A comparison of the Diamed PaGIA with an ELISA method for detection of HIT antibodies

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Abstract

The aim of this study was to determine whether the Diamed ID-PaGIA (particle gel immunoassay) screening test for heparin-induced thrombocytopenia (HIT) could be used as a reliable replacement for ELISA methodology (the Asserachrom HPIA kit, Bayer Healthcare). The potential advantages of the ID-PaGIA method over the ELISA technology are that it is simple to perform, the test can be completed in twenty minutes, no specialised training is required, it could be available 24 hours a day, is cheaper and is reproducible. Potentially the ID-PaGIA method could lead to savings in the pathology laboratory and possibly better outcomes for patients. This study was undertaken on 39 patient samples collected for routine HIT screening. The results obtained with the ID-PaGIA method were similar to the more commonly used ELISA method with no false positive results reported. The results of this pilot study suggest that the Diamed ID-PaGIA method would appear to be suitable as a screening test for HIT.

Keywords: heparin-induced thrombocytopenia, Diamed ID-PaGIA, HIT testing, Type II HIT, Type 1 HIT

Introduction

Heparin-induced thrombocytopenia (HIT) is a common and potentially fatal immune-mediated drug reaction (Stewart *et al* 1995). As heparin is frequently used in medicine for the treatment and prevention of venous thromboembolism, HIT diagnosis is of great importance in limiting the potential deadly consequences of undiagnosed HIT.

Unlike other drug induced causes of thrombocytopenia, heparin causes thrombosis not bleeding (Chong 2003). Approximately 1-3% of all patients exposed to heparin for more than five days develop HIT and a further 1% of these will develop HIT with thrombosis (DeBois *et al* 2003). In those patients who develop thrombocytopenia there is a higher risk of the development or extension of thrombotic events which include stroke, acute myocardial infarction and peripheral occlusion.

Two clinically different forms of HIT have been described: Type I HIT and Type II HIT. Type I HIT is generally a milder form of HIT. It usually presents after a few days of exposure to heparin (Parvathaneni *et al* 1999). Usually there is a mild thrombocytopenia with the platelet count rarely falling below $100x10^9$/L. However, Type I HIT can be present without the platelet count falling below the normal range (NR: $150\text{-}400x10^9$/L for adults). The platelet count can also return to its original levels without the cessation of heparin, thus the diagnosis of Type I HIT can be challenging. Spontaneous recovery of the platelet count can occur in just a few days. The clinical course is generally asymptomatic and is often undiagnosed.

In contrast, Type II HIT has a more severe clinical presentation and disease course. Type II HIT usually occurs after 4 to 5 days of continuous exposure to heparin but can require up to 14 days of continuous exposure (Warkentin *et al* 1995). Clinical presentation can occur earlier than this if there have been previous exposures to heparin. In Type II HIT the thrombocytopenia can be severe. A decrease in platelet count of more than 50% is considered the accepted requirement for clinical diagnosis. In contrast to Type I HIT, the platelet count does not spontaneously recover but requires the cessation of heparin to facilitate platelet recovery. These drops in platelet count are associated with the formation of thrombi (Warkentin *et al* 1998). The resolution of thrombocytopenia in Type II HIT usually takes

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5 to 7 days to occur but may take up to one month after the removal of heparin (Parvathaneni *et al* 1999). Thrombocytopenia can recur promptly on rechallenge with heparin.

Despite the presence of thrombocytopenia and petechial rash, bleeding or increased risk of bleeding is not usually associated with either type of HIT. The activation of platelets in the presence of the HIT antibody causes the formation of thrombi. It is the presence of thrombi that complicates the disease course of Type II HIT. These thromboembolic complications can result in an extension of the clot for which the heparin was used to treat, or the formation of a new thrombus. Until such times as the heparin is withdrawn, there can be continued formation of new thrombi. In Type II HIT patients, a resistance to the anticoagulant affect can be seen. The rate of mortality in Type II HIT sufferers with thrombosis is approximately 20% and the rate of limb amputations (usually a leg) in the same group of patients is approximately 12%. It is therefore important to ensure that accurate and timely diagnosis of HIT is made.

