

Accepted Manuscript

Detection of Autoantibodies against Platelet Glycoproteins in Patients with Immune Thrombocytopenic Purpura by Flow Cytometric Immunobead Array

Yang He, Yun-xiao Zhao, Ming-qing Zhu, Qingyu Wu, Chang-geng Ruan

PII: S0009-8981(12)00502-5
DOI: doi: [10.1016/j.cca.2012.10.035](https://doi.org/10.1016/j.cca.2012.10.035)
Reference: CCA 12870

To appear in: *Clinica Chimica Acta*

Received date: 10 September 2012
Revised date: 8 October 2012
Accepted date: 18 October 2012



Please cite this article as: He Yang, Zhao Yun-xiao, Zhu Ming-qing, Wu Qingyu, Ruan Chang-geng, Detection of Autoantibodies against Platelet Glycoproteins in Patients with Immune Thrombocytopenic Purpura by Flow Cytometric Immunobead Array, *Clinica Chimica Acta* (2012), doi: [10.1016/j.cca.2012.10.035](https://doi.org/10.1016/j.cca.2012.10.035)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Detection of Autoantibodies against Platelet Glycoproteins in Patients with Immune Thrombocytopenic Purpura by Flow Cytometric Immunobead Array

Yang He ^{a*}, Yun-xiao Zhao ^a, Ming-qing Zhu ^a, Qingyu Wu ^b, Chang-geng Ruan ^{a*}

^aKey Lab of Thrombosis and Hemostasis, Jiangsu Institute of Hematology, the First Affiliated Hospital of Soochow University, Suzhou, China

^bCyrus Tang Hematology Center, Jiangsu Institute of Hematology, Soochow University, Suzhou, China

*Corresponding authors: Thrombosis and Hemostasis Key Lab, Jiangsu Institute of Hematology, the First Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215006, China. Tel: +86-512-65182812. E-mail address: heyang1963@163.com (Y. He) or changgengruan@hotmail.com (C. Ruan).

ABSTRACT

Background: The goal of this study is to develop a flow cytometric immunobead array (FCIA) assay to detect platelet autoantibodies commonly present in bleeding patients with immune thrombocytopenic purpura (ITP).

Methods: Polystyrene microbeads coated with antibodies against human platelet glycoproteins (GPs) IX(SZ1), Ib(SZ2), IIIa(SZ21), IIb(SZ22), and P-selectin(SZ51) were incubated with platelet lysate from 50 ITP patients and 86 controls. The platelet antigen-autoantibody complexes were detected by flow cytometry using an FITC-labeled antibody. The results were compared with that of a monoclonal antibody immobilization of platelet antigen (MAIPA) assay.

Results: By FCIA, platelet autoantibodies against GPIb, GPIIb, GPIIIa, GPIX and P-selectin were detected in ITP patients. Mean fluorescent intensity values with antibodies SZ1, SZ2, SZ21, SZ22 and SZ51 were all higher in ITP patients than controls (p values <0.01). In ROC analysis, values of the area under the curve were 0.89, 0.82, 0.93, 0.94 and 0.95, respectively. In ITP diagnosis, the FCIA assay with these five antibodies had better sensitivity and accuracy than the MAIPA assay (96% vs. 44% in sensitivity; 80.9% vs. 64.7% in accuracy, $p <0.01$).

Conclusion: FCIA assays with multiple antibodies against platelet GPs may be used to improve the diagnosis of ITP in hospitals.

Keywords: flow cytometry; idiopathic thrombocytopenic purpura; platelet glycoprotein; thrombocytopenia

1. Introduction

Immune thrombocytopenic purpura (ITP), also called idiopathic thrombocytopenic purpura or immune thrombocytopenia, is a common bleeding disorder characterized by abnormally low platelet counts of unknown cause [1-6]. Studies indicate that autoimmune is a major contributing factor in the pathogenesis of ITP. Autoantibodies against platelet antigens are frequently detected in patients with ITP [7,8]. These autoantibodies often recognize platelet glycoproteins (GP) such as GPIb, GPIIb-IIIa and GP IX that are abundant on the platelet surface [2,9]. The formation of the antigen and autoantibody complex on the platelet surface may promote platelet destruction by macrophage-mediated phagocytosis in the spleen and other organs, which reduces platelet counts in the blood [1-3,5 6]. Autoantibodies or cytotoxic T cells also target megakaryocytes, which may impair megakaryocyte differentiation and maturation in the bone marrow, thereby preventing platelet production [1-3,5,6].

