bjh short report

# Detection of platelet autoantibodies to identify immune thrombocytopenia: state of the art

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Received 22 November 2017; accepted for publication 9 April 2018

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## Summary

Immune Thrombocytopenia (ITP) is diagnosed by exclusion of other causes for thrombocytopenia. Reliable detection of platelet autoantibodies would support the clinical diagnosis of ITP and prevent misdiagnosis. We optimized our diagnostic algorithm for suspected ITP using the direct monoclonal antibody immobilization of platelet antigens assay (MAIPA), which evaluates the presence of platelet autoantibodies on the glycoproteins (GP) IIb/IIIa, Ib/IX and V bound on the patient platelets. The direct MAIPA was shown to be a valuable technique for the detection of platelet autoantibodies and could possibly become a guide for optimizing therapy towards a more personalized treatment of ITP.

**Keywords:** Immune thrombocytopenia, autoantibodies, ITP, MAIPA.

Platelet autoantibodies are regarded to be the major underlying cause of immune thrombocytopenia (ITP), although a role for cytotoxic T cells is also described (Cines *et al*, 2014). Screening for platelet autoantibodies however, is not part of the recommended diagnostic and therapeutic work-up (Neunert *et al*, 2011). Currently, the latter is due to the low sensitivity (60–70%) and specificity (≤60%) of the different types of platelet autoantibody tests (Helmerhorst *et al*, 1983; Hagenström *et al*, 2000) and ITP is therefore diagnosed by exclusion of other causes for thrombocytopenia (Neunert *et al*, 2011). To prevent misdiagnosis, the reliable detection of platelet autoantibodies would be of great value for the clinical diagnosis of ITP. In this respect, we re-evaluated our diagnostic algorithm for suspected ITP using the direct monoclonal antibody immobilization of platelet antigens assay (MAIPA).

Platelet autoantibodies in ITP are predominantly directed against the platelet glycoproteins (GP) IIb/IIIa (CD41/61; fibrinogen receptor), GPIb/IX (CD42c/CD42a) or GPV (CD42d) (Joutsi & Kekomäki, 1997; McMillan, 2003). The presence of platelet antibodies directed against any of these targets can be investigated by enzyme-linked immunosorbent assay-based GP specific assays, such as MAIPA and Luminex beads assays (Kiefel *et al*, 1987; Porcelijn *et al*, 2014). While the indirect MAIPA and commercially available GP-specific assays are known for their high sensitivity and specificity for identification of human platelet antigen (HPA)-specific alloantibodies (Porcelijn *et al*, 2008), platelet autoantibodies in ITP serum or plasma are less easily detected (McMillan, 2003). Also, the direct MAIPA, developed to directly detect platelet-bound antibodies, previously showed a sensitivity for autoantibodies ranging from only 29 to 54% (Joutsi & Kekomäki, 1997; McMillan, 2003).

A more accurate detection of platelet autoantibodies in this respect would be of great value and for this reason, we validated the detection of platelet autoantibodies by the direct MAIPA in known ITP and non-ITP mediated thrombocytopenic patients as well as non-thrombocytopenic controls. Subsequently, we tested the direct MAIPA for its discriminatory power between ITP and non-ITP patients in consecutively diagnostic samples sent to our laboratory.



First published online 29 May 2018
doi: 10.1111/bjh.15404

Healthy control and patient platelets, platelet eluates and sera were tested within 24 h of sampling, with the direct and indirect platelet immunofluorescence test (PIFT) (as described by von dem Borne *et al*, 1978), for the presence of platelet-associated and free circulating autoantibodies of the immunoglobulin (Ig)G and IgM classes, and with the direct MAIPA (as described by Kiefel *et al*, 1987), for the presence of IgG class platelet-associated autoantibodies.

Statistical analyses were performed using SPSS 21 for Windows statistical package (SPSS Inc., Chicago, IL, USA). A $P < 0{\cdot}05$ was considered significant.

Healthy control ($n = 462$) platelets, tested with the direct MAIPA, produced a range of normally distributed very low extinctions [Fig 1, between E = 0·048 and E = 0·052 (range 0·01–0·16 ± 0·023–0·026) for all five autoantibody targets: GPIIb/IIIa (CD41/CD61), GPIb/IX (CD42c/CD42a), GPV (CD42d), GPIa/IIa (CD49b/CD29) and GPIV (CD36)]. With the calculated cut-off value E = 0·13 [mean + 3 × standard deviation (SD)], only one of the healthy controls was positive [optical density (OD) 0·137] by direct MAIPA for only CD41/61 (GPIIb/IIIa) (specificity of 99·8%: Fig 1, Table I).

Six of the 462 healthy controls showed positive results in the direct PIFT by the presence of antibodies of the IgM class.

None of the non-immune thrombocytopenic patients ($n = 43$, Table SIA) showed a positive direct MAIPA result, whereas 16 of these 43 (37%) non-immune thrombocytopenia samples were positive, both with direct PIFT and eluate PIFT (Table I).

