Evaluation of a new automated panel of assays for the detection of anti-PF4/heparin antibodies in patients suspected of having heparin-induced thrombocytopenia

Cristina Legnani; Michela Cini; Caterina Pili; Ottavio Boggian; Mirella Frascarco; Gualtiero Palareti

Department Angiology and Blood Coagulation "Marino Golinelli", University Hospital S. Orsola-Malpighi, Bologna, Italy

Summary

Heparin-induced thrombocytopenia (HIT) is a life-threatening complication of heparin treatment; the prognosis depends on early accurate diagnosis, and prompt start of alternative anticoagulants. Because of high sensitivity, the commercially available immunologic assays are widely used, though not suited to be run on single samples and with a turnaround time of 2–3 hours. We evaluated two new, rapid, automated, semi-quantitative chemiluminescent immunoassays in HIT suspected patients: HemosIL® AcuStar HIT-IgG(PF4-H) (specific for IgG anti-PF4/heparin antibodies) and HemosIL® AcuStar HIT-Ab(PF4-H) (detecting IgG, IgM and IgA anti-PF4/heparin antibodies) (both from Instrumentation Laboratory). A total of 102 patients with suspected HIT were included; HIT was diagnosed in 17 (16.7%). No false negative cases were observed using either the HemosIL AcuStar HIT-IgG(PF4-H) or the HIT-Ab(PF4-H) assay (sensitivity and negative predictive values = 100%; negative likelihood ratios <0.01). The specificity was higher for the HemosIL AcuStar HIT-IgG(PF4-H) in comparison with that of the HemosIL AcuStar HIT-Ab(PF4-H) (96.5% vs. 81.2%). Higher values of the HemosIL AcuStar HIT-IgG(PF4-H) were associated with increased probability of HIT. Patients with confirmed HIT and thrombotic complications had significantly higher levels of HemosIL AcuStar HIT-IgG(PF4-H) than those without thrombotic complications. The HemosIL AcuStar HIT-IgG(PF4-H) and HIT-Ab(PF4-H) assays showed a very high sensitivity, and therefore they can reliably be used to rule out HIT in suspected patients. The diagnostic specificity was greatly increased by using the HemosIL AcuStar HIT-IgG(PF4-H). Both the assays are reproducible (CVs <6%), rapid (turnaround time 30 minutes), automated, and semi-quantitative, and they can be run for single sample testing.

Keywords

Diagnosis, heparin-induced thrombocytopenia, HIT, immunoassay

Introduction

Heparin-induced thrombocytopenia (HIT) is a life-threatening complication of heparin treatment occurring in up to 3% of patients receiving heparin for more than five days, although, patients with prior heparin exposure may experience complications sooner (1–5). HIT is typically caused by heparin-dependent, platelet-activating IgG antibodies that recognise complexes of platelet factor 4 (PF4) bound to heparin with subsequent platelet activation and thrombin generation leading to a prothrombotic state (6). HIT is associated with a high risk of venous and/or arterial thrombosis during heparin treatment. The prognosis depends on early recognition and diagnosis of HIT, and prompt start of alternative anticoagulants (1–5). HIT diagnosis should also be as accurate as possible since the anticoagulant drugs used to replace heparin are expensive, difficult to manage, and associated with high bleeding risk (7, 8).

In the daily practice, diagnosis of HIT – currently based on clinical and laboratory criteria – can be problematic, especially in patient populations with high incidence of thrombocytopenia. Though HIT cannot be diagnosed on clinical criteria alone, the clinical likelihood of HIT can be evaluated in every patient with suspected HIT using a scoring system such as Warkentin’s “4Ts” score (9).

As clinical diagnosis of HIT is difficult, readily available laboratory confirmation of diagnosis is highly desirable, and sensitive and specific assays are needed. Unfortunately, no single laboratory test has 100% sensitivity and specificity; therefore, the results of laboratory tests should be interpreted in the appropriate clinical context.

