INCREASED RISK OF PREGNANCY COMPLICATIONS IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA CARRYING THE JAK2 (V617F) MUTATION

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Short title: JAK2 (V617F) mutation and pregnancy

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Authorship contributions. F.P. and M.L conceived the study, collected, analyzed and interpreted data, wrote the paper; M.L.R. and M.C. analyzed and interpreted data; E.R. collected and analyzed data; E.P., C.E., L.A., F.T., F.F., E.M. collected clinical data; D.P. and M.S. performed JAK2 mutation analysis; R.M. performed thrombophilic tests; and C.P. did statistical analyses. The authors have no potential conflict of interest to disclose

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Abstract

Essential thrombocythemia (ET) may occur in women of childbearing age. To investigate the risk of pregnancy complications, we studied 103 pregnancies occurred in 62 women with ET. Two-tailed Fisher exact test showed that pregnancy outcome was independent from that of a previous pregnancy. The rate of live birth was 64%, and 51% of pregnancies were uneventful. Maternal complications occurred in 9%, while fetal complications occurred in 40% of pregnancies. The Mantel-Haenszel method showed that fetal loss in women with ET was 3.4-fold higher (95% CI 3.1-3.9; \( P < .0001 \)) than that of the general population. Half of the women studied carried the \( JAK2 \) (V617F) mutation, and multivariate logistic regression model identified this mutation as an independent predictor of pregnancy complications \( (P = .01) \). Neither the platelet count nor the leukocyte count were risk factors. \( JAK2 \) (V617F)-positive patients had an odds ratio of 2.02 (95% CI: 1.1-3.8) of developing complications in comparison with \( JAK2 \) (V617F)-negative patients. Aspirin did not prevent complication in \( JAK2 \) (V617F)-positive patients and appeared to worsen outcome in \( JAK2 \) (V617F)-negative. A relationship was found between \( JAK2 \) (V617F) and fetal loss \( (P = .05) \). This study indicates that patients carrying the \( JAK2 \) (V617F) mutation have higher risk of developing pregnancy complications.
Introduction

Essential thrombocythemia (ET) is a chronic myeloproliferative disorder with an increased risk of vascular complications. Despite these events, life expectancy of patients with ET is not significantly affected by the disease in any age category. Patients with ET are predominantly women and part of them are diagnosed in the child-bearing age. Decision making on pregnancy is therefore a common issue in the clinical management of young women with ET.

There are limited information regarding the outcome of pregnancy in patients with ET, mainly from case reports. Papers reviewing published studies on pregnancies in patients with ET report live birth rates of 50% to 70% and spontaneous abortion rates of 25% to 50%. Concerning risk factors, the study of Wright and Tefferi on 43 pregnancies indicates that preconception platelet count and aspirin therapy do not predict the risk of abortion.

The JAK2 (V617F) mutation has been recently identified in about half of patients with ET. It has been suggested that the presence of the mutation in patients with ET characterizes a disease with a higher risk of vascular events. To date, the relationship between JAK2 mutational status and the outcome of pregnancy in women with ET is unknown.

We studied 103 pregnancies occurring in 62 patients with ET to investigate the risk of complications and to find predictors of pregnancy outcome.
Patients, materials, and methods

Patients

This study includes 103 consecutive pregnancies occurred in 62 patients with ET followed between 1980 and 2006 at the Division of Hematology of the Fondazione Policlinico San Matteo, University of Pavia; the Division of Internal Medicine of the University of Padova and the Division of Hematology of the Niguarda Ca’ Granda Hospital of Milan, Italy. The study was approved by the institutional ethics committee of Pavia and the procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2000. Samples for molecular analysis were obtained after patient provided written informed consent.

Diagnostic criteria of ET were those in use at the time of the first observation. Patients who received a cytoreductive treatment during ET were those defined as at high risk. A complete medical history was obtained, including abortion risk factors (parity, outcome of previous pregnancies, weight, hypertension, high cholesterol level, diabetes, current smoking, thyroid diseases), and disease-related risk factors (hematologic features at diagnosis, time elapsed from diagnosis, history of thrombosis or hemorrhage, type and duration of treatments, blood cell counts at conception). Fetal outcome was defined as live birth, induced abortion, fetal loss (spontaneous abortion and stillbirth), intrauterine growth retardation. Stillbirth was defined as fetal loss after 23 weeks of gestation, intrauterine growth retardation as a birth weight below the 5th percentile for gestational age. Preeclampsia was defined by a blood pressure higher than 160/110 mm Hg and urinary protein loss greater than 3 g /24 hours. Arterial hypertension was defined by a blood pressure ranging from normal value to 150/100 mm Hg. Starting from 2005, postpartum anticoagulation was adopted in all ET patients.

