Antiplatelet antibody testing in thrombocytopenic pregnant women

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OBJECTIVE: The purpose of the study was to attempt to distinguish pregnant women with gestational thrombocytopenia from those with idiopathic immune thrombocytopenia by eight different platelet antibody assays.

STUDY DESIGN: Sera from pregnant women with presumed gestational thrombocytopenia ($n = 160$) and idiopathic immune thrombocytopenia ($n = 90$) were prospectively tested for indirect and platelet-associated immunoglobulins G and M and complement C3, as well as for serotonin release. After the results were analyzed, a subset of patients were subsequently analyzed for circulating antiplatelet antibody directed against platelet membrane glycoprotein GPIIb/IIIa.

RESULTS: Indirect immunoglobulin G was significantly greater in the 85 women with idiopathic immune thrombocytopenia than in the 129 women with gestational thrombocytopenia ($p < 0.001$). Platelet-associated immunoglobulin G was elevated in the majority of women, both those with gestational thrombocytopenia and those with idiopathic immune thrombocytopenia. There were also no statistically significant differences in the values for platelet-associated C3 or indirect immunoglobulin M and C3. Levels of platelet-associated immunoglobulin M showed a tendency to be higher in women with gestational thrombocytopenia ($p = 0.04$), as did the values in the serotonin release assay ($p = 0.06$).

CONCLUSION: Our data demonstrate that patients with gestational thrombocytopenia had surprisingly high levels of platelet-associated immunoglobulin despite mild thrombocytopenia. Comparison of a relatively large number of patients with idiopathic immune thrombocytopenia and gestational thrombocytopenia indicates that women with idiopathic immune thrombocytopenia cannot be distinguished from those with gestational thrombocytopenia by means of one or more of the prototypic platelet antiglobulin tests currently in use. Our preliminary data with glycoprotein-specific assays indicate that they may be more useful. (Am J Obstet Gynecol 1996;174:1014-8.)

Key words: Thrombocytopenia, pregnancy, antiplatelet antibody, platelet autoimmunity

The two most common causes of thrombocytopenia during pregnancy are chronic idiopathic thrombocytopenia purpura (ITP) and gestational thrombocytopenia.[1] The distinction between these two entities is important because the pregnancies of mothers with ITP are complicated by severe neonatal thrombocytopenia (platelet count <50,000/μl) in 9% to 15% of all cases. Among infants of mothers with ITP, neonatal intracranial hemorrhage has been identified in 2 of 88[2] and 0 of 61[3] cases for a collective incidence of 1% to 2%.[4] In contrast, women with gestational thrombocytopenia had only 1 in 488 reported pregnancies complicated by severe neonatal thrombocytopenia,[1, 2, 5-11] and no neonate has been described who had an intracranial hemorrhage. Therefore the identification before birth of which neonates of thrombocytopenic mothers will be severely thrombocytopenic has as its first step the distinction of gestational thrombocytopenia from ITP.

Unfortunately the distinction between these two entities is not always apparent. There are three generally agreed on diagnostic criteria for gestational thrombocytopenia. Gestational thrombocytopenia is diagnosed in (1) women without a history of thrombocytopenia, (2) women who are asymptomatic, and (3) women in whom mild thrombocytopenia (platelet counts between 70 to 100 and 150×10^9/L) is detected, usually during the third trimester of an uncomplicated pregnancy.[12] A fourth criterion is that this thrombocytopenia is expected to resolve post partum and that the neonate will have a normal platelet count.[8] In contrast, women with ITP have a his-

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Table I. Platelet-antiglobulin tests in pregnant thrombocytopenic patients

