The allele burden of *JAK2* mutations remains stable over several years in patients with myeloproliferative disorders

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ABSTRACT

In a retrospective single center study we determined the time course of the *JAK2*-V617F or *JAK2* exon 12 allele burden in DNA from purified granulocytes from 48 patients with myeloproliferative disorders. The percentage of change between the first and last sample in *JAK2*-V617F positive patients without cytoreductive therapy (n=16) was only +9% during a follow-up of 36±13 months, reflecting a remarkably stable mutant allele burden. When treatment with hydroxyurea was initiated during the course of the study, we observed a significant decrease of the *JAK2*-V617F allele burden (n=6). However, in *JAK2*-V617F positive patients who were already on hydroxyurea treatment before the first blood sampling (n=14), we observed stable allelic ratios with a variance of only +3% during a follow-up of 34±16 months. Our data suggest that in untreated myeloproliferative disorders patients, from whom samples at diagnosis are not available, the *JAK2* allele burden determined at later stages could be equally informative.

Key words: Janus kinase, *JAK2*-V617F, hydroxyurea, interferon.

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Introduction

Myeloproliferative disorders (MPD) are a heterogeneous group of diseases characterized by increased hematopoiesis leading to elevated numbers of non-lymphoid cells and/or platelets in the peripheral blood. An acquired somatic mutation in the *JAK2* gene resulting in a valine to phenylalanine substitution at position 617 (*JAK2*-V617F) is frequently found in patients with MPDs.[1-4] Using a sensitive allele-specific PCR assay, the *JAK2*-V617F mutation is detectable in >90% of patients with polycythemia vera (PV), >50% of essential thrombocythemia (ET) and >50% of primary myelofibrosis (PMF).[2,5,6] Recently mutations in exon 12 of *JAK2* were found in *JAK2*-V617F negative PV patients.[7] We developed a quantitative PCR assay to measure the allelic ratios of *JAK2* exon 12 mutations.[8] Hydroxyurea is the most frequently used cytoreductive treatment in MPD. PV and ET patients who carry the *JAK2*-V617F mutation are more sensitive to hydroxyurea compared to *JAK2*-V617F negative patients.[9,10] In a phase 2 study, treatment with pegylated interferon-α2a in *JAK2*-V617F positive PV patients was shown to reduce the mutant allelic ratio significantly and lead to molecular remission in one patient.[11] However in another study, treatment with pegylated interferon-α2b induced only a modest decrease in the allelic ratio of *JAK2*-V617F.[12] Here we performed a retrospective single center study and analyzed the allelic ratios of *JAK2* mutations at several time points in purified granulocytes from 48 MPD patients during a follow-up of at least 12 months.

Design and Methods

Patients

We performed a retrospective single center study on patients with MPD diagnosed according to the criteria of the World Health Organization.[13-15] Inclusion criteria for this study were the presence of a *JAK2* mutation (*JAK2*-V617F or exon 12 mutation), availability of at least two blood samples drawn with a minimal interval of at least 12 months and

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information about the treatment during this time period. The collection of patient samples was approved by the local ethics committee (Ethik Kommission Beider Basel). Informed consent from all patients was obtained in accordance with the Declaration of Helsinki.

Samples and analyses

Drawing of blood, purification of granulocytes and preparation of DNA was performed as described.[16] The quantitative PCR assays for *JAK2*-V617F and *JAK2* exon 12 mutations were performed as described.[8] The sensitivity of the assays is around 0.5% allelic ratio. Repeated analysis in a test cohort of 22 patients revealed that the standard deviation between 2 measurements of the same DNAs was ± 1.08% mutated allele (*data not shown*). To indicate the relative change in the allelic ratios between the first and last blood sample in each patient, the following ratio was determined: *JAK2 variance*^{MUT} = (%T last *JAK2*-V617F - %T first *JAK2*-V617F) : %T first *JAK2*-V617F x 100. The same calculation was used for patients with *JAK2* exon 12 mutations.

Statistical analyses

To compare continuous variables among the groups we used the Mann-Whitney U-test and the Kruskal Wallis-Test.

