Int J Hematol (2009) 90:522–525
DOI 10.1007/s12185-009-0411-5

ORIGINAL ARTICLE

# Cytogenetic abnormalities in essential thrombocythemia at presentation and transformation

**Matjaz Sever · Hagop Kantarjian · Sherry Pierce · Nitin Jain · Zeev Estrov · Jorge Cortes · Srdan Verstovsek**

Received: 8 July 2009 / Revised: 28 July 2009 / Accepted: 6 August 2009 / Published online: 29 August 2009


**Abstract** Cytogenetic abnormalities in patients with essential thrombocythemia (ET) are infrequent. Their role in survival of patients and disease transformation is not extensively studied. We describe cytogenetic abnormalities in 172 patients with ET at a single institution. At presentation nine (5.2%) patients had cytogenetic abnormality and three (1.7%) additional patients acquired them during follow-up. Survival of patients with cytogenetic changes at presentation did not differ when compared to the patients with normal karyotype. The more common were abnormalities of chromosome 9 ($n = 4$), 20 ($n = 2$), 5 ($n = 2$), and complex abnormalities ($n = 2$). Forty-one patients (23.8%) had additional cytogenetic tests performed for monitoring purposes during follow-up. Five patients (2.9%) with normal karyotype transformed to myelofibrosis (MF) without developing new cytogenetic changes at transformation. Two patients (1.2%) with normal karyotypes at presentation transformed to myelodysplastic syndrome and acute myeloid leukemia, respectively. Both acquired complex cytogenetic changes at the time of transformation. There is no rationale for repeating cytogenetic tests in ET patients on follow up, unless blood cell count changes suggest possible transformation.

**Keywords** Essential thrombocythemia · Cytogenetic abnormality · Survival · Myelofibrosis · Acute myeloid leukemia · Myelodysplastic syndrome

M. Sever · H. Kantarjian · S. Pierce · N. Jain · Z. Estrov · J. Cortes · S. Verstovsek (✉)
Department of Leukemia, The University of Texas MD Anderson Cancer Center, Unit 428, 1515 Holcombe Blvd., Houston, TX 77030, USA
e-mail: sverstov@mdanderson.org

## 1 Introduction

Essential thrombocythemia (ET) is chronic myeloproliferative neoplasm (MPN) characterized by persistent elevated platelet count (>450 × $10^9$/L) in peripheral blood, bone marrow megakaryocytic proliferation, and presence of $JAK2^{V617F}$ clonal marker in 50% of patients [1]. The course of the disease is benign as patients have close to normal life expectancy, but complicated in some patients by thrombotic and hemorrhagic events. Only small proportion of patients transforms to secondary myelofibrosis (post-ET MF), myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) [2–6]. The discovery of acquired $JAK2^{V617F}$ mutation in MPN caused substantial step forward in our understanding of the disease pathogenesis. The presence of mutation in ET patients has been associated with increased leukocyte and red blood cell counts, reduced platelet count, increased age, higher frequency of thrombotic events and transformation to polycythemia vera [7–11]. On the other hand, cytogenetic abnormalities have been observed in some ET patients, but have contributed little to our understanding of ET pathogenesis and prognosis. This is primarily the result of the fact that cytogenetic abnormalities in ET are rare and heterogeneous; they have been reported in every chromosome and no consistent genetic marker has been identified [12]. Their frequency at diagnosis is between 1.2 and 7.0%, and includes both structural changes, such as unbalanced translocations, and numerical gains and losses [13–18]. Recently, Gangat et al. [14] reported the association of cytogenetic abnormality at diagnosis with palpable splenomegaly, current tobacco use, venous thrombosis and anemia, but no influence on the survival of patients. Hsiao et al. [15] reported association between transformation and unfavorable survival with de novo appearance of cytogenetic abnormalities in ET

patients. It seems that majority of ET patients who transform to MF do not gain a cytogenetic abnormality at transformation, while the majority of those who transform to AML do acquire cytogenetic abnormality at the time of transformation [14, 15]. We analyzed cytogenetic abnormalities in 172 ET patients with informative cytogenetic results at their presentation and during follow up, and assessed their impact on transformation and survival.

