Usefulness of BCSH Criteria for Diagnosing Japanese Polycythemia Vera -Comparative Analysis with WHO 2008 Criteria-

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Objectives: The World Health Organization (WHO) classification (2008) is widely used to diagnose polycythemia vera (PV). However, some patients clinically suspected of PV may not be definitely diagnosed because they do not have a high hemoglobin (Hb) level, one of the main and essential diagnostic criteria, or because of the lack of or unfilled minor criteria such as bone marrow biopsy, endogenous erythroid colonies, and serum erythropoietin. The British Committee for Standards in Haematology (BCSH) employed hematocrit (Ht), but not Hb, as an indicator in the PV diagnostic guidelines. Furthermore, if the presence of the *JAK2* gene mutation is demonstrated, PV can be diagnosed with Ht only by BCSH. Therefore, we evaluated the usefulness of BCSH criteria for diagnosing PV in Japanese patients.

Methods: We determined hematological and clinical differences in 99 patients who met the BCSH criteria (Group A) and 69 PV patients who met the WHO criteria (Group B).

Results: All patients in Group B also met the BCSH criteria. Thirty patients in Group A (43%) did not fulfill the WHO criteria. No significant differences were observed in white blood cell and platelet counts, serum erythropoietin levels, the *JAK2V617F* allele burden, or the risk of thrombosis and transformation into myelofibrosis between Groups A and B.

Conclusions: PV cases diagnosed by the BCSH criteria showed the same clinical picture as those diagnosed by the WHO criteria. In view of actual clinical settings, the diagnostic criteria of BCSH criteria may be useful for the diagnosis of PV in Japanese patients.

Key words: myeloproliferative neoplasms (MPN), polycythemia vera (PV), *JAK2* mutation, The British Committee for Standards in Haematology (BCSH), The World Health Organization (WHO)

Introduction

Myeloproliferative neoplasm (MPN) is a neoplastic disease characterized by an abnormal increase in hematopoietic cells. Philadelphia chromosome (Ph)-negative MPN, excluding chronic myeloid leukemia, mainly includes polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Previous studies reported that the *JAK2V617F* gene mutation was frequently detected in Ph-negative MPN cases in 2005 (>95% in PV and 50-60% in ET and PMF)¹⁻⁵⁾. The prognosis of patients with PV is greatly influenced by thrombosis and leukemic transformation⁶⁾. Therefore, PV should be managed in a manner that depends on an early accurate diagnosis before the

development of complications.

PV is currently diagnosed using the WHO classification (2008)⁷⁾ which specifically employs two main criteria: (1) evidence of either an increase in hemoglobin (Hb) levels (male: >18.5 g/dL; female: >16.5 g/dL) or the RBC count and (2) the presence of either *JAK2V617F* or exon 12 mutations, and three minor criteria: (1) findings of three types of hyperplasia in bone marrow biopsy samples, (2) low serum erythropoietin (EPO) levels, and (3) the formation of endogenous erythroid colonies (EEC). Of these, two main criteria and one minor criterion, or first one of the two main criteria and two minor criteria should be met for a definite PV diagnosis. Hb levels have been employed in the WHO classification as the main criterion reflecting red cell

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mass (RCM), which has traditionally been used as a diagnostic criterion by the Polycythemia Vera Study Group (PVSG) utilizing radioactive elements. However, it currently remains unclear whether Hb can be used as a surrogate for RCM^{(8) (9)} because the number of early PV cases may be underestimated by the WHO Hb criteria, causing diagnosis delays^{(8) (10) (11)}. A tissue diagnosis with a bone marrow biopsy specimen has been used to effectively diagnose PV⁽¹²⁾. However, some patients do not undergo bone marrow biopsies, and diagnoses may differ according to the pathologists' skill levels and expertise. Furthermore, the formation of EEC, which is employed as a minor criterion in the WHO classification, can only be examined in a small number of facilities.

On this background, diagnostic criteria for PV were proposed as convenient diagnostic criteria by the British Committee for Standards in Haematology (BCSH) of the United Kingdom in 2005 and 2007^{(9) (13)}. Since the *JAK2* gene mutation is frequently detected in PV, the BCSH guidelines recommended that PV can be diagnosed based on Ht levels (>52% in males and >48% in females) if the presence of *JAK2*V617F or exon 12 gene mutations can be demonstrated, and also that *JAK2* gene mutation-negative cases can be diagnosed as PV based on exclusion items, such as the absence of splenomegaly and secondary polycythemia. Furthermore, a bone marrow examination is not necessarily required by the BCSH criteria.

