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

A Pich,1 L Riera,2 F Sismondi,2 L Godio,1 L Davico Bonino,1 F Marmont,3 P Francia di Celle2

**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\textsuperscript{V617F} mutation can be found in a large percentage of patients with myeloproliferative neoplasms.

**Aims:** To investigate the association between JAK2\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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 diluted sinusoids and reactive lymphoid nodules.

**Conclusions:** The results indicate that JAK2\textsuperscript{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.

**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. 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 formalin for 24 h, decalcified in Osteodec (EDTA, HCl mixture; Bioptica, Milan, Italy) for 6 h, dehydrated, and embedded in paraffin. Serial sections (3 \(\mu\)m 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), dianobenzidine as a chromogen, and the monoclonal antibodies anti-CD34 (Clone QBEnd/10), anti-CD31 (clone JC70A), anti-CD41 (Clone QBC69.1), anti-glycophorin A (Clone JC1S9), 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. The different cell lineages were...
semiquantitatively evaluated by a score that was mainly based on their frequency in the normal BM: 10, 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, LC) 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.

**JAK2V617F mutation analysis**

JAK2V617F 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. 10 JAK2V617F mutation was assessed in all cases using direct sequencing of exon 14 cRNA: PCR primers (forward: 5'-GTAGGAGACTACGTCCAATCTG-3'; reverse: 5'-TGCATGGCCCATGCCAACT-3') were designed to amplify a 275 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 JAK2V617F point mutation on DNA by amplifying JAK2 exon 14. 7

**Statistical analysis**

The independence between categorical variables and JAK2V617F status was estimated by the Fisher’s exact test. Continuous variables were compared by the Mann–Whitney U test. All data were processed with BMDF selected programs (2D, 3D, 7D, 4F). 16

**RESULTS**

JAK2V617F mutation was identified in 8 of 78 cases (10.2%). The results of the association between JAK2V617F 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 JAK2V617F 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 JAK2V617F-negative patients (p = 0.001). Patients with JAK2V617F mutation had also significantly higher red blood cell count (4.747 ± 10¹²/l versus 3.993 ± 10¹²/l, p = 0.01), haemoglobin level (15.1 g/dl versus 10.8 g/dl, p = 0.02) and neutrophil count (22.624 ± 10¹⁰/l versus 8.267 ± 10¹⁰/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 JAK2V617F 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 20 of 70 (28.6%) non-mutated cases. A marked megakaryocytic hyperplasia was seen in 42 of 78 (54%) cases, while in 35 of 78 (45%) cases only small numbers of megakaryocytes were present.

**Table 1** Association between JAK2V617F mutation and clinical and haematological features in chronic myelomonocytic leukaemia (n = 78)

<table>
<thead>
<tr>
<th>Variable</th>
<th>JAK2V617F positive (n = 8)</th>
<th>JAK2V617F negative (n = 70)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>68.9 (8.3)</td>
<td>70.2 (10.9)</td>
<td>0.4</td>
</tr>
<tr>
<td>Haemoglobin, g/dl</td>
<td>13.1 (2.7)</td>
<td>10.8 (2.2)</td>
<td>0.02</td>
</tr>
<tr>
<td>WBC count, ×10⁹/l</td>
<td>28.386 (29.77)</td>
<td>18.039 (19.73)</td>
<td>0.1</td>
</tr>
<tr>
<td>Platelets, ×10⁹/l</td>
<td>205.5 (277)</td>
<td>139.1 (141)</td>
<td>0.6</td>
</tr>
<tr>
<td>Neutrophils, ×10⁹/l</td>
<td>22.624 (27.33)</td>
<td>8.267 (8.62)</td>
<td>0.02</td>
</tr>
<tr>
<td>Lymphocytes, ×10⁹/l</td>
<td>2.435 (1.047)</td>
<td>3.230 (4.910)</td>
<td>0.5</td>
</tr>
<tr>
<td>Monocytes, ×10⁹/l</td>
<td>2.890 (2.382)</td>
<td>3.631 (4.653)</td>
<td>0.8</td>
</tr>
<tr>
<td>Splenomegaly, n (%)</td>
<td>8 (100)</td>
<td>29 (41.4)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are mean (SD), unless otherwise indicated.