	*Indirect IgG**	*Anti-GP IIB/IIIa†*	*Platelet-associated IgG**	*Platelet-associated C3**	*Platelet-associated IgM**	*Indirect C3**	*Indirect IgM**	*Serotonin release*
ITP	(n = 85)	–	(n = 79)	(n = 65)	(n = 55)	(n = 2)	(n = 8)	(n = 5)
≤2 SD	12	–	28	34	32	2	1	4
2-5 SD	35	4/12	23	22	14	0	3	1
≥5SD	38	–	28	9	9	0	4	0
Gestational	(n = 121)	–	(n = 118)	(n = 89)	(n = 116)	(n = 28)	(n = 74)	(n = 39)
≤2 SD	48	–	36	41	51	13	20	17
2-5 SD	44	1/28	36	23	27	11	22	9
≥5 SD	29	–	46	25	38	4	32	13
Significance	p = 0.001	p = 0.022	p = 0.24	p = 0.09	p = 0.04	p = 0.11	p = 0.86	p = 0.06

*Values are expressed as Number of patients within listed range of standard deviations from mean of normal controls (normal, low-positive, high-positive).

†Values are expressed as ratio of patients with positive results in this assay over total number tested.

tory of thrombocytopenia antedating the pregnancy and have symptoms including petechiae, bruises, epistaxis, and other signs and symptoms of bleeding with isolated thrombocytopenia (typically marked, $<50{,}000 \times 10^9$/L). ITP will persist post partum and, as discussed, is associated with neonatal thrombocytopenia in a percentage of cases.

Notwithstanding these criteria it can be difficult to diagnose gestational thrombocytopenia in certain settings. In some women a previous platelet count is not known or there are bleeding symptoms that may or may not be unrelated to the thrombocytopenia. More critical is that the platelet count separating ITP from gestational thrombocytopenia has been analyzed only at the time of delivery. Therefore it would be desirable to have a simple method by which ITP and gestational thrombocytopenia could be distinguished, for prediction not only of neonatal thrombocytopenia but also of the maternal course. Unfortunately the only currently reliable antepartum means of identifying fetal thrombocytopenia is fetal blood sampling.[13] Fetal blood sampling has small but significant risks of fetal morbidity and mortality that almost certainly exceed the risk of managing all ambiguous patients as if they had ITP. In the current study we used eight different platelet antibody measurements to determine whether any one or a combination of tests can be used to distinguish women with gestational thrombocytopenia from those with ITP.

Material and methods

Patients. During the period from 1979 to 1991, 250 consecutive pregnant women from the Hospital of the University of Pennsylvania and the New York Hospital–Cornell Medical Center with otherwise unexplained thrombocytopenia were included in this study. Except for the assay detecting circulating antiplatelet antibody directed against specific platelet membrane glycoprotein, all platelet antibody testing was performed at the time of the patient's entry into the study. Approval from the institutional review boards was obtained before inclusion. The clinical and serologic features of 162 of these patients were previously reported.[2] Patients with pregnancy-induced hypertension, disseminated intravascular coagulation, systemic lupus erythematosus, or other systemic illness were excluded as were patients taking medications known to cause thrombocytopenia. Of the 90 patients with ITP, 86 had a history of ITP antedating the pregnancy, whereas 4 had repeated platelet counts <70,000/μl, including at the time of delivery and for at least 3 months post partum. Examination of the bone marrow was not performed in all patients. One hundred sixty patients were considered to have gestational thrombocytopenia because during pregnancy they had more than one platelet count between 70 and 150×10^9/L without evidence of bleeding. More than 80% of these patients had normal platelet counts documented before pregnancy or during the first trimester. In the remainder of these patients platelet counts were never below 100×10^9/L during pregnancy and returned to normal after delivery. None of these patients with gestational thrombocytopenia were known to have had a thrombocytopenic newborn.