## 2 Methods

Institutional review board approval was obtained for this study from the University of Texas MD Anderson Cancer Center. MPN database at MD Anderson Cancer Center was searched for ET patients, evaluated from January 1976 to December 2007, who had informative cytogenetics. Clinical and histopathological results in identified patients were reviewed and the diagnosis of ET was confirmed according to the WHO criteria [19]. Conventional cytogenetic analysis was performed on metaphases obtained from unstimulated bone marrow aspirate cultures using standard techniques. Results were reported using International System for Human Cytogenetic Nomenclature. Cytogenetic result was considered pathologic when at least two abnormal metaphases were identified with structural abnormality or chromosome gain, or when at least three metaphases of the same chromosome loss were identified. Cytogenetic testing was considered to be 'at initial diagnosis' if the patient was referred and assessed at our center within 4 months of confirmation of ET; all other testing was considered 'beyond initial diagnosis'. JAK2 mutation status was analyzed in stored bone marrow specimens when possible, using previously published assay [20].

Prism 5 for Windows (GraphPad Software, Inc.) was used for statistical analysis. Survival curves were estimated by Kaplan–Meier plots and homogeneity of survival curves over different groups was tested by log-rank test.

## 3 Results

At presentation at our institution 172 ET patients had adequate metaphases for interpretation on cytogenetic testing. Their general characteristics are presented in Table 1. Among them 82 (47.7%) were newly diagnosed or referred to our institution within 4 months since the diagnosis had been established. Median number of cytogenetic tests per patient performed in our 172 patients was 1 (range 1–9). Forty-one patients (23.8%) had two or more cytogenetic studies performed during follow-up, with median time of 12.5 (range 1–169) months between tests. More common abnormalities involved chromosome 9 ($n = 4$), 20 ($n = 2$), 5 ($n = 2$), and complex abnormalities ($n = 2$), with other detected on only one occasion (Table 2). Nine (5.2%) patients had abnormal cytogenetic studies at presentation. In two (1.2%) additional patients −Y was detected and considered to be normal variant. Three patients (1.7%) with normal karyotype at presentation developed cytogenetic abnormality during follow-up: complex karyotype in two and −X in one patient. The two patients with complex karyotype had their tests performed at the time of transformation to AML and MDS after 216 and 55 months from presentation, respectively. Survival of patients with or without cytogenetic abnormality did not differ significantly (median survival of 15 years and not reached, respectively; $p = 0.7$). Similarly, characteristics of patients with and without cytogenetic abnormality at diagnosis did not differ.

**Table 1** Patient characteristics

| | |
|---|---|
| Total no. of patients | 172 |
| Age in years (median, range) | 51 (16–83) |
| No. of male patients (%) | 63 (36.6) |
| No. with B symptoms (%) | 70 (40.7) |
| No. with palpable spleen (%) | 9 (5.2) (4 splenectomies) |
| Leukocyte count (median, range; $\times 10^9$/L) | 8.4 (1.9–21.9) |
| No. with leukocyte count $\geq 10 \times 10^9$/L (%) | 51 (29.7) |
| No. with leukocyte count $\geq 15 \times 10^9$/L (%) | 12 (7.0) |
| Hemoglobin level (median, range; g/dL) | 13.4 (7.1–16.8) |
| No. with hemoglobin <12 g/dL (%) | 29 (16.9) |
| Platelet count (median, range; $\times 10^9$/L) | 711.5 (153–3065) |
| No. with platelet count $\geq 1{,}000 \times 10^9$/L (%) | 39 (22.7) |
| No. with abnormal cytogenetic test at diagnosis (%) | 9 (5.2) |
| No. with JAK2 test performed (%) | 56 (32.6) |
| No. with JAK2 test positive (%) | 32 (57.1) |
| No. with age ≥60 years (%) | 51 (29.7) |
| No. with hypertension (%) | 51 (29.7) |
| No. with diabetes mellitus (%) | 8 (4.7) |
| No. with hyperlipidemia (%) | 15 (8.7) |
| No. of smokers (%) | 55 (32.0) |
| No. with major thrombosis (%) | 39 (22.7) |
| No. with major hemorrhage (%) | 30 (17.4) |
| No. with transformation to MF ($n = 5$), AML ($n = 1$), MDS ($n = 1$) (%) | 7 (4.1) |
| Median follow up (median, range; years) | 3.22 (0–18.28) |
| No. died (%) | 18 (10.5) |