Results and Discussion

Of 134 MPD patients (62 PV, 51 ET, 21 PMF) initially considered, 51 fulfilled the inclusion criteria (33 PV, 15 ET, 3 PMF). Due to the low number of PMF patients, only PV and ET patients were analyzed in more detail. The patient characteristics are summarized in Table 1. In 3 *JAK2*-V617F negative PV patients a mutation in *JAK2* exon12 was found; all other patients carried the *JAK2*-V617F mutation. The time course of allelic ratios for *JAK2*-V617F and *JAK2* exon 12 determined by allele-specific PCR is shown in Figure 1. The allelic ratios in most patients remained remarkably stable over the observation period (Figure 1A and B). As expected, PV patients had higher allelic ratios of *JAK2*-V617F (63±25%T) than ET patients (23±15 %T, $p<0.005$) (Figure 1A and B).[6,17] The allelic ratios in PV patients with *JAK2* exon 12 mutations were markedly lower (mean=21.3% mutated allele) than in PV patients with *JAK2*-V617F (Figure 1D and G). Interestingly, three *JAK2*-V617F positive patients became negative for the mutation (Figure 1C): One PV patient with secondary myelofibrosis underwent allogeneic hematopoietic stem cell transplantation (HSCT). Conversion to *JAK2*-V617F negativity was reported in most MPD cases successfully treated with HSCT.[18,19] A second PV patient transformed to acute myeloid leukemia (AML) with blast cells negative for *JAK2*-V617F. This phenomenon has been found in a substantial proportion of AML secondary to MPD.[20-23] Finally, one ET patient became *JAK2*-V617F negative during treatment with interferon-α2a. Decrease in the *JAK2*-V617F allelic ratios and occasional complete remission has been reported in PV patients treated with interferon-α2a.[11] The 3 patients who converted to *JAK2*-V617F negativity were excluded from the following statistical analyses.

To assess the effects of cytoreductive treatment on the *JAK2*-V617F allelic ratios, we subdivided the patients according to presence or absence of cytoreduction (Figure 1 D-L). Hydroxyurea was used most frequently (n=26), whereas one ET patient was treated with anagrelide and one above-mentioned patient received interferon-α2a. Interestingly, untreated patients (n=18, Figure 1D and E) and patients who were already on cytoreductive treatment at the time of the first blood sampling (n=16, Figure 1G and H) showed a remarkably stable allelic ratio during the follow-up. The latter group of patients had been on treatment for an average of 90±54 months before entering the study. To quantify the relative changes of the allelic ratios of *JAK2*-V617F we defined the *JAK2 variance*^{MUT} as the percentage of change between first and last blood sample of each patient. We found no significant differences in the *JAK2 variance*^{MUT} between untreated PV and ET patients (Figure 1F) and between treated PV and ET patients (Figure 1I). However, when we looked at PV and ET patients in whom cytoreductive therapy was initiated during the course of the study (n=6), a decrease in the absolute values of the *JAK2*-V617F allele burden was observed in 5/6 patients within six months after hydroxyurea had been started. These differences are more pronounced when expressed as the relative change, i.e. *JAK2 variance*^{MUT} (Figure 1L). None of the 6 patients showed signs of leukemic transformation during follow-up. Conversely, patients who discontinued therapy during the course of the study showed a trend towards an increase in allelic ratios (Figure 1K). One patient showed an increase in allelic ratio of +37% seven months after discontinuation of cytoreduction with hydroxyurea, a treatment that he had been on for more than ten years. The comparison of the *JAK2 variance*^{MUT} between the four subgroups of patients is summarized in Figure 1L. Only the newly treated patients showed a significant deviation from the baseline (no change), when compared to untreated ($p=0.027$) and already treated patients ($p=0.021$). Patients who discontinued therapy showed a trend towards an increase in the allelic ratios, but the differences were not significant ($p=0.4$). Untreated and already treated patients were very stable, with a *JAK2 variance*^{MUT} of only 9% and 3% during a follow-up of 36±13 and 34±16 months respectively (Figure 1L).

Table 1. Clinical characteristics of polycythemia vera and essential thrombocythemia patients.

	PV n=33	*range*	*ET n=15*	*range*
Median age (years)	59	18-78	43	20-84
Gender (m/f)	23/10		5/10	
Median disease duration* (months)	77	0-576	18	0-227
Median interval between first-last sample (months)	34	12-57	23	12-46
Median number of blood samples per patient	5	2-17	3	2-9