The detection of platelet autoantibodies is an important step in the diagnosis of ITP [5,10,11]. Methods that are commonly used to detect platelet autoantibodies in ITP patients include ELISA-based monoclonal antibody immobilization of platelet antigen (MAIPA) assay and immunobead-based radioimmune assay (RIA) [7,8,12,13]. In general, however, these assays are cumbersome and time-consuming, which may limit their wide use in hospital settings [14].

Flow cytometric immunobead array (FCIA) is a recently developed technique, in which flow cytometry detects antibody-coated polystyrene microbeads that bind to specific antigens [15]. In this technique, each type of microbeads that are coated with a specific antibody has distinguishable fluorescent intensity. As a result, different types of microbeads can be mixed in a single tube to detect multiple antigens simultaneously. As such, the technique reduces sample volumes and assay times compared to more traditional methods such as ELISA and RIA. To

date, FCIA-based assays are being developed to measure a variety of proteins in biological samples [15-17].

In this study, we developed an FCIA assay using 5 monoclonal antibodies against human platelet GPs. We showed that the FCIA assay can be used to detect autoantibodies in ITP patients that target platelet GPIb, GPIIb, GPIIIa, GPIX and P-selectin. More importantly, our results showed that the FCIA assay with these monoclonal antibodies improved the sensitivity and accuracy for the diagnosis of ITP.

2. Materials and methods

2.1. Patient samples

Blood samples were from 50 ITP patients who visited the First Affiliated Hospital of Soochow University in Suzhou, China between October 2011 and March 2012. Among them, 15 were males and 35 females, aged from 4 to 78 y (average 39 y). Their platelet counts were $<100 \times 10^9/l$. The diagnosis of ITP was based on the guideline by the American Society of Hematology [10]. Control blood samples were from 37 non-ITP patients, whose diagnosis included leukemia (31 cases), anemia (5 cases), and myelodysplastic syndrome (1 case). Additional blood samples were from 49 normal individuals (24 males and 25 females, 22-51 y (average 37 y) who underwent routine health check-ups at the hospital. Blood platelet counts in non-ITP patients and normal controls were all $>100 \times 10^9/l$. This study was approved by the ethic committee of the hospital. All participants gave written informed consent.

2.2. Isolation of platelets

Venous blood (2 ml) was collected in tubes containing spray-coated EDTA as an anticoagulant (BD Diagnostics, Franklin Lakes, NJ). Blood samples were centrifuged at 200 g for 5 min at room temperature. Platelet-rich plasma (PRP) was transferred to new tubes that were centrifuged at 3,000 x *g* for 2 min. Platelet pellets were washed twice with a phosphate-buffered saline (PBS) containing 0.05% EDTA. Washed platelets were lysed in a PBS buffer (110 μ L) containing 1% Triton X-100. Samples were centrifuged at 3,000 x *g* for 20 min and soluble platelet lysate was collected.

2.3. Antibodies and polystyrene beads

Monoclonal antibodies against human platelet GPIX (SZ1), GPIb (SZ2), GPIIb (SZ22), GPIIIa (SZ21) and P-selectin (SZ51) were prepared in our laboratory, as described previously [18-23]. Fluorescein isothiocyanate (FITC)-labeled goat anti-human IgG (FITC-GAH) and anti-mouse IgG (FITC-GAM) polyclonal antibodies were from Beckman-Coulter (Suzhou, China). Polystyrene microbeads (4 μ m in diameter with 8 fluorescent intensities) were from Spherotech (Lake Forest, IL).

