



Saint Luke's Regional Laboratories Clinical Laboratory Letter



September 2010

New MDRO Alert

Recently, the CDC reported that a new type of multi-drug resistant organism (MDRO) has been detected in the United States (MMWR 2010; 59[24]:750). Three isolates of enteric gram negative bacilli, from 3 different states have been found to produce an enzyme called New Delhi metallo-beta-lactamase (NDM-1). This enzyme confers resistance to all beta-lactam anti-microbials, including carbapenems. The three organisms identified to date included an *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*. All were isolated from patients who recently received medical care in India. These organisms were resistant to most other anti-microbials as well, including aztreonam.

Carbapenem resistance of the NDM-1 type is detected by standard susceptibility test methods. Saint Luke's Regional Laboratories currently performs 3 different types of susceptibility testing on any enteric isolate suspicious for carbapenem resistance. Enteric organisms suspicious for NDM-1 would be forwarded to the CDC for confirmation. Physicians should be aware of the possibility of NDM-1 producing organisms in patients who have received medical care in India or Pakistan in the last 6 months.

CISH vs. Infectious Disease

A recent study explored the genetics of susceptibility to infectious diseases (NEJM 2010;362:2092-2101). Inflammatory cytokine production is the host immune response to infection by many pathogens including bacteria, mycobacteria, and malaria. The *CISH* gene codes for cytokine-inducible SRC homology 2 domain protein (CISH) which controls cytokine signaling, in particular interleukin-2. Interleukin-2 is critical to immunity in that it mediates T-cell response, proliferation of B cells and natural killer cells, and promotes maturation of macrophages.

Researchers analyzed data on 8402 persons from Kenya, Malawi, Hong Kong, Gambia, and Vietnam with various infections including bacteremia,

tuberculosis, and malaria. Peripheral blood samples were collected for *CISH* gene sequencing and SNP (single nucleotide polymorphism) analysis. There were 5 *CISH* SNPs identified that conferred increased susceptibility to bacterial, mycobacterial and malarial infections. The overall risk of infection was increased by 18% in those carrying a single SNP, and by 81% in those with 4 or more risk alleles.

This study indicates that genetic factors in addition to environmental factors, such as malnutrition and HIV disease, are responsible for individual susceptibility to infection. Future clinical management of infectious diseases may include alterations of genetic pathways in addition to antimicrobial agents.

Heparin Induced Thrombocytopenia

Heparin induced thrombocytopenia (HIT) is an antibody mediated adverse effect of heparin that is strongly associated with venous and arterial thrombosis. Major risk factors include exposure to unfractionated high molecular weight heparin for more than 5 days, undergoing surgery and female gender. Diagnostic criteria include:

- Thrombocytopenia
- Onset 5 to 12 days after exposure to heparin
- Exclusion of other causes of thrombocytopenia
- Positive laboratory test for HIT

The incidence of HIT is 2.6% of patients exposed to unfractionated heparin and 0.2% exposed to low molecular weight heparin. Approximately 25% of these patients will develop thrombosis within 30 days. Most cases arise in patients who have not been previously exposed to heparin. In this situation, thrombocytopenia usually occurs 5 to 12 days after heparin initiation. In patients who have been previously sensitized to heparin, platelet counts may decrease within the first three days or even hours after re-exposure. Platelet counts usually decrease more than 50% from baseline and typically fall to 20,000 - 150,000/uL. The nadir is usually reached 5 days after onset of the decline. The thrombocytopenia associated with HIT is not

as severe as other drug induced thrombocytopenia and is seldom associated with spontaneous bleeding.

Venous thrombosis is 3 times more common than arterial, except for patients undergoing cardiovascular surgery. Venous thrombosis most often results in deep vein thrombosis and pulmonary embolism. Arterial thrombosis may cause limb gangrene, myocardial infarction or stroke. Skin necrosis is associated with heparin dependent platelet antibodies but usually not thrombocytopenia.

Any heparin compound can induce antibody formation, but those forms with the highest molecular weight and highest degree of sulfation are associated with the highest incidence of HIT. The types of heparin reported to cause HIT in order of decreasing frequency are bovine heparin > porcine heparin > low molecular weight heparin > heparinoids. Low molecular weight heparin appears to induce antibody formation about one fourth as often as bovine heparin and seldom causes thrombocytopenia. HIT can be induced by any dose or route of heparin administration, including heparin flushes and heparin coated intra-arterial lines. High dose IV heparin is more likely to induce antibody formation than low dose subcutaneous heparin. Long duration of heparin administration is more likely to cause HIT, but the syndrome can occur after a single bolus.

The antibody that causes this syndrome is not directed against heparin alone, but a complex of heparin with platelet factor 4 (PF4), which is a heparin-neutralizing protein contained in the alpha granules of platelets that is released upon activation. Unlike traditional immune responses, IgM, IgA and IgG antibodies are produced simultaneously, but only IgG is associated with thrombosis.

Heparin-PF4 antibodies cause thrombosis by binding to the surface of activated platelets and inducing platelet aggregation. Aggregated platelets are removed prematurely from the circulation leading to thrombocytopenia and the generation of platelet microparticles, which stimulate thrombin generation and thrombosis.

Laboratory tests that detect IgG PF4 antibodies are the most sensitive (96-100%). A negative result strongly suggests the absence of HIT, but a positive test does not necessarily mean that a patient has antibodies capable of activating platelets. Many more patients form antibodies than have thrombosis. The strength of the reaction is predictive of the risk of thrombosis, as seen in the following table.

| Strength of Reaction | Probability of Thrombosis |
|----------------------|---------------------------|
| <0.40 | 0% |
| 0.40-1.00 | 5% |
| 1.01-1.40 | 20-30% |
| 1.41-2.00 | 50-60% |
| >2.00 | >90% |

A PF4 antibody result of >2.00 is most often associated with antibodies capable of activating platelets. The platelet activation capability of intermediate-strength PF4 antibodies can be determined by a confirmatory serotonin release assay. This test is difficult to perform and only available at a few reference laboratories, but has the highest specificity (94-100%) for clinically significant antibodies.

Heparin should be discontinued in any patient with a clinical presentation consistent with HIT. Heparin flushes and heparin coated catheters should also be avoided. After discontinuing heparin, platelet counts begin to rise within 2-3 days and usually return to normal within 10 days. PF4 antibody decreases to undetectable levels within 2-3 months after cessation of heparin therapy. Platelet transfusions are contraindicated because they may contribute to thrombus formation or extension.

Coagulation Screen Change

Currently, the coagulation screen panel includes PT, aPTT, fibrinogen and platelet count. When a coagulation screen and CBC are ordered together, the laboratory has to cancel the platelet count from the coagulation screen to prevent duplicate billing. To alleviate this potential compliance issue, platelet count will be deleted from the coagulation screen.