Clinical Significance of a Borderline Titer in a Negative ELISA Test for Heparin-Induced Thrombocytopenia

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Key Words: Heparin; Heparin-induced thrombocytopenia; Platelet count; Thrombosis; Thrombocytopenia; ELISA; HIT titer

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Abstract

We studied the usefulness of repeated enzyme-linked immunosorbent assay testing for heparin-induced thrombocytopenia (HIT) when the initial test result was negative and sought to determine whether the titer of the initial negative result correlated with the likelihood of obtaining a positive test result in repeated testing. We divided 150 patients who underwent HIT testing into 3 groups (50 patients each): (1) very low titer negative (0.0%-33.3% of the threshold for a positive test); (2) low titer negative (33.4%-66.6% of the threshold); and (3) high titer negative (66.7%-99.9% of the threshold). Among the patients who underwent a repeat test, 5% (1/20) of group 1 patients, 13% (4/32) of group 2 patients, and 43% (13/30) of group 3 patients tested positive in the repeat test (P = .0026). Thus, nearly half of patients with initially negative HIT test results had positive results in the repeat test if the negative titer was 66.7% or more of the threshold. If laboratories report the HIT titer, rather than just negative or positive, the titer might help clinicians predict which patients have HIT despite a negative initial test, and the overall sensitivity for diagnosing HIT might be improved.

Heparin-induced thrombocytopenia (HIT) can be a devastating complication of heparin exposure. Up to 8% of patients exposed to heparin develop the antibody that causes HIT without becoming thrombocytopenic. In another 1% to 5% of patients receiving heparin, HIT with thrombocytopenia develops, and of those, at least one third develop venous and/or arterial thrombosis. Thrombosis usually occurs only in patients with HIT who are thrombocytopenic. However, thrombosis has been reported in patients with HIT with normal platelet counts. Thrombosis in HIT has a high mortality of approximately 20% to 30%, with an equal percentage of patients becoming permanently disabled by amputation, stroke, or other causes. HIT can develop from even small amounts of heparin such as line flushes or heparin-coated catheters.

Among the assays that are used commonly in the United States, platelet aggregation assays are not as sensitive as other methods, and serotonin-release assays are sensitive but are limited to specialized laboratories because they require radioactive serotonin. Enzyme-linked immunosorbent assays (ELISAs) are sensitive and easy to perform. HIT ELISAs detect the antibody that causes HIT, an antibody that forms against heparin–platelet factor 4 (PF4) complexes. The antibody-heparin-PF4 complex binds to and activates platelets, leading to thrombocytopenia and thrombosis. With the Stago HIT ELISA (Diagnostica Stago, Asnieres-sur-Seine, France), patient serum or plasma samples are added to a microtiter plate that is coated with heparin–PF4 complexes. If the HIT antibody is present in the patient sample, the antibody will bind to the plate, leading to a color-generating reaction that increases the optical density (OD) at 492 nm when a second antibody is added. HIT ELISA results are reported as...
positive if the observed OD titer for a patient is above the specified threshold for the assay or negative if the OD is below the threshold. The actual OD obtained for the patient typically is not reported by the laboratory. Whether the actual titer of the assay has clinical significance is unknown.

In our institution, clinicians often submit repeated HIT tests when initial results are negative. The present study investigated the usefulness of such repeated testing by assessing the frequency of positive results among the repeated tests. In addition, since whether the HIT ELISA titer has clinical significance is unknown, we also sought to determine whether the titer of the initial negative result correlated with the likelihood of obtaining a positive HIT test result in repeated testing.

**Materials and Methods**

HIT testing was performed using the HPIA ELISA assay according to the manufacturer’s instructions. Since the OD threshold for positivity varies from kit to kit, the patient titers were converted into percentages of the OD threshold for analysis. The assay threshold for positivity is determined by the manufacturer. A negative test result is any value below that defined as the threshold for positive for the kit in use. The negative range of values below the positive value threshold, defined for this study as 0% to 100% of the positive threshold, was arbitrarily divided into 3 percentage ranges (0.0%-33.3%, 33.4%-66.6%, and 66.7%-99.9%). Any value of 100% or more is positive.

Consecutive patients who underwent HIT testing and tested negative (n = 150) were divided into 3 groups: (1) very low titer negative (0.0%-33.3% of the threshold for positive); (2) low titer negative (33.4%-66.6% of the threshold); and (3) high titer negative (66.7%-99.9% of the threshold). Results for consecutive patients were reviewed until 50 patients were obtained for each of the 3 groups. All subsequent HIT test results then were reviewed for each patient.

Repeated tests were ordered at the discretion of the patients’ physicians. The physicians were unaware of the platelet counts, for which the $t$ test (2-tailed) was used. Institutional review board approval was obtained for the study.

