

PEARLS OF LABORATORY MEDICINE

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TITLE: Heparin-Induced Thrombocytopenia

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Slide 1:

Hello, my name is Kristi Smock. I am an Associate Professor of Pathology at the University of Utah Department of Pathology and Medical Director for the Hemostasis/Thrombosis Laboratory at ARUP Laboratories. Welcome to this Pearl of Laboratory Medicine on “**Heparin-Induced Thrombocytopenia.**”

Slide 2:

Heparin-induced thrombocytopenia (HIT) is an immune-mediated syndrome that occurs in 1 to 5% of patients who receive heparin. HIT is caused by development of IgG antibodies to heparin-platelet factor 4 (PF4) complexes. In patients with HIT, immune complexes result in platelet activation, leading to thrombocytopenia and increased risk of arterial or venous thrombosis. If not recognized and treated appropriately, 50% of patients with HIT will develop thrombosis.

Slide 3:

Let's take a closer look at the pathogenesis of HIT. PF4 is a positively charged substance released from platelet granules upon platelet activation. Due to its positive charge, PF4 can form complexes with negatively charged heparin molecules when these are encountered in the bloodstream. In some patients, the heparin-PF4 complexes are antigenic and trigger an immune response that results in production of antibodies and immune complex formation with immune complexes composed of IgG, heparin and PF4. The immune complexes may activate platelets via the platelet Fc gamma (Fc γ) receptor for IgG, resulting in platelet aggregation and accelerated thrombin generation. HIT is commonly described using an iceberg model. Only a subset of heparin-exposed patients will develop antibodies to heparin-PF4 and only a subset of these will have detectable platelet-activating properties and thrombocytopenia and only a subset of these will develop thrombosis.

Slide 4:

Heparin-exposed surgical patients carry a greater risk for HIT than medical patients due to higher amounts of circulating PF4 released as part of the platelet response to surgical injury. Risk is also related to the type and dose of heparin the patient receives. Treatment with unfractionated heparin (UFH) carries greater risk (approximately 10-fold greater) than treatment with low-molecular-weight heparin (LMWH) since unfractionated heparin carries a greater negative charge and is more likely to combine in complexes with PF4 creating the HIT antigen. The highest risk occurs among patients receiving unfractionated heparin after major surgery.

Slide 5:

HIT diagnosis begins with clinical suspicion, specifically when thrombocytopenia develops several days after exposure to heparin, characteristic of an immune response. This is why heparin anticoagulation protocols for many indications require baseline platelet counts prior to heparin initiation. Clinical pre-test probability is evaluated using clinical scoring systems, such as the common 4T's scoring system. The score takes into account factors such as the degree and timing of thrombocytopenia, presence of thrombosis, and other possible causes for thrombocytopenia. Clinical scoring systems have been shown to have excellent negative predictive value (NPV) of 97-99% for low scores (< 4 points) but unacceptably poor positive predictive value (PPV) for intermediate (4-5 points, PPV 10-20%) or high scores (6-8 points, PPV 40-80%).

Slide 6:

This slide shows additional detail of the 4T's clinical scoring system. The maximum number of points is obtained for a fall in platelet count of at least 50% with nadir platelet counts of $20 \times 10^9/L$ or above and with the thrombocytopenia developing 5-10 days after heparin exposure as long as there has been no additional previous heparin exposure within the past 30 days. In addition, maximum points are given for proven new thrombosis or skin necrosis or acute systemic reaction to heparin upon heparin administration and when no other causes for thrombocytopenia are evident.

Slide 7:

Due to the poor positive predictive value of intermediate or high clinical scores, these patients require laboratory testing to further evaluate the possibility of HIT, which is a clinicopathologic diagnosis. There are two main categories of HIT laboratory tests that are performed on patient serum: Immunoassays and functional assays. The most commonly used immunoassays are commercially available enzyme-linked immunosorbent assay (ELISA) kits that detect antibodies to heparin-PF4 complexes. Functional assays detect platelet activation by HIT immune complexes. Technical aspects and performance characteristics of each assay type will be discussed on upcoming slides.

Slide 8:

HIT ELISA assays utilize plates coated with heparin-PF4 (or an acceptable substitute) and are classified as polyspecific (detect IgG, IgM, and IgA antibodies) or monospecific, which detect only IgG antibodies. The ELISA assays have excellent negative predictive value (~99%) since lack of the pathogenic antibodies at the time of platelet decline excludes the possibility of HIT. However, the positive predictive value of ELISA is poor. Although positive ELISA results are highly sensitive for HIT, indicating the presence of heparin-PF4 antibodies, they are not specific since they cannot determine whether the antibodies have platelet activating properties, which is required for development of the syndrome. Specificity is somewhat improved with use of ELISAs that detect only IgG antibodies since HIT is caused by IgG antibodies but not IgM or IgA. Thus, IgG-specific assays avoid detection of antibodies that are not clinically relevant.

