Heparin induced thrombocytopenia (HIT) is a severe, potentially limb- and life-threatening immune-mediated adverse drug reaction to unfractioned heparin and/or low molecular weight heparin which may occur in up to 3% of treated patients. In spite of thrombocytopenia, (most commonly a 50% fall in platelet count, beginning most usually between 5-14 days after initial exposure to any dose or type of heparin) [1-3], bleeding is uncommon, while thromboembolic complications are the main clinical problem in patients with HIT.

HIT is caused by the formation of antibodies that activate platelets following heparin administration, with a complex of heparin and platelet factor 4 (PF4) as a principal antigen. HIT is a clinicopathological syndrome and the diagnosis of HIT remains primarily clinical, while it should be supported by confirmatory laboratory testing.

Thrombocytopenia in HIT is generally modest, with platelet counts of 50-70 X10^9/L, while severe thrombocytopenia (<10 X10^9/L) is unusual. After an initial exposure to heparin, platelet decrease starts on day 4 or 5 after the formation of IgG antibodies. However, patients exposed to heparin within the last 3 months (100 days), develop abrupt thrombocytopenia within 24 hours after re-exposure to heparin. A gradual decline in platelet count beginning on the first day of heparin therapy, with a decrease in platelet count to 50% of baseline over the first 4-5 days of therapy, is less consistent with a HIT diagnosis [4].

The American College of Chest Physicians (ACCP) recommends that platelet count should be monitored every 2 to 3 days (every second day for postoperative prophylaxis with unfractioned heparin (UFH)) with beginning on the 4th day after the initiation of heparin therapy, until the therapy is discontinued or until the 14th day of heparin exposure in the following clinical settings: any therapeutic dosing of UFH and low molecular weight heparin (LMWH), surgical and medical prophylaxis with UFH and LMWH, UFH prophylaxis in obstetrical patients and in postoperative patients receiving prophylactic-dose LMWH, or intravascular catheter UFH “flushes”. Monitoring is not recommended for prophylactic medical/obstetrical use of LMWH, or for medical patients receiving UFH flushes. If there is a history of heparin exposure within the last 100 days, a platelet count is recommended within 24 hours of heparin re-exposure [5].

HIT is one of the most common adverse drug reactions, but there are many other, more common causes for thrombocytopenia, especially in the setting of severe illness and major surgery [6]. Clinically, HIT is a diagnosis made after the exclusion of more likely causes of thrombocytopenia. Recovery of platelet counts after cessation of heparin therapy is also significant for diagnosis of HIT.

Occurrence of new thrombosis during the administration of heparin anticoagulation should raise significant suspicion for the diagnosis of HIT, while development of allergic reaction to the heparin treatment could also be of importance. Assessment of clinical aspects of a suspected case of HIT utilizing the scoring system may guide the appropriate use and interpretation of antibody testing. Therefore the pre-test clinical probability scoring system (4T's) seems to be a valuable tool for HIT diagnosis (Table 1) [7].

ABSTRACT

The laboratory diagnosis of heparin-induced thrombocytopenia (HIT) is based on the identification of antibodies against the complex between heparin and platelet factor 4 (PF4) by functional and/or immunological methods. These methods are complicated, time- and labour intensive. Therefore an introduction of a rapid, easy to perform method for the identification of circulating HPA is of high importance. ID-PaGIA heparin/PF4 assay may be an appropriate candidate. It should be emphasized that the diagnosis of HIT remains the primarily clinical, using pre-test clinical probability scoring system (4T’s) while laboratory results should be considered as an additional tool in confirming or ruling out clinical diagnosis. An algorithm for HIT diagnosis with combination of clinical and laboratory findings is presented.

Key words: Heparin induced thrombocytopenia (HIT), ID-PaGIA, 4T’s score.
LABORATORY DIAGNOSIS OF HIT

Detection of HIT antibodies is necessary, but not sufficient, for the diagnosis of HIT. Laboratory diagnosis of HIT relies on the detection of antibodies against heparin/PF4 complex in plasma or serum with functional and/or immunological methods.