2.4. Antibody coating

Monoclonal antibodies SZ1, SZ2, SZ21, SZ22 or SZ51 (160 μ g each) in a carbonate coating buffer (pH 9.5) were incubated with 1×10^6 microbeads of distinguishable fluorescent intensities on a shaker at 4°C overnight. Antibody-coated microbeads were washed three times with PBS containing 0.05% Tween-20 and then stored in PBS containing 0.02% sodium azide as a preservative. The stored microbeads were tested with FITC-GAM antibodies on days 1, 11, 21,

31, 41 and 181, respectively, for their stability. In this test, FITC-GAH antibodies were used as a negative control.

2.5. FCIA

Monoclonal antibody-coated microbeads (1×10^4) were added to platelet lysate (150 μ L) from ITP patients or control individuals and incubated on a shaker at room temperature for 1 h. Samples were centrifuged at 500 x *g* for 20 min, washed once with PBS, and incubated with an FITC-GAH antibody at room temperature for 30 min. The microbeads were washed once with PBS, suspended in 0.5 ml of PBS, and analyzed by flow cytometry using an FITC-GAH antibody (Cytomics FC-500, Beckman-Coulter). Data of fluorescent intensity from 1,500-2,000 microbeads were analyzed by the CXP software (Beckman-Coulter) to calculate mean fluorescent intensity (MFI) values for individual samples.

2.6. MAIPA

ELISA-based MAIPA assay was performed according to published methods [24]. Five monoclonal antibodies against human platelet GPs (SZ1, SZ2, SZ21, SZ22 and SZ51) were tested individually with samples from ITP patients and non-ITP and normal controls.

2.7. Statistical analysis

Analysis was done using the SPSS 16.0 software (SPSS, Chicago, IL). All data are presented as the mean $\pm$ SD. Student's *t*-test was used to compare MFI values between two groups. Chi-square test was used to compare the diagnostic sensitivity, specificity and accuracy between FCIA and MAIPA assays or to compare the results of five monoclonal antibodies

combined with that of individual antibodies. A $p < 0.05$ is considered to be statistically significant. Receiver operating characteristic (ROC) curve analysis was done to determine cut-off values of detected platelet autoantibodies that yielded the highest combined sensitivity and specificity for predicting ITP.

3. Result

3.1. Antibody coating and MFI

Polystyrene microbeads were coated with monoclonal antibodies SZ1, SZ2, SZ21, SZ22 and SZ51, separately. The coated microbeads were detected by flow cytometry using an FITC-GAM antibody. The MFI values for microbeads coated with these monoclonal antibodies were 15.7 ± 1.2 (SZ1), 23.7 ± 1.5 (SZ2), 26.1 ± 1.2 (SZ21), 12.1 ± 1.2 (SZ22), and 17.4 ± 1.5 (SZ51), respectively.

3.2. Stability

The antibody-coated microbeads were stored at 4°C and tested over time (up to 181 days) with an FITC-GAM antibody for the stability. The results showed that the MFI values for these antibody-coated microbeads varied ~10% (SZ1: 11.1%; SZ2: 11.1%; SZ21: 11.0%; SZ22: 9.4% and SZ51: 11.2%) within six months, indicating that the antibody-coated microbeads were stable when stored at 4°C.

3.3. Assay variations

We tested intra- and inter-assay CVs for the MFI value for microbeads coated with each antibody. Intra-assay CVs and MFI values for these antibodies were 9.3% and 3.21 ± 0.30 (SZ1),

5.3% and 4.18 ± 0.22 (SZ2), 3.3% and 2.51 ± 0.08 (SZ21), 2.3% and 2.17 ± 0.05 (SZ22), and 2.5% and 2.21 ± 0.06 (SZ51), respectively, and inter-assay CVs and MFI values for these antibodies were 10.9% and 2.78 ± 0.30 (SZ1), 6.6% and 3.84 ± 0.25 (SZ2), 8.7% and 2.41 ± 0.21 (SZ21), 7.1% and 2.01 ± 0.14 (SZ22), and 8.0% and 1.87 ± 0.15 (SZ51), respectively. These CV values indicated that the results from the antibody-coated microbeads were reproducible.

3.4. Platelet autoantibodies in ITP patients

We used the FCIA assay to examine platelet autoantibodies in ITP patients. All microbeads coated by 5 antibodies had higher fluorescent intensities in samples from ITP patients than that of normal controls, indicating the presence of platelet autoantibodies in ITP patients (Fig. 1). The MFI values for these five antibody-coated microbeads were significantly higher in ITP patients than those in non-ITP patients or normal controls (Table 1). No statistically significant difference in MFI values was found between non-ITP patient and normal control groups (Table 1).

