PLATELET ASSOCIATED AUTOANTIBODIES AS DETECTED BY A SOLID-PHASE MODIFIED ANTIGEN CAPTURE ELISA TEST (MACE) ARE A USEFUL PROGNOSTIC FACTOR IN IDIOPATHIC THROMBOCYTOPENIC PURPURA

by

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Running title: platelet autoantibodies in ITP

Key Words: ITP, platelet, autoantibodies, PAIgG, antigen capture assay, MACE, clinical worsening-free survival

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ABSTRACT

50 consecutive ITP adult patients (platelet count < 100 x 10^9/L) were grouped according to positivity or negativity of a solid-phase modified antigen capture ELISA (MACE) against GPIIb/IIIa, Ib/IX and IIa/IIIa. Observation started on the day of MACE assay and lasted at least six months. Clinical worsening was defined as the need for starting or modifying therapy because of thrombocytopenia < 20 x 10^9/L or patient admission due to bleeding symptoms.

MACE-positive patients had a higher probability of clinical worsening than MACE-negatives (p<0.004). The proportion of patients worsening was 18/25 (72%) among MACE-positives and 8/25 (32%) in MACE-negatives. The median time to clinical worsening was 2.1 months for MACE-positive patients and 27.7 months for MACE-negatives.

The assay of specific platelet autoantibodies may be a useful prognostic tool for the clinical course of ITP.
INTRODUCTION
The diagnosis of immune thrombocytopenia, both primary and secondary, is generally accepted to be one of exclusion. However, three prospective studies indicate that when platelet autoantibodies are detectable, they have a highly positive diagnostic value for immune thrombocytopenia. However the utility of platelet autoantibodies assay in the clinical course of ITP is still a matter of debate. Several studies showed that platelet autoantibodies are associated with the severity of the disease. Likewise reduction or even disappearance of autoantibodies was reported during pharmacological therapy or splenectomy.

We then found it useful to verify by a prospective cohort follow-up study the possible correlation between the presence or absence of platelet autoantibodies, as detected by a solid-phase modified antigen capture ELISA test (MACE), and the clinical course of ITP.

PATIENTS AND METHODS

Cohort
50 consecutive ITP adult patients were referred to us between March 1998 and June 2002, 43 primarily and 7 after the diagnosis of ITP had been made elsewhere. At the time of enrollment, 8 patients were on therapy (steroid, danazol and azathioprine, each alone or in combination); 3 had previously undergone splenectomy and 39 had no treatment. Three patients had received platelet concentrates for active bleeding and/or severe thrombocytopenia 3-12 months prior to enrollment.

The diagnostic criteria for ITP were the following: a) acquired and isolated thrombocytopenia with platelet count ≤ 100 x 10⁹/L; b) normal or increased number of bone marrow megakaryocytes; c) normal spleen size; d) exclusion of secondary immune and drug-related thrombocytopenia; e) exclusion of laboratory features of EBV, HBV, HCV, HIV infections; f) normal thyroid function test.
ITP was classified as acute or chronic depending on whether it persisted for more or less than 6 months since diagnosis. Thrombocytopenia was defined as mild for platelet count between 50 and 100 x 10⁹/L, moderate between 20 and 50 and severe below 20 x 10⁹/L.

All patients had a thorough medical history, underwent complete physical examination and gave informed consent to the study.

**Laboratory determination of specific platelet associated autoantibodies**

A solid-phase modified antigen capture ELISA was used for the detection of specific platelet-associated autoantibodies against glycoproteins IIb/IIIa, Ib/IX and Ia/IIa (MACE Auto, GTI, Brookfield, WI, USA), as previously described.11

MACE assay was performed in all patients independently on their treatment. The result of MACE assay was unknown to the clinicians until the end of the study, thus being not used for diagnostic or treatment purposes. Patients were categorized as MACE-positives and MACE-negatives according to the presence or absence of demonstrable specific platelet-associated autoantibodies.

**Clinical outcome and follow-up**

For each patient, the observation period started on the day of the assay of platelet autoantibodies and lasted at least six months.

