

Significance Of Microcytosis In The Clinical Course Of Erythrocytosis

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Citation

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Abstract

Aim: Polycythemia vera (PV) usually occurs in people aged 60–79 years. It is difficult to diagnose PV among patients with erythrocytosis, because the test of JAK2

V617F mutation was not regularly performed in general or geriatric hospitals. Therefore, we noticed microcytosis in PV and retrospectively studied the clinical course of microcytosis in patients with erythrocytosis. **Methods and subjects:** The medical data of 19 patients with erythrocytosis (Hb: males, >18.5 g/dL; females, >16.5 g/dL) collected between August 2005 and December 2009 were retrospectively reviewed for parameters such as age, gender, laboratory test results (hematology, serum ferritin, and the JAK2

V617F mutation), and the day of microcytosis onset during a 2-year period. The patients were divided into 2 groups depending on the presence or absence of the JAK2

V617F mutation. Microcytosis was defined as low mean corpuscular volume (MCV; <79 fL). **Results:** MCV was significantly lower in the JAK2

V617F -positive (PV, N=10) than in the negative erythrocytosis group (N=9). Microcytosis was noted in 2 among PV patients at initial presentation, whereas all patients exhibited microcytosis within 2 years. On the other hand, microcytosis was not noted at initial presentation in the JAK2

V617F-negative erythrocytosis group, and only 1 patient exhibited microcytosis within 2 years because of gastrointestinal bleeding. Microcytosis in the clinical course was associated with the presence of the JAK2

V617F

mutation ($P < 0.05$). **Conclusion:** Microcytosis may be a characteristic of the clinical course of PV and a good marker of the presence of the JAK2

V617F mutation.

INTRODUCTION

Polycythemia vera (PV) occurs in all age groups, although the incidence increases with age. Berlin NI reported the median age at diagnosis to be 60 years¹⁾, while Anía B et al found that the highest incidence was in people aged 70–79 years²⁾.

Because JAK2 V617F mutations are noted in approximately 95% of the patients with PV, testing for this mutation is important for the diagnosis of this disease³⁾. However, the test is not regularly performed in general or geriatric hospitals in several countries including Japan⁴⁾. Before publication of the World Health Organization (WHO) criteria in 2008 that included the presence of the JAK2 V617F mutation³⁾, definite diagnosis of PV was based on various guidelines^{5–7)}. Splenomegaly and the number of leukocytes and platelets are very useful markers in the initial

clinical diagnosis of PV⁶⁾. PV is occasionally accompanied by iron deficiency, which results in microcytosis (i.e., low mean corpuscular volume [MCV])⁸⁾. Therefore, we investigated the occurrence of microcytosis from the perspective of initial diagnosis of PV.

Phlebotomy is used as an initial treatment for PV⁹⁾. This procedure was performed for unclassified polycythemia before the development of tests for the JAK2 V617F mutation, serum erythropoietin level, etc. Venesection alone is known to cause iron deficiency in cases of PV¹⁰⁾.

However, patients with this blood disorder may or may not exhibit hematological microcytosis. Microcytosis in adulthood is known to occur in conjunction with anemia because of iron deficiency, chronic diseases, hemoglobinopathy, etc.^{11, 12)} Mutations in the ank/ank gene induce microcytosis that is unrelated to the JAK2 V617F

mutation¹³⁾. Moreover, the detailed clinical course of microcytosis in patients with PV remains unclear. Therefore, we retrospectively studied the clinical course of microcytosis in patients with PV. Microcytosis occurs more frequently during the clinical course of PV than during that of JAK2 V617F-negative erythrocytosis. First, we determined the significance of microcytosis in the clinical course of PV. Microcytosis during the clinical course of PV may be a result of iron deficiency or excessive erythropoiesis due to the JAK2 V617F mutation. Furthermore, it may be a good marker of the presence of the JAK2 V617F mutation.

