Platelet Count in First Trimester of Pregnancy as a Predictor of Perinatal Outcome

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Abstract

AIM: To rule out maternal and pregnancy factors that may contribute to platelet count (PLT) changes in the first trimester of gestation and examine if there is any association between its levels and adverse perinatal outcome.

METHODS: The study population included all patients from the first-trimester visit between 2013-2015 with pregnancy results. Linear multiple regression was constructed to rule out variables that may have a significant contribution to PLT. For each adverse outcome at birth, multiple logistic regression analysis was implemented to estimate the PLT effect.

RESULTS: PLT was measured in 6092 patients. There was the significant contribution on PLT in the first trimester from maternal weight, the presence of rheumatologic disease, BHCG levels and MPV. There was a significant association between PLT and abnormal cardiotocography at delivery (OR 1.004; IC95% 1.001 to 1.007) and C-Section due to abnormal CTG (OR 1.005; IC95% 1.002 to 1.008). When adjusted for factors that interact with PLT there was also a significant association with pH at birth < 7.10 and gestational diabetes.

CONCLUSIONS: Maternal and pregnancy factors can poorly predict relevant changes in PLT at the first trimester of gestation. PLT at first trimester of pregnancy might predict adverse perinatal outcome in combination with other markers.

Introduction

Prediction of perinatal outcome from early stages of pregnancy has become a priority and a line of research in the last decades as a way to improve both maternal and neonatal healthcare. Several methods of screening have been implemented using maternal characteristics and obstetric history, biophysical and biochemical tests under the assumption that this may enhance obstetrical results through pharmacological intervention and a closer follow-up [1, 2].

Normal pregnancy is characterised by an increase in platelet aggregation and a slight decrease in the mean platelet count than in healthy non-pregnant women [3-5]. This can be of no significance or clinical importance and may be due to increased PLT turnover, immune-related mechanisms, plasma dilution, or a complication of a more severe underlying gestational disorder. Moreover, histological examination of the human placenta revealed that during first stages of maternal pregnancy platelets are trapped by endovascular trophoblast aggregates that are formed inside the spiral arteries [6].

Platelets are likely to be activated and release several soluble factors, promoting the invasive capacity of extravillous trophoblasts. Hence, maternal platelets might be a candidate that attracts extravillous trophoblasts into the spiral arteries and encourages maternal vascular remodelling during early placentation process [7].
Mild decreases in platelet count occur in about 3 to 5% of pregnant women (gestational thrombocytopenia, incidental thrombocytopenia). Gestational thrombocytopenia is characterised by mild asymptomatic low platelet count in a patient without any history of such condition and most frequently during the third trimester. It is not associated with maternal or neonatal sequelae and spontaneously resolves after delivery [8, 9]. Platelet counts are typical > 75,000/μL, with about two-thirds being 130,000 to 150,000/μL.

Longitudinal studies showed that in women with the adverse perinatal outcomes such as Preeclampsia (PE) and intrauterine growth restriction (IUGR) there is a reduction in platelet count and this may predate their development by 3 to 5 weeks [10].

Some others showed that hypertensive disorders cannot be predicted based on platelet count during early stages of pregnancy. Nevertheless, an increased mean platelet volume (MPV) reflects enhanced platelet activation which may be caused by impairment in uteroplacental circulation. When MPV of 10.1 fl or more is used as a threshold, the pregnancies that are destined to develop IUGR and PE can be predicted with considerably high sensitivity and specificity combined with other biochemical markers such as low PAPP-A [11,12].

Several reports investigating changes in PLT number, function and MPV indicate increased PLT turnover following activation within the maternal vasculature in impaired placental conditions such as preeclampsia [13].

The main goal of our research is trying to understand PLT changes in the first trimester of gestation and its relation to pregnancy outcome in an era when sophisticated tests using biochemical or genetic markers are developed. We consider that there might be a place for the simple blood test that can be performed for the identification of women at stake of presenting gestational complications and then be sent to more complex surveillance.

Material and Methods

A retrospective population-based study was performed between 2013 and 2015 to examine whether platelet levels in the first trimester of pregnancy, 8-14 weeks of gestation, are associated with obstetric complications. The second aim is to rule out variables that may have a significant contribution on platelet levels during the first trimester.

Maternal and obstetrical characteristics were collected for patients attending the first-trimester clinic. We assessed the following perinatal outcomes: fetal gender, birth weight, gestational age at birth, bad perinatal outcome defined as: preeclampsia (PE), intrauterine growth retardation as birthweight below the 10th centile (IUGR), perinatal/antenatal death, non-reassuring cardiotocography (CTG), cesarean section (CS) due to non-reassuring CTG, spontaneous delivery before 37 weeks of gestation, Ph at birth < 7.10, newborn resuscitation > 3, Apgar score at 5 min ≤ 7.