3.5. ROC analysis

ROC analysis was done for each type of antibody-coated microbeads to determine cutoff values that yielded the highest combined sensitivity and specificity for detecting platelet autoantibodies in blood samples (Fig. 2). For antibodies SZ1, SZ2, SZ21, SZ22 and SZ51, the actual cutoff values were 2.79, 3.42, 1.71, 1.77, and 1.47, respectively, and the corresponding area under the ROC curve (AUC) were 0.91, 0.81, 0.97, 0.95 and 0.96, respectively. We also did experiments with the MAIPA assay to detect platelet autoantibodies in ITP patients and analyzed the results similarly with ROC (Fig. 2). In the MAIPA assay, the actual cutoff values for

antibodies SZ1, SZ2, SZ21, SZ22 and SZ51 were 0.68, 0.55, 0.47, 0.52, and 0.47, respectively, and the corresponding AUC values were 0.71, 0.66, 0.64, 0.62, and 0.65, respectively.

3.6. Diagnosis of ITP

We examined the sensitivity, specificity, and accuracy in the diagnosis of ITP by the FCIA and MAIPA assays using these monoclonal antibodies. The results from 136 samples (50 ITP and 86 controls) are shown in Table 2. The FCIA assay with SZ1, SZ21, SZ22 and SZ51 antibodies, when used individually, had better sensitivity than the MAIPA assay using the same individual antibodies. Both assays using these antibodies had similar specificities. Overall, The FCIA assay with these four antibodies had better accuracy than the MAIPA assay in diagnosing ITP. SZ2 antibody had similar sensitivity, specificity and accuracy in both FCIA and MAIPA assays (Table 2). When all five antibodies were used together for the diagnosis of ITP, the FCIA assay had significantly better sensitivity and accuracy than the MAIPA assay (96 vs. 44% and 80.9 vs. 64.7%, respectively, both p values <0.01) (Table 2).

4. Discussion

The detection of plasma platelet autoantibodies is critical for the diagnosis of ITP [2, 3, 5]. Changes of platelet autoantibody levels in ITP patients also are important in assessing patients' responses to treatment [25]. Methods commonly used to detect platelet autoantibodies include ELISA and RIA. These methods, however, are not widely used in hospitals because of technical challenges and low assay sensitivity [14]. More recently, flow cytometric methods using single monoclonal antibody have been developed to measure platelet associated autoantibodies in ITP patients, which showed better sensitivity and specificity [26]. FCIA assay is a more advanced

technique that uses microbeads and a panel of antibodies to increase assay sensitivity. The technique allows measuring multiple target molecules in a single test, thereby simplifying experimental procedures and shortening assay time [15].

In this study, we developed an FCIA assay to detect platelet autoantibodies in human blood. In this assay, we used 5 monoclonal antibodies against human platelet GPs, including GPIb, GPIIb, GPIIIa, GPIX and P-selectin that are abundant in platelets. The antibodies were coupled to microbeads that had distinguishable fluorescent intensities. We showed that the antibody-coupled microbeads were stable for at least six months when stored at 4°C. Our data also showed that this assay had relatively small intra- and inter-CVs, and that the results were reproducible. These antibody-coupled microbeads allowed us to detect autoantibodies in ITP patients. Our results showed that the levels of autoantibodies against platelet GPs were significantly higher in ITP patients than that in control individuals (Figure 1 and Table 1).

Studies have shown that autoantibodies developed in different individual ITP patients may recognize different platelet antigens. Platelet GPIb-IX complex, GPIIb-IIIa complex and P-selectin are major molecules on the platelet surface and commonly targeted by autoantibodies in ITP patients. Approximately, 75% autoantibodies identified in ITP patients were found to target platelet GPIb-IX and GPIIb-IIIa complexes [1,2,14,27,28]. Other platelet surface molecules such as GPIa-IIa, GPIV and GPV may also be targeted by autoantibodies in some ITP patients [9,27,29].

