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Recommendations for the implementation of platelet autoantibody testing in clinical trials of immune thrombocytopenia

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Introduction

The utility of platelet autoantibody testing in the management of patients with immune thrombocytopenia (ITP) remains uncertain. Because of low sensitivity reported in prospective studies [1–3], the routine use of platelet autoantibody testing has not been endorsed in recent evidence-based guidelines [4]; however, the 'ITP syndrome' [5], which includes primary and secondary forms, is a disorder that may comprise groups of patients with distinct clinical and serological profiles. To better understand the diagnostic, prognostic and pathogenic role of platelet autoantibodies in ITP, further systematic evaluation is required.

An efficient way to study platelet autoantibodies in ITP is to incorporate testing into the design of large clinical trials. Multinational proficiency testing has shown that most laboratories can readily identify platelet alloantibodies from coded samples [6]; however, platelet autoantibodies may be more difficult to discern. In addition, large-scale implementation would require standard methods of sample collection, processing and testing. This official report of the Platelet Immunology Scientific Subcommittee of ISTH describes a standardized approach to the implementation of platelet autoantibody testing in ITP clinical trials.

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Disclosure of Conflict of Interests
The authors state that they have no conflict of interest.

Methods

Conception of this project

After discussions among trial investigators and members of the Platelet Immunology SSC, it was agreed that methods of implementing platelet autoantibody testing in clinical trials needed to be addressed as an SSC standardization project.

Item generation

A preliminary list of key elements was drafted by the authors and then discussed with all members of the Platelet Immunology SSC in person and by written communication. After discussion, five key questions were identified by the committee: (1) Which anticoagulant should be used for sample collection? (2) What is the minimum volume of sample collection? (3) What method of testing should be employed? (4) Which monoclonal antibody should be used? (5) Should testing be carried out at a central laboratory?

Reaching consensus

Preliminary recommendations were developed through discussions held in an open forum at the SSC meeting in Cairo in 2010. A preliminary draft was circulated to SSC committee members for feedback. Final consensus was achieved after a follow-up group discussion at the Kyoto 2011 meeting. The authors collated the consensus recommendations into this manuscript (Table 1).

Recommendations

(1) Which anticoagulant should be used for sample collection?

Recommendation:acid citrate dextrose solution-A(ACD-A).

ACD-A was the preferred anticoagulant for platelet autoantibody sample collection. ACD is efficient at preserving platelets over several days and thus is suitable for sustaining samples during transit. Other anticoagulants were felt to be less suitable: sodium citrate allows platelets to activate and reduces the platelet yield; ethylenediaminetetraacetic acid is associated with platelet shape change, altered glycoprotein integrity and reduced platelet harvest due to red cell changes; and heparin is known to activate platelets.

(2) What is the minimum volume of sample collection?

Recommendation: 30 mL for adults; 10 mL for children.

A volume of whole blood collection of 30 mL for adults and 10 mL for children was recommended for most platelet autoantibody assays. These volumes of collection were felt to be sufficient to ensure an adequate platelet yield even for patients with severe thrombocytopenia without exceeding acceptable norms. Additional studies would be of value to confirm the suitability of this sample volume for patients with extreme thrombocytopenia (platelet count less than 5×10^9 per L).

(3) What method of testing should be employed?

Recommendation: a direct test that detects glycoprotein-specific antibodies.

Direct tests for platelet autoantibodies, (which measure antibodies on platelets), have higher sensitivity than *indirect* tests, (which measure antibodies free in plasma), and should be used in ITP trials. The method of testing should also be able to detect glycoprotein-specific antibodies, using assays such as monoclonal antibody-specific immobilization of platelet antigen (MAIPA) (2;3), monoclonal antigen capture ELISA (MACE) or variations thereof (1;2). These assays have higher specificity than platelet-associated IgG detected by flow cytometry [7]. Assays using platelet eluates may also be possible [8], but require further validation.

For the MAIPA assay, intact platelets are incubated with an antiplatelet monoclonal antibody (MAb), lysed, then immobilized by a capture antibody in a microtitre well. The amount of IgG bound to platelet glycoprotein is quantified by optical density (OD). A modification of the MAIPA with similar performance characteristics involves the formation of platelet lysates first, followed by incubation with MAb [2]. One advantage of this approach is that platelet lysates can be frozen for batch testing, permitting reporting of relative OD readings across samples and improved standardization across laboratories. The committee felt that assays allowing for batch testing were preferred, but did not recommend one test method over another. Additional quality assurance testing may be needed to determine acceptable storage times of frozen platelet lysates.