Blood samples were obtained by antecubital venepuncture between 8 to 14 weeks of gestation before the clinical visit for routine blood test assessment. Platelet count was measured by an automated hematologic analyser.

Regarding the statistical analysis, values were reported as percentages or means and standard deviations or, for non-normal distributions, as medians and interquartile ranges (IQRs).

Differences of means between two groups were calculated by the Student’s t-test for independent samples if the normal distribution could be assumed. In the Student’s t-test for independent samples, we used the Levene’s test for homogeneity of variances. If normality was not valid, we used the nonparametric Mann–Whitney U-test. Differences of more than two means between groups were calculated by the ANOVA test if the normal distribution could be assumed or by the nonparametric Kruskal–Wallis test if normality was not valid. The Scheffé test was used for multiple comparisons of means. We firstly performed a simple linear regression with 15 different variables that might have a significant contribution to the platelet count in the first trimester of pregnancy selected by authors criteria. Ten of the above mentioned fifteen variables were used in the multivariate analysis.

The relation between platelets count as the predictor and a bad perinatal outcome was analysed firstly by simple logistic multiple regression. For each adverse outcome at birth, a multiple logistic regression analysis was implemented to estimate the platelets levels effect. All tests were two-tailed. P-values below 0.05 were considered statistically significant. Statistical analysis was performed using the SPSS version 18 (SPSS Inc, Chicago, IL, USA).

The present study was approved by the Ethical Committee of Hospital General Universitario Gregorio Marañon de Madrid (Comité ético de Investigacion clinica, reference number OBS05042016, date of approval 05.04.2016).

Results

Platelet count was measured in 6092 patients in the first trimester of pregnancy. The distribution did not follow a normal pattern in our sample (Figure 1); of
these, 22 had thrombocytopenia (0.36%) with PLT below 50,000/μL.

**Predictive linear multivariate regression analysis of platelet count in the first trimester of gestation resulted in a significant model (Table 1, p < 0.001) with an explanatory capacity of 28% (Adjusted R-squared = 0.28).**

**Table 1: Linear multivariate regression analysis of PLT at first trimester of pregnancy**

| Coefficient | 95% CI | p-value |
|-------------|-------|---------|
| Rheumatological disease Yes/No | -17.941 | <0.001 |
| BHCG (Mom) | -2.208 | 0.027 |
| MPV (μL) | -25.462 | <0.001 |
| Maternal weight (kg) | 324 | 0.001 |

From the total population, 1129 pregnancies (26.8%; IC95% 25.5 to 28.0%) were complicated by any of the following adverse perinatal outcomes as summarised in Table 2. The most frequent ones were abnormal CTG in labour (7.2%; IC95% 6.4 to 7.9%) and IUGR (7.1%; IC95% 6.3 to 7.8%).

**Table 2: Perinatal adverse outcomes**

| Total | Absolute frequency | Relative frequency |
|-------|-------------------|--------------------|
| (n)   | (%)               | (n)                |
| Preeclampsia | 4223 | 81 | 1.9 | 1.5 | 2.3 |
| Perinatal/antenatal death | 4223 | 15 | 0.4 | 0.2 | 0.5 |
| Abnormal CTG | 4223 | 392 | 7.2 | 6.4 | 7.9 |
| CS due to abnormal CTG | 4223 | 250 | 5.9 | 5.2 | 6.6 |
| IUGR | 4223 | 298 | 7.1 | 6.3 | 7.8 |
| Preterm birth < 37 sem | 4221 | 125 | 3.0 | 2.4 | 3.5 |
| Ph ≤ 7.10 | 4220 | 95 | 2.3 | 1.8 | 2.7 |
| Resuscitation > 3 | 4222 | 293 | 6.9 | 6.2 | 7.7 |
| Appar at 5 min ≤ 7 | 4221 | 54 | 1.3 | 0.9 | 1.6 |
| Gestational diabetes | 4231 | 239 | 5.6 | 5.0 | 6.3 |
| Any adverse outcome | 4243 | 1139 | 26.8 | 25.5 | 28.0 |

According to simple binary logistic regression, there was a significant association between platelets levels in the first trimester (1000/μL) as the predictor and the following adverse outcomes as seen in Table 3.

