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JAK2V617F mutation and allele burden are associated with distinct clinical and morphological subtypes in patients with essential thrombocythaemia

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Essential thrombocythaemia (ET) is a chronic myeloproliferative neoplasm (MPN) that involves primarily the megakaryocytic lineage.[1] The $JAK2^{V617F}$ mutation has been detected in 50-60% ET, and homozygous mutation in only 5%.[2]

$JAK2^{V617F}$ mutation has been associated with a PV-like phenotype [3] and increased risk of thrombosis,[4] especially in homozygous patients.[5] Increasing mutant allele load correlated with older age, splenomegaly, microvessel symptoms and higher frequency of arterial thrombosis at diagnosis.[2]

Little is known on the association between $JAK2^{V617F}$ mutation and histological changes in bone marrow biopsy (BMB) of ET patients.

One hundred and three consecutive patients with newly diagnosed ET, admitted to the Division of Haematology, S. Giovanni Hospital and University of Turin, Italy, from 2006 to 2010 were included in the study. Diagnosis of ET was performed according to WHO criteria.[1] A general informed consent was obtained according to the local ethical committee guidelines. There were 56 females and 47 males; the mean age was 59.1 years (range, 25 to 86). Splenomegaly was detected in 38 patients (36.9%). Venous or arterial thrombosis (confirmed by ultrasound examination or CT Scan and D-dimer assessment) were found in 21% and 7% of patients, respectively. None was under cytoreductive therapy. The mean duration of the follow-up was 43.4 months (range, 12 to 66). A complete clinical history, blood count and available molecular biology data were given to the pathologist at initial diagnosis; when reassessing ET cases, pathologists were also informed on the size of the spleen, thrombotic or haemorragic complications, $JAK2^{V617F}$ status, lactate dehydrogenase (LDH) level, autoimmunity tests and patient therapy.

Serial sections from Bouin's fixed, paraffin embedded BMB, were stained with haematoxylin-eosin, Dominici, Perls and Gomori staining, and immunostained with monoclonal antibodies anti CD34, CD31, von Willebrand Factor, glycophorin A, and the polyclonal antibody anti human myeloperoxidase (all from Dako, Glostrup, Denmark). Marrow cellularity, hyperplasia and dysplasia of the erythroid, myeloid and megakaryocytic lineages, percentage of CD34 positive blasts, fibrosis, dilated sinusoids and haemosiderin were evaluated. The number of total, large, "stag horn" megakaryocytes, micromegakaryocytes and clusters of megakaryocytes was assessed in 10 HPF (400x) in each case. $JAK2V^{617F}$ mutation was assessed by direct sequencing of exon 14 from peripheral blood or bone marrow samples. The load of $JAK2V^{617F}$ mutation was measured in all samples by semi-quantitative real time PCR allelic discrimination assay. The mutant allele burden was estimated by six-scaled standards of $JAK2V^{617F}$ mutant

allele (2%, 5%, 12.5%, 31%, 50%, and 78%) comparing the mean ratio value obtained for unknown samples with Reference Scale mean ratio values.

$JAK2^{V617F}$ mutation was identified in 59 of 103 cases (57.3%). The mean mutant allele burden was 14.4% (median, 8.7%; range, 0.5 to 76%). A mean allele burden greater than 50% (homozygous mutation) was found in three patients (5.6%).

ET patients with $JAK2^{V617F}$ mutation were younger and presented with higher haematocrit, RBC count, haemoglobin level and lower platelet count than patients without mutation (Table 1). As compared to non-mutated cases, BMB of ET with $JAK2^{V617F}$ mutation (Table 1) showed a higher marrow cellularity (Fig 1a), more micromegakaryocytes (Fig 1c) and more frequent erythroid, myeloid and sinusoid hyperplasia (Fig 1d); all had megakaryocyte dysplasia (Fig 1b). Non-mutated ET displayed more megakaryocytes, with a greater number of large, “stag-horn” (Fig 2a and 2b) and clustered megakaryocytes (Fig 2c and 2d).

Patients were divided into two groups based on 12.5% scaled standard, which was the nearest value to the mean allele burden for the whole series. Patients with high $JAK2^{V617F}$ mutation load had lower haemoglobin level and platelet count, higher LDH level, larger spleen and more frequent venous and arterial thrombosis than patients with low mutation load (Table 2).

BMB of ET with high $JAK2^{V617F}$ mutation load showed more micromegakaryocytes (Fig 1c) and dysplasia of the erythroid and myeloid lineages, and less haemosiderin than cases with low mutation load (Table 2).

The three patients with homozygous $JAK2^{V617F}$ mutation had a marked splenomegaly (mean diameter: 19.5 cm), high LDH levels (629 UI/L), high number of large megakaryocytes (36/10HPF) and megakaryocyte clusters (10.3/10HPF). All showed erythroid dysplasia and altered thrombophilia screening; two had myeloid dysplasia.