**Time between diagnosis of the MPD and the first sampling for the study.*

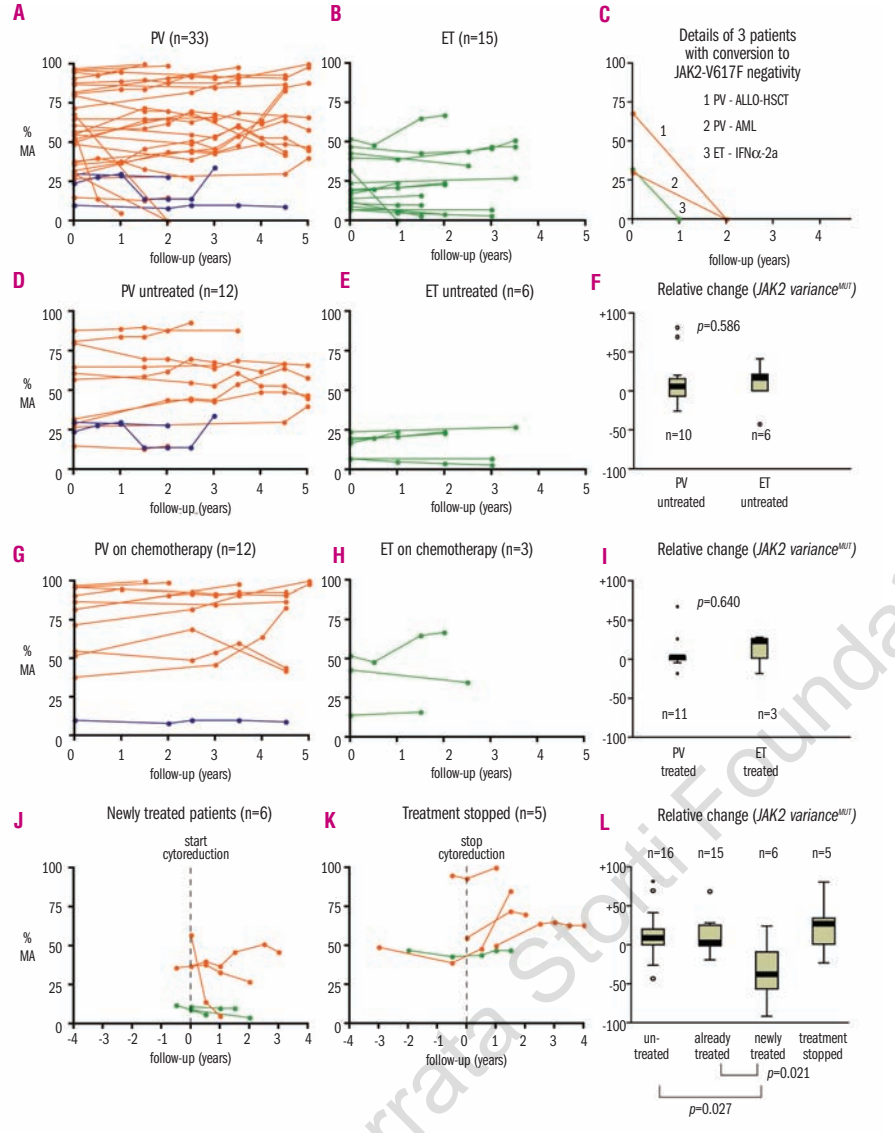

Figure 1. Time course of the *JAK2* mutant allele burden in patients with PV and ET. %MA (mutated allele) indicates the allelic ratio of *JAK2*. Orange dots and lines: PV patients with *JAK2*-V617F mutation. Blue dots and lines: PV patients with *JAK2* exon 12 mutations. Green dots and lines: ET Patients with *JAK2*-V617F mutation. n=number of patients. (A) All PV patients. (B) All ET patients. (C) Details of 3 patients with conversion to *JAK2*-V617F negativity. One underwent allo-HSCT, one transformed to acute myeloid leukemia and one was treated with interferon-α2a. (D) PV patients without cytoreductive treatment. (E) ET patients without cytoreductive treatment. (F) Boxplots representing the relative change of *JAK2*-V617F %T (*JAK2 variance*^{MUT}) during the follow-up period in untreated PV and ET patients. (G) PV patients already treated with hydroxyurea when they entered the study. (H) ET patients already treated with hydroxyurea or when they entered the study. (I) Boxplots representing the *JAK2 variance*^{MUT} during the follow-up period in already treated PV and ET patients. (J) Absolute change of the allelic ratio of *JAK2*-V617F in patients who were newly treated during the follow-up period. Start = initiation of cytoreduction. (K) Absolute change of the allelic ratio of *JAK2*-V617F after cytoreductive therapy was discontinued. (L) Boxplots representing the *JAK2 variance*^{MUT} during the follow-up period. Untreated patients: patients without cytoreductive therapy. Already treated: patients who already took cytoreductive therapy when they entered the study. Newly treated: cytoreductive therapy was initiated during the study. Treatment stopped: cytoreductive therapy was discontinued during the study.

