Fetal Thrombocytopenia: A Retrospective Survey of 5,194 Fetal Blood Samplings

By Patrick Hohlfeld, François Forestier, Cécile Kaplan, Jean-Daniel Tissot, and Fernand Daffos

Fetal platelet counts were retrospectively studied in a series of 5,194 consecutive fetal blood samplings (FBS). The mean value was $245 \pm 65 \times 10^9/L$, without significant variation between 17 and 41 weeks' gestation. After exclusion of false thrombocytopenia due to contamination with amniotic fluid, 247 fetuses had platelet counts less than $150 \times 10^9/L$. In 70 cases, thrombocytopenia was due to congenital infectious diseases (toxoplasmosis, rubella, and cytomegalovirus). It was related to immune causes in 45 cases: anti–HPA-1a (n = 23), anti–HPA-5b (n = 2) or possible anti–HLA (n = 2) alloimmunizations, and immune thrombocytopenic purpura (n = 18). Chromosomal abnormality was the etiology in 43 cases (trisomy 13, 18, and 21, Turner’s syndrome, triploidy).

The purpose of this retrospective study is to describe the frequency and causes of thrombocytopenia observed in 5,194 fetal blood samples.

MATERIALS AND METHODS

In this study, 5,215 FBS performed between 1985 and 1992 were considered. Main indications for prenatal diagnosis included infectious diseases, cytogenetic analysis, and hematologic disorders. FBS was performed under ultrasound guidance between 17 and 41 weeks' gestation, without maternal sedation or fetal paralysis.

Hematologic assessment and evaluation of fetal blood samples for purity were performed as previously described. Platelet counts were established within 5 minutes of the sampling on a Coulter S Plus II (Coulter, Hialeah, FL), and values less than $150 \times 10^9/L$ were verified by a review of the peripheral blood smear and confirmed by phase-contrast microscopy using Unopette (Becton-Dickinson, Rutherford, NJ). Fetuses presenting a platelet count less than $150 \times 10^9/L$ were included in the study, because it is the generally admitted value under which thrombocytopenia is diagnosed. When iterative samplings were performed, the lowest platelet count was considered and counted as only one fetus.

All fetal blood samples were checked for contamination with amniotic fluid. When contamination was important, the sample appeared diluted, other hematologic indices were below normal values for gestational age (ie, hemoglobin, red blood cells, hematocrit), and β-human chorionic gonadotropin (HCG) in fetal blood was highly increased. Due to the procoagulant properties of amniotic fluid, slight contaminations were demonstrated by careful examination of blood smear for the presence of platelet aggregates and amniotic cells. Hemostasis parameters also showed abnormalities such as increased factors Vc and VIIc associated with the presence of d-dimers and F1 + 2 fragments of prothrombin. In some cases, the presence of TAT complexes thrombin-antithrombin III was useful. Twenty-one cases were excluded from the study, because thrombocytopenia was due to contamination with amniotic fluid, as demonstrated by activation of the coagulation.

The indication for the prenatal diagnosis of congenital infections (n = 3,088) was primary maternal infection occurring during pregnancy. Classical criteria were used for the prenatal diagnosis of congenital toxoplasmosis, rubella, and cytomegalovirus infections. Briefly, fetuses were considered infected when parasites or viruses could be demonstrated in fetal blood or amniotic fluid, and/or when serologic testing detected specific IgM in fetal blood.

In cases of specific platelet alloimmunization (anti–HPA-1a, anti–HPA-5b; n = 43), the indication for FBS was the previous delivery of an affected child in 29 cases, and a positive family history associated with antigen incompatibility in the mother and the progenitor in 14 cases (anti–HPA-1a alloimmunizations). In two cases, anti–HLA immunization was the presumed cause of neonatal thrombocytopenia in a previous pregnancy.

Standard criteria were used for the diagnosis of maternal immune thrombocytopenic purpura (ITP; n = 81): normal blood counts except for thrombocytopenia, increased platelet volume, normal bone marrow with adequate megakaryocytes, normal red blood cell and white blood cell morphology, normal clotting studies, except for those affected by thrombocytopenia, and no other observed cause for thrombocytopenia, such as other autoimmune diseases, infectious diseases, drug-induced thrombocytopenia, pre-eclampsia, hemolysis, elevated liver enzymes, and low platelet count (HELLP syndrome), or disseminated intravascular coagulation. Additionally, when platelet autoimmune disease was suspected, circulating and platelet-associated IgG were measured using a radioimmunologic assay. In 48% of cases, the diagnosis of ITP was established before the pregnancy, and 32% of the mothers had a previous splenectomy.

