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Abstract

Introduction: Mutations in the JAK2, CALR, and MPL genes have been shown to have prognostic value in essential thrombocythaemia (ET), but no clear association with morphological changes has been reported so far. We investigated the possible correlation between gene mutations and histopathological features in bone marrow (BM) biopsies of patients with ET. Methods: Marrow cellularity, fibrosis, and the number of total and dysmorphic megakaryocytes and clusters of megakaryocytes were compared to gene mutations in 90 cases of ET at diagnosis. Results: The JAK2V617F mutation was found in 58.9%, CALR in 28.9%, and MPL in 4.4% of the cases, and 7.8% were triple-negative. JAK2V617F-mutated ET showed a high BM cellularity, the lowest number of clusters of megakaryocytes and the highest number of dysmorphic megakaryocytes; CALR-mutated ET showed a reduced BM cellularity, many clusters of large megakaryocytes, and very few dysmorphic megakaryocytes; MPL-mutated ET showed the lowest BM cellularity, the highest number of clustered and large megakaryocytes, and the lowest number of dysmorphic megakaryocytes. Triple-negative ET cases had the highest BM cellularity. Conclusions: Distinct morphological patterns were associated with gene mutations in ET, supporting the classification of ET into different subtypes.

Introduction

Essential thrombocythaemia (ET) is a chronic myeloproliferative neoplasm that involves primarily the megakaryocytic lineage [1].

Patients with ET show a mutation of the JAK2V617F gene in approximately 60–65% of cases, of the calreticulin gene (CALR) in about 20–25%, and of the gene encoding the thrombopoietin receptor (MPL) in 5% of cases; a small group of ET patients (5–10%) do not carry any of these somatic mutations and are therefore regarded as being “triple-negative” (TN) [2, 3]. The JAK2V617F-activating mutation has been associated with a polycythaemia vera (PV)-like phenotype and an increased risk of thrombosis [4-7].

ET patients with CALR mutations experience fewer vascular complications and a milder clinical course than those with JAK2V617F or MPL mutations, despite significantly higher platelet (Plt) levels. ET patients with the MPL mutation have significantly lower haemoglobin (Hb) and haematocrit (Hct) values, higher erythropoietin values, and significantly higher rates of transformation to secondary myelofibrosis (MF) and acute myeloid leukaemia than patients harbouring either JAK2V617F or CALR mutations [7].

Mutations in JAK2, CALR, and MPL have been shown to have prognostic value in ET [6, 7]. However, no clear association with morphological changes has been reported so far.
In this work, we investigated the possible correlation between the various gene mutations and the histopathological features from the bone marrow (BM) biopsies of 90 patients with ET, to verify whether morphology could support the distinction of ET into different subtypes.

Materials and Methods

Patients

Ninety consecutive patients with newly diagnosed ET, admitted to the Department of Haematology, Città della Salute e della Scienza and University of Turin, Italy, in 2006–2010, were included in the study. Diagnosis of ET was performed according to WHO criteria [1]. There were 48 females and 42 males; the mean age was 60.8 (median 64; range 25–86) years. Splenomegaly (mean size 14.9 cm; range 12.2–26 cm) was detected in 27 patients (30%). None of the patients was under cytoreductive therapy. BM biopsies were taken from the posterior-superior iliac crest during the initial investigation, using a Jamshidi needle. Samples were numerically identified, maintaining patients' anonymity.

Histology

Serial sections (3-µm-thick) from Bouin's solution-fixed, paraffin-embedded BM biopsies were stained with haematoxylin-eosin (HE), Dominici, Perls, and reticulin, and immunostained with an automated stainer device (Ventana-Ultra, Ventana Medical Systems, Tucson, AZ, USA) using polyclonal antibodies against myeloperoxidase (#A0398; Dako; Agilent Technologies, Inc., Santa Clara, CA, USA) at 1: 1,000 dilution at 37°C for 20 min, and von Willebrand Factor (#760–2642, Ventana Medical Systems) undiluted at 37°C for 36 min, and monoclonal antibodies against glycophorin A (clone JC159, # M0819; Dako) at 1: 50 dilution at 37°C for 20 min, CD61 (clone 2f2; # 760–4249; Ventana Medical Systems) undiluted at 37°C for 32 min, CD34 (clone QBEnd/10; #NCL-L-END; Novocastra; Leica Microsystems, Milton Keynes, UK) at 1: 50 dilution at 37°C for 36 min, and CD71 (clone MRQ-48, Ventana Medical Systems) undiluted at 37°C for 32 min.
Marrow cellularity and fibrosis were evaluated on the entire histological section. The percentage of CD34-positive blasts, the number of total, "staghorn," and "cloudy" megakaryocytes, and the number of clusters of megakaryocytes were assessed in 10 HPF, in each case using a standard light microscope (×40).

Mutational Analyses

All mutational analyses were performed on DNA from BM or peripheral blood samples at diagnosis. The JAK2V617F mutation was assessed using direct sequencing of exon 14 mRNA: PCR primers (forward: 5′-GTAGGAGACTACGGTCAACTG; reverse: 5′-TGCATGGCCCATGCCAACT) were designed to amplify the codon for amino acid 617. All samples sequenced were compared to published germ-line sequences using the Basic Local Alignment Search Tool (BLAST) on the Internet. We then determined the JAK2V617F mutant allele burden using a JAK2 MutaScreenTM kit (Ipsogen, Marseille, France). The mutant allele burden was estimated by 6-scaled standards of JAK2V617F 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. MPLW515L/K was assessed by allelic discrimination RT-Q-PCR (an MPL MutaScreen kit, Ipsogen). CALR exon 9 mutations were detected by PCR fragment analysis and Sanger sequencing as previously described [8].