In this study, we tested the FCIA assay with monoclonal antibodies against GPIb-IX, GPIIb-IIIa and P-selectin to measure autoantibodies against these major platelet antigens in blood samples from ITP patients. We also compared the results with that of a traditional MAIPA assay that was used in our previous studies [19]. Our results indicated that the FCIA assay with

antibodies SZ1, SZ21, SZ22 and SZ51, when tested individually, all had better sensitivity and accuracy than the MAIPA assay using the same antibodies (Table 2). On the other hand, these two assays had similar specificity for each antibody, indicating that the improved accuracy in the FCIA assay was mainly due to the improved sensitivity. It appears, therefore, that the antibodies on microbeads under flow conditions were more sensitive in detecting platelet autoantibodies than under static conditions in an MAIPA assay. Most likely, the microbeads under flow conditions increased the probability for the antibodies to encounter platelet autoantibody-antigen complexes in solution.

In our FCIA assay using microbeads with five antibodies, the sensitivity (96%) and accuracy (80.9%) of predicting ITP were significantly improved compared to that in the MAIPA assay (44% and 64.7%, respectively). In both assays, the specificity remained similar (72.1% in FCIA and 76.7% in MAIPA) (Table 2). In previous MAIPA or RIA studies, reported sensitivity and specificity ranged from ~49-84% and ~80-93%, respectively [7, 8, 14, 30], which was comparable to our results. More recent flow cytometric studies using antibodies against GPIIb-IIIa and GPIb-IX, individually or in combination, showed improved sensitivity (~82-86%) and specificity (~95-100%) [16,26]. Thus, these data are consistent with our results, indicating that flow cytometric methods are more sensitive than MAIPA and RIA assays in detecting autoantibodies in ITP patients. By using a panel of monoclonal antibodies against major platelet antigens in our FCIA assay, we showed that the sensitivity may be further improved. Technically, the FCIA assay is based on a similar principle to flow cytometry based-MAIPA using a single antibody [26]. By using 5 antibodies targeting different platelet antigens, our FCIA assay increased the probability of capturing more diverse platelet autoantibodies that were present in different individual ITP patients. Together, our studies show that more sensitive and

accurate FCIA tests can be developed to measure platelet autoantibodies in ITP patients. Such an assay can be easily adapted for the use at hospital settings. Our results should encourage further studies with more ITP patient samples to assess the clinical application of FCIA-based assays in the diagnosis of ITP.

Abbreviations:

FCIA, flow cytometric immunobead array; FITC, fluorescein isothiocyanate; GAH, goat anti-human; GAM, goat anti-mouse; GP, glycoprotein; ITP, idiopathic thrombocytopenic purpura; MAIPA, monoclonal antibody immobilization of platelet antigen assay; MFI, mean fluorescent intensity; PRP, platelet-rich plasma

Acknowledgement: This work was supported in part by grants from the National Natural Science Foundation of China (81270593) and the Priority Academic Program Development of Jiangsu Higher Education Institutions of China.

References

- [1] Chong BH, Ho SJ. Autoimmune thrombocytopenia. *J Thromb Haemost* 2005;3:1763-1772.
- [2] Cines DB, Blanchette VS. Immune thrombocytopenic purpura. *N Engl J Med* 2002;346:995-1008.
- [3] Cines DB, Bussel JB, Liebman HA, et al. The ITP syndrome: pathogenic and clinical diversity. *Blood* 2009;113:6511-6521.
- [4] George JN. Definition, diagnosis and treatment of immune thrombocytopenic purpura. *Haematologica* 2009;94:759-762.
- [5] Neunert C, Lim W, Crowther M, et al. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. *Blood* 2011;117:4190-4207.
- [6] Stasi R, Evangelista ML, Stipa E, et al. Idiopathic thrombocytopenic purpura: current concepts in pathophysiology and management. *Thromb Haemost* 2008;99:4-13.
- [7] Brighton TA, Evans S, Castaldi PA, et al. Prospective evaluation of the clinical usefulness of an antigen-specific assay (MAIPA) in idiopathic thrombocytopenic purpura and other immune thrombocytopenias. *Blood* 1996;88:194-201.
- [8] McMillan R, Wang L, Tani P. Prospective evaluation of the immunobead assay for the diagnosis of adult chronic immune thrombocytopenic purpura (ITP). *J Thromb Haemost* 2003;1:485-491.
- [9] McMillan R. Autoantibodies and autoantigens in chronic immune thrombocytopenic purpura. *Semin Hematol* 2000;37:239-248.