Functional and immunologic assays for PF4-heparin (PF4/H) antibodies are available (1, 2, 10, 11). Functional assays, including [14C] serotonin release assay and platelet aggregometry, evaluate aggregation capacity or aggregation activity of heparin-dependent
antibodies after incubation of platelets of healthy donors with patient’s serum and heparin. The highly sensitive and specific [\(^{14}C\)] serotonin release assay is still considered to be the gold-standard. Unfortunately, it is relatively laborious and it requires radioisotope and technical expertise. These requirements restrict this technique to research labs only. Platelet aggregometry has poor sensitivity though it is rather specific. Sensitivity and specificity can be improved by using washed platelets from normal donors (12).

Conversely, the immunologic assays, which detect antibodies to PF4 complexed with heparin or other polyanions, are commercially available and therefore widely used. The sensitivity of immunologic tests for the anti-PF4/H antibodies (both pathologic and non-pathologic) is greater than that of functional assays, although their sensitivity for clinical HIT is believed to be similar to that of washed platelet activation assays (13, 14). Indeed, the specificity of immunologic tests is lower, and a positive result may not denote the magnitude of thrombotic risk as these assays are more likely to detect clinically insignificant antibodies. Up to 50% of patients receiving unfractionated heparin (UFH) during and after open heart surgery demonstrate anti-PF4/H antibodies by immunologic assays, but HIT remains an infrequent diagnosis in this setting (15, 16). Furthermore, the immunologic assays currently offered (based on enzyme immunoassay [EIA] technique) take several hours (h) for the results to be available (turnaround time = 2–3 h). They are also suited to be run in batches rather than on single samples, as it is in the case of routine emergency use in suspected HIT patients.

We report on an evaluation of two new, rapid, automated, semi-quantitative chemiluminescent immunoassays in a series of patients suspected of HIT. The HemosIL AcuStar HIT-IgG\(_{[PF4\text{-H}]}\) (Instrumentation Laboratory, Bedford, MA, USA) is specific for IgG anti-PF4/H antibodies, while the HemosIL AcuStar HIT-Ab\(_{[PF4\text{-H}]}\) (Instrumentation Laboratory) detects IgG, IgM, and IgA anti-PF4/H antibodies.

**Materials and methods**

**Patients**

From January 2008 to October 2008, 102 patients ([55 females; 73 years (1–99)]) were referred to our laboratory for suspected HIT and included in the study. All patients were treated with UFH or low-molecular-weight heparin (LMWH) and had developed a thrombocytopenia with platelet count < 150 X 10⁹/L and/or a ≥30% decrease in platelet count from baseline (platelet count before starting heparin treatment).

The study also included 33 subjects with normal platelet count and no HIT symptoms; 14 [9 females; 76 years (38–94)] were patients who were sampled while on LMWH treatment and 19 [11 females; 44 years (31–62)] were healthy subjects from the laboratory staff who had never received heparin.

The study was approved by the local Ethics Committee and all individuals included in the study gave informed consent.

**Blood sampling**

Blood was collected from the antecubital vein into tubes without anticoagulant; serum was prepared by centrifugation for 20 minutes (min) at 2,800 x g at controlled temperature (20°C) and used immediately. Serum aliquots were also snap frozen and stored at −80°C for further analysis.

For testing with the platelet aggregation assay, blood of 4–5 normal donors who had not taken aspirin or other antiplatelet drugs for at least 10 days was collected from the antecubital vein into 0.109 mM trisodium citrate; platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared by centrifugation for 20 min at controlled temperature at 150 and 2,500 x g, respectively. Platelet aggregation assay was performed using fresh serum heated at 56°C for 30 min to inactivate complement and traces of thrombin; any precipitates formed were removed by sedimentation centrifugation for 5 min at 5,000 x g at controlled temperature.