The pharmacological regimens of ITP were the following: oral prednisone 1 mg/kg/d for 4-6 weeks tapered until the minimal useful dose to maintain platelets above 20 x 10⁹/L; intravenous immunoglobulins (IVGG) 0.4 mg/kg over 3-5 consecutive days; danazol 400 mg/d for at least 30 consecutive days; azathioprine 100 mg/d for at least 6 weeks tapered to 50 mg/d. Treatment was begun or modified whenever platelet count was < 20 x 10⁹/L and/or the patient complained of major bleeding (epistaxis requiring nasal packing, menometrorrhagia, hematuria, gastro-intestinal tract bleeding, cerebral bleeding, or hemorrhages requiring red cell and/or platelet transfusions) or
minor mucocutaneous bleeding associated with thrombocytopenia < 10 x 10⁹/L.
Clinical worsening (CW) of thrombocytopenia was defined as the need for starting or modifying therapy or for hospital admission due to major or minor bleeding associated with thrombocytopenia < 10 x 10⁹/L as above described.

**Statistical analysis**
The Mann-Whitney and chi-square test were used to compare means and proportions. The cumulative proportion of overall CW-free survivors as a function of time was calculated by the Kaplan-Meier estimator.

**RESULTS**

**Platelet associated autoantibodies**
Twenty-five patients (50%) had demonstrable specific platelet associated autoantibodies (MACE-positives) and 25 had no autoantibodies (MACE-negatives).
10 out 25 (40%) of demonstrable platelet autoantibodies were directed against GPIIb/IIIa, 7 (28%) against GPIb/IX and 8 (32%) against both glycoproteins. Anti-GP1a/IIa antibodies were present in two patients together with the anti-GPIIb/IIIa and anti-GPIb/IX antibodies.
MACE-positives and negatives were comparable for age, gender, platelet count, disease duration and therapy regimens (Table 1). Twelve patients (9 MACE-positive and 3 MACE-negative) had acute ITP at the time of enrollment, and 38 patients (16 MACE-positive, 22 MACE-negative) had chronic ITP.

**Clinical worsening analysis**
The Kaplan-Meier curve analysis showed that MACE-negative patients had a lower probability of CW than the MACE-positive ones (p<0.004, Figure 1A). CW probability was lower in MACE-negative patients even when considering those with a platelet count lower than 50 x 10⁹/L (14/25 vs. 18/25), (p<0.05, Figure
or those with chronic thrombocytopenia at the time of study start (22/25 vs. 16/25), (p=0.01, Figure 1C).
The proportion of patients with CW was 72% (18 patients) among MACE-positives and 32% (8 patients) among MACE-negatives. The median time to CW was 2.07 months (range, 0.03 to 47.21) for MACE-positive patients, and 27.35 months (range, 0.03 to 41.98) for MACE-negatives after enrollment.
The specificity of platelet autoantibodies was 7/18 (39%) anti-GPIIb/IIIa, 4/18 (22%) anti-GPIb/IX, 7/18 (39%) toward both glycoproteins in those with CW and 3/7 (43%) anti-GPIIb/IIIa, 3/7 (43%) anti-GPIb/IX, 1/7 (14%) toward glycoproteins IIb/IIIa, Ib/IX, Ia/IIa in those without CW.
Among the 18 MACE-positive patients who had CW, 11 had hospital admission related to thrombocytopenia, 2 had therapy changed and 5 were started on therapy because of progressive falling of platelet count. All 3 MACE-positive patients on therapy at enrolment had CW.
Among the 8 MACE-negative patients with CW, 4 had hospital admission related to ITP, 1 had therapy changed and 3 were started on therapy because of severe thrombocytopenia. 3 out of 5 MACE-negatives on therapy at the time of enrollment and those who had undergone splenectomy had CW.