METHODS AND SUBJECTS

CLINICAL EVALUATION

Tokyo Metropolitan Bokutoh Hospital is a 729-bed acute-care hospital located in eastern Tokyo. We retrospectively reviewed the medical data of 19 patients with erythrocytosis (Hb: males, >18.5 g/dL; females, >16.5 g/dL) collected between August 2005 and December 2009. The patients were divided into 2 groups according to the presence or absence of the JAK2 V617F mutation. Microcytosis was defined as low mean corpuscular volume (MCV) <79 fL.

The patients' charts were reviewed for age, gender, gastrointestinal bleeding events, laboratory test results (i.e., hematology, serum ferritin level, and the JAK2 V617F mutation), and the number of phlebotomies received during 2 years. The presence of the JAK2 V617F mutation was determined from venous blood from patients with erythrocytosis by the SRL company (Tokyo, Japan). The diagnostic criteria for PV were based on the revised WHO classification¹⁾. All JAK2 V617F mutation-positive patients (n = 10) satisfied the WHO criteria. On the other hand, the mutation-negative patients (n = 9) could not be diagnosed with PV despite high hemoglobin concentrations (serum erythropoietin: 15±2 mIU/mL). Patients with secondary polycythemia due to chronic obstructive pulmonary or cyanotic heart diseases or with high serum erythropoietin (>29 mIU/mL) were excluded from this study. Phlebotomies were performed in our hospital if patients had high hematocrit levels (>55%) and were repeatedly performed until hematocrit levels were <45%⁹⁾. The amount of phlebotomized whole blood was 400 mL per patient per phlebotomy. Hydroxycarbamide was administered for phlebotomy-resistant elderly patients (>60 years old) or high-risk groups including those with previous thrombosis⁹⁾. However, all 19 patients had neither previous thrombosis nor episodes of vascular events over the past 2 years. All study participants provided informed consent, and the study design

was approved by the ethics review board of our institution.

STATISTICAL ANALYSIS

We compared differences between groups using Wilcoxon analysis. The data represent the mean ± standard error (SE). The transient incidence of microcytosis in the clinical course between the groups was determined by Chi squared analysis (log rank). All statistical tests were conducted using JMP version 8.0 (SAS Institute, Inc., Cary, NC, USA). The level of statistical significance was set at P < 0.05.

RESULTS

Clinical features of PV or JAK2 V617F-negative erythrocytosis

Among the 19 patients with erythrocytosis, 10 were positive for the JAK2 V617F mutation and were diagnosed with PV by meeting the WHO criteria. However, 9 patients with high hemoglobin concentrations tested negative for the JAK2 V617F mutation and were not diagnosed with PV. The patients with JAK2 V617F-positive erythrocytosis, namely PV (age, 66 ± 3 years), were significantly older than those without the JAK2 V617F mutation (56 ± 4 years); however, no gender difference was noted between groups (male/female: PV, 6/4; JAK2 V617F-negative erythrocytosis, 8/1) (Table 1). None of the patients with PV had gastrointestinal bleeding during the 2-year period; only 1 patient who did not have the mutation experienced this.

In the laboratory tests performed at initial presentation, the leukocyte, erythrocyte, and platelet counts were significantly higher in the PV than in the JAK2 V617F-negative erythrocytosis (Table 1). Hemoglobin concentration and hematocrit were similar between groups (Table 1).

Clinical course of microcytosis in PV and JAK2 V617F-negative erythrocytosis

Among the 19 patients with erythrocytosis, only 2 with PV (2/10, 20%) exhibited microcytosis at the initial presentation, whereas none among the mutation-negative erythrocytosis did (0/9, 0%). Moreover, MCV was significantly lower in the PV than in the mutation-negative erythrocytosis (Table 1). The level of serum ferritin at the initial presentation was significantly lower in the PV (27.0 ± 4.8 µg/L, n=8) than in the mutation-negative erythrocytosis (126.7 ± 26.8 µg/L, n=7), although we only examined serum ferritin levels in 15 among 19 subjects at the initial presentation.

Within the 2 year period, 8 patients with normocytic PV at

the initial presentation exhibited transient microcytosis (8/8; 100%) throughout the initial presentation to days ~23–492 (Figure 1); However, only 1 mutation-negative patient exhibited this (1/9, 11%) at day 265.