The clinical manifestations of the HIT syndrome are initiated by antibodies directed against platelet factor 4 (PF4) (Kelton *et al* 1994). When bound to heparin, PF4 forms a multimolecular complex that is the major target antigen. The heparin-PF4 complex binds to the platelet FcγII receptor, which leads to platelet aggregation (Amiral *et al* 1996). This causes the activation of prothrombotic micro particles and thrombin generation. The presence of these antibodies can also lead to the expression of a prothrombotic endothelial surface by interacting with endothelial cells (Baglin 2001). This may explain why HIT occurs more commonly in the sick patient who has tissue damage as a result of surgery or previous thrombosis. The thrombocytopenia can be caused by the reticuloendothelial clearance of activated or antibody bound platelets.

The risk of developing HIT while on a low molecular weight heparin (LMWH) is considered lower than with exposure to unfractionated heparin (UHF). LMWH is essentially the same as UFH but is produced by enzymatic or chemical (Greer 1999) depolymerisation processes and yields fragments that have a molecular weight of about 5,000d (Hirsch 2001). It is believed that LMWH is less reactive with platelets than UHF. Examples of LMWH include Enoxaparin, Fragmin, Reviparin and Tinzaparin.

Newman and Chong (2000) were able to purify the anti-PF4 heparin IgG complex (HIT IgG), thought to be responsible for HIT syndromes, from two patients. They observed that in the presence of heparin, the HIT IgG antibody was responsible for platelet aggregation showing that the HIT IgG antibody was dependent upon heparin for platelet aggregation (Amiral *et al* 1996). The discovery and identification of this anti-PF4 heparin IgG antibody has allowed the development of more specific and sensitive tests for HIT diagnosis (Amiral *et al* 1996). It should be remembered however, that while the majority of HIT presentations with thrombosis are caused by anti-PF4 heparin IgG antibody there are also cases that are IgM or IgA in origin (Alving 2003).

Laboratory testing for heparin dependent antibodies can be by a functional or antigenic assay. While the use of functional and platelet aggregation tests are useful to support the diagnosis of HIT, their negative predictive value is less than 50% (De Bois *et al* 2003). In contrast the antigenic tests are claimed to be more sensitive but less specific (Elalamy *et al* 2000).

The most sensitive and specific test for the laboratory confirmation of HIT is the [^{14}C]-Serotonin release assay (SRA). This test involves the measurement of serotonin released from donor platelets incubated with patient's serum in the presence of heparin. Because SRA uses radioactivity and is not easily set up, it is generally performed only by reference laboratories. It is usually used as the confirmatory test on patients who have a positive screening test or for those patients for whom there is a high suspicion of HIT but a negative screening test result.

The most widely used functional assay for the detection of heparin dependent antibodies is platelet aggregation testing (PAT). This test measures heparin-dependent serum induced platelet aggregation. It is dependent on the ability of fresh donor platelets to respond to the presence of HIT antibodies and unfractionated heparin. It can take specially trained staff 3-6 hours to perform which can make after hours and weekend testing unlikely. The specificity of the PAT system is less than that of the SRA and it has a sensitivity of around 30% (Walenga *et al* 1999)

The ELISA method has been devised as a more sensitive test to detect antibodies directed against heparin-PF4 complexes, which have been demonstrated to be produced in approximately 85% of HIT cases (Greinacher *et al* 1994). However, these test results should always be interpreted in conjunction with the clinical state of the patient and of their platelet kinetics. The thrombocytopenia that is induced in 15% of Type II HIT cases is associated with antibodies directed against other entities such

as interleukin-8 and neutrophil-activating pepetide-2 (NAP-2). A sudden drop of platelet count of 30 to 50% is generally considered as an alarm signal that HIT is occurring. While no interference by heparin (either UFH or LMWH) has been observed up to a concentration of 2.5 IU/mL, it is not theoretically excluded that heparin at still higher concentrations may dissociate the heparin-PF4 complexes, and aberrant test results may be produced in these instances.

