Increased risk of pregnancy complications in patients with essential thrombocythemia carrying the JAK2 (617V>F) mutation

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Essential thrombocythemia (ET) may occur in women of childbearing age. To investigate the risk of pregnancy complications, we studied 103 pregnancies that occurred in 62 women with ET. The 2-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% confidence interval [CI]: 3.3-9; P < .001) than in the general population. Half of the women studied carried the JAK2(617V>F) mutation, and a multivariate logistic regression model identified this mutation as an independent predictor of pregnancy complications (P = .01). Neither the platelet count nor the leucocyte count was a risk factor. JAK2(617V>F)-positive patients had an odds ratio of 2.02 (95% CI: 1.1 - 3.8) of developing complications in comparison with JAK2(617V>F)-negative patients. Aspirin did not prevent complication in JAK2(617V>F)-positive patients and appeared to worsen outcomes in JAK2(617V>F)-negative patients. A relationship was found between JAK2(617V>F) and fetal loss (P = .05).

This study indicates that patients carrying the JAK2(617V>F) mutation have higher risk of developing pregnancy complications. (Blood. 2007;110:485-489)

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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.1 Patients with ET are predominantly women, and some of them are diagnosed at childbearing age.2 Decision-making on pregnancy is therefore a common issue in the clinical management of young women with ET.

There is limited information regarding the outcome of pregnancy in patients with ET, mainly from case reports. Papers reviewing published studies on pregnancies in patients with ET3-5 report live birth rates of 50% to 70% and spontaneous abortion rates of 25% to 50%. Concerning risk factors, the study of Wright and Tefferi6 on 43 pregnancies indicates that preconception platelet count and aspirin therapy do not predict the risk of abortion.

The JAK2(617V>F) mutation has been recently identified in approximately half of patients with ET.7-10 It has been suggested that the presence of the mutation in patients with ET characterizes a disease with a higher risk of vascular events.9 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 that occurred in 62 patients with ET who were 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 patients provided written informed consent.

Diagnostic criteria of ET were those in use at the time of the first observation.11-13 Patients who received a cytoreductive treatment during ET were those defined as at high risk.14 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 classified as live birth, induced abortion, fetal loss (spontaneous abortion and stillbirth), and intrauterine growth retardation. Stillbirth was defined as fetal loss after 23 weeks of gestation, and intrauterine growth retardation was defined as a birth weight below the fifth percentile for gestational age. Pre-eclampsia was defined by a blood pressure higher than 160/110 mmHg and urinary protein loss greater than 300 mg/24 h.

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3 g per 24 hours. Arterial hypertension was defined by a blood pressure ranging from normal value to 150/100 mmHg. Starting from 2005, postpartum anticoagulation was adopted in all patients with ET.3

Assessment of JAK2 (617V>F) 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 617V>F mutation of the JAK2 gene.15 In the Padova cohort, the detection of JAK2 (617V>F) mutation in peripheral blood granulocyte DNA was based on allele-specific PCR, as previously described.15

Assessment of thrombophilia

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

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 univariate and multivariate logistic regression models. The risk of fetal loss in this cohort was compared with that in the Italian population by the Mantel-Haenszel method. It allowed us to estimate an age-adjusted odds ratio (OR) using the available data on number of live births, stillbirths, and spontaneous abortions 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 (Redmond, WA) and Statistica 7.0 for Windows (StatSoft, Tulsa, OK).

Results

At diagnosis of ET, the median age was 28 years (range, 18 to 44 years), and the median platelet count was 710 × 10^9/L (range, 620 to 3000 × 10^9/L). The median hemoglobin level was 133 g/L (13.3 g/dL) (range, 110 to 153 g/L [11 to 15.3 g/dL]), and median leukocyte count was 8.1 × 10^9/L (range, 4 to 11.1 × 10^9/L). The Mann-Whitney U test showed that patients carrying the JAK2 (617V>F) mutation had a significantly higher hemoglobin level at diagnosis (median, 136 g/L [13.6 g/dL]) than those without the mutation (median, 129 g/L [12.9 g/dL]; P = .01). A total of 11 (19%) patients were at high risk14; 8 patients had a platelet count higher than 1500 × 10^9/L, and 3 patients had thrombosis.

Pregnancy data

Of 103 pregnancies, 7 (7%; 4 women) underwent provoked abortion for the following reasons: patient’s concern for disease evolution or complications in 4 (2 were receiving hydroxyurea), personal reasons in 3. 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 to 15 years). One patient had a 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 × 10^9/L (range, 250 to 1660 × 10^9/L) in the first trimester, 505 × 10^9/L (range, 220 to 1700 × 10^9/L) in the second trimester, and 429 × 10^9/L (range, 219 to 2000 × 10^9/L) in the third trimester. The Wilcoxon matched-pair test showed a significant reduction of platelet count during pregnancy (P < .007). A significant fall in the platelet count was shown in both JAK2 (617V>F)–positive (P = .003) and JAK2 (617V>F)–negative patients (P = .001), without differences between the 2 groups.