**Confirmation/exclusion of HIT**

Confirmation or exclusion of HIT was based on the flow chart proposed by Pouplard et al. (17). The pretest probability of HIT (low = 0–3, intermediate = 4–5, high = 6–8) was estimated by using the “4Ts” clinical score as previously described (9). The information needed to calculate the clinical score was obtained by a standard questionnaire that had to be completed by the referring physician and sent to the lab with the blood sampling. The clinical score was calculated by the lab personnel (C.L.); in case of lacking/incomplete information, the referring physician was interviewed by phone.

A rapid particle gel immunoassay that detects IgG, IgA, and IgM specific to PF4-heparin complexes (ID-Heparin PF4 PaGIA, Diamed SA, Cressier sur Morat, Switzerland) was immediately performed in all patients. The ID-Heparin PF4 PaGIA was carried out according to the manufacturer’s instructions. Briefly, the test system employs red-dyed polystyrene beads coated with PF4-heparin complexes. Results are qualitative (positive/negative) and can be read after 10 min of centrifugation. Absence of antibodies against PF4/H complexes results in a lack of agglutination and beads can be entirely centrifuged to the bottom of the gel column; presence of agglutinates at the top of the gel column or dispersed through the gel matrix is read as positive. The test was performed by well-trained technicians who were blinded to the pretest probability result. In a few cases, no clear agglutinates were observed; these indeterminate results were considered as positive.

In patients with negative ID-Heparin PF4 PaGIA test and low/intermediate pretest probability, the presence of HIT was excluded without performing other tests. In patients with positive ID-Heparin PF4 PaGIA test or with negative ID-Heparin PF4 PaGIA but with high pretest probability, a platelet aggregation test was also performed.

Platelet aggregation was performed according to Chong et al. (18) using a four channel Chrono-Log platelet aggregometer (model 540, Chrono-Log Corp., Havertown, PA, USA); the platelet count in PRP was standardised to 300 X 10⁹/L and PRP was stored at room tem-

© Schattauer 2010

**Thrombosis and Haemostasis 104.2/2010**
Temperature in capped tubes until use. After blanking the aggregometer with PPP, 290 μl of pre-heated serum was added to 160 μl of pre-warmed PRP in a stir bar containing cuvette. After 5 min to rule out spontaneous aggregation, 10 μl of UFH (1 and 100 IU/ml final concentration) was added to the cuvette and the aggregation response was monitored for 20 min. The presence of HIT was confirmed if the aggregation was > 20% with 1 IU/ml UFH and completely inhibited or < 20% with 100 IU/ml UFH. The presence of HIT was excluded if the platelet aggregation was < 20% with 1 IU/ml UFH or was not inhibited in the presence of 100 IU/ml UFH.

Following this procedure, the presence of HIT was confirmed in patients with: 1) positive ID-Heparin PF4 PaGIA + positive platelet aggregation test (for all levels of pretest probability) or 2) high pretest probability + negative ID-Heparin PF4 PaGIA + positive platelet aggregation test (see Fig. 1).

**HemosIL AcuStar HIT-IgG(PF4-H) and HIT-Ab(PF4-H)**

The HemosIL AcuStar HIT-IgG(PF4-H) and HIT-Ab(PF4-H) are two chemiluminescent two-step immunoassays consisting of magnetic particles coated with PF4 complexed to polyvinyl sulfonate (PVS) which capture the anti-PF4/H antibodies from the sample. After incubation, magnetic separation, and a wash step, reagents that trigger the luminescent reaction are added, and the emitted light is measured as relative light units (RLUs) by the ACL AcuStar optical system (Instrumentation Laboratory). The RLUs are directly proportional to the anti-PF4/H IgG or IgG/IgA/IgM concentration in the sample.