Immunological methods, such as an ELISA are available in most clinical laboratories and they detect circulating IgG, IgA and IgM antibodies against heparin/PF4 complex. The most usual threshold for a positive test result in the available commercial kits is an optical density (OD) of 0.400. Since only some antibodies (e.g. IgG) are of clinical importance, sensitivity to all three subclasses of antibodies decreases assay specificity (50-93%) \[8, 9\]. Increasing cut-off values of OD (to 1 or 1.4) could improve assay specificity \[10\]. At the same time, the sensitivity of this method is high (>97%) with the negative predictive value of > 95% \[11, 12\]. Therefore negative results obtained with ELISA may be used for ruling out a HIT diagnosis, while positive results should be combined with clinical findings and/or functional assay. The specificity of this method could be improved using IgG specific ELISA assay which is currently under evaluation in several laboratories. We have shown potentially better specificity of this assay in comparison with standard ELISA in recent pilot study \[13\].

Functional methods measure the activation of platelets of healthy donors after the addition of patients’ plasma and heparin (conc. 0.1 – 1 IU/mL). Heparin induced platelet aggregation assay performed in donor’s platelet rich plasma is most commonly used in clinical laboratories. The sensitivity of this method is good (>90%) while the specificity for detecting clinically relevant (pathogenic) antibodies is higher than with the commercially available antigen assay (77-97%) as a consequence of the fact it exclusively detects platelet-activating antibodies of immunoglobulin IgG class (only IgG antibodies can activate platelets via their Fc or IgG-receptors) \[11\]. Another assay, the serotonin release assay (SRA), utilizes donor platelets in which serotonin is radiolabeled by C14. The addition of heparin results in platelet activation and release of radiolabeled serotonin which is detected. This assay has the advantage of proving that an anti-PF4-heparin antibody in the patient’s sample can actually stimulate platelet activation and therefore this assay expresses the highest specificity and is considered the “gold standard” \[1, 8\]. However, the use of radioactive isotopes, special equipment requirements and lack of experienced staff limit this method to the very few highly specialized reference laboratories.

No single assay has 100% sensitivity and specificity and therefore it seems that the optimal laboratory diagnostic approach is a combination of both functional and antigen assays. However, both ELISA and functional assays are complicated, time consuming and labor intensive and could be performed in most laboratories only during daily working hours. Therefore, one rapid, easy to perform method for the detection of circulated heparin/PF4 antibodies is highly desirable because the decision about the possible interruption of heparin treatment should not be delayed.

ID-PAGIA HEPARIN/PF4 ASSAY

ID-PaGIA heparin/PF4 (DiaMed) is a rapid particle gel immunoassay that detects IgG, A and M specific to heparin/PF4 complexes. The principle behind this method is widely used in blood group serology determination in transfusion medicine. Briefly, 10 μl of plasma are placed in the reaction chamber of the test ID-card (containing a buffered sephacryl gel matrix) followed by 50 μl of polymer particles (red high density polystyrene beads coated with heparin/PF4 complexes which serve as the solid-phase in a particle agglutination assay). After 5 minutes of incubation at room temperature the ID-card is centrifuged for 10 minutes in the appropriate ID-centrifuge.
Results are presented qualitatively (positive/negative) and can be read directly without the employment of additional equipment. When anti-heparin-PF4 antibodies are present in the plasma, the particles are cross-linked and remain at the top of the gel chamber (positive results). If there is no significant level of anti-heparin/PF4 antibodies, all the particles sink to the bottom of the gel chamber (negative result).

Negative ELISA is used to adequately and safely rule out HIT diagnosis. A very good overall agreement between ELISA and PaGIA (86%) was described in one previously published study [14]. We have recently observed similar results (90% of overall agreement) [15]. However, one out of 82 samples was considered falsely negative on ID-PaGIA since both ELISA and platelet aggregation assay were positive and clinical criteria for HIT were fulfilled. It was also previously calculated that there was 16% probability for HIT diagnosis in high risk patients even if the ID-PaGIA assay was negative [16]. This is further supported by findings of potentially lower sensitivity of ID-PaGIA in comparison to ELISA (94 vs 100%) observed in one study [17]. Lack of information about the clinical status in patients from that study rendered the relevance of those findings uncertain. Nevertheless, it seems that the interpretation of negative results should be cautious in patients with a high pre-test clinical probability score.