Our current protocol for HIT usually involves the ELISA test. Positive results undergo PAT to determine whether there is cross reactivity with Clexane, Fragmin and Orgaran. Since the usual treatment of Type II HIT is to cease heparin or change the type of heparin used, the cross reactivity studies allow the laboratory to recommend an alternative form of heparin that will not react with the antibodies that are already present. In samples where the result is not conclusive after ELISA and PAT testing, the SRA is performed which provides the conclusive result.

A recent development in HIT laboratory testing is the introduction of the Diamed ID -PaGIA test kit. This is another antigenic assay system and involves particle gel immunoassay (PaGIA) technology to detect antibodies against the ID-heparin/PF4 complex. A positive test result is indicative of the presence of antibodies to the heparin/PF4 complex. It appears technically easy, provides standardised results and can be completed in 20 min. It is the first available kit that allows for use in the emergency situation.

The purpose of this pilot study was to determine whether the Diamed ID-PaGIA test kit for HIT would be suitable as a replacement screening test over the ELISA method.

Materials and methods

Ethical approval was obtained from Ethics in Human Research Committee, Charles Sturt University. Liverpool Hospital allowed exemption from ethical approval.

Samples from 39 consecutive patients who were suspected of having HIT were tested. Of the 39 patients tested, 34 had thrombocytopenia at time of collection. Blood samples were collected by a combination of clinical, nursing and pathology staff and stored according to the manufacturers' instructions prior to testing. The samples were collected as either serum or 3.2% tri-sodium citrate plasma by venepuncture. The blood samples were separated into two portions, one for routine testing and the other for the current study. The patients in this study group were from the six hospitals that are serviced by the South Western Area Pathology Service (SWAPS).

All patient samples were tested by the ELISA Asserachrom HPIA kit (Bayer Healthcare) method to diagnose HIT at the request of the treating clinician - this established test was deemed to provide the "target" result for this study. The Diamed ID-PaGIA testing was performed on the same samples. Both tests were undertaken according to the manufacturers' instructions.

Asserachrom HPIA ELISA test (Bayer Healthcare) – principle of the test

Samples are incubated in plastic micro-wells precoated with heparin-PF4 complexes. If any anti-heparin-PF4 antibodies are present, they are captured by the complexes. Goat anti-human IgG, IgA, IgM antibodies are then coupled with peroxidase, bind to the available antigenic determinates of the immobilised antibodies, forming a "sandwich". The bound enzyme peroxidase is then revealed by its activity in a predetermined time on the substrate ortho-phenylenediamine in the presence of hydrogen peroxide. After stopping the reaction with sulphuric acid, the intensity of the colour produced is related to the autoantibody level initially present in the sample. In this kit, all samples with an absorbance value that was greater than 26% of the internal reference absorbance are considered positive.

Diamed ID-PaGIA test - principle of the test

This test involves incubating 10μl of test serum with 50μl of polymer particles in a microtube of the ID-Card for 5 min at room temperature (18-25°C). The ID-Card is then centrifuged for 10 min. The presence of anti-HPF4 antibodies in a patient sample is indicated by agglutination of the polymer particles. As a result agglutinates are unable to be centrifuged through the sephacryl gel matrix. The agglutinates can be clearly seen dispersed through or on top of the gel matrix. This result is read as positive. In the absence of anti-HPF4 antibodies there is no agglutination of the polymer particles. As a result the centrifugation of the ID-Card results in the deposition of the polymer particles and sample in the bottom of the gel column, with no residual agglutinates in the gel matrix. This is read as a negative test result.

Platelet Aggregation Testing - Principle of test

Heparin dependent antibodies will produce a measurable aggregation of ABO group compatible platelets in the presence of heparin. Aggregation is measured by the transmittance of light. As platelets aggregate the light transmittance increases. The amount of transmittance and the rate of increase are dependant on the platelet reactivity to the added agonist (heparin).

Results

Table 1 indicates that the two tests gave the same result for 34 of the 39 samples. However 5 samples were negative with the Diamed ID-PaGIA system and moderately or strongly positive with the Asserachrom HPIA ELISA test. Each of these 5 samples were tested by PAT and one was further tested by SRA. Further testing of these samples showed that three may have been contaminated with heparin as they had prolonged APTTs. Each of these three also had normal PAT. Another sample gave equivocal PAT studies and returned a negative SRA after referral to another laboratory. The last sample in this group was positive by PAT - this was the only sample of the five that was considered to be positive for HIT. Therefore the Diamed ID-PaGIA system produced one false negative result and the Asserachrom HPIA ELISA system produced four false positive results. The adjusted results are shown in Table 2.