*MF* myelofibrosis, *AML* acute myeloid leukemia, *MDS* myelodysplastic syndrome

## 4 Discussion

In the present study, we analyzed cytogenetic abnormalities in 172 ET patients seen at our institution. The frequency of

**Table 2** Cytogenetic abnormalities in the patient group ($n = 172$) at diagnosis ($n = 9$) and follow up ($n = 3$)

| Cytogenetic abnormality | Number of lesions |
|---|---|
| Abnormality of chromosome 5 | |
| 46,XX,del(5)(q?)[3]/46,XX[26] | 1 |
| 46,XX,del(5)(q13q33)[2]/46,XX[28] | 1 |
| Abnormality of chromosome 9 | |
| 46,XX,inv(9)qh (reported as clonal abnormality) | 1 |
| 46,XY,inv(9)(p11q12)[20] | 1 |
| 46,XX,inv(9)(p11q13)c[5]/46,XX[25] | 1 |
| 47,XX,+9[2]/46,XX[17] | 1 |
| Abnormality of chromosome 13 | |
| 45,XX,der(13;22)(q10;q10)[20] | 1 |
| Del (20) | |
| 46,XY,del(20)(p12)[2]/46,XY[27] | 1 |
| 46,XY,del(20)(q12)[7]/46,XY[18] | 1 |
| Del X | |
| 45,X,-X[3]/46,XX[17] | 1 |
| Complex abnormalities | |
| 46,XY,+1,der(1, 7)(q10;p10)[8]/ 47,XY,+1,der(1, 7)(q10;p10),+8[2][a] | 1 |
| 47,XY,+1,der(1;17)(q10;q10)[9]/ 47,XY,+1,der(1;17)(q10;q10),+8[11][a] | 1 |

[a] Cytogenetic abnormality discovered at the time of transformation to AML and MDS

cytogenetic abnormality at presentation was 5.2%, similar to previous reports by other investigators [13–18]. Different chromosomal abnormalities have been identified, similar to a report by Gangat et al. [14], who analyzed cytogenetics in the largest group of ET patients to date. 5q− deletion in ET patients has been described in several reports. Since clinical picture of 5q− syndrome (MDS subtype) may overlap with that of ET, some argue that there is continuity between ET and development of 5q− MDS [21–24]. Our patients with 5q− abnormality had no dysplasia in the bone marrow, but typical findings of ET; one had $JAK2^{V617F}$ mutation. We observed der(1;7)(q10;p10) abnormality in one patient at the time of transformation to AML. This abnormality has been associated with leukemic transformation and unfavorable prognosis in ET patients by Hsiao et al. In MDS and AML patients, it carries poor prognosis with median survival of 23 months [25]. In general, however, ET patients who transform to MDS and AML have poor prognosis not only because of the acquired cytogenetic abnormality. On the other hand, it appears that cytogenetic abnormalities are not acquired upon transformation to MF, and post-ET MF patients have no different outcome than patients with primary MF. The pericentric inversion of chromosome 9 is commonly considered constitutional abnormality, occurring in 1–3% of general population. Recently, however, cases of patients with hematological diseases and acquired inv(9)(p11q13)c have been reported, including newly diagnosed patient with ET [26–28], rising a question of its role in the pathogenesis of the disease. Because of that, we report all chromosome 9 inversion abnormalities among other identified abnormalities in Table 2, similar to a report by Gangat et al. [14].

