Współczesna Onkologia (2008) vol. 12; 10 (452–454)

Introduction: JAK2V617F, a point mutation involving the JAK2 tyrosine kinase gene, occurs in nearly all patients with polycythaemia vera (PV) and in a variable subset of patients with other myeloproliferative neoplasms (MPN).
Aim: We addressed the issue of whether the presence of JAK2 V617F correlates with clinical phenotype.
Material and methods: In a single institution study we screened for the JAK2 point mutation in 60 consecutive patients with PV and essential thrombocythaemia (ET). The mutation was detected in peripheral blood granulocyte DNA using allelic discrimination polymerase chain reaction (PCR).
Results: In total, 42 (70%) patients were positive for this mutation, including 21 subjects with PV (100%), and 21 with TE (54%). The frequency of homozygosity was 21% in PV patients and 0% in ET patients. We found no difference in age at diagnosis, gender or disease duration between patients with and without the mutation. However, a significantly higher haemoglobin concentration and white blood cell count were observed in cases with the JAK2 point mutation. Conversely, the platelet count was significantly lower in V617F-positive cases. Thrombotic complications were more common in patients with V617F.
Conclusions: Our results support the previous observation that JAK2 point mutation is associated with some clinical and haematological features in patients with PV and ET.

Key words: JAK2 V617F, polycythaemia vera, essential thrombocythaemia.

The JAK2 V617F point mutation correlates with clinical phenotype in patients with polycythaemia vera and essential thrombocythaemia

Obecność mutacji punktowej JAK2 V617F koreluje z typowym obrazem klinicznym u chorych z nadkrwistością prawdziwą i nadpłytkowością samoistną

Grzegorz Helbig, Agata Wieczorkiewicz, Małgorzata Krawczyk, Dariusz Kata, Marek Seweryn, Włodzimierz Mendrek, Małgorzata Kopera, Beata Stella-Hołowiecka, Sławomira Krzemień

Department of Haematology and Bone Marrow Transplantation, Silesian Medical University

Introduction

The JAK2 V617F tyrosine kinase mutation results from the substitution of valine by phenylalanine at codon 617 and occurs in nearly all patients with polycythaemia vera (PV) and in a variable proportion of patients with other Philadelphia-negative chronic myeloproliferative neoplasms (MPN) [1]. The association between mutational status and clinical phenotype is still under debate. Due to the small number of JAK2-negative patients with PV, a comparison between positive and negative cases is not possible. The confounding results were demonstrated for patients with essential thrombocythaemia (ET) when comparing the JAK2 positive and negative cases [2, 3]. In our study, we collected data from a series of 60 patients with PV and ET and addressed the issue of whether the presence of JAK2 V617F point mutation is associated with clinical and haematological profile.

Material and methods

Sixty subjects with PV and ET admitted to our Department in 2007 were eligible for the study. The diagnosis of myeloproliferative neoplasm (MPN) was established according to the criteria of the World Health Organisation (patients diagnosed after 2001) and of the Polycythemia Vera Study Group (patients diagnosed before 2001). At admission the following studies were performed: physical examination, complete blood count (CBC) with differential, chemistry, chest X-ray, ultrasound, bone marrow aspirate/biopsy and conventional cytogenetics. The history of thrombotic events was collected.

The JAK2 V617F mutation was detected in peripheral blood granulocyte DNA using allelic discrimination polymerase chain reaction (PCR) with the commercially available kit MutaScreen™ provided by Ipsogen, France. Samples were scored as homozygous if the proportion of the mutant allele was greater than 50%.

Categorical variables were compared between patients who were V617F-positive and V617F-negative using the Mann-Whitney U test. Fisher's exact test was used for nominal variables.

Results

We screened 60 patients (PV, n=21; TE, n=39) at median age of 45 years (range 17-83), 38 female/22 male, for the presence of JAK2 V617F point

Wstęp: Mutacja punktowa JAK2 V617F dotyczy genu JAK2 o aktywności kinazy tyrozynowej i występuje u prawie wszystkich chorych z nadkrwistością prawdziwą (PV) oraz z różną częstotliwością w pozostałych nowotworach mieloproliferacyjnych (MPN).
Cel: Celem badania była analiza korelacji pomiędzy obecnością mutacji JAK2 V617F a obrazem klinicznym.
Materiał i metody: U 60 kolejnych chorych z nadkrwistością prawdziwą i nadpłytkowością samoistną (ET) wykonano analizę mającą na celu wykrycie obecności mutacji punktowej V617F. Mutację badano w DNA uzyskanym z granulocytów krwi obwodowej przy zastosowaniu metody dyskryminacji allelicznej polimerazowej reakcji łańcuchowej.
Wyniki: Mutację wykryto u 42 chorych (70%), w tym u 21 pacjentów z PV (100%) oraz 21 z ET (54%). Homozygotyczność V617F wykazano u 21% chorych z PV i 0% z ET. Nie obserwowano różnic pomiędzy pacjentami JAK2+ i JAK2 – w odniesieniu do wieku przy rozpoznaniu, płci i czasu trwania choroby. W grupie JAK2+ odnotowano znacząco większe stężenie hemoglobiny oraz liczbę białych krwinek, podczas gdy liczba płytek była znamiennie mniejsza. Powikłania zakrzepowe częściej obserwowano u chorych z mutacją V617F.
Wnioski: Wyniki potwierdzają wcześniejsze doniesienia o korelacji pomiędzy obecnością mutacji V617F a określonymi cechami klinicznymi u chorych z PV i ET.