Table 1

Summary of results with the Asserachrom HPIA ELISA test being used as the target value (n=39).

	Number of Positive Diamed ID-PaGIA samples	Number of Negative Diamed ID-PaGIA samples
Number of Positive ELISA samples	3	5
Number of Negative ELISA samples	0	31

Values for specificity, sensitivity and negative predictive value (NPV) were calculated using two criteria, - the first directly compared the Diamed ID-PaGIA test to the Asserachrom HPIA ELISA testing system and the second compared the Diamed ID-PaGIA system against the PAT result issued by the laboratory. These values are displayed in Table 3.

Table 2

Results obtained from the Diamed ID-PaGIA and Asserachrom HPIA ELISA methods compared to the final diagnosis (the number of positive HIT samples was 4).

	Number of samples	***%***
Positive ELISA and Diamed	3	8
Negative ELISA and Diamed	31	79
False Positive (Diamed)	0	0
False Positive (ELISA)	4	10
False Negative (Diamed)	1	3
False Negative (ELISA)	0	0
Total	39	100

Table 3

Sensitivity, specificity and predictive values of the Diamed ID-PaGIA results compared to the Asserachrom HPIA ELISA results and the clinical diagnosis (n=39 with 4 positive HIT samples).

	Asserachrom HPIA ELISA	***Clinical Diagnosis***
Sensitivity	38%	75%
Specificity	100%	100%
Negative predictive value	86%	97%
Positive predictive value	100%	100%
Accuracy	87%	97%

Discussion

In clinical practice the diagnosis of HIT is based on the analysis of clinical features and laboratory tests (Elalamy *et al* 2000). The SRA is considered to be the reference method with the ELISA and PAT tests being used in routine practice. These are both time consuming and have variable sensitivity (Chong 1995). The results of this preliminary study suggest that the Diamed ID-PaGIA HIT screening method could be a useful replacement test for HIT as it was sensitive and had a high NPV, both of which are requirements for a good screening test. In this study the Diamed ID-PaGIA method had an NPV of 97% and a sensitivity of 75% when compared to the final result issued. Increased sensitivity is likely to be obtained if further testing of known HIT positive samples was undertaken. Nonetheless, the results from this study are consistent with those of Meyer and co-workers (1999), who reported a correlation of 86% between the PF4 heparin ELISA assay and the ID-PaGIA assay. The positive predictive value (PPV) for this current test was 100%.

Another study undertaken by Tazzari and colleagues (2002) reported a novel flow cytometric method of HIT determination where 70 patient samples were tested. Their flow method used the heparin/PF4 coated beads from the Diamed ID-PaGIA kit in combination with IgG, IgM and IgA FITC. The beads were visualised and gated. The presence of an antigen antibody complex was clearly apparent in their study. As part of their research they compared the performance of Asserachrom HPIA, ID-PaGIA and their own flow method. All methods used showed complete concordance, highlighting the usefulness of the ID-PaGIA technique as a screening test for Type II HIT detection.

In this current study it was shown that the Asserachrom HPIA ELISA system produced a false positive rate of 10% compared to PAT, while there were none with the Diamed ID-PaGIA method (Table 2). While the false positive rate may be as high as 10% for Asserachrom HPIA ELISA, it is possible that the lower sensitivity of the PAT means that true positives may have been missed by both the PAT and ID-PaGIA methods. Further studies would be needed to confirm this. This correlates with an earlier study by Eichler and colleagues (2002) who compared the ID-PaGIA test against PAG, SRA, PF4/Heparin ELISA (Stago method) and the PF4/polyanion ELISA method. The screening system in place in many laboratories requires PAT testing on all positive ELISA tests. The PAT is used to determine if there is cross reactivity between the antibody detected and the alternative to standard heparin. High numbers of false positive ELISA tests therefore create extra unnecessary work. False positive ELISA results may also lead to samples being tested by SRA at a reference laboratory, which may be unnecessary and lead to a delay in diagnosis.

