhibitors, and other abnormalities are sometimes found. Platelet ultrastructural examination in these patients has revealed various abnormalities, including reduced, fused, or giant granules (storage pool deficiency); dilated canaliculi, and aberrant microtubules. Abnormal platelet membrane glycoprotein expression has also been described. A platelet function abnormality should be expected when the clinical bleeding manifestations are unusually severe for the degree of thrombocytopenia.

Final Diagnosis: Myelodysplastic syndrome with thrombocytopenia and acquired platelet function defect.
References:


22. Mufti GJ, Bennett JM, Goasguen J, et al. Diagnosis and classification of myelodysplastic syndrome: International Working Group on Morphology of myelodysplastic syndrome (IWGM-MDS) consensus proposals for the definition and enumeration of myelo-


<table>
<thead>
<tr>
<th>Factor</th>
<th>Other Names</th>
<th>Molecular Weight (Daltons)</th>
<th>Plasma Concentration</th>
<th>Minimal Hemostasis (%)</th>
<th>Major Surgery (%)</th>
<th>Half-Life</th>
<th>Vitamin K Dependent</th>
<th>Function</th>
<th>Major Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Fibrinogen</td>
<td>333,000</td>
<td>150-400 mg/dL</td>
<td>50-100 mg/dL</td>
<td>&gt;100 mg/dL</td>
<td>3-6 Day</td>
<td>No</td>
<td>Fibrin precursor</td>
<td>Forms fibrin clot after cleavage by thrombin, fibrin clot stabilized by factor XIII</td>
</tr>
<tr>
<td>II</td>
<td>Prothrombin</td>
<td>72,000</td>
<td>100 µg/mL</td>
<td>10-20%</td>
<td>30-40%</td>
<td>50-80 Hr</td>
<td>Yes</td>
<td>Serine protease</td>
<td>Thrombin precursor, activated by prothrombinase complex on surface of activated platelets</td>
</tr>
<tr>
<td>III</td>
<td>Tissue factor, thromboplastin, CD142</td>
<td>47,000</td>
<td>Tissue protein</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Cofactor</td>
<td>Cell membrane glycoprotein on subendothelial cells, platelets, monocytes, cofactor for factor VII, member of cytokine receptor class II family.</td>
</tr>
<tr>
<td>IV</td>
<td>Calcium</td>
<td>-</td>
<td>9-10.5 mg/dL</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Cofactor</td>
<td>Essential component of the tenase and prothrombinase complexes</td>
</tr>
<tr>
<td>V</td>
<td>Proaccelerin, labile factor, accelerator (Ac-) globulin</td>
<td>300,000</td>
<td>7-10 µg/mL</td>
<td>5-15%</td>
<td>25%</td>
<td>4.5-36 Hr</td>
<td>No</td>
<td>Cofactor</td>
<td>Protein cofactor in the activation of prothrombin by factor Xa; activated by thrombin</td>
</tr>
<tr>
<td>VI</td>
<td>FVa, Accelerin</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VII</td>
<td>Proconvertin, serum prothrombin conversion accelerator (SPCA), cothromboplastin</td>
<td>50,000</td>
<td>0.5-1 µg/mL</td>
<td>5-10%</td>
<td>10-20%</td>
<td>2-5 Hr</td>
<td>Yes</td>
<td>Serine protease</td>
<td>Endopeptidase with gla residues; activated by thrombin in presence of Ca²⁺</td>
</tr>
<tr>
<td>VIII-AHF</td>
<td>Anthemophilic factor A, anthemophilic globulin (AHG)</td>
<td>293,000</td>
<td>0.2 µg/mL</td>
<td>1-5%</td>
<td>50-60%</td>
<td>8-12 Hr</td>
<td>No</td>
<td>Cofactor</td>
<td>Activated by thrombin; cofactor in activation of factor X by factor IXa</td>
</tr>
<tr>
<td>IX</td>
<td>Christmas Factor, antihemophilic factor B, plasma thromboplastin component (PTC)</td>
<td>56,000</td>
<td>4-5 mg/mL</td>
<td>10-20%</td>
<td>50-60%</td>
<td>18-24 Hr</td>
<td>Yes</td>
<td>Serine protease</td>
<td>Endopeptidase with gla residues; activated by factor Xla in presence of Ca²⁺</td>
</tr>
<tr>
<td>X</td>
<td>Stuart-Prower Factor</td>
<td>56,000</td>
<td>10 µg/mL</td>
<td>5-10%</td>
<td>15-20%</td>
<td>20-42 Hr</td>
<td>Yes</td>
<td>Serine protease</td>
<td>Endopeptidase with gla residues; activated on surface of activated platelets by tenase complex and by factor VIIIa in presence of tissue factor and Ca²⁺</td>
</tr>
<tr>
<td>XI</td>
<td>Plasma thromboplastin antecedent</td>
<td>160,00</td>
<td>2-7 µg/mL</td>
<td>10-15%</td>
<td>15-30%</td>
<td>40-80 Hr</td>
<td>No</td>
<td>Serine protease</td>
<td>Endopeptidase; activated by factor XIIa</td>
</tr>
<tr>
<td>XII</td>
<td>Hageman Factor</td>
<td>76,000</td>
<td>30-40 µg/mL</td>
<td>&lt;10%</td>
<td>10%</td>
<td>50 Hr</td>
<td>No</td>
<td>Transpeptidase</td>
<td>Endopeptidase; binds to exposed collagen at site of vessel wall injury, activated by high-MW kininogen and kallikrein</td>
</tr>
<tr>
<td>XIII</td>
<td>Protransglutaminase, fibrinoligase, fibrin stabilizing factor (FSF)</td>
<td>320,000</td>
<td>30 µg/mL</td>
<td>1%</td>
<td>5%</td>
<td>7-14 Day</td>
<td>No</td>
<td>Serine protease</td>
<td>Transpeptidase; activated by thrombin in presence of Ca²⁺; stabilizes fibrin clot by covalent cross-linking</td>
</tr>
<tr>
<td>Prekallikrein</td>
<td>PK, Fletcher factor</td>
<td>86,000</td>
<td>40</td>