Our results confirm that $JAK2^{V617F}$ mutated ET has a phenotype similar to PV. Indeed, patients with the mutation presented with higher haemoglobin level, haematocrit, RBC count and lower platelet count than non-mutated patients, in accordance with previous reports.[3] Furthermore, the BMB histological pattern of $JAK2^{V617F}$ mutated cases was rather similar to PV, with high cellularity, frequent hyperplasia of the erythroid and myeloid lineages and a large number of dilated sinusoids. On the contrary, BMB of non-mutated ET showed a greater number of total, large, “stag horn” and clustered megakaryocytes.

ET with a high allele burden displayed lower haemoglobin level and platelet count, higher LDH level, larger spleen and greater incidence of venous and arterial thrombosis than cases with low allele burden. Our results are partly in agreement with previous reports [2,6] and suggest that high $JAK2^{V617F}$ mutation load is associated with a more severe disease. Furthermore, BMB

of ET with high mutation load more frequently displayed dysplasia of the erythroid, myeloid and megakaryocytic lineages. These morphological changes are also suggestive of a more severe disease, and could explain the lower haemoglobin level and platelet count in patients with high allele burden. Homozygous $JAK2^{V617F}$ mutation was found in only 3 mutated cases (5.6%), in accordance with the literature.[2] With the limitation due to the small number of cases, patients with homozygous $JAK2^{V617F}$ mutation seem to have a particularly severe disease, with marked splenomegaly, high LDH level, altered thrombophilia screening and dysplasia of the erythroid and myeloid lineages.

No difference in venous thrombosis between mutated and non-mutated cases was found, contrary to previous reports [4] but in accordance with others.[7] However, venous thrombosis was directly related to the mutation load, like in other studies.[2,8]

In conclusion, our results confirm that ET patients with $JAK2^{V617F}$ mutation have a PV-like phenotype. $JAK2^{V617}$ mutational status is associated with BMB morphological changes, mainly of the megakaryocyte lineage. Therefore, $JAK2^{V617}$ mutation and allele burden may identify distinct clinical and morphological subtypes of ET.

Table 1. Association between JAK2^{V617F} mutation and clinical and haematological features and bone marrow histology in ET (N = 103)

	JAK2^{V617F} Positive cases (N=59)	JAK2^{V617F} Negative cases (N=44)	
Variable	Mean ± SD	Mean ± SD	*P*-value
Age (years)	56.4 ± 15.8	62.8 ± 15.7	0.04
Haematocrit (%)	43.6 ± 5.2	39.8 ± 3.8	0.0002
Hb level (g/dL)	14.2 ± 1.8	13 ± 1.8	0.0004
RBC count (x10^{12}/L)	5.083 ± 0.6	4.435 ± 0.7	<0.0001
WBC count (x10^{9}/L)	10.23 ±8.5	7.88 ± 2.3	0.08
Plt count (x10^{9}/L)	693.2 ± 240	926.8 ± 271	<0.0001
Spleen (cm)	12 ± 3.4	11.5 ± 1.8	0.2
Venous thrombosis	11/49 (22.4%)	7/37 (18.9%)	0.45
Arterial thrombosis	4/48 (8.3%)	2/37 (5.4%)	0.46
Bone marrow cellularity (%)	70.1 ± 10.2	61.8 ± 12.36	0.0009
CD34+ blasts (%)	2.58 ± 1.5	2.3 ± 1.2	0.5
Megakaryocytes*	78.6 ± 26.4	100.9 ± 30.7	0.0002
Large Megakaryocytes*	18.7 ± 8.9	37.8 ± 17.1	<0.0001
"Stag horn" Megakaryocytes*	2.6 ± 2.8	12.7 ± 5.1	<0.0001
Micromegakaryocytes*	8.5 ± 6.3	3.6 ± 4.6	<0.0001
Clusters of Megakaryocytes **	5.3 ± 4.1	13.7 ± 4.7	<0.0001
Erythroid hyperplasia	54 (91.5%)	9 (20.5%)	<0.0001
Myeloid hyperplasia	56 (94.9%)	36 (81.8%)	0.03
Megakaryocytic dysplasia	59 (100%)	36 (81.8%)	0.0007
Sinusoid hyperplasia	36 (61%)	16 (36.4%)	0.01
Fibrosis	41 (69.5%)	36 (81.8%)	0.2

Values are means (SD), unless otherwise indicated.