The assays utilise a four-parameter logistic curve fit data reduction method to generate a master curve. The master curve is predefined and lot dependent and it is stored in the instrument through the cartridge barcode. With the measurement of the two calibrators contained in the kit (Calibrator 1 and 2 HIT-IgG(PF4-H) and Calibrator 1 and 2 HIT-Ab(PF4-H)), the predefined master curve is transformed to a new, instrument specific working curve; the working curve is valid until a new reagent lot is used. However, each working day two quality control materials, (Low and High HIT Controls, Instrumentation Laboratory) which are assigned to both the HIT-IgG(PF4-H) and the HIT-Ab(PF4-H) assays, are analysed; if the controls fall out of specifications, the assay is re-calibrated. The results are reported in arbitrary units (U/ml) and are available in 30 min. The clinical cut-off for HIT for both assays of 1.00 U/ml is suggested by the manufacturer based on preliminary data of a previously performed evaluation of the analytical performance studying a population of apparently healthy individuals and patients suspected of HIT. In a separate internal study completed by the manufacturer, the two assays compared well with other commercially available ELISA assays (PF4 Enhanced® and PF4 IgG® Solid Phase ELISAs, GTI Diagnostics, Waukesha, WI, USA) resulting in overall agreement of 96.0% (95% CI 90.9% – 98.7%). According to the manufacturer, the assays do not show interference for haemoglobin up to 500 mg/dl, bilirubin up to 18 mg/dl, triglycerides up to 1,250 mg/dl, and hepato...
rin (LMWH and UFH) up to 1 IU/ml. In patient samples with concentrations within the range -0.00 U/ml to -1.7 U/ml the assays do not show interference for rheumatoid factor up to 800 IU/ml. It is noted that RF positive samples may increase the value of an already positive HIT antibody sample.

In this study, the patient samples were tested using thawed, previously frozen serum aliquots over six different working days. Samples were numerically coded and tested blindly. Each day of analysis, the two controls (Low and High HIT Controls) were tested in duplicate for the HIT-IgG(PF4-H) and the HIT-Ab(PF4-H) assays before and after the serum samples of the patients. The results were used to calculate the run-to-run and day-to-day variations (total number of replicates n=24).

**Statistical analysis**

Results are presented as median and range. Comparisons were performed by the Mann Whitney U test and Kruskal Wallis test. A p-value < 0.05 was considered statistically significant. Sensitivity, specificity, negative and positive predictive values, and positive and negative likelihood ratios were calculated according to standard methods for proportions; the 95% confidence interval limits (95% CI) were computed according to the binomial distribution.

**Results**

The characteristics of the patients with suspected HIT included in the study are reported in Table 1. UFH or LMWH had been administered in 40 and 62 patients, respectively. Heparin was administered for thromboembolism prevention in 50 medical and 32 surgical patients (including 10 patients who had undergone cardiac surgery with cardiopulmonary bypass); 20 patients had been treated with heparin for thrombotic events. Before heparin treatment, the median platelet count was 189 X 10^9/l (47–702 X 10^9/l). The presence of HIT was suspected at a median interval of nine days (1–100 days) after initiation of heparin, and the median platelet count nadir was 79 X 10^9/l (10–396 X 10^9/l).

**“4Ts” score pretest probability and ID-Heparin PF4 PaGIA to diagnose HIT**

Pretest probability resulted low (score 0–3), intermediate (score 4–5) or high (score 6–8) in 35 (34.3%), 49 (48.0%), and 18 (17.7%) patients, respectively. The ID-Heparin PF4 PaGIA test was positive in 20 cases (19.6%).

In the 73 patients with a negative ID-Heparin PF4 PaGIA test and low/intermediate pretest probability, the presence of HIT was excluded without performing other tests (Fig. 1). On the contrary, platelet aggregation test was performed in the 20 patients with a positive ID-Hepar
symptoms who were sampled while on LMWH treatment had levels higher than 1 U/ml.