<td>N/A</td>
<td>N/A</td>
<td>?</td>
<td>No</td>
<td>Serine protease</td>
<td>Single chain gamma globulin; role in early stage contact factor activation, kinin formation, and fibrinolysis</td>
</tr>
<tr>
<td>High Molecular Weight Kininogen</td>
<td>HMWK, Contact activation cofactor, Fitzgerald, Flaujeac Williams factor</td>
<td>120,000</td>
<td>100</td>
<td>N/A</td>
<td>N/A</td>
<td>?</td>
<td>No</td>
<td>Cofactor</td>
<td>Source of kinin; links prekallikrein to negative surface for activation by surface bound factor Xlla; forms complex with factor XI and accelerates activation</td>
</tr>
<tr>
<td>Factor</td>
<td>Other Names</td>
<td>Molecular Weight</td>
<td>Plasma Concentration</td>
<td>Half-Life</td>
<td>Vitamin K Dependent</td>
<td>Function</td>
<td>Major Activity</td>
<td></td>
<td></td>
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<td>-------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
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<td>--------------------------------------------------------------------------------------------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>von Willebrand Factor</td>
<td>vWF</td>
<td>260,000 - &gt;10,000,000</td>
<td>Variable</td>
<td>N/A</td>
<td>N/A</td>
<td>12 hours, No, Binding to proteins and cells</td>
<td>Variable multimers of 2050 amino acid (260 kDa) monomer; protects factor VIII from degradation in circulation; links collagen and GPIb; binds to many substances</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vWF-Cleaving Protease</td>
<td>vWFCP; ADAMTS-13; ADAM metallopeptidase with thrombospondin type 1 motif, 13</td>
<td>190,000</td>
<td>1 µg/mL</td>
<td>N/A</td>
<td>N/A</td>
<td>? No, Cleaves plasma and platelet surface vWF multimers into smaller forms</td>
<td>Cleaves vWF at the 842-Tyr-</td>
<td>-Met-843 in the A2 domain of the vWF subunit; TTP results from ADAMTs-13 deficiency or anti-ADAMTS-13 antibody</td>
<td></td>
</tr>
<tr>
<td>Antithrombin</td>
<td>AT, antithrombin III, AT III, serine peptidase inhibitor, serine protease inhibitor</td>
<td>58,000</td>
<td>0.12 mg/mL</td>
<td>3 days</td>
<td>No</td>
<td>Serine protease inhibitor</td>
<td>Inhibits thrombin, factor Xa, factor IXa, factor Vlla, factor XIa, and factor XIla; activity greatly enhanced by heparin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein C</td>
<td>Autoprothrombin IIA Anticoagulant protein C Blood coagulation factor XIV</td>
<td>62,000</td>
<td>25 mg/L</td>
<td>8-12 Hr</td>
<td>Yes</td>
<td>Serine protease</td>
<td>Activated (activated protein C, APC, protein Ca) by thrombin bound to thrombomodulin; inactivates factors Va and Vlla in the presence of calcium ions and phospholipid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein S</td>
<td>-</td>
<td>84,000</td>
<td>25 mg/L</td>
<td>?</td>
<td>Yes</td>
<td>Cofactor</td>
<td>Cofactor of protein degradation of coagulation factors Va and Vlla.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombomodulin</td>
<td>TM, CD141, BDCA-3, fetomodulin</td>
<td>74,000</td>
<td>Membrane protein</td>
<td>N/A</td>
<td>No</td>
<td>Type I membrane protein, endothelial cell receptor</td>
<td>Forms complex with thrombin that activates protein C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue factor pathway inhibitor</td>
<td>TFPI, serpin A10</td>
<td>34,000-40,000</td>
<td>75-120 ng/mL</td>
<td>?</td>
<td>No</td>
<td>Endopeptidase inhibitor</td>
<td>Circulates in association with plasma VLDL lipoproteins, reversibly inhibits factor Xa; FVlla-TF complex is inhibited by TFPI-Xa complex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein Z</td>
<td>-</td>
<td>62,000</td>
<td>1400 ng/mL</td>
<td>?</td>
<td>Yes</td>
<td>Cofactor for ZPI</td>
<td>Binds thrombin and promotes its association with phospholipid vesicles, reduces rate of coagulation factor Xa inhibition by anti-thrombin, enhances inhibition of factor Xa mediated by ZPI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein Z-Related Protease Inhibitor</td>
<td>ZPI, serpin peptidase inhibitor, PZ-dependent protease inhibitor, human leuserpin-2</td>
<td>72,000</td>
<td>4 ug/mL</td>
<td>?</td>
<td>No</td>
<td>Serine protease inhibitor</td>
<td>Inhibits factor Xa activity in the presence of protein Z, calcium and phospholipid, inhibits factor Xla</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heparin Cofactor II</td>
<td>Heparin cofactor A, antithrombin BM, derrman sulfate cofactor, human heuserpin-2</td>
<td>65,600</td>
<td>90 ug/mL</td>
<td>?</td>
<td>No</td>
<td>Serine protease inhibitor</td>
<td>Inhibits thrombin; increased activity in presence of heparin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Contemporary Issues in Diagnostic Hemostasis and Thrombosis
ASCP Annual Meeting, October 2011
### Components of Fibrinolytic System