Frequency of $JAK2^{V617F}$ mutation has been extensively studied in ET patients. However, among studies examining cytogenetic changes in ET [13–18] only Gangat et al. [14] examined the correlation between the presence of $JAK2^{V617F}$ mutation and cytogenetic abnormality. The frequency of mutation overall was 58.5% with no statistically significant difference between patients with cytogenetic changes and those with normal karyotype. In our patient population testing for $JAK2^{V617F}$ mutation was done in 56 patients, of which only four had cytogenetic abnormalities at presentation and three of them tested positive.

Bone marrow examination in patients with ET is usually performed at presentation for diagnostic purpose, primarily to exclude any other hematological disease, particularly early phase MF. While a good number of reports have now established a lack of abnormal cytogenetics influence on patient outcome, cytogenetic testing should be performed at the time of bone marrow test. For example, patients with 5q− abnormality could be treated with lenalidomide. In 23.8% of our patients cytogenetic test was performed more than once during their follow-up, for monitoring purpose. However, we do not see any advantage of such a practice. There appears to be no rationale to repeat cytogenetic test after initial assessment, unless changes in blood cell count indicate possible transformation.

In summary, cytogenetic abnormalities at presentation and during follow-up in ET patients are rare. There is no difference in survival of patients with cytogenetic abnormalities and those with diploid karyotype at presentation. Monitoring patients by repeating cytogenetic test at follow-up is not indicated. This should be, however, done at the time of suspected transformation.

## References

1. Tefferi A, Thiele J, Orazi A, Kvasnicka HM, Barbui T, Hanson CA, et al. Proposals and rationale for revision of the World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis: recommendations from an ad hoc international expert panel. Blood. 2007;110(4):1092–7.
2. Chim CS, Kwong YL, Lie AK, Ma SK, Chan CC, Wong LG, et al. Long-term outcome of 231 patients with essential thrombocythemia: prognostic factors for thrombosis, bleeding, myelofibrosis, and leukemia. Arch Intern Med. 2005;165(22):2651–8.