Laboratory testing. Platelet antiglobulin tests were performed in all patients between 32 and 36 weeks' gestation and analyzed at a single laboratory with a radioimmunoassay as previously described.[4, 14, 15] The quantity of platelet-associated immunoglobulin (Ig) G, IgM, and complement C3 bound to platelets and the quantity of platelet-bindable or indirect IgG, IgM, and C3 in plasma were determined by measuring the percentage binding (mean ± SD) of each radiolabeled reagent to the platelets of each patient compared with the results from >200 normal nonpregnant donors and from 26 women in the third trimester of uncomplicated pregnancy studied concurrently. All of the latter were in the normal range determined for the nonpregnant normal control population. In some cases there was insufficient sample to perform all tests, which explains the differing patient numbers in Table I. Fewer patients had determinations of

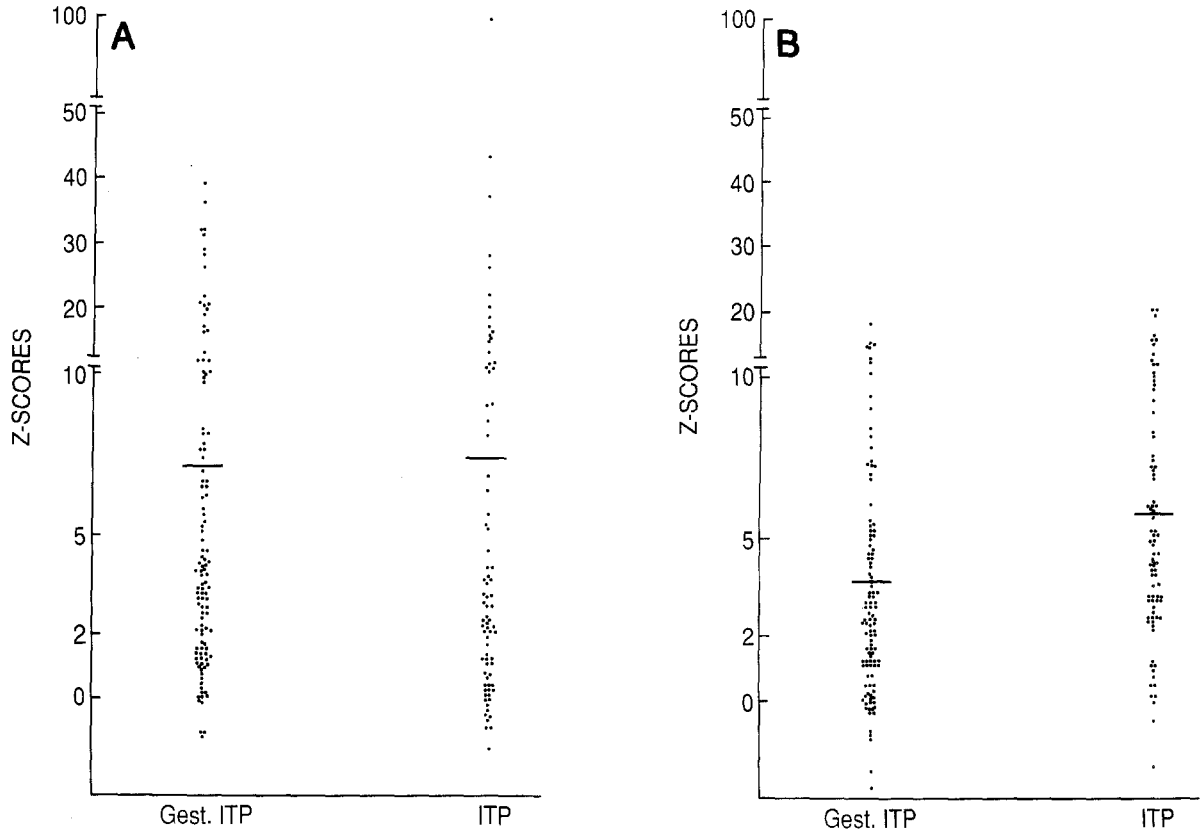

Fig. 1. A, Scatter plot illustrating individual patient z scores (number of standard deviations from the normal mean) of platelet-associated IgG (*y* axis) for gestational thrombocytopenia and ITP patients (*x* axis). Note extensive degree of overlap among individual patients of the two groups. **B,** Scatter plot illustrating z scores of indirect IgG (*y* axis) for gestational thrombocytopenia and ITP patients (*x* axis). Although the mean value of indirect IgG is significantly greater in the ITP patients, a threshold value to indicate to which group a patient would belong could not be established.