The diagnosis of gestational thrombocytopenia (n = 20) was considered when unexpected mild to moderate thrombocytopenia was associated with growth retardation, rhesus disease, or gestational thrombocytopenia in 62 cases. No specific cause for the low platelet count could be established in 27 fetuses (range, 115 to $149 \times 10^9/L$). Severe thrombocytopenia ($\leq 50 \times 10^9/L$) occurred mainly in immune cases (16%), congenital infectious diseases (7%), and chromosomal abnormalities (1%). Diagnosis, prognosis, and management of fetal thrombocytopenia are presented in the different clinical situations. In this series, FBS was never associated with serious bleeding, and no fetal exsanguination was observed.

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developed in otherwise healthy, nonhypertensive pregnant patients without a previous history of ITP.

In most cases, cytogenetic analyses (n = 1,245) were performed after detailed ultrasonographic examination had shown fetal malformations, growth retardation, or both. It was also used to clarify chromosomal mosaicism discovered after amniocentesis or chorionic villi sampling. In some cases, maternal age was the indication in association with other suspected disorders requiring FBS.

Other indications for FBS included prenatal diagnosis of hemophilia A and B, sickle cell anemia, beta- and alpha-thalassemia, other coagulation disorders, and red blood cell alloimmunization.

RESULTS

Normal values for fetal platelet count in these high-risk pregnancies were determined from the whole population (n = 5,194). The mean value ± SD was 245 ± 65 × 10^9/L (95% mean confidence interval, 243 to 247; 5th percentile [P], 138; 10th P, 170; 90th P, 518; and 95th P, 344). There was no significant variation according to the gestational age (correlation coefficient: r = .013, P = .38).

Thrombocytopenia (≤150 × 10^9/L) was demonstrated in 247 fetuses (Fig 1). The main etiologies encountered were fetal infectious disease (n = 70), followed by platelet immune disease (n = 45), chromosomal abnormality (n = 43), and nonchromosomal malformation (n = 31). Miscellaneous causes included intrauterine growth retardation (IUGR; n = 15), rhesus disease (n = 11), and gestational thrombocytopenia (n = 5). In this series, FBS was never associated with serious bleeding, and no fetal exsanguination was observed.

Infections. In congenital infectious diseases (Table 1), thrombocytopenia was present in 36% of cytomegalovirus, 26% of toxoplasmosis, and 20% of rubella cases. The most severe cases occurred with cytomegalovirus (three of eight having platelets <10 × 10^9/L). In congenital toxoplasmosis, nine of 51 had values less than 50 × 10^9/L. Hemostasis was studied in 22 of 51 thrombocytopenic fetuses, and thrombocytopenia was linked to disseminated intravascular coagulation in six cases in which circulating soluble fibrin monomer complexes, fibrin degradation products, and F1 +2 prothrombin fragments could be demonstrated in association with fibrinogen levels less than 0.5 g/L. Severe thrombocytopenia was never demonstrated in congenital rubella syndrome. The only case of congenital varicella syndrome diagnosed in this series had a normal platelet count.

Immune causes. Among cases of immune origin (n = 45), alloimmunization against HPA-1a antigen was the most common cause of thrombocytopenia, demonstrated in 23 of 35 sampled fetuses (66%) (Table 2). Iterative samplings were performed in this group, with a mean of 2.14 FBS per case. Most of the affected fetuses had platelet counts less than 100 × 10^9/L (18 of 35), and 17 had values less than 50 × 10^9/L. The mean value was 51 × 10^9/L (range, 3 to 145 × 10^9/L) (Table 2). Management of alloimmune thrombocytopenia included corticosteroids in seven cases and intravenous gamma globulins (IVIg) in five cases. Predelivery platelet transfusions were performed in 12 cases. The response to medical therapy was variable (platelet counts improving in two of seven fetuses with corticosteroids, and in three of five with IVIg), but the outcome of pregnancy was good, and healthy newborns were delivered in all but one case. This fetus presented severe thrombocytopenia (6 × 10^9/L) as early as 21 weeks’ gestation, and died in utero at 23 weeks. Postmortem examination showed disseminated foci of intracranial hemorrhage. In the other immune cases (anti-HPA-5b or possible anti-HLA alloimmunization), no thrombocytopenia less than 50 × 10^9/L was demonstrated.