Our study found surprisingly little change in the allelic ratios of *JAK2* mutations in patients with or without cytoreductive treatment. We used solely purified peripheral blood granulocytes with a purity of ≥95% as the source of DNA, which helps to avoid problems that may result from variable cellular composition of whole blood or bone marrow samples. As has been shown by several groups, the *JAK2*-V617F and exon 12 mutations are invariably present in DNA from granulocytes, but the presence of these mutations in other lineages, in particular in lymphocytes, can show large inter-individual differences,[8,24,25] which could introduce false variability in apparent allelic ratios. In 6 patients of the untreated group, the first available sample coincided with the time of the MPD diagnosis, whereas in the remaining 10 patients the first sample was obtained later during the course of the disease. However, there was no difference in the *JAK2 variance*^{MUT} between these two groups of patients (not shown). These data suggest that the MPD clone size, defined by the allelic ratios of the *JAK2* mutations, increases before laboratory and clinical signs of the MPD become manifest to reach a certain level at the time of diagnosis, which depends on the disease entity (ET *vs.* PV) and inter-individual determinants that are currently not understood. Once the disease is manifest, the size of the MPD clone appears to remain relatively stable in the majority of cases. Furthermore, in the steady state, treatment with hydroxyurea did not change the allelic ratios, resulting in a low *JAK2 variance*^{MUT}. However, in individual patients we observed changes in allelic ratios when treatment with hydroxyurea was initiated or terminated (Figure 1J and K). A significant reduction in the *JAK2*-V617F allelic ratio in PV and ET patients treated with hydroxyurea has recently been reported.[26] Similarly, a decrease in the allelic ratio in PV patients who were treated with pegylated interferon-α2a was found.[11] Prospective studies will determine if serial determinations of the *JAK2* allele burden can be used to assess the response to newly initiated cytoreductive therapy. The study of Vannucchi *et al.* suggests that the allelic ratio of *JAK2*-V617F determined at MPD diagnosis correlates with the likelihood of complications such as thrombosis.[27,28] However, for patients with MPD diagnosed before the discovery of the *JAK2* mutations blood samples from

the time of diagnosis are not available. Our data suggest that allelic ratios determined later during the course of the disease may be equally informative in patients who have not been on cytoreductive therapy before the first quantitative determination of *JAK2* mutations. However, this might not be applicable in patients who were already treated as therapy with hydroxyurea appears to induce an initial decrease in mutant allele burden before it reaches a steady state. Currently, no data are available on the effects of anagrelide. Ultimately, prospective studies are needed to validate our observations.

Authorship and Disclosures

AT performed research, analyzed data and wrote the paper; JRP analyzed data, MM, SL, HHS and ASB performed research; AG and AT analyzed data and RCS designed research, analyzed data and wrote the paper.

The authors reported no potential conflicts of interest.