Risch and co-workers (2003) also assessed the usefulness of ID-PaGIA systems as a negative predictor of HIT in cardiac patients. They reported a perception that there was a lower sensitivity of ELISA methods to determine HIT in cardiac patients. They concluded that in cardiac surgery patients, the ID-PaGIA system was a useful tool in excluding rapid onset and typical onset of HIT and provided further valuable information in the confirmation of rapid onset of HIT. While this current study did not directly look at this issue, 12% of the patients had cardiac related illnesses. It would appear that the underlying cause for heparin use did not affect the sensitivity of the Diamed test method. As in other immunologically based assays, antibodies against less typical HIT antibodies such as interleukin - 8 (IL-8) cannot be detected by the Diamed PaGIA test (Eichler *et al* 2002). This and the fact that only 80% of patients diagnosed on clinical grounds have a positive HIT screening test (Paravathaneni *et al* 1999), may explain the false negative result obtained in this study. From the preliminary data provided here, this institution will perform further correlation studies during the introduction of the Diamed ID-PaGIA test for HIT.

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References

Alving BM. 2003. How I treat heparin-induced thrombocytopenia and thrombosis. *Blood*; 101: 31-37.

Amiral J, Wolf M, Fischer A, Boyer-Neumann C, Vissac A, Meyer D. 1996. Pathogenicity of IgA and /or IgM antibodies to heparin-PF4 complexes in patients with heparin-induced thrombocytopenia. *Br J Haematol*; 92: 954-959.

Baglin TP. 2001. Heparin-induced thrombocytopenia (HIT/T) syndrome: diagnosis and treatment. *J Clin Pathol*; 54: 272-274.

Chong BH. 1995. Heparin-Induced Thrombocytopenia. *Br J Haematol*; 89: 431-439.

Chong BH. 2003. Heparin-induced thrombocytopenia. *J Thromb Haemost*; 1: 1471-1478.

DeBois WJ, Liu J, Lee LY, Girardi LN, Mack C, Tortolani A, Krieger KH, Isom OW. 2003. Diagnosis and treatment of heparin-induced thrombocytopenia. *Perfusion*; 18: 47-53.

Eichler P, Raschke R, Lubenow N, Meyer O, Schwind P, Greinacher A. 2002. The new ID-heparin/PF4 antibody test for rapid detection of heparin-induced antibodies in comparison with functional and antigenic assays. *Br J Haematol*; 116: 887-897.

Elalamy I, Lecrubier C, Horellou MH, Conrad J, Samama MM. 2000. Heparin-induced thrombocytopenia: laboratory diagnosis and management. *Ann Med*; 32(Suppl 1): 60-67.

Greer IA. 1999. Thrombosis in pregnancy: maternal and fetal issues. *Lancet*; 353;1258-1265.

Greinacher A, Potzsch B, Amiral J, Dummel V, Eichner A, Mueller-Eckhardt C. 1994. Heparin-associated thrombocytopenia: isolation of the antibody and characterization of a macromolecular PF4-heparin complex as the major antigen. *Thromb Haemost*; 71: 247-251.

Hirsch J, Warkentin TE, Shaughnessy SG, Anand SS, Halperin JJ, Raschke R., Granger C, Ohman ME & Dalen JE. 2001, Heparin and low-molecular-weight heparin: mechanisms of action, pharmacokinetics, dosing, monitoring, efficacy and safety. *Chest*; 119 (suppl 1), 64s-94s.

Kelton JG, Smith JW, Warkentin TE, Hayward CP, Denomme GA, Horsewood, P. 1994. Immunoglobulin G from patients with heparin-induced thrombocytopenia binds to a complex of heparin and platelet factor 4. *Blood*; 83: 3232-3239.

Meyer O, Salama A, Pittet N, Schwind P. 1999. Rapid detection of heparin-induced antibodies with particle gel immunoassay (ID-HPF4). *Lancet*; 354: 1525-1526.