Slide 9:

ELISA specificity is also improved by considering the optical density (OD) value of positive results since the OD value is related to antibody titer and higher OD values are more likely to represent antibodies capable of platelet activation. For instance, the HIT literature indicates that weakly positive ELISA OD values (0.4-1.0) carry only a ~5% probability of HIT and the probability increases to only ~25% with OD values in the 1.0-1.5 range. OD values of 2.0 or greater carry a much higher risk of approximately 90%. However, it should be remembered that these are general statements and there is between-laboratory variability in assay performance and OD values. Some laboratories further investigate positive ELISA results by repeating the reaction in the presence of a high heparin concentration. If a positive reaction is inhibited by high heparin, it suggests that the reactivity is heparin-dependent, possibly modestly increasing the specificity of positive results. However, this step is not thought to be a reliable differentiator between clinically significant and non-significant antibodies and may result in false-negative results since very high-titer strongly reacting antibodies may not be inhibited. Taking all of these variables into account, HIT ELISA assays are often described as 95-100% sensitive but with specificity often well below 90%.

Slide 10:

In contrast to HIT immunoassays, functional platelet activation assays have >90% sensitivity and specificity for HIT since they are capable of identifying the platelet-activating properties that cause the syndrome. The most common functional assay and golden standard is the serotonin release assay (SRA), which is considered the gold-standard test for HIT due to high sensitivity and specificity. Common uses of the SRA are to further evaluate unexpected or positive ELISA results.

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The SRA is performed by incubating patient serum (source of antibodies) with donor platelets (reagent) and heparin (reagent). These conditions allow the pathogenic immune complexes to form and serum from a patient with HIT will result in platelet activation. Activated platelets

release a variety of proteins and other substances from their granules, including serotonin, which is released from the platelet dense granules. The end-point of the SRA assay is detection and quantification of any serotonin released from the reagent platelets into the reaction supernatant. In other words, the appearance of large quantities of serotonin in the reaction supernatant is an indicator of platelet activation. A variety of methods can be used to quantify released serotonin. The classic method utilizes radiolabeled serotonin, while more recent versions use nonradioactive methods such as high-performance liquid chromatography (HPLC), mass spectrometry, or immunoassay. In addition, other types of platelet activation assays utilize different endpoints for identifying platelet activation. These include platelet aggregation, release of other substances, such as ATP, from platelet granules, or flow cytometric detection of markers expressed on the surface of activated platelets. These alternative methods, of course, are not classified as serotonin release assays.

Slide 12:

This slide discusses the reporting and interpretation of SRA results. In SRA reports, released serotonin, which is the indicator of whether platelet activation did or did not occur, is usually expressed as percent release (% release). The percent release value indicates what percentage of total possible platelet serotonin was released in the reaction. The total possible platelet serotonin is determined by measuring serotonin in a lysed platelet preparation. Greater than or equal to 20% serotonin release is commonly used as the cutoff value for platelet activation, although different cutoffs can be used. Also, each patient specimen is tested with two different heparin concentrations (low and high) and percent release for both reactions are reported. The lower concentration represents a typical therapeutic heparin concentration and the higher concentration is markedly supra-therapeutic. In positive samples, platelet activation occurs in the presence of the therapeutic heparin concentration, due to efficient HIT immune complex formation, but the activation is inhibited in the presence of the high heparin concentration, due to disruption of the IgG-heparin-PF4 immune complexes by excess heparin. Evaluation of the low and high heparin patterns improves the specificity of the assay for HIT, which is a heparin-dependent platelet activation disorder.

Slide 13:

This slide summarizes the different reaction patterns to low- and high- heparin environments that result in classification of SRA specimens as positive, negative, or indeterminate. The final classification and an interpretive comment should be provided with the result. Note that positive samples demonstrate platelet activation with low heparin and inhibition with high heparin, while negative specimens do not demonstrate platelet activation at either heparin concentration, and indeterminate samples demonstrate activation regardless of the heparin concentration. The differential activation response in positive specimens demonstrates that HIT is a heparin-dependent phenomenon, which is why HIT patients are treated by discontinuing all heparins and implementing anticoagulation with a non-heparin anticoagulant such as a direct thrombin

inhibitor. A positive SRA result supports a diagnosis of HIT while HIT is very unlikely if the SRA result is negative. Indeterminate results do not demonstrate a heparin-dependent pattern since the reaction is not impacted by changes in heparin concentration. These usually represent HLA antibodies, platelet autoantibodies, or other types of immune complexes with *in vitro* platelet activating properties. Indeterminate results are not common, occurring in less than 5% of SRA specimens, but they may necessitate repeat testing or reliance on other information to make or exclude a HIT diagnosis.

Slide 14:

This slide depicts a stepwise clinicopathologic approach to HIT diagnosis. Use of a clinical scoring system helps to avoid over-testing due to the high negative predictive value of low clinical scores. When HIT is not ruled out by clinical scoring, many centers perform ELISA testing first and are able to exclude negative patients from further testing due to the high ELISA negative predictive value. Although interpretation of HIT ELISA results has various nuances, many centers reflex all positive ELISA specimens to SRA to either confirm or exclude HIT. Overall, this type of approach reserves the more technically complex and expensive functional testing for a smaller subset of samples. While many larger centers offer HIT ELISA testing, platelet activation assays like the SRA are laboratory-developed tests offered by only a limited number of laboratories. Since HIT is an urgent clinical condition due to the high risk of thrombosis, treatment decisions, including discontinuing heparin and implementation of alternative anticoagulation, must be made while the laboratory results are still pending. Treatment plans are subsequently modified when laboratory results become available.

Slide 15: References

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Slide 16: Disclosures

I am employed by the University of Utah Department of Pathology and ARUP Laboratories.

Slide 17: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on “**Heparin-Induced Thrombocytopenia.**”