- [10] George JN, Woolf SH, Raskob GE, et al. Idiopathic thrombocytopenic purpura: a practice guideline developed by explicit methods for the American Society of Hematology. *Blood* 1996;88:3-40.
- [11] Stasi R. Immune thrombocytopenia: pathophysiologic and clinical update. *Semin Thromb Hemost* 2012;38:454-462.
- [12] Kiefel V, Santoso S, Weisheit M, et al. Monoclonal antibody--specific immobilization of platelet antigens (MAIPA): a new tool for the identification of platelet-reactive antibodies. *Blood* 1987;70:1722-1726.
- [13] McMillan R, Tani P, Millard F, et al. Platelet-associated and plasma anti-glycoprotein autoantibodies in chronic ITP. *Blood* 1987;70:1040-1045.
- [14] McMillan R. Antigen-specific assays in immune thrombocytopenia. *Transfus Med Rev* 1990;4:136-143.
- [15] Morgan E, Varro R, Sepulveda H, et al. Cytometric bead array: a multiplexed assay platform with applications in various areas of biology. *Clin Immunol* 2004;110:252-266.
- [16] Liu XG, Li JL, Qin P, et al. Determination of platelet-bound glycoprotein-specific autoantibodies by flow cytometric immunobead assay in primary immune thrombocytopenia. *Eur J Haematol* 2011;86:339-346.
- [17] Weerkamp F, Dekking E, Ng YY, et al. Flow cytometric immunobead assay for the detection of BCR-ABL fusion proteins in leukemia patients. *Leukemia* 2009;23:1106-1117.
- [18] Gu JM, Xu WF, Wang XD, et al. Identification of a nonsense mutation at amino acid 584-arginine of platelet glycoprotein IIb in patients with type I Glanzmann thrombasthenia. *Br J Haematol* 1993;83:442-449.

- [19] Jiang H, Wu G, Zhang W, et al. Antiplatelet granule membrane protein (GMP-140) autoantibodies detected in plasma from patients with idiopathic thrombocytopenic purpura. *Nouv Rev Fr Hematol* 1993;35:487-490.
- [20] Ruan C. Monoclonal antibodies against platelets and its clinical application. *Bull Acad Natl Med* 2009;193:723-726.
- [21] Ruan CG, Du XP, Xi XD, et al. A murine antiglycoprotein Ib complex monoclonal antibody, SZ 2, inhibits platelet aggregation induced by both ristocetin and collagen. *Blood* 1987;69:570-577.
- [22] Wu G, Xi X, Li P, et al. Preparation of a monoclonal antibody, SZ-51, that recognizes an alpha-granule membrane protein (GMP-140) on the surface of activated human platelets. *Nouv Rev Fr Hematol* 1990;32:231-235.
- [23] Xi X, Zhao Y, Jiang H, et al. Competitive binding of a monoclonal antibody SZ-21 with anti-PLA1 antibodies and its potential for clinical application. *Nouv Rev Fr Hematol* 1992;34:239-242.
- [24] Stockelberg D, Hou M, Jacobsson S, et al. Detection of platelet antibodies in chronic idiopathic thrombocytopenic purpura (ITP). A comparative study using flow cytometry, a whole platelet ELISA, and an antigen capture ELISA. *Eur J Haematol* 1996;56:72-77.
- [25] Berchtold P, Wenger M. Autoantibodies against platelet glycoproteins in autoimmune thrombocytopenic purpura: their clinical significance and response to treatment. *Blood* 1993;81:1246-1250.
- [26] Tomer A, Koziol J, McMillan R. Autoimmune thrombocytopenia: flow cytometric determination of platelet-associated autoantibodies against platelet-specific receptors. *J Thromb Haemost* 2005;3:74-78.

