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JAK2^{V617F} activating mutation is associated with the myeloproliferative type of chronic myelomonocytic leukaemia

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ABSTRACT

Background: Chronic myelomonocytic leukaemia (CMML) is a haematopoietic malignancy with heterogeneous clinical and morphological features. It is classified in the World Health Organization myeloproliferative-myelodysplastic overlap category. JAK2^{V617F} mutation can be found in a large percentage of patients with myeloproliferative neoplasms.

Aims: To investigate the association between JAK2^{V617F} mutation and clinical, haematological and bone marrow histological features in CMML and to verify whether the mutation is associated with the myeloproliferative type of the disease.

Methods: 78 consecutive patients with newly diagnosed CMML from 2004 to 2008 were included in the study. JAK2^{V617F} mutation was assessed using direct sequencing of exon 14 or by allele-specific PCR from total peripheral blood or bone marrow samples.

Results: JAK2^{V617F} mutation was identified in eight cases (10.2%). All patients with the mutation presented with splenomegaly and had a significantly higher haemoglobin level and neutrophil count than patients without the mutation. All bone marrow biopsies of JAK2^{V617F}-mutated CMML showed increased erythropoiesis, a marked myeloid and megakaryocytic hyperplasia with occasionally clustered megakaryocytes, and a mild or moderate (grade 1 or 2) fibrosis; six cases showed an increased number of dilated sinusoids and reactive lymphoid nodules.

Conclusions: The results indicate that JAK2^{V617F} mutation is associated with clinical and morphological features of the myeloproliferative type of CMML. Therefore, JAK2 mutation analysis together with bone marrow morphology could help in a more appropriate classification of the disease.

Chronic myelomonocytic leukaemia (CMML) is a clonal disorder of a bone marrow stem cell, characterised by a persistent peripheral blood monocytosis. The clinical, haematological and morphological features of the disease are heterogeneous and vary from predominantly myelodysplastic to mainly myeloproliferative patterns.[1–3] According to the World Health Organization (WHO) classification of myeloid neoplasias, CMML is placed in the myeloproliferative-myelodysplastic overlap category.[4] However, a more appropriate classification of the disease would be desirable.

A somatic point mutation (V617F) in a highly conserved residue of the pseudokinase domain of the Janus kinase 2 (JAK2) has been detected in a large percentage of patients with myeloproliferative disorders, such as polycythaemia vera (65–97%), essential thrombocythaemia (23–79%) and primary myelofibrosis (35–78%).[5–9] JAK2^{V617F} mutation has been rarely observed in patients with myelodysplastic/myeloproliferative diseases or myelodysplastic syndromes.[10–12]

The aim of this study was to search for JAK2^{V617F} mutation in CMML and investigate the relationship with clinical, haematological and bone marrow (BM) histological features, to verify whether the JAK2^{V617F} mutation is associated with the myeloproliferative type of CMML.

METHODS

Patients

A total of 78 consecutive patients with newly diagnosed CMML, admitted to the Division of Haematology, San Giovanni Hospital and University of Turin, Italy, from 2004 to 2008 were included in the study. Diagnosis of CMML was performed according to WHO criteria.[4] There were 24 women and 54 men; the mean age was 70 years (range 36–88 years). The study was carried out with the approval of the local ethics committee. BM biopsies were taken during initial investigation, from posterior–superior iliac crest, using a Jamshidi needle.

Histology

Specimens were immediately fixed in buffered acid formol for 24 h, decalcified in Osteodec (EDTA, HCl mixture; Bioptica, Milan, Italy) for 6 h, dehydrated, and embedded in paraffin. Serial sections (3 μm thick) were stained with H&E, Dominici, Perls and Gomori stains. Immunohistochemistry was performed with an automatic stainer device (Dakoautostainer; Dako, Glostrup, Denmark), using the Labelled Streptavidin-Biotin 2 System detection kit (Dako), diaminobenzidine as a chromogen, and the monoclonal antibodies anti-CD34 (Clone QBEnd/10), anti-CD31 (clone JC70A), anti-von Willebrand Factor (Clone F8/86), anti-glycophorin A (Clone JC159), anti-CD68 (clone PG-M1), and the polyclonal antibody anti-human myeloperoxidase (all from Dako, Glostrup, Denmark). The following histological parameters were evaluated: marrow cellularity, hyperplasia and dysplasia of the erythroid, myeloid and megakaryocyte lineages, percentage of monocytes and CD34-positive blasts, marrow fibrosis, presence of dilated sinusoids and reactive lymphoid nodules. Bone marrow cellularity was determined taking into account the age-related changes in the study population.[13] The different cell lineages were