References

1. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature 2005;434:1144-8.
2. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 2005; 365:1054-61.
3. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell 2005;7:387-97.
4. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005;352:1779-90.
5. Antonioli E, Guglielmelli P, Pancrazzi A, Bogani C, Verrucci M, Ponziani V, et al. Clinical implications of the JAK2 V617F mutation in essential thrombocythemia. Leukemia 2005;19:1847-9.
6. Lippert E, Boissinot M, Kralovics R, Girodon F, Dobo I, Praloran V, et al. The JAK2-V617F mutation is frequently present at diagnosis in patients with essential thrombocythemia and polycythemia vera. Blood 2006;108:1865-7.
7. Scott LM, Tong W, Levine RL, Scott MA, Beer PA, Stratton MR, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med 2007;356: 459-68.
8. Li S, Kralovics R, De Libero G, Theocharides A, Gisslinger H, Skoda RC. Clonal heterogeneity in polycythemia vera patients with JAK2 exon12 and JAK2-V617F mutations. Blood 2008;111:3863-6.
9. Campbell PJ, Scott LM, Buck G, Wheatley K, East CL, Marsden JT, et al. Definition of subtypes of essential thrombocythaemia and relation to polycythaemia vera based on JAK2 V617F mutation status: a prospective study. Lancet 2005;366: 1945-53.
10. Sirhan S, Lasho TL, Hanson CA, Mesa RA, Pardanani A, Tefferi A. The presence of JAK2V617F in primary myelofibrosis or its allele burden in polycythemia vera predicts chemosensitivity to hydroxyurea. Am J Hematol 2008;83:363-5.
11. Kiladjian JJ, Cassinat B, Turlure P, Cambier N, Roussel M, Bellucci S, et al. High molecular response rate of polycythemia vera patients treated with pegylated interferon alpha-2a. Blood 2006;108:2037-40.
12. Jones AV, Silver RT, Waghorn K, Curtis C, Kreil S, Zoi K, et al. Minimal molecular response in polycythemia vera patients treated with imatinib or interferon alpha. Blood 2006;107:3339-41.
13. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood 2002;100:2292-302.
14. Murphy S. Diagnostic criteria and prognosis in polycythemia vera and essential thrombocythemia. Semin Hematol 1999;36(1 Suppl 2):9-13.
15. Pearson TC. Evaluation of diagnostic criteria in polycythemia vera. Semin Hematol 2001;38(1 Suppl 2): 21-4.
16. Kralovics R, Teo SS, Li S, Theocharides A, Buser AS, Tichelli A, et al. Acquisition of the V617F mutation of JAK2 is a late genetic event in a subset of patients with myeloproliferative disorders. Blood 2006;108: 1377-80.
17. Passamonti F, Rumi E, Pietra D, Della Porta MG, Boveri E, Pascutto C, et al. Relation between JAK2 (V617F) mutation status, granulocyte activation, and constitutive mobilization of $CD34^+$ cells into peripheral blood in myeloproliferative disorders. Blood 2006;107:3676-82.
18. Kroger N, Badbaran A, Holler E, Hahn J, Kobbe G, Bornhauser M, et al. Monitoring of the JAK2-V617F mutation by highly sensitive quantitative real-time PCR after allogeneic stem cell transplantation in patients with myelofibrosis. Blood 2007;109: 1316-21.
19. Steckel NK, Koldehoff M, Ditschkowski M, Beelen DW, Elmaagacli AH. Use of the activating gene mutation of the tyrosine kinase (VAL617Phe) JAK2 as a minimal residual disease marker in patients with myelofibrosis and myeloid metaplasia after allogeneic stem cell transplantation. Transplantation 2007; 83:1518-20.
20. Jelinek J, Oki Y, Gharibyan V, Bueso-Ramos C, Prchal JT, Verstovsek S, et al. JAK2 mutation 1849G>T is rare in acute leukemias but can be found in CMML, Philadelphia chromosome-negative CML, and megakaryocytic leukemia. Blood 2005;106: 3370-3.
21. Campbell PJ, Baxter EJ, Beer PA, Scott LM, Bench AJ, Huntly BJ, et al. Mutation of JAK2 in the myeloproliferative disorders: timing, clonality studies, cytogenetic associations, and role in leukemic transformation. Blood 2006;108:3548-55.
22. Theocharides A, Boissinot M, Girodon F, Garand R, Teo SS, Lippert E, et al. Leukemic blasts in transformed JAK2-V617F-positive myeloproliferative disorders are frequently negative for the JAK2-V617F mutation. Blood 2007;110: 375-9.
23. Pasqualucci L, Li S, Meloni G, Schnittger S, Gattenlohner S, Liso A, et al. NPM1-mutated acute myeloid leukaemia occurring in JAK2-V617F+ primary myelofibrosis: de-novo origin? Leukemia 2008;22: 1459-63.
24. Delhommeau F, Dupont S, Tonetti C, Masse A, Godin I, Le Couedic JP, et al. Evidence that the JAK2 G1849T (V617F) mutation occurs in a lymphomyeloid progenitor in polycythemia vera and idiopathic myelofibrosis. Blood 2007;109:71-7.
25. Ishii T, Bruno E, Hoffman R, Xu M. Involvement of various hematopoietic-cell lineages by the JAK2V617F mutation in polycythemia vera. Blood 2006;108:3128-34.
26. Ricksten A, Palmqvist L, Johansson P, Andreasson B. Rapid decline of JAK2V617F levels during hydroxyurea treatment in patients with polycythemia vera and essential thrombocythemia. Haematologica 2008;93:1260-1.
27. 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;110:840-6.
28. Antonioli E, Guglielmelli P, Poli G, Bogani C, Pancrazzi A, Longo G, et al. Influence of JAK2V617F allele burden on phenotype in essential thrombocythemia. Haematologica 2008;93:41-8.