indirect platelet-associated C3, indirect platelet-associated IgM, and serotonin release measured because these assays were incorporated into the testing panel only recently. With the use of frozen stored banked samples a subset of patients (12 with ITP and 29 with gestational thrombocytopenia) were subsequently analyzed for circulating antiplatelet antibody directed against platelet membrane glycoprotein GPIIb/IIIa with the modified antigen capture enzyme-linked immunoassay.[16, 17]

Analysis of data. The data for seven of the eight tests (platelet-associated IgG, IgM, and C3; indirect IgG, IgM, and C3; and serotonin release) are expressed as negative (a value ≤2 SD), positive (a value >2 but <5 SD), and highly positive (a value ≥5 SD). Anti-GPIIb/IIIa is expressed as a ratio of those patients who tested positive in this assay to the total number tested. Statistical analyses for platelet-associated IgG, IgM, and C3; indirect IgG, IgM, C3; and serotonin release were performed with the Wilcoxon rank sum test. The results of the anti-GPIIb/IIIa assay were analyzed with the Fisher exact test. Linear regression analysis was used to compare the additive effects of the indirect IgG test with platelet-associated IgM. Statistical significance was defined by a p value of <0.025 in a two-tailed test to correct for the several analyses performed.

Results

Platelet antibody tests were performed on samples from 250 consecutive women with thrombocytopenia in pregnancy including 90 with ITP and 160 with gestational thrombocytopenia. Table I illustrates the results for each of the eight platelet antibodies with the probability of difference between patients with gestational thrombocytopenia and those with ITP.

Platelet-associated IgG was comparably elevated in the majority of women with gestational thrombocytopenia, as well as in those with ITP. There were also no differences in either the frequency or the level of positive results between the two groups (Fig. 1, *A*). Similarly there were no statistically significant differences in the values for platelet-associated C3 or indirect IgM or C3. Levels of platelet-associated IgM showed a tendency to be higher in women with gestational thrombocytopenia ($p = 0.04$) as did the values in the serotonin release assay ($p = 0.06$).

The only statistically significant difference between the two patient groups, other than in the modified antigen capture enzyme-linked immunoassay, was in the level of indirect IgG, which was significantly greater in the 85 women with ITP than in the 129 women with gestational thrombocytopenia ($p < 0.001$). Nevertheless, as shown in Fig. 1, *B*, there was extensive overlap among individual patients of the two groups so that no threshold value could be established that would provide clinically useful resolution between the two populations. Moreover, combinations of test results (i.e., indirect IgG and platelet-associated IgM) provided no greater discrimination of the two groups.

Four of 12 women with ITP had demonstrable antibody to the platelet glycoprotein complex GPIIb/IIIa. In contrast, only 1 of 28 women with gestational thrombocytopenia had detectable anti–GPIIb/IIIa antibody ($p < 0.022$).

Comment

Our current data, derived from a relatively large number of patients with each condition, indicate clearly that women with ITP cannot be distinguished from those with gestational thrombocytopenia with one or more of the currently available prototypic platelet antiglobulin tests. Our results are consistent with previously reported data from several smaller series of elevated levels of circulating and platelet-associated antibodies as depicted in Table II.

While no fewer than 30 patients were studied for any given assay, not all platelet antibody studies were performed with large numbers of patients. Therefore extensive comment cannot be made concerning indirect C3 and indirect IgM in part because of the small sample size. There may be a trend toward significance with increased serotonin release in patients with gestational thrombocytopenia; studies of additional patients are in progress.