Regarding the hematology of the microcytosis patients, erythrocyte count, hemoglobin concentration, and hematocrit (RBC: $6780 \pm 190 \times 10^9/L$, Hb: 16.7 ± 0.6 g/dL, Hct: $52 \pm 2\%$) were significantly lower than those measured at the initial presentation. However, the leukocyte ($13.2 \pm 0.2 \times 10^9/L$) and platelet ($500 \pm 100 \times 10^9/L$) counts were not significantly different from those at the initial presentation. Serum ferritin levels significantly decreased in the 8 patients with microcytosis (17.0 ± 2.9 µg/L).

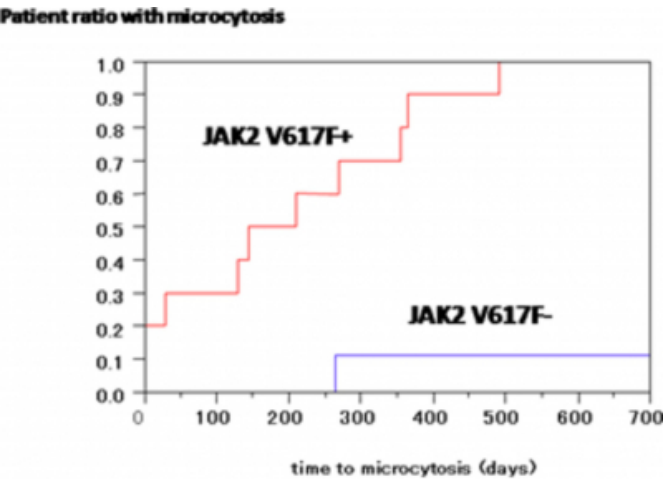
Phlebotomies were similarly required in both the PV (10/10, 7 ± 2 times) and mutation-negative erythrocytosis (9/9, 6 ± 2 times) during the 2-year period.

Hydroxycarbamide administration was required by 5 out of the 10 patients with PV (50%). After hydroxycarbamide therapy, 4 of 5 patients (80%) exhibited normocytosis ranging from days 73–221. Serum ferritin levels increased in the 5 patients with normocytosis (50.0 ± 8.7 µg/L). Two of the four patients who exhibited normocytosis again exhibited microcytosis at the initial presentation. Six of 10 patients with PV still had microcytosis during the 2-year period. On other hand, 1 out of the 9 patients with JAK2 V617F mutation-negative erythrocytosis who exhibited microcytosis on day 265, presented with normocytosis again on day 724 because of the resolution of gastrointestinal bleeding without iron supplementation. Therefore, all JAK2 V617F mutation-negative erythrocytosis patients finally exhibited normocytosis by the end of the 2-year period.

Microcytosis among the patients with erythrocytosis at the initial presentation was not associated with the presence of the JAK2 V617F mutation ($P = 0.26$). However, transient microcytosis in the clinical course was significantly associated with the presence of the JAK2 V617F mutation ($P < 0.05$).

Figure 1

Figure 1. Transient incidence of microcytosis in the clinical course of erythrocytosis



Two patients with polycythemia vera (2/10, 20%) exhibited microcytosis upon initial presentation. Eight patients with normocytic polycythemia vera upon initial presentation exhibited temporal microcytosis (8/8; 100%) throughout the initial presentation to days ~23–492. On other hand, 1 of 9 patients with JAK2 V617F mutation-negative erythrocytosis exhibited temporal microcytosis at day 265. Chi squared analysis (log rank) revealed a significant difference between JAK2 V617F mutation-positive and mutation-negative erythrocytosis ($P < 0.05$).

Figure 2

Table 1. Clinical features and laboratory findings of erythrocytosis at the initial presentation.