Assessment of JAK2 (V617F) mutational status

In the Pavia and Milan cohorts, granulocytes were obtained from the neutrophil fraction by osmotic lysis of red cells. Genomic DNA was obtained by using the Puregene Blood DNA isolation kit (Gentra Systems, Minneapolis, MN). A quantitative real-time polymerase chain reaction (qRT-PCR)-based allelic discrimination assay was used to detect the V617F
mutation of the \textit{JAK2} gene.\textsuperscript{8} In the Padova cohort, the detection of \textit{JAK2} (V617F) mutation in peripheral blood granulocyte DNA was based on allele-specific PCR, as previously described.\textsuperscript{15}

\textit{Assessment of thrombophilia}

Molecular diagnosis of factor V Leiden mutation was performed as described by Bertina et al.\textsuperscript{16} The mutation in the methylenetetrahydrofolate reductase (MTHFR) gene was detected as described by Frosst et al.\textsuperscript{17} The mutation in the prothrombin gene was detected as described by Poort et al.\textsuperscript{18} Levels of free protein S (immunoassay, HemosIL, Instrumentation Laboratory, Lexington, USA), protein C activity (chromogenic assay, Dade Behring, Marburg, USA), plasmatic homocysteinemia (chemiluminescent Hcy assay, Bayer ADVIA Centaur) and antithrombin III activity (chromogenic assay, Dade Behring, Marburg, USA) were evaluated outside pregnancy as well as antiphospholipid antibodies (immunoassay, Orgentec Diagnostika GmbH, Mainz, Germany).

\textit{Statistical analysis}

Demographic and disease characteristics of the patients were summarized using descriptive statistics. The analysis of risk factors associated with pregnancy complications was carried out by means of univariate and multivariate logistic regression models. The risk of fetal loss in this cohort was compared to that in the Italian population by means of the Mantel-Haenszel method. It allowed to estimate an age-adjusted Odds Ratio (OR) using the available data on number of live births, stillbirths, and spontaneous abortion by 5-year age bands in the years 1998 and 1999 as published by ISTAT (Italian Statistical Institute). All statistical analyses were performed using Microsoft Excel 2000 and Statistica 7.0 for Windows.
Results

At diagnosis of ET, the median age was 28 years (range, 18-44 years) and the median value of platelet was 710 x 10^9/L (range, 620-3000). The median value of hemoglobin was 13.3 g/dL (range, 11-15.3), and that of leukocyte was 8.1 x 10^9/L (range, 4-11.1). Mann-Whitney U test showed that patients carrying the JAK2 (V617F) mutation had a significantly higher hemoglobin at diagnosis (median value 13.6 g/dl) than those without the mutation (median value 12.9 g/dl) (P=.01). Eleven (19%) patients were at high risk: eight had a platelet count over 1500 x10^9/L and three had thrombosis.

Pregnancy data

Within 103 pregnancies, seven (7%; four women) underwent provoked abortion for the following reasons: patient’s concern for disease evolution or complications in four (two were receiving hydroxyurea), personal reasons in three. Therefore, we evaluated 96 pregnancies in 58 women for the analysis of pregnancy complications.

The median time elapsed from diagnosis to first pregnancy was 2.6 years (range, 0-15 years). One patient had diagnosis of ET while pregnant. Demographic and clinical characteristics at first pregnancy are summarized in Table 1. No evidence of polycythemia vera or iron deficiency was present at the time of pregnancy. The median platelet count was 646 x 10^9/L (range, 250-1660) at first-trimester, 505 x 10^9/L (range, 220-1700) at second-trimester and 429 x 10^9/L (range, 219-2000) at third-trimester. Wilcoxon matched pair test showed a significant reduction of platelet count during pregnancy (P<.000). A significant fall in the platelet count was shown in both JAK2 (V617F)-positive (P=.003) and in JAK2 (V617F)-negative patients (P=.001), without differences between the two groups.