The median levels according to the “4Ts” clinical score pretest probability were 0.26 (0.12–3.47), 0.30 (0.08–33.28) and 2.19 (0.14–130.0) U/ml in the low-, intermediate- and high-probability groups (p=0.0084), respectively. The number of patients with the levels higher than the cut-off level was 2/35 (5.7%), 9/49 (18.4%) and 9/18 (50.0%) in the low-, intermediate- and high-pretest probability groups, respectively.

The results obtained in the healthy subjects [0.26 U/ml (0.16–0.78)], in patients without HIT symptoms who were sampled while on LMWH treatment [0.31 U/ml (0.13–0.58)] and in those with suspected and excluded [0.25 U/ml (0.08–1.38)] or confirmed HIT [6.70 U/ml (1.64–130.0)] are reported in Table 2. The median level of results obtained in the 17 patients with confirmed HIT was significantly higher in comparison with those of the other groups (p<0.0001). HIT was excluded in all patients with results < 1.0 U/ml; the number of patients with confirmed HIT was 1/4 (25.0%) in the group of patients with results in the range 1–2 U/ml and 16/16 (100%) with values > 2 U/ml. Among patients with confirmed HIT, those with thrombotic complications (n=7) presented significantly higher levels in comparison with those without thrombotic complication (n=10) [51.1 U/ml (16.4–130.0) vs. 4.46 U/ml (1.64–33.3); p=0.004).

Since the assay was negative (≤ 1 U/ml) in 82 patients in which HIT was excluded according to the procedure used in our lab, the negative predictive value was 100% (95% CI: 95.6–100) and the negative likelihood ratio was excellent, resulting <0.01. The test gave positive results (> 1 U/ml) in all 17 patients with confirmed HIT [no false negative cases; sensitivity = 100%; 95% CI: 80.5–100]. The assay was positive in 20 patients: 17 with confirmed HIT and three in which the presence of HIT had been excluded [specificity: 96.5%; 95%CI: 90.0–99.3]. The positive predictive value was therefore 85.0% (95%CI: 62.1–96.8) with a positive likelihood ratio of 28.6 (Table 2).
Performance of the HemosIL AcuStar HIT-Ab(PF4-H) assay

The HemosIL AcuStar HIT-Ab(PF4-H) levels resulted higher than the pre-defined cut-off (1 U/ml) in 33 (32.4%) suspected HIT patients; levels above the cut-off were also found in one healthy subject and in one patient without HIT symptoms who was sampled while on LMWH treatment.

The median level of results obtained in the 17 patients with confirmed HIT was significantly higher [16.7 U/ml (2.54–130.0)] in comparison with those of the other groups [0.44 U/ml (0.14–3.20) in patients with excluded HIT; 0.38 U/ml (0.16–1.14) in healthy subjects; 0.44 U/ml (0.17–1.13) in patients without HIT symptoms who were sampled while on LMWH treatment; (p<0.0001)].

As shown in Table 2, none of the patients with confirmed HIT had results below the cut-off; the negative predictive value was therefore 100% (95%CI: 94.8–100) and the negative likelihood ratio was <0.01; the sensitivity was 100% (95% CI: 80.5–100). The test was positive in 16/85 patients in which the presence of HIT was excluded [specificity: 81.2% (95%CI: 71.3–88.8)]. The positive predictive value was therefore 51.5% (95%CI: 33.5–69.2) with a positive likelihood ratio of 5.32.

Analysis of discordant results

The clinical and laboratory records of the three suspected HIT patients with positive HemosIL AcuStar HIT-IgG(PF4-H) assay (> 1 U/ml) in which the presence of HIT was excluded by the procedure used in our lab were reviewed blindly.

In two of these patients for whom pretest probability was low and ID-Heparin PF4 PaGIA negative, the LMWH treatment was continued with correction of platelet count in the following days; no arterial/venous thrombotic complications were registered until platelet normalisation.