Newman PM, Chong BH. 2000. Heparin-induced thrombocytopenia: new evidence for the dynamic binding of purified anti-PF4-heparin antibodies to platelets and the resultant platelet activation. *Blood*; 96: 182-187.

Parvathaneni S, Alapati K, Tuchek M, Bakhos M, Blakeman B. 1999. Recurrent iliac venous thrombosis and heparin induced thrombocytopenia and thrombosis syndrome. *Chest*; 116 (suppl 2): 419S-420S.

Risch L, Bertschmann W, Heijnen IA, Huber AR. 2003. A differentiated approach to assess the diagnostic usefulness of a rapid particle gel immunoassay for the detection of antibodies against heparin-platelets factor 4 in cardiac surgery patients. *Blood Coagul Fibrinolysis*; 14: 99-106.

Stewart MW, Etches WS, Boshkov LK, Gordon PA. 1995. Heparin-induced thrombocytopenia: an improved method of detection based on lumi-aggregometry. *Br J Haematol*; 91: 173-177.

Tazzari PL, Ricci F, Vitale M, Malferrari F, Salama A, Schwind P, Conte R. 2002. Heparin-induced thrombocytopenia: detection of antiheparin/PF4 antibodies by means of a heparin/PF4-coated beads and flow cytometry. *Transfus Med*: 12: 193-198.

Walenga JM, Jeske WP, Wood JJ, Ahmad S, Lewis BE, Bakhos M. 1999 Laboratory tests for heparin-induced thrombocytopenia: a multicenter study. *Semin Hematol*; 36(1 Suppl 1): 22-28.

Warkentin TE, Chong BH, Greinacher A. 1998. Heparin-induced thrombocytopenia: towards consensus. *Thromb Haemost*; 79: 1-7.

Warkentin TE, Levine MN, Hirsch J, Horsewood P, Roberts RS, Gent M, Kelton JG. 1995. Heparin-induced thrombocytopenia in patients treated with low-molecular-weight heparin or unfractionated heparin. *New Engl J Med*; 322: 1330-1335.

ERRATUM

In the May 2006 issue of the AJMS, an error occurred in the article "Uncertainties in measurement of uncertainty measurements: a commentary from the perspective of a specialist haemostasis laboratory" by Dr Emmanuel Favaloro.

Figure 1, "Correlation analysis of MU" showed the same key for two different symbols. The correct figure is shown below.

The AJMS apologises sincerely to Dr Favaloro for this error.

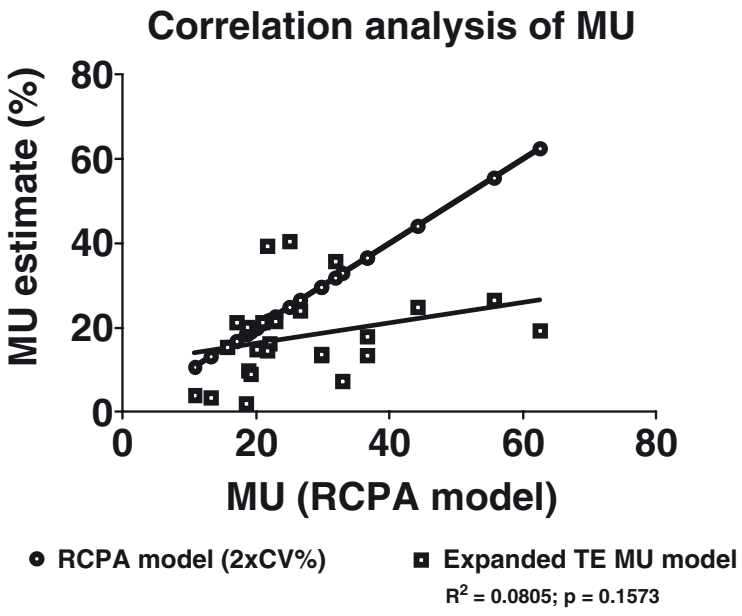

Figure 1

Correlation between MU estimated using the RCPA model (as 2xCV%) versus that estimated using an expanded TE concept model as proposed by Mina (2005). There was limited correlation, and the relationship was not statistically significant (p=0.1573).