- [27] Kunicki TJ, Newman PJ. The molecular immunology of human platelet proteins. *Blood* 1992;80:1386-1404.
- [28] van Leeuwen EF, van der Ven JT, Engelfriet CP, et al. Specificity of autoantibodies in autoimmune thrombocytopenia. *Blood* 1982;59:23-26.
- [29] He R, Reid DM, Jones CE, et al. Spectrum of Ig classes, specificities, and titers of serum antiglycoproteins in chronic idiopathic thrombocytopenic purpura. *Blood* 1994;83:1024-1032.
- [30] Warner MN, Moore JC, Warkentin TE, et al. A prospective study of protein-specific assays used to investigate idiopathic thrombocytopenic purpura. *Br J Haematol* 1999;104:442-447.

Figure Legends

Fig. 1. Representative detection of platelet autoantibodies in ITP patients. Platelet lysates from ITP patients (black peak) or normal controls (open peak) were incubated with microbeads coupled with monoclonal antibodies SZ1, SZ2, SZ21, SZ22 or SZ51. Autoantibodies were detected by an FITC-labeled goat anti-human (GAH) antibody by flow cytometry.

Fig. 2. ROC curves from FCIA and MAIPA tests for the diagnosis of ITP. Platelet autoantibodies were measured in samples from 50 ITP patients and 86 controls. ROC curves from microbeads coupled with each monoclonal antibody are shown.

Table 1. MFI values from ITP patient, non-ITP patient and normal control groups

Group	case (n)	SZ1	SZ2	SZ21	SZ22	SZ51
ITP	50	5.03 ± 3.90* [†]	4.67 ± 3.66* [†]	2.70 ± 1.31* [†]	2.52 ± 1.19* [†]	2.33 ± 1.19* [†]
Non-ITP	37	1.91 ± 0.64	2.36 ± 1.16	1.08 ± 0.32	1.10 ± 0.41	0.96 ± 0.40
Normal	49	1.88 ± 0.84	2.16 ± 0.93	1.14 ± 0.41	1.28 ± 0.57	1.06 ± 0.67

* $P < 0.01$ vs. non-ITP patient group of the same antibody; t values for SZ1, SZ2, SZ21, SZ22 and SZ51 were 5.930, 4.724, 8.727, 8.404 and 8.086, respectively.

[†] $P < 0.01$ vs. normal group of the same antibody; t values for SZ1, SZ2, SZ21, SZ22 and SZ51 were 5.514, 4.197, 8.599, 7.459 and 6.736, respectively.

Table 2. Sensitivity, Specificity and Accuracy of Diagnosing ITP by FCIA and MAIPA

Ab	Case (n)	Sensitivity (%)		Specificity (%)		Accuracy (%)	
		FCIA	MAIPA	FCIA	MAIPA	FCIA	MAIPA
SZ1	136	66.0 (33/50)*	38.0 (19/50)	90.7 (78/86) [†]	89.5 (77/86)	81.6 (111/136)*	70.6 (96/136)
SZ2	136	50.0 (25/50) [†]	36.0 (18/50)	89.5 (77/86) [†]	90.7 (78/86)	75.0 (102/136) [†]	70.6 (96/136)
SZ21	136	78.0 (39/50)**	42.0 (21/50)	88.4 (76/86) [†]	82.6 (71/86)	84.6 (115/136)**	67.7 (92/136)
SZ22	136	72.0 (36/50)**	26.0 (13/50)	91.9 (79/86) [†]	90.7 (78/86)	84.6 (115/136)**	66.9 (91/136)
SZ51	136	90.0 (45/50)**	34.0 (17/50)	91.9 (79/86) [†]	90.7 (78/86)	91.2 (124/136)**	69.9 (95/136)
five abs							
combined	136	96.0 (48/50)**	44.0 (22/50)	72.1 (62/86) [†]	76.7 (66/86)	80.9 (110/136)**	64.7 (88/136)

* $P < 0.05$; ** $P < 0.01$ FCIA vs. MAIPA of the same group;

[†] $P > 0.05$ FCIA vs. MAIPA of the same group; abs, antibodies.