<table>
<thead>
<tr>
<th>Factor</th>
<th>Other Names</th>
<th>Molecular Weight</th>
<th>Plasma Concentration</th>
<th>Half-Life</th>
<th>Vitamin K Dependent</th>
<th>Function</th>
<th>Major Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasminogen</td>
<td>Profibrinolysin</td>
<td>92,000</td>
<td>200 ug/mL</td>
<td>0.8-2.2 Days</td>
<td>No</td>
<td>Plasmin proenzyme, serine protease</td>
<td>Activated by tPA, uPA, thrombin, fibrin, and factor XII; degrades fibrin and other plasma proteins</td>
</tr>
<tr>
<td>Tissue-type plasminogen activator</td>
<td>t-PA, tPA, PLAT, Alteplase</td>
<td>68,000</td>
<td>5 ug/mL (total)</td>
<td>5 minutes</td>
<td>No</td>
<td>Plasminogen activator</td>
<td>Largely circulates in complex with PAI-1; converts plasminogen to plasmin</td>
</tr>
<tr>
<td>PAI-1</td>
<td>PLANH1, PAI1, Endothelial plasminogen activator inhibitor</td>
<td>45,000</td>
<td>5-10 ng/mL</td>
<td>1 hour</td>
<td>No</td>
<td>Serine protease inhibitor</td>
<td>Activated by vitronectin; principal inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA)</td>
</tr>
<tr>
<td>Alpha 2-antiplasmin</td>
<td>Alpha-2 plasmin inhibitor, primary plasmin inhibitor or anti-plasmin</td>
<td>58,700</td>
<td>70 ug/mL</td>
<td>?</td>
<td>No</td>
<td>Serine protease inhibitor</td>
<td>Major inhibitor of circulating serine proteases, including plasmin, trypsin, and chymotrypsin; cross-linked to fibrin by factor Xlla; inhibits plasmin by binding irreversibly to active catalytic site, inhibits binding of plasmin to fibrin</td>
</tr>
<tr>
<td>Thrombin-activatable fibrinolysis inhibitor</td>
<td>TAFI, plasma carboxypeptidase B, pCPB, carboxypeptidase U, CPU, carboxypeptidase B-like protein, thrombin-activatable fibrinolysis inhibitor</td>
<td>60,000</td>
<td>2.5 ug/mL</td>
<td>?</td>
<td>?</td>
<td>Fibrinolysis inhibitor</td>
<td>Activated by thrombin-thrombomodulin; down-regulates fibrinolysis by decreasing fibrin binding for t-PA and plasminogen</td>
</tr>
</tbody>
</table>