In 13 (14%) of 96 pregnancies, patients had been receiving a cytoreductive treatment (interferon in 8 pregnancies, hydroxyurea in 5 pregnancies) in the 6 months before conception. Hydroxyurea was withdrawn in all patients, and interferon was continued in 3 patients. In 44 (46%) of 96 pregnancies, patients were receiving antiplatelet therapy at conception. Aspirin at a daily dose of 100 mg was administered in 60 (62%) of 96 pregnancies. Among the 13 pregnancies conceived while on cytoreductive therapy, 5 (40%) occurred in JAK2 (617V>F)–positive patients, and 8 (60%) in JAK2 (617V>F)–negative patients. Among the 68 pregnancies conceived while not on cytoreductive therapy, 35 (51%) occurred in JAK2 (617V>F)–positive patients, and 33 (49%) in JAK2 (617V>F)–negative patients. The 2-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 = .12) between JAK2 (617V>F)–positive patients (median, 501 × 10^9/L; range, 200 to 1350 × 10^9/L) and JAK2 (617V>F)–negative patients (median, 650 × 10^9/L; range, 250 to 1300 × 10^9/L). Of the 47 complications, 38 (80%) involved the fetus, and 9 (20%) involved the mother. Maternal complications resolved after delivery. An abortion was complicated by deep venous thrombosis 2 weeks later.

Table 1. Demographic and hematologic characteristics at first pregnancy of 58 women with ET

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients</th>
<th>No. of pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age at diagnosis, y (range)</td>
<td>28 (18-44)</td>
<td>96</td>
</tr>
<tr>
<td>Median age at conception, y (range)</td>
<td>32 (18-44)</td>
<td></td>
</tr>
<tr>
<td>No. with at least 1 abortion risk factor (%)</td>
<td>10/58 (17)</td>
<td></td>
</tr>
<tr>
<td>No. with thrombophilia</td>
<td>15/46 (33)</td>
<td></td>
</tr>
<tr>
<td>Factor V Leiden mutation: +/- †</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Methylenetetrahydrofolate reductase mutation: +/- †</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Prothrombin mutation: +/- †</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Hyperhomocysteinemia</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Antiphospholipid antibody</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

*Abortion risk factors include overweight, hypertension, high cholesterol level, diabetes, current smoking, and thyroid diseases.
†+/− indicates homozygous; +/−, heterozygous.
‡Hyperhomocysteinemia, more than 13.9 μM.
A total of 9 (60%) of 15 patients with thrombophilia had complications in first pregnancy: abortion in 6 patients (5 with MTHFR mutation and 1 with prothrombin gene mutation), pre-eclampsia in 1 patient (MTHFR mutation), and intrauterine growth retardation in 2 patients (1 with Factor V Leiden mutation and 1 with MTHFR mutation). A total of 17 (71%) of 24 patients carrying the JAK2 (617V>F) mutation had complications at first pregnancy (abortion in 8 patients, stillbirth in 2 patients, intrauterine growth retardation in 3 patients, preeclampsia in 2 patients, and hypertension in 2 patients).

Of 13 pregnancies conceived while patients were receiving a cytoreductive treatment, 9 (70%) were complicated (6 abortions and 3 preeclampsia). According to treatment at conception, complications occurred in 4 (80%) of 5 pregnancies on hydroxyurea and in 5 (62%) of 8 pregnancies on interferon. Of the 3 patients who continued interferon during pregnancy, 1 (33%) had preeclampsia.

The impact of a previous pregnancy was investigated in 31 patients who had 2 pregnancies. The outcome of pregnancies was concordant in 19 (61%) patients (both pregnancies uncomplicated or complicated), and discordant in 12 (39%). The 2-tailed Fisher exact test showed that pregnancy outcome was not significantly influenced by the outcome of a previous pregnancy. We further analyzed the 24 patients with multiple pregnancies who had JAK2 mutational status assessed (15 positive and 9 negative). Of the patients who had complications with all pregnancies, 6 (40%) of 15 carried the JAK2 (617V>F) mutation, and 2 (22%) of 9 were without the mutation (P = .19).

We investigated as potential predictors of complications for the first pregnancy both maternal characteristics (age, parity, presence of abortion risk factors, presence of thrombophilia), and disease characteristics (hemoglobin level, platelet and leukocyte counts, history of thrombosis, platelet counts lower than 1000 × 10^9/L, white blood cell [WBC] count higher than 10 × 10^9/L at conception, JAK2 mutational status, and antiplatelet and antineoproliferative therapy before and during pregnancy). A univariate logistic regression model showed that the JAK2 (617V>F) mutation was a significant risk factor (P = .01) for complications. A multivariate logistic regression model confirmed the JAK2 (617V>F) 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 (617V>F) mutation had an OR equal to 2.02 (95% confidence interval [CI]: 1.1 to 3.8) of developing complications during pregnancy. To find whether the JAK2 (617V>F) mutation compounded the effect of thrombophilia, a multivariate logistic regression analysis with JAK2 (617V>F) mutational status and thrombophilia as covariate was applied. We found that the JAK2 (617V>F) mutation was an independent predictor of pregnancy outcome (P = .03) without any significant interaction between the 2 parameters (P = .37).