The *JAK2* gene mutation is included in both the WHO and BCSH criteria for PV, and has become essential in the diagnosis of PV. We previously developed a *JAK2*V617F allele burden quantification method (ABC-PCR method) using peripheral blood⁽¹⁴⁾. In the present study, we utilized this *JAK2* mutation detection method and examined the usefulness of the BCSH criteria for diagnosing PV in Japanese patients.

Patients and Methods

1. Study population

A total of 194 patients from 20 facilities, for whom consent was obtained after approval (No. 21,076) by the Juntendo University Ethics Committee between April 2010 and December 2013, were included. Peripheral blood was collected from patients to be used in the *JAK2* gene analysis. The

following clinical data entered by the attending physicians were collected: date of the first visit, blood counts at the time of the diagnosis or sample submission for a genetic analysis (WBC, differential WBC, RBC, and platelet counts, Hb, Ht, serum EPO, bone marrow biopsy, splenomegaly [palpation and imaging (ultrasonography or computed tomography (CT))], and a history of thrombosis or prior treatment until blood sample submission. Blood count data collected at the first visit or diagnosis were used. Bone marrow biopsies were histologically diagnosed in reference to pathologists' findings at each facility.

2. *JAK2*V617F mutation analysis

Five milliliters of peripheral blood was collected from the patients, followed by DNA extraction from the whole blood. The *JAK2*V617F allele burden was quantified using the ABC-PCR method⁽¹⁴⁾. An allele burden of >10% was regarded as positive (sensitivity: 5–10%). Patients with cut-off values of approximately 10% were re-examined using the AS-qPCR method.

3. *JAK2* exon 12 mutation analysis

DNA was extracted from the EEC of *JAK2*V617F-negative cases clinically suspected of having PV, followed by an analysis for the exon 12 mutation⁽¹⁵⁾.

4. Statistical analysis

All statistical analyses were performed by JMP[®] 10 (SAS Institute Inc., Cary, NC, USA). The level of significance was two-sided $p < 0.05$. A *t*-test or Fisher's exact test was used to compare the two groups, depending on the type of data. After grouping depending on the presence or absence of a history of thrombosis, the odds ratio of thrombosis was estimated using a logistic model including age, sex, *JAK2*V617F allele burden, blood counts, EPO, splenomegaly, and cardiovascular risk factors (smoking, hypertension, dyslipidemia, and diabetes) as explanatory variables. The final model was selected using the minimum AICc criterion (AICc is the finite corrected Akaike's information criterion)⁽¹⁶⁾.

Results

1. PV patient profiles

As shown in Table-1, 99 patients were diagnosed with PV based on the criteria of the BCSH guidelines (Group A) while 69 patients were diagnosed according to the WHO criteria (Group B). All patients in Group B fit the PV BCSH criteria. Although bone marrow biopsy is required for the detection of hyperplasia¹⁷⁾ which is included in the WHO minor criteria, it could not be conducted in some patients who had been administered an anti-platelet agent or were of an advanced age. In the present study, bone marrow biopsy was not conducted in 20 out of 30 cases that met the PV criteria of the BCSH criteria, but did not fulfill the WHO criteria. This

was one of the major reasons why patients having both of the major criteria did not meet the WHO criteria. Another main reason was the lack of serum EPO data (Table-2). Figure-1 shows the schema of Group A (BCSH (+)), Group B (BCSH (+)/WHO (+)), and Group C ((BCSH (+)/WHO (-))).