semiquantitatively evaluated by a score that was mainly based on their frequency in the normal BM:[14] 0, no increase in comparison with the normal state; +1, mild increase (corresponding to a mild hyperplasia); +2, moderate increase (moderate hyperplasia); +3, marked increase (marked hyperplasia). BM fibrosis was graded according to the criteria of the European consensus on grading bone marrow fibrosis[13] and a semiquantitative scale was used: 0, scattered linear reticulin with no intersections, corresponding to normal bone marrow; 1, loose network of reticulin with many intersections, especially in perivascular areas (corresponding to a mild fibrosis); 2, diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen (corresponding to a moderate fibrosis). All biopsy specimens were independently examined by two pathologists (AP, LG) who had no knowledge of the mutational status. Disagreement between the observers was found in less than 10% of the cases. In these cases, a consensus interpretation was reached after re-examination of the slides with a double-headed microscope.

JAK2^{V617F} mutation analysis

JAK2^{V617F} mutation testing was performed on peripheral blood or bone marrow samples. RNA and/or DNA were automatically extracted by using Maxwell 16 blood DNA purification kit, Wizard SV 96 genomic DNA purification system or SV96 total RNA isolation system following the manufacturer's instructions (Promega Corporation, Madison, Wisconsin, USA). cDNA was prepared by reverse transcription following the standardised BIOMED-1 protocol.[15] JAK2^{V617F} mutation was assessed in all cases using direct sequencing of exon 14 cRNA: PCR primers (forward: 5′-GTAGGAGACTACGGTCAACTG-3′; reverse: 5′-TGCATGGCCCATGCCAACT-3′) were designed to amplify a 273 bp segment of JAK2 encompassing the codon for amino acid 617. The sequencing reaction was carried out using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, USA), and the analysis was performed on an ABI 310 automated capillary system, following the manufacturer's instructions. All sequences of samples were compared with published germ-line sequences using basic local alignment search tool (BLAST) on the Internet.

In selected cases, allele-specific PCR was also utilised to screen for the JAK2^{V617F} point mutation on DNA by amplifying JAK2 exon 14.[5]

Statistical analysis

The independence between categorical variables and JAK2^{V617F} status was estimated by the Fisher's exact test. Continuous variables were compared by the Mann–Whitney U test. All data were processed with BMDP selected programs (2D, 3D, 7D, 4F).[16]

RESULTS

JAK2^{V617F} mutation was identified in 8 of 78 cases (10.2%). The results of the association between JAK2^{V617F} mutation and clinical, haematological and BM histological features in patients with CMML are summarised in tables 1 and 2. Of 37 patients that had palpable splenomegaly, 22 underwent an ultrasound or CT scan that showed a maximum diameter of the spleen ranging from 14 to 25 cm. All patients with JAK2^{V617F} mutation presented with splenomegaly (palpable spleen or echographically/CT enlarged spleen more than 14 cm in the maximum diameter), while splenomegaly was found in only 29 of 70 JAK2^{V617F}-negative patients ($p = 0.001$). Patients with JAK2^{V617F} mutation had also significantly higher red blood cell count (4.747×10^{12}/l versus 3.593×10^{12}/l, $p = 0.01$), haemoglobin level (13.1 g/dl versus 10.8 g/dl, $p = 0.02$) and neutrophil count (22.624×10^{9}/l versus 8.267×10^{9}/l, $p = 0.02$) than patients without the mutation. No association was found for patient age, sex, white blood cell count, or platelet, monocyte or lymphocyte count. BM biopsies of CMML with JAK2^{V617F} mutation showed a mean cellularity of 85% (range 70–100), a mean percentage of CD34-positive blasts of 5.75% (range 1–15) and a mean percentage of monocytes of 18.1% (range 15–25); these values were not different to BM biopsies of non-mutated cases. Erythroid hyperplasia was found in all mutated cases, but in only 20 of 70 (28.6%) non-mutated cases ($p = 0.0001$). A marked myeloid (fig 1) and megakaryocytic hyperplasia was seen in all mutated cases, but in only 60% and 50% of non-mutated cases ($p = 0.02$ and 0.006, respectively). Megakaryocytes were occasionally clustered and large, with hyperlobulated nuclei (fig 1, inset). Mild or moderate fibrosis (grade 1 or 2)[13] (fig 2) was seen in all mutated cases, but in only 13 of 70 (18.6%) non-mutated cases ($p<0.00001$). An increased number of dilated sinusoids and reactive lymphoid nodules was seen in six of mutated cases (75%), but in only 4 (5.7%) and 15 (21.4%) of non-mutated cases ($p<0.0001$ and 0.004, respectively).