**Results**

Of all the consecutive negative HIT cases reviewed, 50% were very low titer, 30% were low titer, and 20% were high titer negative. The first 50 patients for each of the 3 groups were included in the present study.

Clinicians ordered a substantial number of repeated tests in all 3 groups, after the initially negative result. Of the patients, 20 of 50 in group 1, 32 of 50 in group 2, and 30 of 50 in group 3 underwent repeated testing. Of these patients, 1 (5%) of 20 group 1 patients, 4 (13%) of 32 group 2 patients, and 13 (43%) of 30 group 3 patients had positive results in the repeated test ($P = .0026$ for group 3 vs group 1).

Table 1 shows the platelet count at the time of the initially negative test result did not differ significantly among the 3 groups ($P = .24$ and $P = .44$ for group 3 vs groups 1 and 2, respectively).

Among the patients whose results were positive in repeated testing, the repeated test was performed a mean (median) of 6 (6) days after the initially very low titer-negative result (n = 1), 16 (18) days after the initially low titer-negative result (n = 4), and 9 (3) days after the initially high titer-negative result (n = 13). Table 1 also shows that the platelet count at the time of the initially negative test result did not differ significantly among the 3 groups.

Table 2 shows the platelet counts and the HIT titers for each of the cases that were positive in repeated testing. In almost all cases, the titer of the positive result was substantially higher than the titer of the negative result. Table 2 and Figure 2 show that the platelet counts were similar at the time of the negative and positive test results. With only 1 exception, patients already were thrombocytopenic at the time of the initially negative test result, suggesting that the antibody was capable of initiating thrombocytopenia before it could be detected in the assay in these cases.

**Table 1**

<table>
<thead>
<tr>
<th>Initial Negative HIT Titer Relative to Threshold for Positive Test (100%)</th>
<th>Mean (Median) Platelet Count at Time of Negative Test Result ($\times 10^{3}$/µL)</th>
<th>No. of Patients Who Received a Repeated HIT Test</th>
<th>No. (%) of Patients With Positive Test Result in Repeated Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0%-33.3% (n = 50)</td>
<td>92 (89)</td>
<td>20</td>
<td>1 (5)</td>
</tr>
<tr>
<td>33.4%-66.6% (n = 50)</td>
<td>119 (96)</td>
<td>32</td>
<td>4 (13)</td>
</tr>
<tr>
<td>66.7%-99.9% (n = 50)</td>
<td>108 (95)</td>
<td>30</td>
<td>13 (43)</td>
</tr>
</tbody>
</table>

HIT, heparin-induced thrombocytopenia.

* Values given are conventional units; values in Système International units are the same and are reported as $\times 10^{9}$/L.

Statistical analysis was performed using the Fisher exact test, except for the platelet counts, for which the $t$ test (2-tailed) was used.

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For the cases with positive results in repeated testing, patient histories were reviewed. In all cases, patients experienced a decrease of more than 50% in the platelet count in association with exposure to heparin, consistent with HIT. Mortality among these patients was 44% (1/1 in group 1, 1/4 in group 2, and 6/13 in group 3). Thrombosis in association with heparin exposure occurred in 28% (0/1 in group 1, 1/4 in group 2, and 4/13 in group 3). These percentages are similar to the rates observed among 91 consecutive HIT-positive patients in our institution (36% mortality and 31% thrombosis).

**Discussion**

To our knowledge, this is the first study investigating the clinical significance of a negative HIT ELISA titer. The results of the present study suggest that the titer of an initially negative HIT result can identify patients who could benefit most from repeated testing. Specifically, if the initially negative titer is very low (<33.3% of the threshold for positivity) or low (33.4%-66.6% of the threshold), repeated testing is less likely to show a positive result than if the initially negative titer is high (66.7% or more of the threshold for positivity). The platelet count itself was not different among the 3 groups in the present study. Therefore, the platelet count at the time of the negative test was not helpful for identifying patients who could benefit from repeated testing.