In 13 (14%) of 96 pregnancies, patients had been receiving a cytoreductive treatment (interferon in 8, hydroxyurea in 5) for the last six months before conception. Hydroxyurea was withdrawn in all patients, while interferon was continued in three. In 44 (46%) of 96 pregnancies, patients were receiving an anti-platelet therapy at conception. Aspirin at a daily dose of 100 mg was administered during 60 (62%) of 96 pregnancies. Among the 13 pregnancies conceived while on cytoreductive therapy, 5 (40%) occurred in JAK2 (V617F)-positive and 8 (60%) in JAK2 (V617F)-negative patients. Among the 68 pregnancies
conceived out of cytoreductive therapy, 35 (51%) occurred in JAK2 (V617F)-positive and 33 (49%) in JAK2 (V617F)-negative patients. Two-tailed Fisher exact test did not reveal a significantly different segregation ($P=.54$).

**Pregnancy complications**

Overall, 47 (49%) of 96 pregnancies were complicated (Table 2). Calendar year at diagnosis and institutional location did not influence pregnancy outcome. Platelet count at the time of complications was not significantly different ($P=0.12$) between JAK2 (V617F)-positive (median 501 x 10⁹/L, range 200-1350) and JAK2 (V617F)-negative patients (median 650 x 10⁹/L, range 250-1300). Of the 47 complications, 38 (80%) involved the fetus and nine (20%) the mother. Maternal complications had resolution after delivery. An abortion was complicated by deep venous thrombosis two weeks later.

Nine (60%) of 15 patients with thrombophilia had complications at first pregnancy: abortion in six (five with MTHFR mutation and one with prothrombin gene mutation), preeclampsia in one (MTHFR mutation), intrauterine growth retardation in two (one with Factor V Leiden mutation and one with MTHFR mutation). Seventeen (71%) of 24 patients carrying the JAK2 (V617F) mutation had complications at first pregnancy (abortion in eight, stillbirth in two, intrauterine growth retardation in three, preeclampsia in two, hypertension in two).

Within 13 pregnancies conceived while patients were receiving a cytoreductive treatment, nine (70%) were complicated (six abortions and three preeclampsia). According to treatment at conception, complications occurred in four (80%) of five on hydroxyurea and in five (62%) of eight on interferon. Of the three patients who continued interferon during pregnancy, one (33%) had preeclampsia.

The impact of a previous pregnancy was investigated in 31 patients who had two pregnancies. The outcome of pregnancies was concordant in 19 (61%) patients (both pregnancies uncomplicated or complicated), and discordant in 12 (39%). Two-tailed Fisher exact test showed that pregnancy outcome was not significantly influenced by that of previous pregnancy. We further analyzed the 24 patients with multiple pregnancies who had JAK2 mutational status assessed (15 positive and nine negative). Patients who had all
pregnancies with complications were six (40%) of 15 carrying the JAK2 (V617F) mutation and two (22%) of nine without the mutation ($P=.19$).

We investigated as potential predictors of complications for the first pregnancy both maternal characteristics (age $>$ 35 years, parity, presence of abortion risk factors, presence of thrombophilia), and disease characteristics (hemoglobin level, platelet and leukocyte count at diagnosis, history of thrombosis, PLT count $>$1000 x10$^9$/L and WBC count $>$10 x10$^9$/L at conception, JAK2 mutational status, anti-platelet and anti-myeloproliferative therapy before and during pregnancy). Univariate logistic regression model showed that the JAK2 (V617F) mutation was a significant risk factor ($P=.01$) for complications. Multivariate logistic regression model confirmed the JAK2 (V617F) mutation as an independent risk factor for pregnancy complications ($P=.01$). Relevant OR for the prevalence of risk factors in patients with pregnancy complications are reported in Figure 1. Patients with ET carrying the JAK2 (V617F) mutation had an OR equal to 2.02 (95% CI: 1.1-3.8) of developing complications during pregnancy. In order to find whether JAK2 (V617F) mutation compounded the effect of thrombophilia, a multivariate logistic regression analysis with JAK2 (V617F) mutational status and thrombophilia as covariate was applied. We found that the JAK2 (V617F) mutation was an independent predictor of pregnancy outcome ($P=.03$) without any significant interaction between the two parameters ($P=.37$).

Within the 40 pregnancies in JAK2 (V617F)-positive patients, complications occurred in 13 (52%) of 25 patients receiving aspirin and in 12 (80%) of 15 without any anti-platelet therapy. The difference between the two proportions was not statistically significant ($P=.08$). Within the 42 pregnancies in JAK2 (V617F)-negative patients, complications occurred in 13 (52%) of 25 patients receiving aspirin and in 4 (23%) of 17 without any anti-platelet therapy ($P=.034$).