**Table 1. Fetal Thrombocytopenia Related to Congenital Infectious Diseases**

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<th>Disease</th>
<th>No.</th>
<th>%</th>
<th>Platelet Count</th>
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<td></td>
<td></td>
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<td>100-150 × 10^9/L</td>
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<td>Toxoplasmosis</td>
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</tr>
<tr>
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<td>Cytomegalovirus</td>
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In ITP, thrombocytopenia was less frequent (18 of 81 fetuses, 22%) and less severe (mean value, 93 × 10^9/L; range, 11 to 148 × 10^9/L), with only three fetuses having values less than 50 × 10^9/L (Table 3). In the beginning of our experience, 22 cases underwent iterative sampling. Corticosteroids were used in eight cases and IVIg in three of our experience, thrombocytopenia was mild (R2 = .43) or platelet-associated IgG and the fetal platelet count (R2 = .84; P = .06). Mean fetal platelet volume was measured in 24 cases, and tended to increase with lower platelet counts (R2 = .257; P = .01).

**Gestational thrombocytopenia.** Maternal gestational thrombocytopenia was the indication for FBS in 20 cases. Most fetuses had normal (n = 15) or slightly decreased (n = 3) platelet counts (range, 130 to 141 × 10^9/L) (Table 3). In two cases, thrombocytopenia was more severe. One fetus sampled at 38 weeks had 55 × 10^9 platelets/L. Maternal thrombocytopenia was mild (108 × 10^9/L), and no platelet-associated IgG or circulating antiplatelet antibodies could be demonstrated. The mother delivered 1 week later, and neonatal thrombocytopenia was confirmed (26 × 10^9/L). No sign of internal bleeding was demonstrated. Prednisone was administered to the mother for 1 month, and her platelet count remained normal during the follow-up period (2 years), but no further investigation could be obtained in this patient. A previous case, considered as gestational thrombocytopenia, was sampled at 41 weeks, and showed a fetal platelet count of 35 × 10^9/L (confirmed after birth). At that time, there was no criteria for an ITP, but the mother was lost to follow-up.

**Chromosomal and nonchromosomal malformations.** Thrombocytopenia is a frequent finding associated with chromosomal abnormalities (Table 4), but is rarely severe (one of 43 fetuses had a platelet count <50 × 10^9/L). It was diagnosed in association with trisomy 18 (86%), trisomy 13 (54%), Turner’s syndrome (31%), and triploidy (3 of 4), whereas it was relatively uncommon in Down’s syndrome (6%).

Thrombocytopenia was diagnosed incidentally in 31 fetuses presenting various nonchromosomal malformations: nonimmune hydrops (n = 10), multiple birth defects (n = 7), cardiac malformation (n = 6), noninfectious hydrocephaly (n = 4), renal malformation (n = 2), and diaphragmatic hernia (n = 1). In one case, bilateral aplasia of the radius was associated with amegacaryocytic thrombocytopenia (thrombocytopenia and absent radius syndrome).

**Miscellaneous.** Fifteen growth-retarded fetuses had thrombocytopenia without evidence of malformation or chromosomal abnormality. This finding was associated with a poor outcome of pregnancy, because only five infants survived. Thrombocytopenia was never isolated, and all fetuses had high erythroblastosis (two cases presenting >1,000 erythroblasts for 100 leukocytes).

FBS was performed in 107 cases of rhesus alloimmunization, and 11 anemic fetuses had mild thrombocytopenia (range, 80 to 137 × 10^9/L).

No specific cause for the low platelet count could be established in 24 otherwise healthy fetuses (platelet values ranged from 115 to 149 × 10^9/L). In two fetuses presenting hemophilia B, and one presenting hemophilia A, the platelet count appeared incidentally decreased (range, 125 to 137 × 10^9/L).