Słowa kluczowe: JAK2 V617F, nadkrwistość prawdziwa, nadpłytkowość samoistna.

Table 1. Patient characteristics
Tabela 1. *Charakterystyka pacjentów*

	JAK2 V617F POSITIVE	JAK2 V617F NEGATIVE	P-value
Number	41	19	
Female/male	23/18	15/4	NS
Median age at diagnosis (years)	50 (22-83)	40 (17-70)	NS
Disease duration (months)	18 (2-300)	40 (5-196)	NS
Median WBC (×10^9 cells/l)	11 (4.4-38.3)	7.1 (4.1-15.9)	<0.001
Median haemoglobin (g/dl)	15.5 (10.3-22)	12.8 (10.5-15.5)	<0.001
Median platelet count (×10^9 cells/l)	603 (231-1688)	862 (202-1720)	0.01
Splenomegaly	22	11	NS
Hepatomegaly	14	6	NS
Thrombotic event	13	1	0.02

NS – not significant

mutation. In total, 42 (70%) patients were positive for this mutation, including 21 subjects with PV (100%), and 21 with TE (54%). The median percentage of mutated JAK2 allele was 57% for PV patients (range 21-73) and 22% for ET cases (range 5-33). The frequency of homozygosity was 21% in PV patients and 0% in ET patients. At diagnosis the median white blood cell (WBC) count was 9.3×10^9 cells/l (range 4.1-38.3), the median haemoglobin concentration was 14.2 g/dl (range 10.3-22.0) and the median platelet count was 721×10^9 cells/l (range 202-1720). Hepatomegaly and/or splenomegaly was present in 88% of patients at diagnosis on ultrasound examination. Thrombotic complications including both arterial and venous events occurred after the disease onset and were present in 14 out of the 60 patients (23%). The clinical characteristics of the study group are shown in Table 1.

Discussion

We screened 60 samples from patients with Ph-negative myeloproliferative neoplasms. In total, 42 cases were positive for JAK2 V617F, giving an overall frequency of the mutation of 70%. In PV, almost 100% of cases express JAK2 V617F point mutation; hence a valid comparison between positive and negative patients is not possible. However, compared with their heterozygote counterparts, homozygote patients had higher haemoglobin level at diagnosis, a higher transformation rate and higher PRV-1 transcript level [4]. In a large retrospective analysis, homozygous patients, irrespectively of their diagnosis, were older and had higher WBC count and haematocrit at diagnosis. They also presented a larger spleen when compared to heterozygotes [5]. In our study due to the small patient population with homozygosity, this comparison was not performed.

In our study group, the V617F mutation was detected in 100% of patients with PV and in 54% with TE, which is similar to the results cited by others [1, 2]. The retrospective study of ET has shown that V617F-positive ET resembles PV and some features suggest that V671F-positive thrombocythemia is a forme fruste of polycythaemia vera [6]. Based on this observation, the V617F-positive ET and PV patients were summarized together and compared to V617F-negative cases.

Clinical correlations of JAK2 V617F were attempted in several previous studies, but the results remained inconclusive. Levine et al. demonstrated an association between presence of the mutation and female gender in patients with PV, but it was not confirmed for ET [3, 7] or for other MPN [8]. We found