The profiles of patients diagnosed by BCSH (Group A, n=99) and WHO (Group B, n=69) were compared (Table-1). As described above, all patients in Group B met the diagnostic criteria of the BCSH criteria, indicating that Group A contained all the patients of Group B. No bias was noted in sex or age distribution between these two groups. No significant difference was noted not only in blood test items (WBC counts, Hb, Ht, platelet counts, or serum EPO), but also in the *JAK2V617F*

Table-1 Profiles of PV patients who met the diagnostic criteria of the BCSH criteria or WHO 2008 classification

	BCSH (A)	A vs. B	WHO 2008 (B)
Number of patients (M : F)	99 (46 : 53)	-	69 (31 : 38)
Age (years, mean $\pm$ SD)	65.1 $\pm$ 12.1 (20-90)	0.9844	65.0 $\pm$ 12.2 (20-90)
RBC ($10^4/\mu\text{L}$, mean $\pm$ SD)	704.6 $\pm$ 95.2	0.2179	722.7 $\pm$ 89.9
M	701.1 $\pm$ 102.5	0.1743	732.6 $\pm$ 93.2
F	707.7 $\pm$ 89.2	0.7156	714.6 $\pm$ 87.4
Hb (g/dL, mean $\pm$ SD)	18.8 $\pm$ 1.8 (14.7-24.3)	0.1165	19.2 $\pm$ 1.7 (16.5-24.3)
M	19.3 $\pm$ 1.6 (16.9-24.3)	0.0464	20.0 $\pm$ 1.4 (18.7-24.3)
F	18.4 $\pm$ 1.9 (14.7-22.2)	0.5363	18.6 $\pm$ 1.8 (16.5-22.2)
Hematocrit (% , mean $\pm$ SD)	58.2 $\pm$ 5.4 (48.1-72.7)	0.1128	59.5 $\pm$ 5.3 (52.6-72.7)
M	59.1 $\pm$ 5.4 (52.5-72.7)	0.0846	61.3 $\pm$ 5.1 (53.2-72.7)
F	57.9 $\pm$ 5.3 (48.1-70.5)	0.5072	58.1 $\pm$ 5.0 (50.8-70.5)
MCV (fL, mean $\pm$ SD)	83.4 $\pm$ 8.3	0.7720	83.0 $\pm$ 7.6
M	85.3 $\pm$ 8.1	0.6121	84.4 $\pm$ 7.2
F	81.8 $\pm$ 8.1	0.9078	81.9 $\pm$ 7.7
WBC ($10^3/\mu\text{L}$, mean $\pm$ SD)	15.1 $\pm$ 7.3 (5.19-53.0)	0.3818	14.2 $\pm$ 6.1 (5.19-33.9)
Platelets ($10^4/\mu\text{L}$, mean $\pm$ SD)	52.4 $\pm$ 24.4 (11.4-181.5)	0.6131	50.4 $\pm$ 25.0 (11.4-181.5)
EPO (mU/mL, mean $\pm$ SD)	8.0 $\pm$ 2.8 (n=76) (1.0-14.8)	0.5199	7.7 $\pm$ 2.6 (n=66) (1.0-12.1)
<i>JAK2V617F</i> , N (%)	96 (97.0%)	-	66 (95.7%)
Allele burden (% , mean $\pm$ SD)	73.3 $\pm$ 24.4 (18.6-100)	0.7138	71.6 $\pm$ 25.8 (18.6-100)
<50%	14 (14.6%)	-	10 (15.1%)
50% $\geq$	82 (85.4%)	-	56 (84.9%)
Exon 12, N (%)	3 (3.0%)	-	3 (4.2%)
Splenomegaly, N (%)	46 (46.5%)	0.6369	29 (42.0%)
Thrombotic event, N (%)	20 (20.2%)	1.0000	14 (20.3%)
Episode N	25	-	18
Arterial	18 (72.0%)	-	11 (61.1%)
Venous	7 (28.0%)	-	7 (38.9%)
Smoking	16 (18.2%) (n=88)	0.8294	10 (16.4%) (n=61)

Table-2 Detailed reasons why 30 patients did not meet WHO 2008 criteria

		WHO classification				
		major criteria		minor criteria		
		Hb	<i>JAK2</i> mutation	EPO	pathological findings	EEC
BCSH n=30	a(n=11)	○	○	8 [§] /3 [¶]	2 [§] /9 [¶]	11 [¶]
	b(n=9)	9 [§]	○	9 [¶]	2 [§] /7 [¶]	9 [¶]
	c(n=2)	2 [§]	○	○	○	2 [¶]
	d(n=3)	3 [§]	○	3 [¶]	○	3 [¶]
	e(n=5)	5 [§]	○	○	1 [§] /4 [¶]	5 [¶]

Open circles: fulfilled the criteria, §: did not fulfill the criteria, ¶: not conducted.