**Table 2
Patients With an Initial Negative HIT Test Result and a Positive Repeated HIT Test Result**

<table>
<thead>
<tr>
<th>Initial Titer/Case No.</th>
<th>Initial Negative HIT Titer (% of Threshold)</th>
<th>Platelet Count at Time of Negative HIT Titer (× 10^9/µL)*</th>
<th>Days to Positive HIT Titer From Negative HIT Titer</th>
<th>Positive HIT Titer (% of Threshold)</th>
<th>Platelet Count at Time of Positive HIT Titer (× 10^9/µL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very low (0.0%-33.3% of the threshold)</td>
<td>1 5.7</td>
<td>109</td>
<td>6</td>
<td>295</td>
<td>28</td>
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<tr>
<td>Low (33.4%-66.6% of the threshold)</td>
<td>1 38.4</td>
<td>74</td>
<td>21</td>
<td>205</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>2 45.9</td>
<td>101</td>
<td>15</td>
<td>107</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>3 63.2</td>
<td>76</td>
<td>1</td>
<td>173</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>4 64.6</td>
<td>106</td>
<td>26</td>
<td>199</td>
<td>38</td>
</tr>
<tr>
<td>High (66.7%-99.9% of the threshold)</td>
<td>1 66.8</td>
<td>123</td>
<td>1</td>
<td>120</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2 68.3</td>
<td>105</td>
<td>1</td>
<td>115</td>
<td>68</td>
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<td></td>
<td>3 72.4</td>
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<td>17</td>
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<td>7 79.8</td>
<td>70</td>
<td>1</td>
<td>183</td>
<td>74</td>
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<td></td>
<td>8 83.0</td>
<td>25</td>
<td>13</td>
<td>167</td>
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</tr>
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thrombocytopenia before it could be detected in the assay in these cases.

We speculate that the reason for the initially negative test results is performance of the test early during HIT, when the HIT antibody titer is still increasing following heparin exposure.

The sensitivity and specificity of HIT ELISA methods can vary substantially among commercially available and home-brew products. The Stago HIT ELISA is approximately 90% sensitive. Although ELISA methods have excellent sensitivity, approximately 10% of cases have a false-negative result. In some cases, this is because the HIT is due to an antigen other than PF4. The present study suggests that the overall sensitivity of the assay could be improved if repeated testing is considered for patients who have high titer-negative results. It is not clear on which day repeated testing should be performed, but because the median time between negative and positive results was 3 days for group 3 patients in this study, we suggest that repeated testing be considered 3 days after a high titer-negative result. Some studies propose that sensitivity of HIT testing also could be improved if more than one type of assay is performed, for example, the serotonin-release assay combined with an ELISA. The serotonin-release assay has a sensitivity similar to that of the ELISA, and it can detect the occasional HIT cases in which the antigen is not PF4. However, serotonin-release assays are not routinely available in the United States outside of specialized coagulation laboratories.

Case histories of the patients whose results were positive on repeated testing showed a high mortality (44%) and high rate of thrombosis (28%). Whether HIT had a direct role in the observed mortality among the patients with an initially negative test result is unknown, because only 1 of the patients who died also had thrombosis. The patients who died all had multiorgan failure. It is possible that HIT could contribute to the development of multiorgan failure due to multiple microthrombi. All except 1 of the thrombotic events occurred after the initially negative HIT test result and before the subsequent positive HIT test result (the exception occurred the day after the positive HIT test result). Thus, it is possible that if the high titer of the negative HIT result had been known by the clinicians, preventive measures (such as discontinuation of heparin) could have been taken in an effort to prevent thrombosis.

Among the patients whose test results were positive in repeated testing, the observed rates of mortality (44%) and thrombosis (28%) were similar to the rates of 36% and 31%, respectively, among 91 consecutive HIT-positive patients in our institution.
One limitation of the present study is that not all patients underwent repeated testing. Repeated tests were ordered at the discretion of the patients’ physicians, who were unaware of the study and unaware of the titer of the initially negative test result. Thus, the titer could not have influenced the decision to order a repeated test. In the present study, all patients except 1 who subsequently had positive results were thrombocytopenic at the time of the initially negative test result. Therefore, we are uncertain whether the titer of the negative result predicts subsequent positivity in repeated testing among patients who have normal platelet counts at the time of their initially negative result. The vast majority of testing for HIT occurs when patients have low and/or decreasing platelet counts. Therefore, this issue is not commonly encountered in the clinical setting. However, 1 of the patients in the study had a normal platelet count at the time of a negative test result, and the repeated test result was positive. The negative titer in this case was high, suggesting that the negative titer could be useful even if the platelet count is normal. However, no conclusions can be drawn from the 1 case.

We studied the effect of titer with negative HIT test results. A few other studies have investigated the effect of titer with positive HIT test results. One study found that high levels of the IgG HIT antibody were more likely to be associated with thrombocytopenia but not thrombosis, compared with lower levels of HIT antibody.20

Findings of the present study suggest that the titer of an initially negative HIT ELISA test result may be informative. Specifically, 43% of high titer-negative HIT results (OD 66.7%-99.9% of the threshold for positivity) were associated with a positive result in repeated testing several days later. Approximately 20% of all negative HIT test results fall into the high titer-negative category. Thus, for patients with a negative HIT test result that is high titer, we recommend repeated testing in several days to identify patients who have HIT despite a negative initial HIT test result.

References