Parameter	Polycythemia vera (n = 10)	JAK2 V617F mutation-negative erythrocytosis (n = 9)
Age (yr)	66 (3)*	56 (4)
Gender (M/F)	6/4	8/1
Phlebotomy (n)	7 (2)*	6 (2)
GI bleeding event (n)	0	1
Hematology		
Leukocytes ($\times 10^9/L$)	11.8 (2.2)*	6.5 (0.4)
Erythrocytes ($\times 10^9/L$)	7110 (230)*	5960 (80)
Hb (g/dL)	19.3 (0.5)	19.3 (0.2)
Hct (%)	58.8 (1.3)	56.1 (1.2)
Platelets ($\times 10^9/L$)	410 (60)*	210 (10)
MCV (fL)	83 (2)	95(1)
Low MCV (n)	2	0

Values are presented as the mean (SE)* $P < 0.05$ by Wilcoxon analysis.

DISCUSSION

CLINICAL FEATURES OF PV

Among the patients of the PV group that exhibited microcytic characteristics (2 and 8 cases during the initial presentation and clinical course respectively), red blood cell, leukocyte, and platelet counts were greater in comparison with those in the JAK2 V617F mutation-negative erythrocytosis group (9 cases, no microcytosis at the initial presentation). There was no significant difference in the

bloodletting number between the groups during the 2-year period. An increased leukocyte count is a risk factor for thrombosis in patients with PV¹⁴. In a previous study, transgenic mice with the JAK2 V617F mutation exhibited polycythemia with leukocytosis but without thrombocytosis¹⁵. We speculate that thrombocytosis in microcytic polycythemia is a manifestation of myeloproliferative syndrome¹⁶ and that platelet counts relatively increase with iron deficiency.

SIGNIFICANCE OF MICROCYTOSIS IN PV

Several studies show that iron deficiency accompanies PV and secondary polycythemia^{8,17}. Rector et al. report that after 15 years, venesection caused iron deficiency in 6 patients with PV¹⁰. There are also reports on an increase in serum-soluble transferrin receptors, which are markers of iron deficiency¹⁷ after autologous blood donation, blood donation by healthy volunteers, and phlebotomy^{18,19}. Flesland et al. report that the number of soluble transferrin receptors increases with the number of blood donations in frequent blood donors¹⁸. We subsequently speculate that bloodletting contributed to part of the iron deficiency observed in our study. However, phlebotomies were similarly required in both PV and mutation-negative erythrocytosis groups during the 2-year period. Therefore, this suggests that the main cause of microcytosis in PV in our study is not iron deficiency due to phlebotomy. On the other hand, hydroxycarbamide therapy changed the hematological data of the PV patients, including MCV. Therefore, we speculate that if there are no events of gastrointestinal bleeding, microcytosis in PV may be a result of iron deficiency and excessive erythropoiesis due to the JAK2 V617F mutation.

A case report suggests that microcytic polycythemia is a combination of PV and α -thalassemia minor¹⁸. Two patients with PV with microcytosis at the initial presentation became normocytic after hydroxycarbamide therapy; the other 8 patients who were originally normocytic became microcytic during the clinical course. Therefore, this suggests that there is no possibility of α -thalassemia in our study.

Microcytosis is not a characteristic of JAK2 V617F mutation-negative erythrocytosis, which does not satisfy the 2008 polycythemia vera criteria by the WHO³. We speculate that JAK2 V617F mutation-negative erythrocytosis, that revealed normal serum erythropoietin concentration, might be idiopathic erythrocytosis²¹. In a group of patients with erythrocytosis, the cause was not identified. In our study,

neither leukocytosis nor thrombocytosis were noted in the JAK2 V617F mutation-negative erythrocytosis group; this group was labeled idiopathic erythrocytosis. However, regarding idiopathic erythrocytosis, other mutations such as the JAK2 exon 12, VHL gene, and hypoxia-inducible factor-2 mutations are known to occur^{21,22}. We cannot exclude the possibility of the presence of these mutations because general hospitals lack appropriate laboratory facilities to determine them.

The test for the JAK2 V617F mutation is important in diagnosing PV, but it is not common in the general or geriatric hospitals of many countries including Japan⁴. MCV is commonly available in clinical laboratories, and low MCV in the clinical course of erythrocytosis may be a good marker for the presence of the JAK2 V617F mutation.

In order to definitively diagnose this blood disorder by using low MCV, general physicians should refer patients to hematologists in specialized hospitals where the test for the JAK2 V617F mutation is available.