### Special Platelet Components

<table>
<thead>
<tr>
<th>Factor</th>
<th>Other Names</th>
<th>Molecular Weight</th>
<th>Plasma concentration</th>
<th>Source/Localization</th>
<th>Function</th>
<th>Major Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-Thromboglobulin</td>
<td>β-TG</td>
<td>35,800, homotetramer of 8800 Mr subunit</td>
<td>18 ng/mL</td>
<td>Platelet (alpha granule)</td>
<td>Biologic function unknown</td>
<td>Heparin-binding protein, plasma marker of platelet activation</td>
</tr>
<tr>
<td>Platelet Factor 4</td>
<td>PF4</td>
<td>29,000</td>
<td>6 ng/mL</td>
<td>Platelet (alpha granule)</td>
<td>Heparin neutralization</td>
<td>Neutalizes anticoagulant activity of heparin, stimulates histamine release from basophils, neutrophil/monocyte chemotactic activity</td>
</tr>
<tr>
<td>Thrombospondin</td>
<td>TSP-1, thrombin-sensitive protein</td>
<td>450,000</td>
<td>440 ng/mL</td>
<td>Platelet (alpha granule), many other cells. Integral membrane component in some cells</td>
<td>Adhesive protein (cell-cell, cell-substrate), multiple functions</td>
<td>Platelet adhesion and aggregation, inhibition of angiogenesis, activation of apoptosis, immune regulation, and activation of TGF-beta. Multiple receptors, including CD36, CD47, integrins</td>
</tr>
</tbody>
</table>

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VCU School of Medicine