RBC, red blood cell; WBC, white blood cell; Plt, platelet; Hb, haemoglobin

* Cell numbers/10 HPF ; ** Number of clusters/10 HPF; Fibrosis (WHO MF-1 or 2)

Table 2. Association between JAK2^{V617F} mutation load and clinical and haematological features and bone marrow histology in ET (N = 53)

	JAK2^{V617F} mutation load >12.5% (N=16)	JAK2^{V617F} mutation load ≤12.5% (N=37)	
Variable	Mean ± SD	Mean ± SD	*P*-value
Age (years)	59.9 ± 20.2	55.1 ± 13.8	0.2
Haematocrit (%)	40.8 ± 7.8	45.2 ± 3	0.2
Hb level (g/dL)	13.3 ± 2.6	14.7 ± 1.3	0.05
WBC count (x10^9/L)	9.42 ± 4.3	10.68 ± 10.4	0.65
Plt count (x10^9/L)	577.7 ± 276	750.6 ± 206	0.01
LDH (UI/L)**	604 ± 132	386 ± 94	0.004
Spleen (cm)	15.4 ± 4.9	11.2 ± 2.1	0.004
Venous thrombosis	6/12 (50%)	3/32 (6.3%)	0.002
Arterial thrombosis	3/12 (25%)	1/31 (3.2%)	0.02
Bone marrow cellularity (%)	72.5 ± 9.6	68.5 ± 11	0.2
Micromegakaryocytes*	12.3 ± 8.9	7.6 ± 4.5	0.05
Erythroid dysplasia	10 (62.5%)	9 (24.3%)	0.01
Myeloid dysplasia	3 (18.8%)	1 (2.7%)	0.04
Excess of haemosiderin	1 (6.2%)	11 (29.7%)	0.005
Sinusoid hyperplasia	7 (43.7%)	25 (67.6%)	0.1

Values are means (SD), unless otherwise indicated. RBC, red blood cell; WBC, white blood cell; Plt, platelet; Hb, haemoglobin; LDH, lactate dehydrogenase

* Cell numbers/10 HPF

** Normal range: 250-450 UI/L

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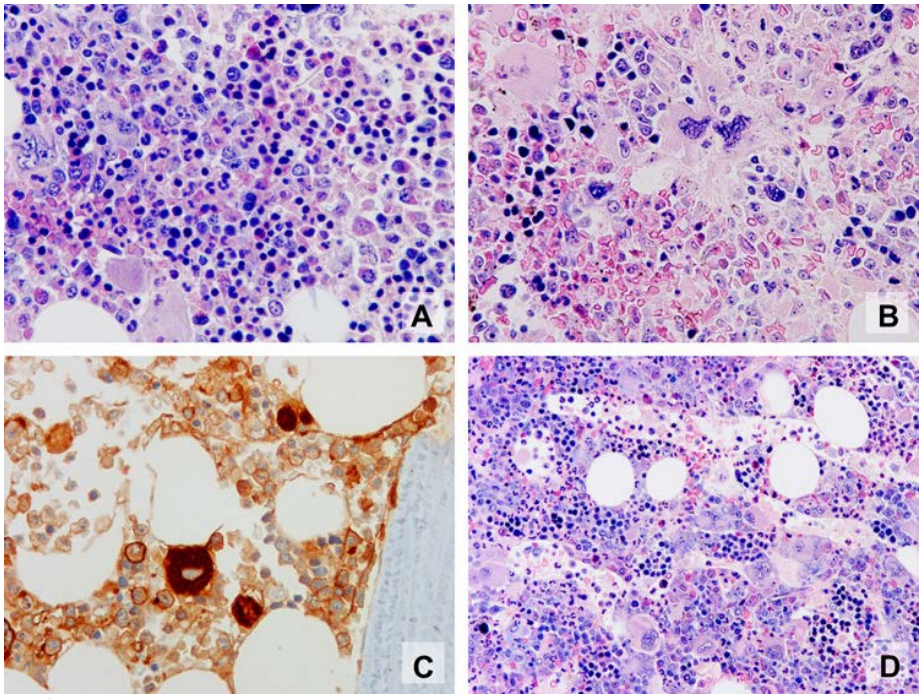
Legends

Figure 1

a) BM biopsy of ET with JAK2^{V617F} mutation showing a hypercellular (90%) marrow with a marked erythroid hyperplasia (Dominici stain, original magnification x400). b) BM biopsy of ET with JAK2^{V617F} mutation showing dysplastic megakaryocytes (Dominici stain, original magnification x400). c) BM biopsy of ET with high mutation load: micromegakaryocytes are strongly stained with anti CD31 monoclonal antibody (CD31 immunostaining, original magnification x400). d) BM biopsy of ET with JAK2^{V617F} mutation showing dilated sinusoids (Dominici stain, original magnification x200).

Figure 2

a) BM biopsy of JAK2^{V617F} non-mutated ET showing a "stag horn" megakaryocyte (H.E. stain, original magnification x600). b) "Stag horn" megakaryocytes are more numerous in non-mutated than in JAK2^{V617F} mutated ET (Dominici stain, original magnification x600). c) BM biopsy of a non-mutated ET showing a large cluster of megakaryocytes (H.E. stain, original magnification x400). d) Clusters of megakaryocytes are more often seen in non-mutated than in mutated ET (H.E. stain, original magnification x



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