no difference in age at diagnosis, gender or disease duration between patients with and without the mutation. However, a significantly higher haemoglobin level and white blood cell count were observed in cases with the V617F point mutation; p-values were <0.001 and <0.001 respectively. The platelet count was significantly lower in JAK2-positive cases (p=0.01). Our results were consistent with those reported by others [9-11]. Thrombotic complications were more common in patients with the mutation; due to the small number of cases a comparison between arterial and venous events was not performed. Several other groups reported that JAK2 V617F mutation is associated with an increased risk of thrombosis when compared to JAK2-negative patients [6, 8, 10]. However, this observation was not confirmed by other large studies [3, 9, 12]. It seems likely that older age, a higher leukocyte count and haemoglobin concentration were associated with an increased risk of thrombosis in JAK2-positive patients [11-13]. Our findings have confirmed these suggestions: WBC count and haemoglobin concentration were significantly higher in patients with the mutation; the JAK2-positive patients were also older, but in respect to age, significance was not reached. It should be emphasized that V617F may identify the clinically undetected myeloproliferative neoplasm in patients with otherwise unexplained intra-abdominal thrombosis. It was demonstrated that JAK2 V617F mutant clone was present in a substantial proportion of patients who did not meet the criteria of MPN at the time of thrombosis onset [14-16]. Conversely, the screening for JAK2 V617F mutation in 295 patients with idiopathic thromboses found this mutation only in one case [17]. In conclusion, the results of our study confirm the previous observations that the presence of JAK2 V617F point mutation correlates with some clinical and haematological features. However, the role of JAK2 mutation in risk stratification for therapy in patients with PV and ET requires further studies.

References

1. Jones AV, Kreil S, Zoi K, et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood 2005; 106: 2162-8.
2. Baxter EJ, Scott LM, Campbell PJ, et al. An acquired translocation in JAK2 Val617Phe-negative essential thrombocythemia associated with autosomal spread of X-inactivation. Lancet 2005; 365: 1054-61.
3. Wolanskyj AP, Lasho TL, Schwager SM, McClure RF, Wadleigh M, Lee SJ, Gilliland DG, Tefferi A. JAK2 mutation in essential thrombocythemia: clinical associations and long-term prognostic relevance. Br J Haematol 2005; 13: 208-13.
4. Tefferi A, Lasho TL, Schwager SM, et al. The clinical phenotype of wild-type, heterozygous, and homozygous JAK2V617F in polycythemia vera. Cancer 2006; 106: 631-35.
5. Vannucchi AM, Antonioli E, Guglielmelli P, et al. Clinical profile of homozygous JAK2V617F mutation in patients with polycythemia vera or essential thrombocythemia. Blood 2007; 110: 840-6.
6. Campbell PJ, Scott LM, Buck G, 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.
7. Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia amd myeloid metaplasia with myelofibrosis. Cancer Cell 2005; 7: 387-97.
8. Kralovics R, Passamonti F, Buser AS, et al. A gain of function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 2005; 352: 1779-90.
9. Antonioli E, Guglielmelli P, Pancrazzi A, et al. Clinical implications of the JAK2 V617F mutation in essential thrombocythaemia. Leukaemia 2005; 19: 1847-9.
10. Cheung B, Radia D, Pantelidis P, Yadegarfar G, Harrison C. The presence of the JAK2 V617F mutation is associated with a higher haemoglobin and increased risk of thrombosis in essential thrombocythaemia. Br J Haematol 2005; 132: 244-50.
11. Kittur J, Knudson RA, Lasho TL, et al. Clinical correlates of JAK2V617F allele burden in essential thrombocythaemia. Cancer 2007; 109: 2279-84.
12. Carobbio A, Finazzi G, Guerini V, et al. Leukocytosis is a risk factor for thrombosis in essential thrombocythemia: interaction with treatment, standard risk factors, and Jak2 mutation status. Blood 2007; 109: 2310-3.
13. Hsiao HH, Yang MY, Liu YC, Lee CP, Yang WC, Liu TC, Chang CS, Lin SF. The association of JAK2V617F mutation and leukocytosis with thrombotic events in essential thrombocythemia. Exp Hematol 2007; 35: 1704-07.
14. De Stefano V, Fiorini A, Rossi E, Za T, Farina G, Chiusolo P, Sica S, Leone G. Incidence of the JAK2 V617F mutation among patients with splanchnic or cerebral venous thrombosis and without overt chronic myeloproliferative disorders. J Thromb Haemost 2007; 5: 708-14.
15. Goulding C, Uttenthal B, Foroni L, et al. The JAK2 (V617F) tyrosine kinase mutation identifies clinically latent myeloproliferative disorders in patients presenting with hepatic or portal vein thrombosis. Int J Lab Hematol 2008; 30: 415-9.
16. Patel RK, Lea NC, Heneghan MA, et al. Prevalence of the activating JAK2 tyrosine kinase mutation V617F in the Budd-Chiari syndrome. Gastroenterology 2006; 130: 2031-8.
17. Remacha AF, Estivill C, Sarda Pilar M, et al. The V617F mutation of JAK2 is very uncommon in patients with thrombosis. Haematologica 2007; 92: 285-6.

Corresponding author

Grzegorz Helbig
Department of Haematology and Bone Marrow Transplantation
Silesian Medical University
25 Dabrowski Street
40-032 Katowice
phone: +48 32 259 12 36
fax: +48 32 255 49 85
e-mail: ghelbig@o2.pl