In spite of this the use of PaGIA as a routine 24-hour available screening test for the diagnosis of HIT seems to be justified. ID-PaGIA may replace the standard ELISA and offers a possibility to rule out HIT diagnosis within minutes after the request for laboratory testing in the majority of patients. That would decrease the amount of unnecessary switches of heparin to other anticoagulant treatments which are both costly and may increase the risk of bleeding complications.

RISK FOR OVER-DIAGNOSIS OF HIT

PF4-heparin antibodies could be detected using described laboratory assays in many different patient populations. Up to 50% of all individuals undergoing cardiac surgery develop HIT antibodies, but only a small percentage actually manifests clinical HIT [9]. Up to 3% of patients receiving UFH for medical prophylaxis will have a positive ELISA for heparin/PF4 antibodies, but only 0.5% of them develop thrombocytopenia [18]. The use of heparin for chronic hemodialysis is associated with a 12% prevalence of heparin/PF4 antibodies, but nearly all cases of HIT during hemodialysis occur within the first 3-4 weeks of initiation [19]. This suggests that HIT is unlikely to develop in the setting of chronic heparin exposure.

Those data suggest that HIT diagnosis should not be based on laboratory tests. Laboratory results should be considered only as an additional tool in confirming or ruling out clinical diagnosis. The pre-test clinical probability scoring system (4Ts) should therefore be combined with laboratory results in establishing HIT diagnosis.

SUMMARY

Focusing on the laboratory diagnosis of HIT, according to our own experience at Karolinska University Hospital, discussions and preliminary recommendations from Nordic Laboratory Group on HIT, ECAT pilot survey and study on HIT diagnostics, as well as most available literature data, it seems that:
- 4Ts pre-test clinical probability score must be used in the HIT diagnostic algorithm;
- High specificity and negative predictive value (>95%) justify ELISA as the most appropriate first line assay for laboratory investigation of HIT (standard Ig G, A, M ELISA may be replaced with IgG specific ELISA according to preliminary data but the absence of false negative results should be confirmed in larger studies).
- In spite of better specificity, due to lower sensitivity and different pitfalls (complexity, different heparin concentration, source of donor platelets) it seems that heparin induced platelet aggregation could not be used in a laboratory diagnosis of HIT alone. Performed in combination with ELISA, this assay should be interpreted together with the pre-test clinical probability score (4Ts’s). Platelet rich plasma (PRP) or “washed” platelets collected from at least 3 donors should be used in aggregation assay, while the concentration of heparin should be 0.1 – 1 IU/mL. Positive samples may be further tested with high heparin concentration (10 or 100 IU/mL) to avoid non-immunological reactivity to heparin.
- The use of a rapid, 24 hour available screening method may be beneficial in routine work. ID-PaGIA may be a good candidate and it expresses slightly lower sensitivity and potentially better specificity than ELISA. However, the interpretation of negative results should be cautious, especially in patients with a high pre-test clinical probability score.

There is no conflict of interest to declare - I do not have any relation with Diamed - a manufacturer of ID-PaGIA.

ALGORITHM FOR HIT DIAGNOSIS:
- HIT diagnosis may be ruled-out with >95% probability if ID-PaGIA/ELISA are negative. A negative ID-PaGIA test should be completed with ELISA in patients with a high pre-test clinical probability score.
Positive ID-PaGIA/ELISA indicates HIT diagnosis in patients with high pre-test clinical probability score. Further testing is not necessary.

Positive ID-PaGIA/ELISA should combine with the functional method – heparin induced platelet aggregation in patients with low and intermediate pre-test clinical probability score:

- Positive heparin induced platelet aggregation test increases the probability for HIT diagnosis in those patients. HIT is probable.
- Negative heparin induced platelet aggregation test increases the probability for ruling out HIT diagnosis. HIT is however still possible in patients with an intermediate pre-test clinical probability score.

LITERATURE