**DISCUSSION**

During FBS, the risk of contamination increases when the needle passes through the amniotic cavity before reaching the umbilical cord, and when sampling is difficult. Assessment of the purity of the samples is essential before diagnosing thrombocytopenia in a fetus, because contamination with amniotic fluid will activate the coagulation. Edetic acid (EDTA) must be used as anticoagulant, and the platelet count should ideally not be delayed beyond 5 minutes. In this study, a reference range for fetal platelet count was established from the whole population. No significant variation was observed during the second and third trimesters of gesta-

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tion, whereas smaller series reported a significant increase between the 15th and 40th week. The risk of complications and fetal death after FBS varies according to the underlying fetal disorder, the gestational age, and the operator’s experience. In low-risk situations, such as prenatal diagnosis of congenital toxoplasmosis, the risk of early fetal loss (within 3 weeks of the sampling) was 0.60%, and the total spontaneous fetal loss was 1.29% in our series of 2,631 consecutive cases. Furthermore, in our experience, no fetal or neonatal death was observed in the case of cases. Moreover, in our experience, no fetal or neonatal death was observed in the case of FBS performed past the 35th week of gestation (n = 365). Sampling should only be considered when the maternal bleeding time is normal and platelet count =50 × 10^9/L. In all situations at risk, pregnant women should be specifically instructed to avoid acetylsalicylic acid and other drugs interfering with platelet function. In this series, thrombocytopenia, however severe, never led to serious funicular hemorrhage after FBS.

Infectious diseases can interfere with the maturation of megakaryocytes and lead to a decrease in platelet production, but an immune origin has also been described. Thrombocytopenia can be related to splenomegaly and the marked pooling of platelets in the enlarged spleen. Finally, intravascular coagulation may also be induced by viremia or parasitemia. Thrombocytopenia is associated with a poor prognosis, and is often found in situations in which termination of pregnancy is discussed. Prenatal thrombocytopenia associated with congenital infections can occasionally be severe and, in most cases, is accompanied by other nonspecific alterations of biologic tests.

The glycoproteins Ib and IIb to IIIa are present, and express the platelet-specific alloantigens as early as 16 weeks’ gestation. explaining the severe thrombocytopenia demonstrated early in pregnancy in the case of alloimmunization. The relatively low percentage (68%) of thrombocytopenic fetuses observed in FBS for HPA-1a alloimmunization is due to the screening of relatives of affected mothers (after phenotyping of maternal and paternal platelets was performed and showed that the fetus was at risk). Among mothers having previously delivered an affected child, the incidence of fetal thrombocytopenia was 95%. FBS can be used to estimate the risk of fetal thrombocytopenia by phenotyping fetal platelets, and to evaluate the need for maternal therapy with IVIg or corticosteroids. Recently, IVIg have been successfully used for direct fetal treatment, but this was not confirmed by other investigators. At the end of pregnancy, in case of failure of the medical treatment to improve the fetal condition, in utero platelet transfusion is considered before the delivery. There is no clear evidence that corticosteroids or IVIg are reliable as prenatal therapy, but in our experience, management including medical therapy, predelivery platelet transfusion, and cesarean section seems to protect from intracranial hemorrhage.

In ITP cases, the incidence of documented fetal thrombocytopenia was 22%, which is similar to that reported by others. Our data confirm that the maternal thrombocytopenia and the level of platelet-associated IgGs do not correlate with the fetal platelet count. The level of circulating antiplatelet antibodies could have a better predictive value, but it is unlikely that an accurate prognosis will be derived from a maternal parameter, when one considers that dizygotic twins born to mothers with ITP can present discordant platelet counts. Moreover, the risk of thrombocytopenia also depends on the capacity of both the fetal megakaryocytes to compensate for the platelet destruction, and of the reticuloendothelial system to destroy IgG-sensitized fetal platelets. Because the efficacy of treatments aimed at the fetus was never demonstrated in controlled studies, FBS should only be used to determine the mode of delivery. When vaginal delivery is considered, pregnant patients with a history of ITP should undergo FBS at 37 weeks’ gestation or at the time of delivery, even when maternal thrombocytopenia is moderate, to exclude severe fetal thrombocytopenia. This approach also seems to decrease the rate of cesarean sections for this indication. When performed by experienced investigators, FBS is a safe and accurate diagnostic tool (in this study, fetal platelet count determined during the third trimester always correlated to the values observed at birth). No complications following FBS for ITP were observed in our series. Some investigators, considering the low risk of severe fetal thrombocytopenia, solely advocate close monitoring of platelet counts after birth. Most authorities allow a vaginal delivery if the fetal platelet count is known to be greater than 50 × 10^9/L, whereas lower counts should be considered as an indication for cesarean section.