3. Gangat N, Wolanskyj AP, McClure RF, Li CY, Schwager S, Wu W, et al. Risk stratification for survival and leukemic transformation in essential thrombocythemia: a single institutional study of 605 patients. Leukemia. 2007;21(2):270–6.
4. Passamonti F, Rumi E, Arcaini L, Boveri E, Elena C, Pietra D, et al. Prognostic factors for thrombosis, myelofibrosis, and leukemia in essential thrombocythemia: a study of 605 patients. Haematologica. 2008;93(11):1645–51.
5. Tefferi A, Gangat N, Wolanskyj AP, Schwager S, Pardanani A, Lasho TL, et al. 20+ yr without leukemic or fibrotic transformation in essential thrombocythemia or polycythemia vera: predictors at diagnosis. Eur J Haematol. 2008;80(5):386–90.
6. Wolanskyj AP, Schwager SM, McClure RF, Larson DR, Tefferi A. Essential thrombocythemia beyond the first decade: life expectancy, long-term complication rates, and prognostic factors. Mayo Clin Proc. 2006;81(2):159–66.
7. Heller PG, Lev PR, Salim JP, Kornblihtt LI, Goette NP, Chazarreta CD, et al. JAK2V617F mutation in platelets from essential thrombocythemia patients: correlation with clinical features and analysis of STAT5 phosphorylation status. Eur J Haematol. 2006;77(3):210–6.
8. Ohyashiki K, Aota Y, Akahane D, Gotoh A, Ohyashiki JH. JAK2(V617F) mutational status as determined by semiquantitative sequence-specific primer-single molecule fluorescence detection assay is linked to clinical features in chronic myeloproliferative disorders. Leukemia. 2007;21(5):1097–9.
9. Rudzki Z, Sacha T, Stoj A, Czekalska S, Wojcik M, Skotnicki AB, et al. The gain-of-function JAK2 V617F mutation shifts the phenotype of essential thrombocythemia and chronic idiopathic myelofibrosis to more "erythremic" and less "thrombocythemic": a molecular, histologic, and clinical study. Int J Hematol. 2007;86(2):130–6.
10. Speletas M, Katodritou E, Daiou C, Mandala E, Papadakis E, Kioumi A, et al. Correlations of JAK2-V617F mutation with clinical and laboratory findings in patients with myeloproliferative disorders. Leuk Res. 2007;31(8):1053–62.
11. Vannucchi AM, Antonioli E, Guglielmelli P, Rambaldi A, Barosi G, Marchioli R, et al. Clinical profile of homozygous JAK2 617V>F mutation in patients with polycythemia vera or essential thrombocythemia. Blood. 2007;110(3):840–6.
12. Steensma DP, Tefferi A. Cytogenetic and molecular genetic aspects of essential thrombocythemia. Acta Haematol. 2002; 108(2):55–65.
13. Bacher U, Haferlach T, Kern W, Hiddemann W, Schnittger S, Schoch C. Conventional cytogenetics of myeloproliferative diseases other than CML contribute valid information. Ann Hematol. 2005;84(4):250–7.
14. Gangat N, Tefferi A, Thanarajasingam G, Patnaik M, Schwager S, Ketterling R, et al. Cytogenetic abnormalities in essential thrombocythemia: prevalence and prognostic significance. Eur J Haematol. 2009;83(1):17–21.
15. Hsiao HH, Ito Y, Sashida G, Ohyashiki JH, Ohyashiki K. De novo appearance of der(1;7)(q10;p10) is associated with leukemic transformation and unfavorable prognosis in essential thrombocythemia. Leuk Res. 2005;29(11):1247–52.
16. Panani AD. Cytogenetic findings in untreated patients with essential thrombocythemia. In Vivo. 2006;20(3):381–4.
17. Panani AD. Single chromosomal abnormalities in Philadelphia-negative chronic myeloproliferative disorders. In Vivo. 2007; 21(5):867–70.
18. Sessarego M, Defferrari R, Dejana AM, Rebuttato AM, Fugazza G, Salvidio E, et al. Cytogenetic analysis in essential thrombocythemia at diagnosis and at transformation. A 12-year study. Cancer Genet Cytogenet. 1989;43(1):57–65.
19. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood. 2002;100(7):2292–302.
20. McClure R, Mai M, Lasho T. Validation of two clinically useful assays for evaluation of JAK2 V617F mutation in chronic myeloproliferative disorders. Leukemia. 2006;20(1):168–71.
21. Koike T, Uesugi Y, Toba K, Narita M, Fuse I, Takahashi M, et al. 5q-syndrome presenting as essential thrombocythemia: myelodysplastic syndrome or chronic myeloproliferative disorders? Leukemia. 1995;9(3):517–8.
22. Reis MD, Sher GD, Lakhani A, Dube ID, Senn JS, Pinkerton PH. Deletion of the long arm of chromosome 5 in essential thrombocythemia. Cancer Genet Cytogenet. 1992;61(1):93–5.
23. Takahashi H, Furukawa T, Hashimoto S, Kanazawa N, Satoh N, Suzuki N, et al. 5q− syndrome presenting chronic myeloproliferative disorders-like manifestation: a case report. Am J Hematol. 2000;64(2):120–3.
24. Welborn J. 5q− in essential thrombocythemia. Cancer Genet Cytogenet. 1993;67(2):150.
25. Sanada M, Uike N, Ohyashiki K, Ozawa K, Lili W, Hangaishi A, et al. Unbalanced translocation der(1;7)(q10;p10) defines a unique clinicopathological subgroup of myeloid neoplasms. Leukemia. 2007;21(5):992–7.
26. Betz JL, Behairy AS, Rabionet P, Tirtorahardjo B, Moore MW, Cotter PD. Acquired inv(9): what is its significance? Cancer Genet Cytogenet. 2005;160(1):76–8.
27. Udayakumar AM, Pathare AV, Dennison D, Raeburn JA. Acquired pericentric inversion of chromosome 9 in acute myeloid leukemia. J Appl Genet. 2009;50(1):73–6.
28. Wan TS, Ma SK, Chan LC. Acquired pericentric inversion of chromosome 9 in essential thrombocythemia. Hum Genet. 2000;106(6):669–70.