Significance of a Borderline Negative ELISA Test for Heparin Induced Thrombocytopenia

Heparin-induced thrombocytopenia (HIT) is a common and potentially devastating complication of heparin exposure, occurring in 1-5% of patients receiving heparin. At least one third of these patients develop venous and/or arterial thrombosis, with an associated mortality rate of approximately 20-30%. HIT is caused by an antibody directed against heparin-PF4 (platelet factor 4) complexes, which bind to and activate platelets, leading to thrombocytopenia and thrombosis. This antibody can be detected by a sensitive ELISA, which has proved useful clinically for diagnosis of HIT. In this assay, presence of the antibody results in a color change, detected as an increase in optical density (OD). The ELISA is reported as positive if the observed OD is above a specific threshold value, and negative if the OD is below the threshold. It has not been known whether the actual OD has clinical significance, and specifically if there is any value in repeating the assay if the result is negative but just below the threshold.

A recent study addressed this issue (Am J Clin Pathol 2003;119:61-65). Consecutive patients who had negative ELISA tests for HIT were divided into 3 equal groups, according to the OD for the negative result (see Table). The high negative group had borderline negative OD readings that were just below the threshold for positivity. Repeated ELISA tests were ordered at the discretion of the patients' physicians, who were unaware of the OD titer values. The results are shown in the following table.

<table>
<thead>
<tr>
<th>Initial negative HIT titer</th>
<th>No. with repeat HIT test</th>
<th>No. (%) with positive repeat test</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. low negative (n=50)</td>
<td>20</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Low negative (n=50)</td>
<td>32</td>
<td>4 (13)</td>
</tr>
<tr>
<td>High negative (n=50)</td>
<td>30</td>
<td>13 (43)</td>
</tr>
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</table>

These results indicate that a significant proportion of patients with a high negative (borderline) HIT ELISA result will convert to positive on repeat testing. Among those that converted to positive in this group, the median interval before the test was repeated was 3 days. The initial platelet counts (low in all except one patient) did not differ significantly among the 3 groups, and platelet counts in those that converted to positive did not change significantly. Among all the patients that converted to a positive HIT result on repeat testing, thrombosis occurred in 28%, and mortality was 44%, confirming the clinical importance of this finding. It is speculated that the initial false negative result in these patients may be due to performance of the test early in the course of HIT, when the antibody concentration may not yet be high enough to be detectable.

In view of these findings, it is recommended that an initially negative HIT ELISA test be repeated after an interval of approximately 3 days, if the result is high negative, or if there is a strong clinical suspicion that HIT is present. The sensitivity of the HIT ELISA test is currently approximately 90%, a value that could probably be improved if repeat testing is undertaken. It should also be kept in mind that the ELISA test is not 100% specific - a positive test may occur in a proportion of patients exposed to heparin who do not develop HIT (specificity has varied in different studies from 86-98%). The ELISA test for HIT has been performed at Saint Luke's Regional Laboratories for several years, with results reported as positive or negative. In the future, if a negative result falls into the “high/borderline negative” category, this will be noted on the chart, with a comment recommending that the test be repeated after approximately 3 days.

Positive Blood Cultures: Significant vs. Contaminant

Last year the Microbiology laboratory processed 12,740 blood cultures, with an overall positive rate of 13.4%. Coagulase-negative staphylococcus (CNS) was the most common isolate accounting for...
40% of all positive cultures. *Staphylococcus aureus*, *E. coli*, and viridans streptococcus were also common isolates. Nationwide, the most common blood culture isolate is also CNS and many studies show these organisms are contaminants 60-80% of the time. Contamination of blood cultures has been shown to increase the cost of hospitalization due to unnecessary antibiotic therapy, repeat cultures, and increased length of stay.

Criteria have been suggested for the determination of true positive versus contaminated blood cultures. Although the number of blood culture sets positive is significant, the number of bottles positive within a set is not useful in predicting true bacteremia. Generally, of the most common blood culture isolates, the following organisms are considered clinically significant, regardless of number of sets positive: *Staphylococcus aureus*, *Streptococcus pneumoniae*, enteric gram negative rods, *Pseudomonas aeruginosa*, and *Candida* species. Enterococci are significant 80% of the time. The most common contaminating organisms include CNS, viridans streptococci, diphteroids, *Propionibacterium*, *Micrococcus*, and *Bacillus* species.

Beginning April 1, Microbiology will begin a new approach to susceptibility testing of the most common blood culture contaminants. Based on the number of cultures drawn and the number of sets positive, the following comments will appear when CNS or viridans streptococci are isolated.

**Single blood culture set drawn within a 24 hour period:** "Coagulase negative staphylococcus isolated from 1 culture only. Susceptibility performed on request. For adult patients, it is recommended that two or three blood cultures be obtained per septic episode for determination of clinical significance."

**One set positive of 2 or more sets drawn within 24 hours:** "Coagulase negative staphylococcus isolated, possible contaminant. Susceptibility performed on request."

When 2 or more sets are positive within a 24 hour period, routine susceptibility testing will be performed. All organisms isolated from blood cultures are frozen and retained for six months, so that susceptibility testing can be performed retrospectively if necessary. This protocol will exclude all oncology and nursery patients, for whom susceptibility testing will continue to be performed routinely on all isolates. It is recommended by the American Society for Microbiology that blood culture contamination rates should not exceed 3%. The overall contamination rate for Saint Luke's Hospital in 2002 was 2.5%. A significant reduction in blood culture contaminants was seen in the Emergency Department last year during a pilot study of a new site prep agent containing chlorhexidine (Chloraprep). During the next few months, a new blood culture site prep procedure utilizing Chloraprep will be introduced to all nursing units.

### Diagnosis of *Clostridium difficile*-Associated Disease

Toxin-producing *Clostridium difficile* is associated with 90-100% of cases of pseudomembranous colitis, 60-75% of antibiotic-associated colitis, and 11-33% of antibiotic-associated cases of diarrhea. Common nonspecific laboratory abnormalities in patients with *Clostridium difficile*-associated disease (CDAD) include leukocytosis and hypoalbuminemia. Fecal leukocytes are detected in 50-60% of cases. Gram stains of fecal specimens are of no value, since *C. difficile* is only a small part of the fecal flora, even among patients with severe colitis. Likewise, anaerobic stool cultures are of little use in the diagnosis, due to the inability to distinguish between toxigenic and nontoxigenic strains.

The tissue culture cytotoxin B assay is the gold standard for diagnosis of CDAD, detecting as little as 10 pg of cytotoxin. However, this assay is labor intensive and results are delayed for up to 48 hours. The most commonly used diagnostic tests for CDAD are enzyme immunoassays (EIA), which detect 100 to 1000 pg of cytotoxin. Saint Luke's Regional Laboratories performs an EIA that detects both cytotoxin A & B. The sensitivity and specificity of the test are 95% and 97% respectively.

A minority of patients (5-20%) requires more than 1 stool assay to detect toxin. In suspected CDAD a single stool specimen should be submitted for testing initially. If the results are negative and diarrhea persists, 1 or 2 additional stool samples should be submitted. Blood, barium sulfate, metronidazole or vancomycin in the sample does not affect results. Stool specimens should be submitted in a clean airtight container with no preservative. Specimens that must be stored prior to testing should be refrigerated.