(4) Which monoclonal antibody should be used?

Recommendation: anti-GPIIb and anti-GPIIIa, or anti-GPIIbIIIa; and anti-GPIbIX.

To detect platelet glycoprotein-specific antibodies, MAbs specific for GPIIb, GPIIIa or the GPIIbIIIa complex and MAbs specific for GPIbIX are recommended. The use of MAbs to each of the GPIIb and GPIIIa subunits will avoid false-negative results due to competition with autoantibodies. MAbs to the intact GPIIbIIIa complex have also been used successfully [9]. While many commercial and in-house MAbs are available, the committee agreed that the same MAbs should be used across test centers or by the central laboratory. Members of the SSC found the following MAbs to be useful for platelet autoantibody testing in ITP: *GPIIb antibodies* –SZ22; *GPIIIa antibodies* – SZ21, AP3; *GPIIbIIIa antibodies* –Gi5, AP2, Raj-1; *GPIbIX antibodies* – AP1, FMC25, TW-1, Beb-1.

(5) Should testing be carried out at a central laboratory?

Recommendation: yes

Centralization of platelet autoantibody testing for clinical trials will reduce variability across laboratories. This might mean that fresh samples would have to be sent to a central laboratory for processing and freezing (lysates) within 3 days of sample collection. For large international trials, more than one central laboratory may be necessary to avoid prolonged transit times and cross-border delays. The preparation and freezing of platelet lysates locally would avoid the need to ship fresh samples, which can be logistically challenging and

expensive; however, local staff would have to be adequately trained in specialized test methods.

Feasibility

Recent experience has shown that implementation of platelet antibody testing in a mutlicenter clinical trial is feasible. In a 60-patient randomized trial of adjuvant rituximab or placebo [10], platelet autoantibody testing was systematically performed at baseline, 1 month, 3 months and 6 months. Thirty-milliliter whole blood samples were collected into ACD-A (trisodium citrate, 22.0 g per L, citric acid, 8.0 g per L, and dextrose 24.5 g per L) and shipped fresh to the central study laboratory, where platelet lysates were prepared and frozen at −70°C until testing. In that trial, 93.1% of 231 samples were successfully collected and processed within 48 h. A modification of the MAIPA assay was used whereby platelet lysates were prepared before the monoclonal antibody was added.

Conclusion

Platelet autoantibody evaluations in large cohorts of well-characterized ITP patients are needed. Clinical trials offer such an opportunity. Such studies will advance understanding of ITP pathophysiology and will clarify the role of platelet autoantibodies in risk stratifying patients, which is especially important in light of the introduction of new therapeutic agents such as rituximab and the thrombopoietin receptor agonists. Implementation of platelet autoantibody testing on a large scale is challenging because of specific sample requirements and the need for centralized testing, but previous experience has shown that it is feasible. This document is meant to serve as a guide for the incorporation of platelet autoantibody testing in future ITP trials.

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Table 1

Consensus recommendations of the Platelet Immunology Scientific Subcommittee for the implementation of platelet autoantibody testing in clinical trials of immune thrombocytopenia

Key element	Consensus recommendations	Rationale
Anticoagulant	ACD-A	ACD-A minimizes platelet loss and prevents platelet activation
Volume of collection	30 mL (adults); 10 mL (children)	Sufficient sample volume is required for adequate platelet yield even in patients with severe thrombocytopenia.
Test method	Direct glycoprotein-specific assay (e.g. MAIPA, MACE)	Direct tests are more sensitive than indirect tests; glycoprotein-specific assays are more specific than PAIgG
Monoclonal antibody	Commercial or in-house anti-GPIIb and anti-GPIIIa, or anti-GPIIbIIIa; *and* anti-GPIbGPIX	The majority of antibodies are directed against GPIIbIIIa or GPIbIX. Sample size, platelet yields, monoclonal antibody availability and cost limit routine testing of other glycoproteins
Centralized testing	Yes	Centralization of processing and testing is necessary to reduce variability across laboratories

ACD-A, acid citrate dextrose solution-A; MAIPA, monoclonal antibody-specific immobilization of platelet antigen assay; MACE, monoclonal antigen capture ELISA; PAIgG, platelet-associated IgG.