**DISCUSSION**

Our results show that JAK2V617F mutation can be detected in a small number (8/78) of CMML, in accordance with previous observations. Of the 8 JAK2V617F-positive cases, 7 were positive for JAK2V617F by allele-specific PCR. The association between JAK2V617F mutation and clinical features is summarised in table 2. The mean white blood cell count in JAK2V617F-positive cases was significantly higher (205.5 ± 277 × 10⁹/l) than in the JAK2V617F-negative cases (139.1 ± 141 × 10⁹/l, p = 0.001). The mean platelet count was also significantly increased in JAK2V617F-positive cases compared to JAK2V617F-negative cases (22.624 ± 27.33 × 10⁹/l versus 8.267 ± 8.62 × 10⁹/l, p = 0.02). The mean percentage of CD34-positive blasts was also significantly higher in JAK2V617F-positive cases compared to JAK2V617F-negative cases (5.75 ± 5.2% versus 3.04 ± 3.46%, p = 0.09). The mean percentage of monocytes was also significantly increased in JAK2V617F-positive cases compared to JAK2V617F-negative cases (18.1 ± 3.72% versus 19.5 ± 8.2%, p = 0.9).

**Table 2** Association between JAK2V617F mutation and bone marrow histology in chronic myelomonocytic leukaemia (n = 78)

<table>
<thead>
<tr>
<th>Variable</th>
<th>JAK2V617F positive (n = 8)</th>
<th>JAK2V617F negative (n = 70)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity*</td>
<td>85 (10.7)</td>
<td>83.3 (10.6)</td>
<td>0.8</td>
</tr>
<tr>
<td>CD34+ blasts*</td>
<td>5.75 (5.2)</td>
<td>3.04 (3.46)</td>
<td>0.09</td>
</tr>
<tr>
<td>Monocytes*</td>
<td>18.1 (3.72)</td>
<td>19.5 (8.2)</td>
<td>0.9</td>
</tr>
<tr>
<td>Fibrosis, n (%)</td>
<td>8 (100)</td>
<td>13 (18.6)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Erythroid hyperplasia, n (%)</td>
<td>8 (100)</td>
<td>20 (28.6)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Myeloid hyperplasia, n (%)</td>
<td>8 (100)</td>
<td>42 (60)</td>
<td>0.02</td>
</tr>
<tr>
<td>Megakaryocytic hyperplasia, n (%)</td>
<td>8 (100)</td>
<td>35 (50)</td>
<td>0.006</td>
</tr>
<tr>
<td>Dilated sinusoids, n (%)</td>
<td>6 (75)</td>
<td>4 (5.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Reactive lymphoid nodules, n (%)</td>
<td>6 (75)</td>
<td>15 (21.4)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Values are mean (SD) percentages, unless otherwise indicated.

Fibrosis, mild or moderate fibrosis (grade 1 or 2). 13
Our results indicate that JAK2V617F mutation is associated with increased hemoglobin level, red blood cell and neutrophil count than patients without the mutation. Bone marrow biopsies of JAK2V617F-mutated CMML showed in all cases an increased erythropoiesis, a marked myeloid and megakaryocytic hyperplasia and a mild or moderate fibrosis.

The JAK2V617F mutation is associated with clinical and morphological features of the myeloproliferative type of CMML. Therefore, JAK2V617F mutation analysis together with bone marrow histopathology could help to provide a more appropriate classification of the disease.

**Take-home messages**

- Chronic myelomonocytic leukemia (CMML) is a haematopoietic malignancy with heterogeneous clinical and morphological features and is classified in the World Health Organization myeloproliferative-myeledysplastic overlap category. JAK2V617F mutation can be found in a large percentage of myeloproliferative neoplasms.
- All patients with CMML and JAK2V617F mutation displayed splenomegaly with higher haemoglobin level and neutrophil count than patients without the mutation. Bone marrow biopsies of JAK2V617F-mutated CMML showed in all cases an increased erythropoiesis, a marked myeloid and megakaryocytic hyperplasia and a mild or moderate fibrosis.
- JAK2V617F mutation is associated with clinical and morphological features of the myeloproliferative type of CMML. Therefore, JAK2V617F mutation analysis together with bone marrow histopathology could help to provide a more appropriate classification of the disease.

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**Competing interests:** None.

**Ethics approval:** Ethics approval was obtained from the Ethics Committee of ASO San Giovanni Battista - ASO CTO/CRF/Maria Adelaide of Turin.

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