Of the 40 pregnancies in JAK2 (617V>F)–positive patients, complications occurred in 13 (52%) of 25 patients receiving aspirin, and in 12 (80%) of 15 patients not receiving any antiplatelet therapy. The difference between the 2 proportions was not statistically significant (P = .08). Of the 42 pregnancies in JAK2 (617V>F)–negative patients, complications occurred in 13 (52%) of 25 patients receiving aspirin and in 4 (23%) of 17 patients not receiving any antiplatelet 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%) occurred at the second trimester.

The Mantel-Haenszel method was used to quantify the rate of fetal loss among patients with ET compared with that of an age-matched general Italian population. We obtained an OR of 3.4 (95% CI: 3 to 3.9; P < .001), which means a 3.4-fold higher risk of fetal loss for patients with ET compared with the 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 (617V>F) 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 ET. The rate of live birth was 64%, and 51% of pregnancies were uneventful. Maternal complications such as preeclampsia and hypertension occurred in 9% of pregnancies 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 pregnancy.

### Table 2. Complications of 96 pregnancies in 58 patients with ET

<table>
<thead>
<tr>
<th>Pregnancy complications</th>
<th>No. 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></td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>9 (9)</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>4</td>
</tr>
</tbody>
</table>

Figure 1. Odds ratios for the prevalence of risk factors in patients with pregnancy complications. ORs were 0.9 (95% CI [bar]: 0.5-1.7) for age 35 years or younger, 0.8 (95% CI: 0.4-1.7) for parity, 1 (95% CI: 0.5-2.0) 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 × 10^9/L, 0.7 (95% CI: 0.4-1.3) for leukocytosis exceeding 10 × 10^9/L, 2 (95% CI: 1.1-3.8) for the presence of JAK2 (617V>F) mutation, and 0.9 (95% CI: 0.5-1.6) for antiplatelet therapy during pregnancy. The JAK2 (617V>F) mutation was a significant risk factor for pregnancy complications.
puerperium. This is in keeping with other studies.\(^6,19-21\) 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.\(^3\) 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 in pregnancy complications in the general population,\(^22,23\) 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 in their first pregnancy. However, thrombophilic state per se did not reach statistical significance as risk factor for complications, probably because it was obscured by stronger disease-related factors. Nevertheless, the inclusion of thrombophilic tests in the work-up of a woman with ET of childbearing age is recommended for individualized therapeutic interventions aimed at improving pregnancy outcome.\(^24\)

The JAK2 (617V>F) mutation assessment is a key tool in the diagnostic work-up of patients with chronic myeloproliferative disorders.\(^25,26\) In our series of pregnant women with ET, the JAK2 (617V>F) mutation was found in 49% of patients, similar to that in other series.\(^8,10,27\) The same concordance was found also in the proportion of mutant alleles, which ranged from 3.9% to 24.2%, 5,28,29 At diagnosis of ET, patients carrying the JAK2 (617V>F) mutation had a significantly higher hemoglobin level than those without the mutation. Concerning the influence of JAK2 (617V>F) 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 2-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.\(^5,20\) The reduction of platelet count was observed in both JAK2 (617V>F)–positive and JAK2 (617V>F)–negative patients without significant differences. This suggests that this phenomenon is independent of the JAK2 (617V>F) mutation.

Concerning treatment of ET during pregnancy, cyto reduction should be avoided, particularly in the first trimester,\(^5\) because teratogenicity of cyoreductive agents cannot be ruled out.\(^20\) Interferon is considered the agent of choice in pregnant women with ET who need platelet count reduction.\(^3\) In this study, 1 of 3 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.\(^31\) Aspirin is commonly used in patients with ET who do not have a history of bleeding.\(^32\) In our series of 96 pregnancies considered as a whole, the use of aspirin did not influence pregnancy outcome, as was also found by Tefferi and coworkers.\(^6\) Grouping patients according to JAK2 (617V>F)–positive/patients, and appeared to worsen outcome in JAK2 (617V>F)–negative patients.

In conclusion, this study on patients with ET indicates that pregnancy may evolve uneventfully in half of the patients. Women carrying the JAK2 (617V>F) mutation have higher risk of developing pregnancy complications.

Acknowledgments

This work was supported by grants from Fondazione Cariplo, Milan; Associazione Italiana per la Ricerca sul Cancro (AIRC), Milan; Fondazione Ferrata Storti, Pavia; and Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.

Authorship

Contribution: F.P. and M.L conceived the study, collected, analyzed, and interpreted data, and 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., and 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.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References

mostly polyclonal and V617F-JAK2 negative.


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