Fetal loss

The live birth rate was 64% (Table 2). Among cases of fetal loss, abortion was more frequent than stillbirth. Of 31 abortions, 27 (87%) occurred at the first-trimester and 4 (13%) at the second-trimester.
The Mantel-Haenszel method was used to quantify the rate of fetal loss among patients with ET compared to that of age-matched Italian population. We obtained an OR of 3.4 (95% CI 3-3.9, \( P < .0001 \)), that means a 3.4-fold higher risk of fetal loss for patients with ET compared to age-matched general Italian population. By univariate logistic regression models, the study of potential predictors of fetal loss among maternal and disease-related risk factors showed a relationship with the \( JAK2(V617F) \) mutation (\( P = .05 \)).
Discussion

We evaluated 103 pregnancies occurring in 62 patients with ET to investigate the risk of complications and to find predictors of pregnancy outcome.

This study shows that pregnancy is not contraindicated in patients with essential thrombocythemia. The rate of live birth was 64%, and 51% of pregnancies were uneventful. Maternal complications such as preeclampsia and hypertension occurred in 9% and resolved after delivery. In this study, patients did not develop vascular complications during pregnancy with the exception of a single case of deep venous thrombosis during puerperium. This is in keeping with other studies. Fetal complications, including abortion, stillbirth, and intrauterine growth retardation occurred in 40% of pregnancies. Abortion accounted for 91% of fetal loss and occurred mostly during the first-trimester. The risk of fetal loss in women with ET was 3.4-fold higher than expected in the age-matched general Italian population. In this series of patients, pregnancy outcome was independent from that of previous pregnancy.

To date, no risk factors have been identified to predict pregnancy outcome in patients with ET. In this study neither the platelet count nor the leukocyte count were risk factors of pregnancy complications. Although thrombophilia is known to play a role on pregnancy complications in the general population, there are no large studies on the impact of thrombophilia in pregnant women with ET. Among 15 patients with thrombophilia in our series, 60% had complications at first pregnancy. However, thrombophilic state per se did not reach statistical significance as risk factor for complications, likely because it was obscured by stronger disease-related factors. Nevertheless, the inclusion of thrombophilic tests in the work-up of a woman with ET in the child-bearing age is recommended for individualized therapeutic interventions aimed at improving pregnancy outcome.

The JAK2 (V617F) mutation assessment is a key tool in the diagnostic work-up of patients with chronic myeloproliferative disorders. In our series of pregnant women with ET the JAK2 (V617F) mutation was found in 49% of cases, similarly to other series. The same concordance was found also in the proportion of mutant alleles, that ranged from 3.9% to 24.2%. At diagnosis of ET, patients carrying the JAK2 (V617F) mutation had
a significant higher value of hemoglobin than those without the mutation. Concerning the influence of JAK2 (V617F) on the outcome of the first pregnancy in patients with ET, this study provides evidence that JAK2 mutational status is an independent risk factor for pregnancy complications. In fact, women with ET carrying the mutation had a two-fold higher risk of developing complications than patients without the mutation. In 24 women with ET who had multiple pregnancies, JAK2 mutational status was not significantly predictive of outcome from pregnancy to pregnancy. As the number of patients with multiple pregnancies grouped by JAK2 mutational status was relatively small, studies on larger series are needed to settle this issue.

A common finding in pregnant women with ET is the fall of the platelet count during pregnancy.\textsuperscript{6,20} The reduction of platelet count was observed in both JAK2 (V617F)-positive and JAK2 (V617F)-negative patients without significant differences. This suggests that this phenomenon is independent from the JAK2 (V617F) mutation.

Concerning treatments of ET during pregnancy, cytoreduction should be avoided, particularly in the first trimester,\textsuperscript{3} because teratogenicity of cytoreductive agents cannot be ruled out.\textsuperscript{30} Interferon is considered the agent of choice in pregnant women with ET who need reduction of platelet count.\textsuperscript{3} In this study, one of three women treated with interferon developed complications. Low dose aspirin during pregnancy has been shown to be safe for the fetus in the general population without an increased risk of bleeding for the mother.\textsuperscript{31} Aspirin is commonly used in patients with ET who do not have history of bleeding.\textsuperscript{32} In our series of 96 pregnancies considered as a whole, the use of aspirin did not influence pregnancy outcome, as also found by Tefferi and coworkers.\textsuperscript{6} Grouping patients according to JAK2 (V617F) mutational status, aspirin did not prevent pregnancy complication in JAK2 (V617F)-positive patients and appeared to worsen outcome in JAK2 (V617F)-negative patients.