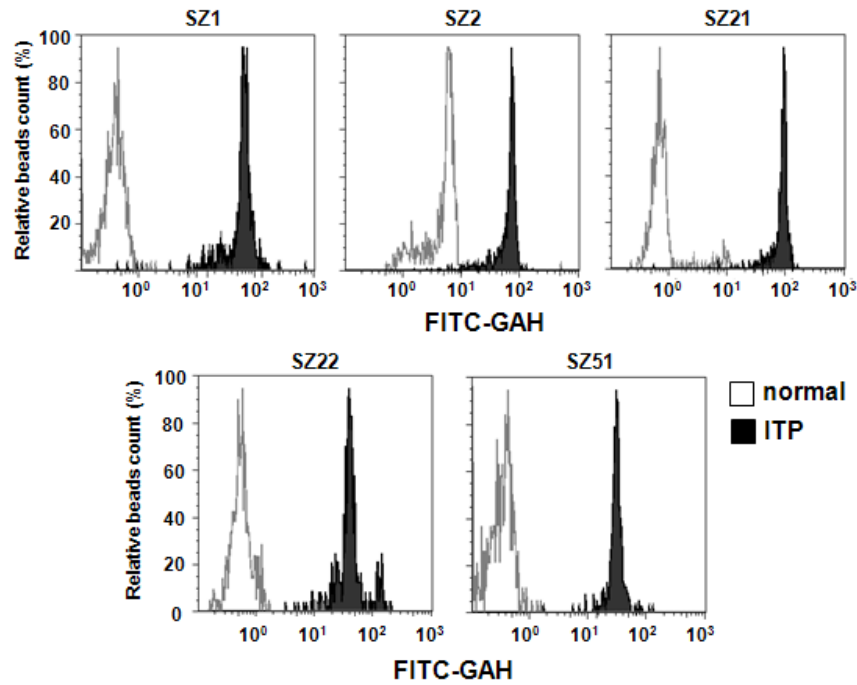


Figure 1

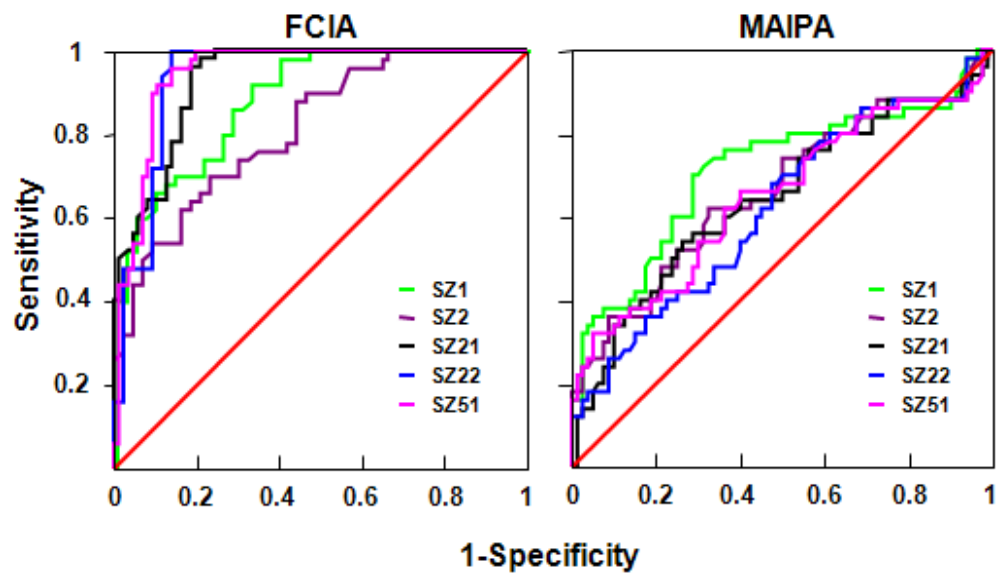


Figure 2

Highlights

- Immune thrombocytopenic purpura (ITP) is a bleeding disorder associated with platelet autoantibodies.
- We developed a flow cytometric immunobead array (FCIA) assay using a panel of antibodies against platelet glycoproteins.
- The FCIA assay detected high levels of platelet autoantibodies in ITP patients.
- The assay significantly improved the sensitivity and accuracy in ITP diagnosis.