Known ITP patients ($n = 60$) were diagnosed - in accordance with the recommendations of the American Society of Hematology (Neunert *et al*, 2011) – by means of the medical history, physical examination, complete blood count, peripheral blood smear, platelet counts between 10 and 50 × $10^9$/l, normal or slightly increased plasma thrombopoietin levels (as described by Folman *et al*, 1997) and did not receive treatment for at least 3 months. The direct MAIPA produced positive reactions for 51/60 (85%) of these known ITP patients (Fig 1, Table I). The PIFT (direct + eluate) produced positive reactions in only 39 (65%) of these samples. Most autoantibodies were directed against GPIIb/IIIa, GPIb/IX and/or GPV. None of the samples had autoantibodies exclusively directed against GPIa/IIa and/or GPIV detected (Table SII).

For 178/204 (86%) thrombocytopenic patients suspected for ITP, the MAIPA was performed without knowledge of the clinical diagnosis. For 26 of these patients, all with a platelet count <10 × $10^9$/l, an insufficient number of platelets could be isolated. Clinical data was obtained for 165 of the remaining 178 tested patients (Figure S1). ITP was excluded based on the clinical data in 76 of these 165 patients (46%) with a mean platelet count of 104 × $10^9$/l (range 9–386, SD ± 71) and a mean plasma thrombopoietin (TPO) level of 106 arbitrary units (AU)/ml (range 5–956, SD ± 166; Figure S2). In 25 of these patients, the platelet counts never fell below 100 × $10^9$/l (Table SIB). Seventy-four of these 76 patients (97%) had a negative direct MAIPA result (Fig 1). Of the two patients with a positive direct MAIPA result, one, suffering from autoimmune haemolytic anaemia; showed GPV (CD42d)-bound platelet autoantibodies (OD 0·199), PIFT negative. The other patient, diagnosed with EDTA-dependent pseudothrombocytopenia (platelet counts in EDTA- and citrate-anticoagulated blood of 60 and 109 × $10^9$/l, respectively) showed GPV- and GPIb/IX-bound platelet autoantibodies (OD 0·176 and 0·186, respectively); the direct PIFT was weak positive for IgM only; the eluate PIFT was negative.

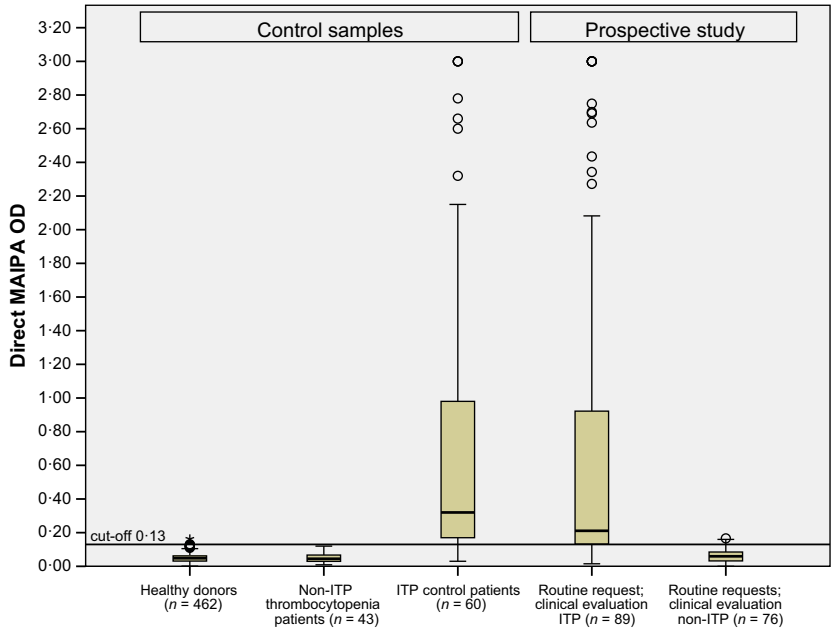


**Fig 1.** Direct MAIPA results for ITP patients and controls. Direct MAIPA OD above 0·13 is considered positive. Control samples: historically well characterized ITP patients. Prospective study: requests for serological ITP diagnostics, after final clinical evaluation classified as ITP or non-ITP. ITP, immune thrombocytopenia; MAIPA, monoclonal antibody immobilization of platelet antigens assay; OD, optical density. [Colour figure can be viewed at wileyonlinelibrary.com]

**Table I.** Test results for: healthy donors ($n$ = 462), ITP patients ($n$ = 60), non-ITP patients ($n$ = 43) and prospective requests for ITP diagnostics ($n$ = 165).