CONCLUSION

Microcytosis in the clinical course of PV may be a result of iron deficiency and excessive erythropoiesis due to the JAK2 V617F mutation. In addition, microcytosis in erythrocytosis may be a good marker for the presence of the JAK2 V617F mutation.

References

1. Berlin, NI. Diagnosis and classification of polycythemias. 1975; *Semin Hematol* 12: 339.
2. An a B, Suman V, Sobell J, Codd M, Silverstein M, Melton L. Trends in the incidence of polycythemia vera among Olmsted County, Minnesota residents, 1935-1989. 1994; *Am J Hematol* 47: 89-93.
3. Vardiman JW, Thiele J, Arber DA et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*. 2009; 114: 937-951.
4. Abel GA, DeAngelo DJ, Sholl JM et al. Clinical JAK2 V617F mutation testing: Limited utility for general hospital patients with venous and arterial thrombosis in common locations. *Am J Haematol*. 2008; 83: 519-520.
5. Berk PD, Goldberg PB, Donovan PB et al. Therapeutic recommendations in polycythemia vera on Polycythemia vera study group protocols. *Semin Hematol*. 1986; 23: 132-143.
6. Turkington RC, Arnold EC, Percy MJ et al. Comparison of diagnostic criteria for polycythemia vera. *Hematology*. 2007; 12: 123-130.
7. Pearson TC, Messinesy M. The diagnostic criteria of polycythemia rubra vera. *Leuk Lymphoma*. 1996; 22: 87-93.
8. Pavic M, Francina A, Vital Durand D et al. Polycythemia and iron deficiency. *Lancet*. 2003; 362: 1624.
9. Finazzi G, Barbui T. How I treat patients with polycythemia vera. *Blood*. 2007; 109: 5104-5111.

10. Rector WG, Fortuin NJ and Coley CL. Non-hematologic effects of chronic iron deficiency. A study of patients with polycythemia vera treated solely with venesections. *Medicine*. 1982; 61: 383-389.
11. Smith DL. Anemia in the elderly. *Am Fam Physician*. 2000; 62: 1565-72.
12. Abdul-Ghaffar NU, El-Sonbaty MR and Kumar R. Microcytosis: guidelines for family doctors. *Trop Doc*. 1996; 26: 20-25.
13. Wang J, Wang C, Tsui HW et al. Microcytosis in ank/ank mice and the role of ANKH in promoting erythroid differentiation. *Exp Cell Res*. 2007; 313: 4120-4129.
14. Barbui T, Carobbio A, Rambaldi A et al. Perspectives on thrombosis in essential thrombocythemia and polycythemia vera: is leukocytosis a causative factor? *Blood*. 2009; 114: 759-763.
15. Zaleskas VM, Krause DS, Lazarides K et al. Molecular pathogenesis and therapy of polycythemia induced in mice by JAK2 V617F. *PLoS ONE*. 2006; 1: e18.
16. Schafer AI. Molecular basis of the diagnosis and treatment of polycythemia vera and essential thrombocythemia. *Blood*. 2006; 107: 4214-4222.
17. Manteiga R, Remacha AF, Sarda MP et al. Serum transferrin receptor in polycythemia. *Haematologica*. 1998; 83: 958-959.
18. Dimonte D, Pepe M, Silletti I et al. Serum transferrin receptor is a marker of iron deficiency in patients included in programs for preoperative autologous blood donation. *Transfusion and Apheresis Science*. 2002; 27: 211-216.
19. Flesland O, Eskelund A-K, Flesland AB et al. Transferrin receptor in serum. A new tool in the diagnosis and prevention of iron deficiency in blood donors. *Transfusion and Apheresis Science* 2004; 31: 11-16.
20. Thomas JP. β thalassemia minor and newly diagnosed polycythemia rubra vera in a 71-year-old. *Hosp Physician*. 2001; 37: 78-83.
21. McMullin MF. Idiopathic erythrocytosis: a disappearing entity. *Hematology*. 2009; 2009:29-635.
22. Scott LM, Tong W, Levine RL et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N Engl J Med*. 2007; 356: 444-445.

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