Table 1 Association between JAK2^{V617F} mutation and clinical and haematological features in chronic myelomonocytic leukaemia (n = 78)

Variable	JAK2^{V617F} positive (n = 8)	JAK2^{V617F} negative (n = 70)	p Value
Age, years	68.9 (8.3)	70.2 (10.9)	0.4
Haemoglobin, g/dl	13.1 (2.7)	10.8 (2.2)	0.02
WBC count, $\times10^9$/l	28.386 (29.77)	18.039 (19.73)	0.1
Platelets, $\times10^9$/l	205.5 (277)	139.1 (141)	0.6
Neutrophils, $\times10^9$/l	22.624 (27.33)	8.267 (8.62)	0.02
Lymphocytes, $\times10^9$/l	2.435 (1.047)	3.230 (4.910)	0.5
Monocytes, $\times10^9$/l	2.890 (2.382)	3.631 (4.653)	0.8
Splenomegaly, n (%)	8 (100)	29 (41.4)	0.001

Values are means (SD), unless otherwise indicated.
WBC, white blood cell.

DISCUSSION

Our results show that JAK2^{V617F} mutation can be detected in a small number (8/78) of CMML, in accordance with previous

Table 2 Association between JAK2^{V617F} mutation and bone marrow histology in chronic myelomonocytic leukaemia (n = 78)

Variable	JAK2^{V617F} positive (n = 8)	JAK2^{V617F} negative (n = 70)	p Value
Cellularity*	85 (10.7)	83.3 (10.6)	0.8
CD34+ blasts*	5.75 (5.2)	3.04 (3.46)	0.09
Monocytes*	18.1 (3.72)	19.5 (8.2)	0.9
Fibrosis, n (%)	8 (100)	13 (18.6)	<0.00001
Erythroid hyperplasia, n (%)	8 (100)	20 (28.6)	0.0001
Myeloid hyperplasia, n (%)	8 (100)	42 (60)	0.02
Megakaryocytic hyperplasia, n (%)	8 (100)	35 (50)	0.006
Dilated sinusoids, n (%)	6 (75)	4 (5.7)	<0.0001
Reactive lymphoid nodules, n (%)	6 (75)	15 (21.4)	0.004

Values are mean (SD) percentages, unless otherwise indicated.
Fibrosis, mild or moderate fibrosis (grade 1 or 2).[13]

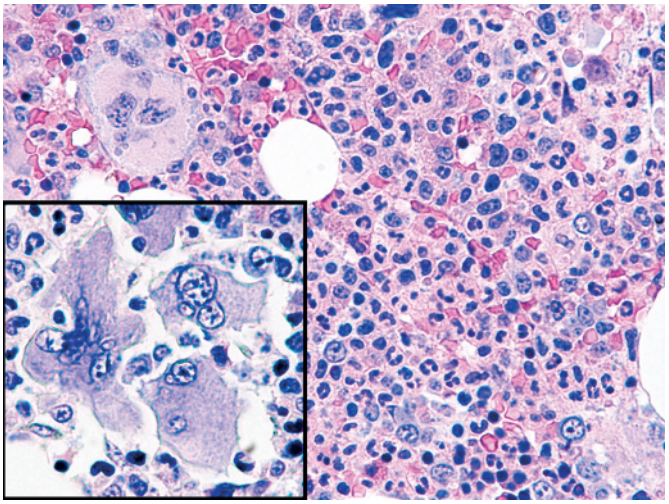

Figure 1 Bone marrow biopsy of a chronic myelomonocytic leukaemia with JAK2^{V617F} mutation showing marrow hypercellularity, marked myeloid hyperplasia and clustered megakaryocytes (inset) (Dominici stain, original magnification ×400).

studies reporting mutations in 3%,[12] 7.8%[10] and 13% of CMML.[11] Interestingly, JAK2^{V617F} mutation was associated with clinical, haematological and BM morphological features suggestive of a myeloproliferative disease. Indeed, the patients with JAK2^{V617F} mutation all presented with splenomegaly and had significantly higher haemoglobin level, red blood cell and neutrophil count than patients without the mutation.