| | Direct MAIPA positive Direct PIFT* positive $n$ (%) | Direct MAIPA positive Direct PIFT* negative $n$ (%) | Direct MAIPA negative Direct PIFT* positive $n$ (%) | Direct MAIPA negative Direct PIFT* negative $n$ (%) |
|---|---|---|---|---|
| Controls ($n$ = 545) | | | | |
| Healthy donors ($n$ = 462) | 0 | 1 (0·2) | 6† (1·3) | 455 (98·5) |
| Non-ITP ($n$ = 43) | 0 | 0 | 16 (37) | 25 (58) |
| ITP ($n$ = 60) | 39 (65) | 12 (20) | 4 (7) | 5 (8) |
| Routine requests ($n$ = 165) | | | | |
| ITP ($n$ = 89) | 60 (67) | 9 (10) | 2 (2) | 18 (20) |
| Non-ITP ($n$ = 76)‡ | 2 (3) | 1 (1) | 26 (34) | 47 62) |
| Total group ($n$ = 268) | | | | |
| ITP ($n$ = 149) | 99 (66) | 21 (14) | 6 (4) | 23 (15) |
| Non-ITP ($n$ = 119)‡ | 2 (2) | 1 (1) | 44 (37) | 72 (61) |

ITP, immune thrombocytopenia; MAIPA, monoclonal antibody immobilization of platelet antigens assay; PIFT, platelet immunofluorescence test.
*PIFT: direct PIFT + eluate PIFT.
†All six positive results were due to antibodies of the IgM class.
‡By clinical data analysis, ITP could be excluded for 76 of the 165 patients, initially suspected for ITP.

In 89 of the 165 tested samples, the diagnosis of ITP was clinically made, with a mean platelet count of 45 × $10^9$/l (range 8–171, SD ± 34·7). A mean plasma TPO level of 38 AU/ml (range 4–381 SD ± 62 AU/ml; Figure S2) was found. The direct MAIPA was positive for 69/89 (78%) of these patients (Fig 1, Table I).

Platelet -associated antibodies of the IgG (and/or IgM) class were detected by direct PIFT in 62/89 (70%) suspected ITP patients; for two of these, no antibodies were detected by the direct MAIPA.

Overall, the direct MAIPA correlated with the clinical diagnosis of ITP with a sensitivity of 81% [95% confidence interval (CI), 73–87%], and a specificity of 98% (95% CI, 94–100%). A positive predictive value of 98% (95% CI, 94–100%) for clinical ITP and a negative predictive value of 80% (95% CI, 72–86%) were obtained.

The direct MAIPA has two limitations. First, approximately 16% of the samples referred for routine ITP diagnostics had insufficient isolated patient platelets to perform a direct MAIPA. Second, no autoantibodies were detected in approximately 20% patients suspected for ITP. Intriguingly, this lack of antibodies might still be considered as an immune-dependent thrombocytopenia i.e. caused by T cell autoimmunity. Additional research in these clinically typical ITP patients without detectable antibodies should reveal the nature of such thrombocytopenias.

Notwithstanding the limitations, the advantages of the direct MAIPA assay are many-fold. Next to its value for enabling a much more reliable ITP diagnosis, the presence and further characterization of the glycoprotein specificity of platelet autoantibodies by the direct MAIPA may be correlated with the severity of bleeding symptoms and additionally lead to a more personalized ITP therapy. For instance, autoantibodies blocking the fibrinogen binding-site of GPIIb/IIIa were associated with more severe bleeding in ITP (De Cuyper *et al*, 2013). Furthermore, platelet autoantibodies binding to platelet GPIb/IX have been shown to induce desialylation of GPIb/IX and, as such, more prevalent destruction of the platelets by the Ashwell-Morell receptor of hepatocytes (Li *et al*, 2015). If so, such findings would make intravenous Ig treatment and splenectomy less likely to be effective. Third the inhibitory effect of platelet autoantibodies on compensatory thrombocytopoiesis might also depend on the glycoprotein specificity of the antibodies and lower response of these patients to TPO analogues (Iraqi *et al*, 2015). Finally, in our assays, the observed changes in antibody presence might steer the continuation, tapering or termination of treatment. Our recently described association of antibody presence and lowering thereof after rituximab nicely underlines the value of our assays (Porcelijn *et al*, 2017).

We conclude that the direct MAIPA not only enables a more reliable diagnosis of ITP but may also help in the choice and continuation of therapy i.e. by monitoring the immune activity in ITP during long term TPO analogues. This will be indispensable for more personalized treatment algorithms for ITP.

## Authorship

L.P. conceptualized and designed the study, conducted the data analysis and statistical analysis, drafted the initial manuscript, and approved the final manuscript as submitted. E.H. and G.O. coordinated and supervised data collection, critically reviewed the manuscript and approved the final manuscript as submitted. M.S. reviewed and revised the manuscript and approved the final manuscript as submitted. J.J.Z. and M.de H. supervised the study, conceptualized and co-drafted the initial manuscript, and approved the final manuscript as submitted.

## Conflict of Interest

None of the authors has a financial conflict of interest.

## Key point

A reliable assay for the detection of platelet autoantibodies to diagnose ITP.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Correlation direct MAIPA results and clinical diagnosis.

**Figure S2.** Platelet counts versus plasma Tpo levels for routine request patients.

**Table SI**. (A) Non-ITP thrombocytopenic patients (n=43). (B) Routine ITP serology request patients, ITP excluded* (n=76).

**Table SII.** (A) Pattern of reactivity of platelet autoantibodies in ITP samples, as determined with the direct MAIPA. (B) Platelet autoantibody reactivity in ITP samples as tested for five platelet glycoproteins with the direct MAIPA.

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