Differential diagnosis between gestational thrombocytopenia and ITP is difficult, but is important, because it appears that in gestational thrombocytopenia, the risk of significant fetal thrombocytopenia is much lower when compared with ITP. In this series, three fetuses of patients with apparent gestational thrombocytopenia had slightly decreased platelet counts, and two presented with significant thrombocytopenia. In the latter cases, there was no evidence that the mothers had ITP. A few years ago, we considered

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gestational thrombocytopenia an indication for FBS. Nowadays, the low fetal risk associated with this disorder is well recognized, and FBS is rarely indicated and should only be considered for unexplained maternal thrombocytopenia less than 75 X 10^9/L.28

Fetal thrombocytopenia is common in trisomy 13 and 18, in the Turner syndrome, and in triploidy, but seems less frequent in trisomy 21. In infants, thrombocytopenia has been associated with trisomy 13,24 and 18,34-45 and could be related to a congenital megakaryocyte anomaly. An isolated thrombocytopenia in a fetus undergoing FBS for reasons other than evaluation of chromosomes does not seem to warrant karyotyping analysis, but thrombocytopenia associated with more specific biologic anomalies would appear to warrant karyotyping.46 Thrombocytopenia was demonstrated in other serious malformations with or without intrauterine growth retardation.

Fetal thrombocytopenia associated with IUGR is generally associated with chronic hypoxia, important erythroblastosis, increased gamma-glutamyltransferase, and polycythemia or anemia.42 The mechanism of thrombocytopenia in this condition is unclear, but could be related to fetal consumptive coagulopathy and platelet destruction.48,49 The poor prognosis of IUGR pregnancies with thrombocytopenia has already been described,47,50 and is confirmed in this study (increased risk of intrauterine death, increased perinatal mortality and morbidity).

When present in erythroblastosis fetalis, thrombocytopenia is usually moderate, but platelet counts in the range of 30 to 40 X 10^9/L may occur in severe cases.10,51-53 Platelet destruction is probably due to their interaction with products of red blood cell breakdown associated with hemolysis.54 Platelets may also be reduced by dilution after intrauterine transfusion (for intrauterine transfusions, tightly packed red blood cells are used, and the blood is leukocyte- and platelet-poor).

Significant progress has been accomplished in the diagnosis of thrombocytopenia. Nowadays, investigations are more precise when compared with the techniques previously available. It is then impossible to exclude that some pathologies, discovered several years ago, would have been diagnosed otherwise with the more recent methods. In this retrospective study including 5,194 FBS in high-risk pregnancies, the incidence of fetal thrombocytopenia is 4.75%. On the other hand, considering the normal values for fetal platelets, and the distribution of cases within this study, significant thrombocytopenia should be considered only when the platelet count is less than the mean minus 2 SD (ie, 115 X 10^9/L). With this limit, 121 fetuses would be concerned, and fetuses with unexplained low platelet counts would not be included. Thus, as far as fetal thrombocytopenia is concerned, the limit of 150 X 10^9 platelets/L should be questioned.

The approach to incidental fetal thrombocytopenia diagnosed on FBS includes a careful determination of contamination with amniotic fluid. When thrombocytopenia is associated with other biologic anomalies, suggesting congenital infection or chromosomal defect, specific investigations must be performed. An isolated incidental thrombocytopenia should always lead to investigations regarding an immune cause. Even when another cause of thrombocytopenia appears to be present, a study of alloimmunization is warranted if the thrombocytopenia is more severe than is typical for that etiology. An accurate diagnosis is important because it influences the management to reduce the risk of intracranial hemorrhage and determines the risk of recurrence in a subsequent pregnancy. In most cases, the etiology of thrombocytopenia can be identified, allowing adaptation of the therapy and estimation of the fetal prognosis.

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