Figure-1 Relationship between BCSH-defined and WHO-defined PV patients

The schema of Group A (BCSH (+)), Group B (BCSH (+)/WHO (+)), and Group C (BCSH (+), WHO (-)) are shown.

allele burden between these groups (mean, Group A: 73.3%, Group B: 71.6%). However, in the male population, Hb levels were significantly lower in Group A than in Group B (mean, Group A: 19.3g/dL, Group B: 20.0 g/dL). More than 80% of cases in Groups A and B showed a $\geq 50\%$ *JAK2*V617F allele burden (mean, Group A: 85.4%, Group B: 84.9%). Clinical findings revealed no significant differences in the frequencies of splenomegaly and thrombosis (Table-1). Myelofibrosis was not observed in patients who underwent bone marrow biopsies (data not shown). Thus, no significant differences were noted in clinical backgrounds between patients who met the BCSH criteria and those who met the WHO classification. All three patients with the exon 12 mutation met the WHO classification (Group B) and accounted for 3.0% and 4.2% of Group A and Group B, respectively. The frequency of the exon 12 muta-

Table-3 Thrombotic events

Thrombosis		N(%)	Male	Female
Arterial	Total	18	7	11
	Stroke	14(56%)	4	10
	AMI/AP	3(12%)	2	1
	Splenic infarction	1(4%)	1	0
Venous	Total	7	3	4
	DVT	5(20%)	3	2
	PE	1(4%)	0	1
	HVT	1(4%)	0	1

AMI: acute myocardial infarction, AP: angina pectoris, DVT: deep venous thrombosis, HVT: hepatic vein thrombosis, PE: pulmonary embolism

tion was previously shown to be high in Asia¹⁸⁾, but was similar to that reported in Europe and the United States¹⁹⁾. These three patients had different mutations¹⁵⁾, all of which had been reported previously¹⁹⁾.

2. Occurrence of thrombosis

A history of thrombosis was observed in twenty (20.2%) and fourteen patients (20.3%) in Group A and Group B, respectively. Thus, there was no significant difference in its frequency between the two groups. This frequency was almost consistent with the findings of previous foreign retrospective studies on PV patients (16–38%)^{20)–23)}.

The results of thrombosis based on the site of onset are shown in Table-3. A previous study reported that arterial thrombosis was commonly observed in PV patients²¹⁾. Arterial thrombosis (18 cases, 72%) was also more common than venous thrombosis (7 cases, 28%) in the present study. The incidence of cerebral infarction was the highest in patients with arterial thrombosis, stroke (14

Table-4 Multivariate analysis of risk factors for thrombotic events in Group A

	χ^2	p-value
Splenomegaly	6.81247929	0.0091
<i>JAK2V617F</i> allele burden	2.36317738	0.1242
Age	0.19852119	0.6559
WBC	0.81803507	0.3658
Smoking	0.81306578	0.3672
Hypertension	0.59577978	0.4402

cases, 56%), followed by myocardial infarction or angina, which required catheterization (3 cases, 12%), and then splenic infarction (1 case, 4%). The incidence of leg vein thrombosis was the highest in patients with venous thrombosis (5 cases, 20%), followed by pulmonary infarction (1 case, 4%), and then relatively rare hepatic vein thrombosis (1 case, 4%). No significant difference was observed in the frequency of arterial or venous thrombosis between Group A ($n=25$, 72.0% vs. 28.0%) and Group B ($n=18$, 61.1% vs. 38.9%) ($p=0.5205$). When 99 PV patients diagnosed by the BCSH criteria were divided into two groups according to the occurrence of thrombosis, splenomegaly was strongly associated with thrombosis (Table-4). No relationship was observed against the *JAK2V617F* allele burden, age, WBC, smoking, or hypertension as variables (Table-4). A high WBC count ($>15,000/\mu\text{L}$) and *JAK2V617F* allele burden ($\geq 50\%$), which have been identified as risk factors for thrombosis^{24)–26)}, were not significantly different in this analysis. Consistent with previous findings in which patients with MPN repeatedly developed thrombosis²³⁾, five out of 20 patients repeatedly developed thrombosis in the present study.