**Table 3: Simple logistic binary regression (predictor PLT 1000/µL)**

| Adverse perinatal outcome | OR | 95% CI lower | 95% CI upper | p-value |
|---------------------------|----|--------------|--------------|---------|
| Preeclampsia | 1.002 | 0.997 | 1.007 | 0.470 |
| Perinatal/antenatal death | 1.007 | 0.997 | 1.017 | 0.165 |
| Abnormal CTG | 1.004 | 1.001 | 1.007 | 0.003 |
| CS due to abnormal CTG | 1.005 | 1.002 | 1.008 | 0.001 |
| IUGR | 1.000 | 0.997 | 1.003 | 0.918 |
| Preterm birth < 37 sem | 1.001 | 0.997 | 1.005 | 0.634 |
| Ph ≤ 7.10 | 1.000 | 0.996 | 1.005 | 0.963 |
| Resuscitation > 3 | 1.002 | 0.999 | 1.005 | 0.144 |
| Appar at 5 min ≤ 7 | 1.005 | 0.999 | 1.010 | 0.101 |
| Gestational diabetes | 1.002 | 0.999 | 1.005 | 0.197 |
| Any adverse outcome | 1.002 | 1.000 | 1.003 | 0.040 |

For each adverse outcome, a multiple logistic binary regression models was constructed and adjusted by rheumatologic disease. BHCG levels, MPV and maternal weight taking into account their interaction with platelets count.

The adjusted effect from PLT resulted into a significant contribution to the following outcomes (Table 4):

**Table 4: Estimative model adjusted by interaction factors**

| Adverse perinatal outcome | OR | 95% CI upper | 95% CI lower | p-value |
|---------------------------|----|--------------|--------------|---------|
| Preeclampsia | 1.002 | 0.997 | 1.007 | 0.470 |
| Perinatal/antenatal death | 1.007 | 0.997 | 1.017 | 0.165 |
| Abnormal CTG | 0.988 x 1.00025 | 0.975 x 1.00006 | 1.005 x 1.00004 | 0.018 |
| CS due to abnormal CTG | 0.986 x 1.00003 | 0.972 x 1.00001 | 0.999 x 1.00002 | 0.007 |
| IUGR | 1.000 | 0.997 | 1.003 | 0.918 |
| Preterm birth < 37 sem | 1.001 | 0.997 | 1.005 | 0.634 |
| Ph ≤ 7.10 | 1.010 x 0.982 | 1.007 x 0.982 | 1.017 x 0.982 | 0.009 |
| Resuscitation > 3 | 1.002 | 0.999 | 1.005 | 0.144 |
| Appar at 5 min ≤ 7 | 1.005 | 0.999 | 1.010 | 0.101 |
| Gestational diabetes | 1.007 x 0.995 | 1.003 x 0.995 | 1.010 x 0.995 | 0.019 |
| Any adverse outcome | 0.987 x 1.002 | 0.978 x 1.002 | 1.000 x 1.002 | 0.002 |

Abnormal CTG: The adjusted effect by platelets was significant (p=0.018) and had interaction with maternal weight as seen in this example:

0.988 x 1.00025<sup>60</sup> = 0.988 x 1.015 = 1.0029

The risk of a non-reassuring CTG was raised by 0.29% for every increase by 1000 platelets/μL in a 60 kg pregnant woman.

CS due to abnormal CTG: The adjusted effect by platelets was significant (p=0.007) and had interaction with maternal weight as seen below:

0.986 x 1.0003<sup>60</sup> = 0.986 x 1.015 = 1.0009

The risk of CS due to abnormal CTG was raised 0.09% for every increase of 1000 platelets/μL in a 60 kg pregnant woman.

Ph ≤ 7.10: The adjusted effect by platelets was significant (p=0.09) and had interaction with BHCG Mom values as seen in the table.

The adjusted effect from PLT resulted into a significant contribution to the following outcomes (Table 4):

Gestational diabetes: The adjusted effect by platelets was significant (p=0.019) and had interaction with MPV values and maternal weight as seen in this example;
1.007 x 0.998^9 x 1.0002^{20} = 1.007 x 0.982 x 1.012 = 1.0009

In a 60 kg patient with an MPV of 9 fl in the first trimester, the risk to develop gestational diabetes is raised 0.09% per every increase by 1000 platelets/μL.

Discussion

A relevant finding of our study is that the PLT in the first trimester of pregnancy is not associated with PE, IUGR or preterm delivery. A significant association was found between abnormal CTG. CS due to abnormal CTG. And low pH at birth and gestational diabetes when adjusted for interaction factors and an increase in PLT. This association remains very mild, and its significance could be noticed as a result of a very large sample of patients. We also hypothesised that patients with worse outcome at birth and late stage of pregnancy could show higher PLT values despite the BHCG increment between 8 to 11 weeks as a consequence of an unclear dysregulation in the inflammatory process that occurs during early placentation. However, its clinical importance seems weak further research combining simple markers such as MPV or PAPPA could potentially improve PLT predicting value.

Even though some of the reports did not find an association between low PLT in pregnancy and hypertensive disorders or increase in the perinatal morbidity and mortality. The same show significantly higher rates of preterm birth before 37 weeks of gestation (OR 1.82, 1.1-2.97, 95% CI) were documented among patients with platelets < 100.000/μL [14].