The last case is that of a woman (T.E.; 30 years old, with type I protein C deficiency) who, after one month of treatment with LMWH followed with warfarin for an acute isolated pulmonary embolism (PE), referred to the emergency room where a deep venous thrombosis in the right leg was diagnosed (the INR was in the therapeutic range). She was then treated with LMWH at therapeutic dosage abandoning warfarin. Three weeks later she presented again to the emergency room for a worsening of dyspnea; a recurrence of PE was then diagnosed together with the presence of a thrombus in the right atrium. Treatment was shifted to continuous infusion of UFH. At that moment the platelet count was 160 X 10^9/L. Two weeks later HIT was suspected because the platelet count had decreased to 65 X 10^9/L. The pretest probability was high, the ID-Heparin PF4 PaGIA was positive but the platelet aggregation test excluded the presence of HIT (the aggregation was 15% and 12% with 1 IU/ml and 100 IU/ml UFH, respectively). The UFH treatment was continued. During the following five days the general clinical conditions markedly worsened; the platelet count further decreased to 42 X 10^9/L and finally the patient underwent bilateral pulmonary endoarteriectomy. A serum aliquot of this patient was sent to the lab of Prof. Andreas Greinacher (Dept. Immunology and Transfusion Medicine, Greifswald University, Germany) who kindly accepted to perform the washed platelet assay (12) and a quantitative IgG specific ELISA test (in-house assay using PF4 purified from platelet concentrates, [19]). The washed platelet assay was negative, while the ELISA test was positive (optical density [OD] = 1.120). These results – also in agreement with those obtained in our lab – are not typical for clinically relevant HIT as the IgG heparin-PF4 antibodies present in the sample did not seem to activate platelets.

Reproducibility of the HemosIL AcuStar HIT-IgG(PF4-H) and HIT-Ab(PF4-H) assays

The reproducibility of the HemosIL AcuStar HIT-IgG(PF4-H) and of the HIT-Ab(PF4-H) assays was evaluated using the results of the low and high HIT Controls. The results of the total variation (run-to-run and day-to-day) obtained over six different working days are reported in Table 3. The reproducibility of the assays was very good, with total CV% < 6%.

Discussion

In this study we evaluated the performance of two new, rapid, automated, semi-quantitative immunoassays in a series of consecutive patients referred to our lab for suspected HIT. The HemosIL AcuStar HIT-IgG(PF4-H) is specific for IgG anti-PF4/H antibodies, while the HemosIL AcuStar HIT-Ab(PF4-H) detects IgG, IgM and IgA anti-PF4/H antibodies. The assays were performed using patient’s serum aliquots collected at presentation and stored at −80°C until use. In the patients included in the study, confirmation or exclusion of the presence of HIT was based on the flow chart proposed by Pouplard et al. (17), which combines the results of the pretest probability of HIT, estimated by the “4Ts” clinical score (9),
and the results of ID-Heparin PF4 PaGIA, a rapid particle gel immunoassay. In patients with positive ID-Heparin PF4 PaGIA test and in those with negative ID-Heparin PF4 PaGIA but with high pretest probability, a platelet aggregation assay (18) was performed using platelets from healthy donors. This approach to testing HIT-suspected patients (ID-Heparin PF4 PaGIA followed by platelet aggregation test in conjunction with clinical score) is representative of the methods used in labs seeking a solution for quick work-up of the diagnostic algorithm for HIT while working outside the restrictions of other labour-intensive or highly technical methods.

No false negative cases were observed for either HemosIL AcuStar HIT assay, therefore, sensitivity and negative predictive values are calculated as 100% with excellent negative likelihood ratios (<0.01). These results support the use of the HemosIL AcuStar HIT-IgG(PF4-H) and HIT-Ab(PF4-H) assays to rule out the diagnosis of HIT without further testing when used with the "4Ts" clinical score. A negative likelihood ratio lower than 0.1 is indeed associated with a decrease in the probability of a disease by at least 45% (20, 21). These results are in line with those already published for EIA immunologic assays which showed a very high sensitivity (~99%). Since HIT is very unlikely in cases with a negative EIA result, a negative test is considered safe to rule out the diagnosis (22, 23).