DISCUSSION
In spite of evidence of the role of platelet autoantibodies as detected by phase III assays, they are so far considered unnecessary in the setting of immune thrombocytopenic purpura (ITP).
In our series, thrombocytopenia recurred in 72% of MACE-positives and only in 32% MACE-negatives after a mean time of 2.1 and 27.7 months, respectively. Platelet autoantibodies were more frequently found in patients with active ITP and a relationship has been reported between their level and response to therapy. The effect of therapy on the following clinical course cannot be evaluated in our series due to the low number of patients receiving drugs prior to enrollment.
Since the degree of thrombocytopenia and its short duration may also affect the clinical course of ITP, we analysed also subgroups of patients with
moderate-severe thrombocytopenia and patients with chronic disease at enrollment. Such further analysis validates the higher incidence of CW in patients with detectable platelet autoantibodies.

In accord with previous reports, 3,11,13,14 40% of demonstrable platelet autoantibodies were against GPIIb/IIIa, 28% were against GPIb/IX and 32% were against both antigens in our series. Finally, it has been suggested that antibody specificity might help predicting the course of ITP: in fact, patients with antibodies against GPIb/IX or multiple antigens have been reported to suffer from more severe bleeding symptoms, lower platelet counts and a poor response to steroids. 6,7,15 In our series, the specificity of autoantibodies was not correlated with the clinical outcome, but this can be due to the relatively low number of our patients.

In conclusion, this study seems to support for the first time evidence that ITP patients with platelet autoantibodies, as detected by MACE, have a CW of thrombocytopenia more frequently and sooner than patients without autoantibodies.
REFERENCES


Table 1. Characteristics of MACE positive and MACE negative patients

<table>
<thead>
<tr>
<th>FEATURES</th>
<th>MACE positives</th>
<th>MACE negatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td><strong>Males/Females</strong></td>
<td>6/19</td>
<td>10/15</td>
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<tr>
<td><strong>Median Age, yr (range)</strong></td>
<td>48 (18-79)</td>
<td>54 (22-83)</td>
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<tr>
<td><strong>Mean Platelet count (x 10^9/L)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-20 x 10^9/L</td>
<td>9 (36)</td>
<td>5 (20)</td>
</tr>
<tr>
<td>21-50 x 10^9/L</td>
<td>9 (36)</td>
<td>9 (36)</td>
</tr>
<tr>
<td>51-100 x 10^9/L</td>
<td>7 (28)</td>
<td>11 (44)</td>
</tr>
<tr>
<td><strong>Mean Duration of disease (months)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>11</td>
<td>51</td>
</tr>
<tr>
<td>&lt; 6 months</td>
<td>9 (36)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.3±1.9</td>
<td>1.5±1.2</td>
</tr>
<tr>
<td>&gt; 6 months</td>
<td>16 (64)</td>
<td>22 (88)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>61±54</td>
<td>61 ± 36</td>
</tr>
<tr>
<td><strong>Patients on therapy at sampling</strong></td>
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<tr>
<td>no. (%)</td>
<td>3 (12)</td>
<td>5 (20)</td>
</tr>
<tr>
<td><strong>Previous splenectomy</strong></td>
<td>1 (4)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>no. (%)</td>
<td>2 (8)</td>
<td>1 (4)</td>
</tr>
<tr>
<td><strong>Previous PC infusion</strong></td>
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* Mean ± SD
Figure 1A. Kaplan-Meier curves of MACE positive and MACE negative patients

![Kaplan-Meier curves](image)

Log-Rank p = 0.0036

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Figure 1B. Kaplan-Meier curves of MACE positive and MACE negative patients with platelet count ≤ 50 x 10⁹/L at the time of enrolment.
Figure 1C Kaplan-Meier curves of MACE positive and MACE negative patients with chronic thrombocytopenia at the time of enrolment

Log-Rank $p = 0.01$
Legend for the figures

Figure 1
Cumulative proportion of clinical worsening among the cohort of patients (Kaplan-Meier estimate) and the subgroups.
The estimate curve of all patients (A) shows in MACE positive patients a higher probability of clinical worsening than that of MACE negatives.
The same results are obtained also by the estimate curves of subgroup of patients with a platelet count lower than 50x10⁹/L at the enrollment (B), and considering only the 38 patients with chronic form of ITP (C).
Platelet associated autoantibodies as detected by a solid-phase modified antigen capture ELISA test (MACE) are a useful prognostic factor in idiopathic thrombocytopenic purpura

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