Indirect IgG (also known as circulating, serum, or platelet-bindable IgG) was the only assay to demonstrate a significant difference between the two groups. Whereas this difference may provide insight into a pathophysiologic divergence of thrombocytopenia in the two conditions, the overlap among values was too extensive to establish a threshold to discriminate gestational thrombocytopenia from ITP by clinical and standard serologic criteria alone in individual cases. In contrast to the findings with indirect antiplatelet IgG, specific anti-GPIIb/IIIa antibodies were rarely detected in women with gestational thrombocytopenia. Although the incidence in the small sample of pregnant women with ITP studied (4/12) was somewhat below the 50% to 70% incidence generally reported in nonpregnant patients with ITP,[19, 20] there are relatively few published data on anti-GPIIb/IIIa in pregnancy. These initial results, if confirmed with larger numbers of patients and extended to include antigen capture assays with other platelet glycoproteins, suggest the possibility of a better test to discriminate serologically between these two conditions.

Table II. Platelet antibody studies in gestational thrombocytopenia with and without comparison with women having chronic ITP

Study	*Results*
1. Matthew et al.[8]	47 patients with gestational ITP Platelet-associated IgG: Mean 15.8 ng/10^6 platelets (normal 2-10) Platelet-associated IgM: Mean 19.6 ng/10^6 platelets (normal <2.5) Platelet-associated C3: Mean 3.3 ng/10^6 platelets (normal <3.5)
2. Freedman et al.[9]	Results of indirect antibody testing Gestational thrombocytopenia: Total patients 20/22 IgG only 1 IgG plus C3 6 C3 only 13 ITP: Total patients 12/13 IgG only 2 IgG plus C3 9 C3 only 1
3. Hart et al.[10]	Comparison of normal women with 28 women with gestational thrombocytopenia Normal pregnant: Platelet-associated IgG 59% Indirect IgG 59% Gestational thrombocytopenia: Platelet-associated IgG 79% Indirect IgG 61%
4. Kaplan et al.[5]	No. of patients with increased platelet-associated IgG Gestational thrombocytopenia: 10/27 ITP: 10/28
5. How et al.[18]	No. of patients with positive results for indirect IgG antiplatelet antibody Gestational thrombocytopenia 6/15

Previously we suggested that indirect IgG antibodies might help to identify which neonates of mothers known to have ITP before pregnancy would have severe thrombocytopenia.[2, 11] Whereas these findings are related to the finding in this study, here we are comparing ITP to gestational thrombocytopenia.

A related issue raised by the results of this study is why such a high proportion of patients with gestational thrombocytopenia have positive platelet-associated and indirect antiglobulin test results. The answer to these two issues, although interrelated, may be different. We hypothesize that the increase in surface-bound IgG on these platelets is a result of platelet activation.[21] The mechanism by which such activation might occur is unknown but might include thrombin generation or altered interactions between platelets and placental trophoblasts. Regardless of the mechanism, platelet activation may stimulate the immunoglobulin normally contained within the alpha granules to be secreted and expressed

on the cell surface, stimulate the expression of FcγRIIA receptors, and/or expose cryptic determinants to which autoantibodies may develop and bind. The latter hypothesis may help to explain the concomitant elevations in platelet-associated IgM and platelet-associated C3 on the platelets of patients with gestational thrombocytopenia and the presence of circulating antibodies detectable in vitro in platelets that may be activated by handling. In addition, antibodies directed to cryptic, especially internal, platelet determinants could explain why antibodies in the maternal plasma detected in vitro might not bind in vivo to fetal (or maternal) platelets. However, the presence of anti–human leukocyte antigens or other broadly reactive antibodies that would bind to platelets in vitro but minimally if at all in vivo cannot be excluded.

Unfortunately, at this time there is not a simple laboratory test or group of tests that are risk-free and can distinguish ITP from gestational thrombocytopenia. Specifically, fetal blood sampling is not without risk in the thrombocytopenic fetus,[22, 23] and fetal scalp sampling requires a special method that may prevent universal availability.[24] The data presented here show that platelet antiglobulin tests should not be performed in thrombocytopenic patients during pregnancy with the misapprehension that positive results would indicate that the patient has "true" ITP. As a result the intrapartum management of certain thrombocytopenic women remains controversial. Development of tests that are more predictive of fetal thrombocytopenia would be useful.

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