Not surprisingly, the two new assays showed a different specificity. As expected, the specificity was higher for the HemosIL AcuStar HIT-IgG(PF4-H) in comparison with that of the HemosIL AcuStar HIT-Ab(PF4-H) (96.5% and 81.2%, respectively). It has been already reported that EIA immunologic assays also detect non-platelet activating antibodies. These non-functional antibodies can also be IgG isotypes, but are more likely to be of IgM and IgA classes (24). The diagnostic specificity for HIT of the immunologic assays is therefore greatly increased when testing is restricted to IgG class by avoiding the detection of nonpathogenic IgM and IgA antibodies (22, 25–27); however, there is no published consensus.

The present study also showed that higher values of the HemosIL AcuStar HIT-IgG(PF4-H) were associated with increased probability of HIT; the percentage of patients with confirmed HIT was, indeed, 25% for values just above the cut-off (1–2 U/ml) and 100% for values higher than 2 U/ml. Furthermore, patients with confirmed HIT and thrombotic complications when referred to our lab had significantly higher levels than those without thrombotic complications. These results are in line with those already reported for EIA immunologic assays. Although the EIA immunologic assays are designed to be reported in a dichotomous fashion (positive/negative), emerging data are showing that an increase in the absolute OD value is associated with increasing probability of HIT (28–32) and that patients with OD values > 1.0 had a six-fold increased risk of thrombotic complication compared with those who had lower OD values (29).

Some limitations of the present study need to be pointed out. First and foremost, differently from the data published by Poulard et al. (17), we could not perform the [14C] serotonin release as functional assay to confirm the presence of HIT in selected patients. Instead, a platelet aggregation test was used. It has been reported that the latter assay is less sensitive than the [14C] serotonin release assay (2, 11, 12); sensitivity is not improved even when the test is performed with platelets from 4–5 healthy donors. We cannot exclude that the platelet aggregation test used in our study may have given some false negative results, although the heparin induced platelet aggregation assay confirmed our result of the discordant case (T.E.) described in the Results section. Second, the study group is relatively small and, consequently, the number of patients with confirmed HIT is low. This study should be considered as a first pilot study on this assay and other studies on a larger population should be performed. Third, in our diagnostic work-up we used a qualitative assay, the ID-Heparin PF4 PaGIA. Recent data have shown some failures of this test in detecting HIT (33). Unfortunately, additional serum aliquots were not available to perform a quantitative ELISA test.

In conclusion, the two new semi-quantitative immunnoassays evaluated in this study, the HemosIL AcuStar HIT-IgG(PF4-H) and HIT-Ab(PF4-H), showed very high sensitivity (100%) and, therefore, they can be used reliably to rule out HIT in suspected patients. In this study, the diagnostic specificity was greatly increased (from 81% to 96%) by using the HemosIL AcuStar HIT-IgG(PF4-H) assay which detects IgG antibodies only. Both the assays are highly reproducible (run-to-run and day-to-day variation <6%), rapid (turnaround time 30 minutes), completely automated, semi-quantitative, and can be run for single sample testing. The assays are performed using ready to use cartridges which can be stored on-board the analyser for up to three months. The specific analyser is based on a highly sensitive technology (chemiluminescence), providing a wider working range over traditional immunnoassay methods.

Further prospective clinical studies are recommended to evaluate if the predictive value of these new assays for HIT can be improved when used in conjunction with the pretest probability.

Acknowledgements

We thank Prof. Andreas Greinacher and Dr. Mirjana Rajkovic (Dept. Immunology and Transfusion Medicine, Greifswald University, Germany) for performing the washed platelet assay and the
quantitative IgG specific ELISA test in the patient (T.E.) with discordant results and inconclusive HIT diagnosis. We thank Instrumentation Laboratory for providing us with the reagents used in the study and Romiya Barry for the English revision.

References