In conclusion, this study on patients with essential thrombocythemia indicates that pregnancy may evolve uneventful in half of cases. Women carrying the JAK2 (V617F) mutation have higher risk of developing pregnancy complications.
Table 1. Demographic and hematologic characteristics at first pregnancy of 58 women with essential thrombocythemia.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>58</td>
</tr>
<tr>
<td>No. of pregnancies</td>
<td>96</td>
</tr>
<tr>
<td>Age at diagnosis, years, median (range)</td>
<td>28 (18-44)</td>
</tr>
<tr>
<td>Age at conception, years, median (range)</td>
<td>32 (18-44)</td>
</tr>
<tr>
<td>No. with at least one abortion risk factor*</td>
<td>10/58 (17%)</td>
</tr>
<tr>
<td>No. with thrombophilia</td>
<td>15/46 (33%)</td>
</tr>
<tr>
<td>Factor V Leiden mutation (+/-)</td>
<td>1</td>
</tr>
<tr>
<td>Methylene tetrahydrofolate reductase mutation (+/+)</td>
<td>7</td>
</tr>
<tr>
<td>Prothrombin mutation (+/-)</td>
<td>1</td>
</tr>
<tr>
<td>Hyperhomocysteinemia</td>
<td>4</td>
</tr>
<tr>
<td>Antiphospholipid antibody</td>
<td>2</td>
</tr>
<tr>
<td>No. with JAK2 (V617F) mutation</td>
<td>24/49 (49%)</td>
</tr>
<tr>
<td>JAK2 (V617F) mutation burden, %, median (range)</td>
<td>10.1 (3.9-24.2)</td>
</tr>
<tr>
<td>WBC count at pregnancy, x 10^9/L, median (range)</td>
<td>7.1 (4.2-15.3)</td>
</tr>
<tr>
<td>Hemoglobin level at pregnancy, g/dL, median (range)</td>
<td>13.1 (11.5-15.4)</td>
</tr>
<tr>
<td>PLT count at pregnancy, x 10^9/L, median (range)</td>
<td>601 (266-1660)</td>
</tr>
</tbody>
</table>

*Abortion risk factor: overweight, hypertension, high cholesterol level, diabetes, current smoking, thyroid diseases. +/-: homozygous; +/- heterozygous; hyperhomocysteinemia: >13.9 µmol/L
Table 2. Complications of 96 pregnancies in 58 patients with essential thrombocythemia.

<table>
<thead>
<tr>
<th>Pregnancy complications</th>
<th>Number (% of pregnancies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total events</td>
<td>47 (49)</td>
</tr>
<tr>
<td>Fetal loss</td>
<td>34 (36)</td>
</tr>
<tr>
<td>First-trimester abortion</td>
<td>27</td>
</tr>
<tr>
<td>Second-trimester abortion</td>
<td>4</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>3</td>
</tr>
<tr>
<td>Intrauterine growth retardation</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Maternal complications</td>
<td>9 (9)</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>5</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>4</td>
</tr>
</tbody>
</table>
Passamonti et al, Figure 1.
Legend to Figure

Figure 1. Odds ratios for the prevalence of risk factors in patients with pregnancy complications.
Values of odds ratio were: 0.9 (95% CI: 0.5-1.7) for age > 35 years, 0.8 (95% CI: 0.4-1.7) for parity, 1 (95% CI: 0.5-2) for the presence of abortion risk factor, 1.2 (95% CI: 0.7-2.3) for the presence of thrombophilia, 1 (95% CI: 0.5-2.2) for thrombocytosis exceeding 1000 x10^9/L, 0.7 (95% CI: 0.4-1.3) for leukocytosis exceeding 10 x 10^9/L, 2 (95% CI: 1.1-3.8) for the presence of JAK2 (V617F) mutation, 0.9 (95% CI: 0.5-1.6) for anti-platelet therapy during pregnancy. JAK2 (V617F) mutation was a significant risk factor of pregnancy complications.
References

Increased risk of pregnancy complications in patients with essential thrombocythemia carrying the JAK2(V617F) mutation

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