Discussion

We recently defined cases clinically suspected of having PV, with low Hb levels in spite of a high RBC count, as “suspected PV.” The *JAK2V617F* allele burdens of these cases were almost the same as those of PV cases that met the WHO classification. Among 40 suspected PV patients, 11 were diagnosed with PV according to the BCSH criteria¹⁵⁾. In the present study, we compared hematological and clinical aspects between patients who met the BCSH criteria (Group A) and those who met the WHO criteria (Group B), and found no significant

differences in clinical backgrounds between Group A and Group B. In addition, the distribution of the *JAK2V617F* allele burden was not significantly different between these groups.

Barbui *et al.* defined PV cases with Hb levels that were lower than the WHO criteria (males: <18.5 g/dL; females: <16.5 g/dL) and whose bone marrow biopsy met the pathological criteria as “masked PV”, thereby emphasizing the importance of bone marrow biopsy¹⁷⁾. In our analysis, five of the 30 patients diagnosed with PV by the BCSH criteria only whose marrow biopsy findings corresponded to those of PV, but did not meet the WHO criteria for Hb, were diagnosed with masked PV (Table-2, column c; $n=2$, column d; $n=3$). Thus, some patients with “masked PV” were diagnosed with PV using the BCSH diagnostic criteria of the BCSH criteria. Silver *et al.* previously characterized “masked PV” patients as follows; predominantly male, higher platelet counts, and more severe BM reticulin fibrosis¹²⁾. Consistent with these findings, the ratio of males to females in our “masked PV” patients was 4 to 1, although the number of patients was very small ($n=5$). However, no significant difference was observed in platelet counts between the five “masked PV” patients and defined PV patients diagnosed by the WHO criteria (mean $\pm$ SD $\times 10^4/\mu\text{L}$; 43.6 ± 9.9 vs 59.5 ± 4.4) ($p=0.1544$).

Although the exon 12 gene mutation was detected at a low frequency (3 cases, 3%), as previously reported abroad¹⁹⁾, some PV cases may be overlooked unless the exon 12 mutation is analyzed in *JAK2V617F*-negative cases; however, all three patients with the exon 12 mutation met the WHO criteria for Hb in the present study.

The causal relationship between thrombosis and the *JAK2V617F* allele burden remains controversial. Vannucchi *et al.* reported that cardiovascular events may occur in patients with a $>75\%$ *JAK2V617F* allele burden²⁷⁾. In the present study, three patients with cardiovascular diseases had a $>75\%$ *JAK2V617F* allele burden. Cerebral infarction was the most common arterial thrombosis, while DVT was the most common venous thrombosis, which is consistent with previous findings²⁵⁾. The prevalence of risk factors for cardiovascular events (smoking, hypertension, dyslipidemia, and diabetes mellitus) was analyzed; however, these were not recognized as risk factors for thrombosis (data not

shown), presumably because of the limited number of patients. The odds ratio of thrombosis was only significant for splenomegaly in a logistic model ($p = 0.0091$) (Table-3). Although this was inconsistent with previous findings²⁴⁾, this discrepancy may be explained by differences in populations between Japan and European countries. In consideration of the similar frequencies (20%) of thrombosis between Groups A and B, it may be beneficial to select patients suspected of having PV using the BCSH criteria for therapeutic interventions to prevent thrombosis.

A recent study demonstrated that PV and ET patients with a $> 50\%$ or increasing *JAK2V617F* allele burden were both more likely to develop myelofibrosis and thrombosis²⁶⁾. Thus, the allele burden should be measured over time, as well as at the initial diagnosis. In addition, according to the BCSH criteria, PV can only be diagnosed according to the *JAK2V617F* mutation and Ht level, and a pathological examination is not required. Thus, mimic ET accompanied by thrombocytosis and initial/pre-polycythemic PV may not be diagnosed by the BCSH criteria in patients with Ht levels that do not meet the diagnostic criteria²⁸⁾. However, in contrast to PV cases, most ET cases showed a $< 50\%$ *JAK2V617F* allele burden²⁹⁾. Furthermore, ET with a high allele burden is more likely to transit to PV²⁹⁾. Our method in which the allele burden was combined with the BCSH criteria may be useful; however, further studies are warranted.

In conclusion, PV cases diagnosed by the BCSH criteria showed the same clinical picture as those diagnosed by the WHO criteria. In view of actual clinical settings, the diagnostic criteria of the BCSH criteria may be useful for the diagnosis of PV in Japanese patients.

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