Furthermore, BM biopsy of CMML with JAK2^{V617F} mutation showed in all cases marked erythroid, myeloid and megakaryocitic hyperplasia, with occasionally large and clustered megakaryocytes. In particular, a mild or moderate fibrosis (grade 1 or 2)[13] was evident in BM biopsy of all mutated CMML, but in only 13 of 70 non-mutated cases ($p<0.00001$); an increased number of dilated sinusoids and reactive lymphoid nodules was seen in six of the mutated cases, but in only 4 and 15 of 70 non-mutated cases ($p<0.0001$ and 0.004, respectively). These findings are in contrast with those of Steensma *et al*[12] who found primarily proliferative features in only one of three patients with JAK2^{V617F}-mutated CMML, and are also partly different from those of Jelinek *et al*[11] who reported splenomegaly and megakaryocytic hyperplasia in only three and five of seven CMML patients, respectively. An appropriate classification of CMML is still controversial, and the use of the peripheral white blood cells at diagnosis ($\leqslant 13\times10^9$/l) as the single criterion for subclassificaton of the disease does not seem fully justified.[17] Our results indicate that JAK2^{V617F} mutation is associated with clinical and morphological features of the myeloproliferative type of CMML. Therefore, JAK2^{V617F} mutation analysis together with bone marrow histopathology could help to provide a more appropriate classification of the disease.

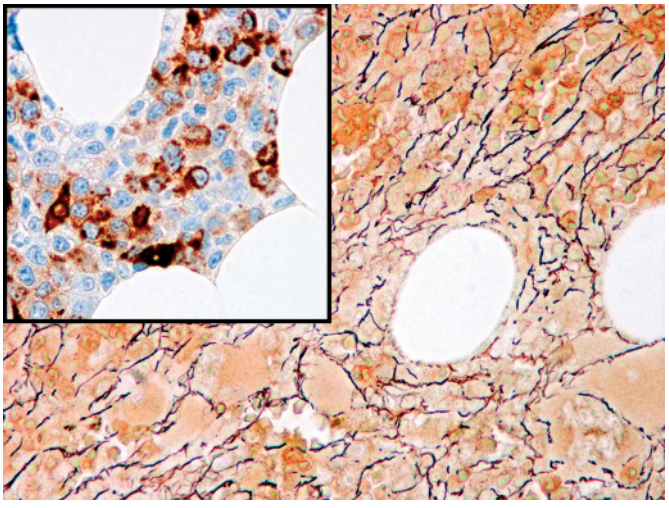

Figure 2 Bone marrow biopsy of a chronic myelomonocytic leukaemia with JAK2^{V617F} mutation showing grade 1 fibrosis (Gomori stain, original magnification ×400) and clusters of monocytes (inset, CD68PGM1 immunostaining, original magnification ×400).

Take-home messages

- Chronic myelomonocytic leukaemia (CMML) is a haematopoietic malignancy with heterogeneous clinical and morphological features and is classified in the World Health Organization myeloproliferative-myelodysplastic overlap category. JAK2^{V617F} mutation can be found in a large percentage of myeloproliferative neoplasms.
- All patients with CMML and JAK2^{V617F} mutation displayed splenomegaly with higher haemoglobin level and neutrophil count than patients without the mutation. Bone marrow biopsies of JAK2^{V617F}-mutated CMML showed in all cases an increased erythropoiesis, a marked myeloid and megakaryocytic hyperplasia and a mild or moderate fibrosis.
- JAK2^{V617F} mutation is associated with clinical and morphological features of the myeloproliferative type of CMML. Therefore, JAK2^{V617F} mutation analysis together with bone marrow histopathology could help to provide a more appropriate classification of the disease.

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