PE and IUGR are thought to be the consequence of impaired placentation due to an inadequate trophoblastic invasion of the spiral arteries. An imbalance between angiogenic and anti-angiogenic proteins [15, 16] and endothelial damage in which platelets may play a role in its pathogenesis [17].

Discordant conclusions have been published regarding platelet number and size variations in normal and complicated pregnancies. Some research groups found no difference in platelet counts and MPV values between complicated and controls, whereas others [18] demonstrated lower platelets and higher MPV in women suffering from PE. However, it seems that early pathogenesis of placental impairment disease during the first weeks of gestation does not have a clear influence on platelet count.

The vascular remodelling that ensures appropriate placental perfusion is an important component of human reproduction and should be secured by several complementary mechanisms. In this respect, promotion of trophoblast invasion by maternal platelets could be one of the multiple mechanisms that regulate this vascular remodelling. Although the vascular changes might occur in the absence of maternal platelets.

The fact that pregnancies with severe platelet defects can achieve an uneventful pregnancy may suggest that maternal platelets are not a fundamental element of human placentation.

The objective of finding a clinically useful first-trimester screening for pregnancy complications is the identification of pregnancies at high-risk of developing gestational and perinatal adverse outcome and through pharmacological intervention and a closer follow-up in this high-risk group reduces the prevalence of the disease or diminishes its deleterious effects [19, 20, 21].

Severe thrombocytopenia with platelet count < 50.000/μL occurs in less than 5 % of preeclamptic women when the disease is established. However, the frequency and severity of thrombocytopenia increase with the severity of PE. and much more increased in patients with HELLP syndrome or those with eclampsia in whom disseminated intravascular coagulation may be a contributing factor [22]. Our research cannot identify or predict patients at risk of developing such problems and presumably not the ones that will have a poorer prognosis once any of these conditions are settled either, but further investigation should question this hypothesis.

The other aim of the study was a method to design a model to predict changes in platelet count in the first trimester by maternal and obstetrical characteristics. Laboratory and ultrasound variables using linear multiple regression shows a mild capacity in explaining platelets variability. Only four variables demonstrated a significant contribution to PLT in the first trimester, but it helped us to understand more precisely what are some of the factors that explicate its variations during early stages of pregnancy.

Thrombocytopenia is very common in pregnant women. Evaluation of blood count has shown that thrombocytopenia is the second most common haematological problem in gestation. It may result from diverse aetiologies, but so far a method to predict and quantify platelet count changes at early stages of pregnancy has not been demonstrated.

Platelet count is lower in pregnant compared to healthy non-pregnant women. It is believed that immunological mechanisms at the time of placentation are involved in the process [23]. However, the major factors that change the PLT in pregnancy are still to be specified. Our analysis only included the presence of rheumatologic conditions as the immunological factor, but its contribution resembles weak.

We also know that in pregnancy the demands on the hemostatic and fibrinolytic systems change to

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preventing excessive placental hemorrhage throughout gestation and especially during placental separation at delivery. A relative hypercoagulable state compared with non-pregnant women is caused by the marked increase in coagulation factors, reduced fibrinolysis, and increased platelet activity.

BHCG is secreted by trophoblast. A layer of cells on the outside of the blastocyst that provides the embryo with nutrients and later forms part of the placenta and the fetal membranes. Extravillous cytrophoblast produces hyperglycosylated hCG (hCG-H). The main form of BHCG present during the first two postconception weeks when implantation is taking place. hCG-H appears to promote invasion of extravillous cytrophoblast into the myometrium wall to form gripping villi (interstitial invasion) and into the spiral arteries (endothelial invasion) to create a high-flow low-resistance vessels.

During pregnancy, BHCG concentration peaks at 93.598 mIU/mL (range 27.300 to 233.000 mIU/mL) at 8 to 11 weeks of gestation and as seen in our study this hormone is a significant contributor to PLT decrease maybe as a booster of platelet aggregation, activation, placental entrapment and hemodynamic changes (Figure 2).

![Figure 2: Relation between BHCG in Mom and PLT at first trimester of pregnancy](image)

It has long been known that platelet volume is a direct indicator of increased platelet synthesis and activation [24]. In normal pregnancies, a mild increase in platelet aggregation was observed, which is compensated by increased synthesis and consequently higher MPV values which are consistent with our findings.

Our study has several weaknesses. Despite the large sample size of women with platelet count measurement, only about one-third of them were eligible to take part as observations in our multiple regression works. Many of the variables that we took into account appeared to be irrelevant which necessitated looking for other ones that might be better predictors of platelet count changes in pregnancy.

In conclusion, maternal and pregnancy factors can predict very mildly clinical relevant changes in PLT at the first trimester of gestation. PLT at first trimester of pregnancy might predict adverse perinatal outcome in combination with other markers, but its clinical use remains worthless as a unique test to choose. Further research should be undertaken to test for this purpose.

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