Statistical Analysis

All clinical and laboratory parameters included in the statistical analyses were gathered at diagnosis. The association of patients or BM characteristics and types of mutation was assessed by one-way analysis of variance (ANOVA). The independence between categorized variables and types of mutation was estimated by the Yates-corrected $\chi 2$ test. All analyses were carried out using SPSS v17 (SPSS Inc., Chicago, IL, USA). As a level of significance, $p < 0.05$ was used.

Results

The JAK2V617F mutation was identified in 53 of 90 cases (58.9%), the CALR mutation in 26 (28.9%), and the MPL mutation in 4 (4.4%); 7 cases (7.8%) were TN. Age of presentation was the highest in MPL-mutated patients (mean ± SD: 80.3±5 years) and the lowest in the JAK2V617F-mutated patients (57.8±15.8 years) ($p = 0.02$).

Patients with the JAK2V617F mutation presented with the highest Hb level (14.1±1.8 g/dL) and the lowest Plt count (687±246 × 109/L); those with the CALR mutation had an intermediate Hb level (13.4±2 g/dL) and Plt count (871±213 × 109/L); MPL-mutated patients had the lowest Hb level (12.6±1 g/dL) and the highest Plt count (mean 993±90 × 109/L) ($p = 0.001$). TN patients had a low Hb level (12.8±1 g/dL), a high Plt count (924±379 × 109/L), the lowest Hct level (37.5±1.7%) ($p = 0.02$) and the smallest spleen size (10.4±0.8 cm) (Table 1).

Cellularity, as stratified by age, was 74% for patients < 40 years of age, 65% for patients aged 40–59 years, 66% for patients aged 60–79 years, and 58% for those > 80 years of age.

JAK2V617F-mutated ET had a high BM cellularity (Fig. 1a, b), the lowest number of clusters of megakaryocytes, the highest number of dysmorphic megakaryocytes (Fig. 1b–d), and very few "staghorn" megakaryocytes; CALR-mutated ET showed a reduced BM cellularity (Fig. 1e), many clusters of large megakaryocytes (Fig. 1f, g) and only a few dysmorphic megakaryocytes; MPL-mutated ET showed the lowest BM cellularity (Fig. 1h), the highest number of clustered, large, and "staghorn" megakaryocytes (Fig. 1i, j), and the lowest number of dysmorphic megakaryocytes. TN patients had the highest BM cellularity (70±8.2%) ($p = 0.01$) and the lowest number of "cloudy" megakaryocytes (mean ± SD/10 HPF: 9±3) (Table 1).

Discussion

It is well known that the type of gene mutation can identify different subtypes of ET. Indeed, JAK2V617F-mutated cases were found to have multiple features similar to PV, suggesting that JAK2V617F-positive ET and PV may form a biological continuum [4]; JAK2V617F mutated ET and PV have been regarded as different phenotypes of a single myeloproliferative neoplasm [6]. It has also been suggested that the phenotypical division of PV and ET should be substituted for a classification based on the type of mutation status to better-match clinical prognosis [7]. In a previous study, we reported an association between JAK2V617F mutation and a number of BM morphological features: mutated ET showed a higher marrow cellularity, hyperplasia of erythroid and granulocytic lineages, and a smaller number of "staghorn" megakaryocytes than non-mutated cases [9]. In this study, JAK2V617F-mutated ET showed the highest level of Hb and Hct, the highest red blood cell count, and the lowest Plt count when compared to the other mutation groups, in line with studies demonstrating that ET cases with the JAK2V617F mutation have features similar to PV, without meeting the diagnostic criteria for this disease [7]. However, in JAK2V617F-mutated ET, we also found the highest white blood cell count, the largest spleen size, a very high marrow cellularity, the lowest number of "staghorn" megakaryocytes, and the highest number of dysmorphic megakaryocytes compared to the other mutation groups. These morphological features, in part, overlap those observed in pre-fibrotic/early primary MF, as defined in the revised 2017 WHO classification of haematological malignancies [10]. MPL-mutated ET showed the lowest Hb level and the highest Plt count, in accordance with previous reports [7, 11, 12]. BM biopsies of MPL-mutated ET in our series showed the lowest marrow cellularity and the highest number of total, cloudy, and "staghorn" megakaryocytes, in line with a previous study [12], and also the greatest number of clusters of megakaryocytes and the lowest number of dysmorphic megakaryocytes. From a morphological point of view, MPL-mutated ET seems to be the group that mostly fits with the commonly accepted definition of ET [1]. Due to the low number of these cases, the hypothesis needs to be verified in larger studies.

Our CALR-mutated ET presented with a higher Plt count and lower Hb level than patients with the JAK2V617F mutation, in line with previous reports [6, 8, 13]; BM biopsies in our series showed a

low cellularity, many clusters of megakaryocytes, and only very few dysmorphic megakaryocytes. This finding is in line with the low rate of leukaemic transformation [6] and the mild clinical course and superior overall survival reported for CALR-mutated patients [7, 8].

TN ET patients are uncommon; they represented 7.8% of cases in our series, in line with the data in the literature [2, 3]. Contrary to TN primary MF patients [14], it has been reported that TN ET patients have a relatively good prognosis, with 80% overall survival at 5-year follow-up [7], and with no transformation to acute leukaemia or secondary MF [15]. BM biopsies of TN ET in our series showed a high cellularity, the lowest number of cloudy megakaryocytes, and a low number of dysmorphic megakaryocytes, all histological features suggesting a favourable evolution.

TN does not exclude that other gene mutations can be present in ET. Indeed, apart from JAK2V617F, MPL, and CALR mutations, several other gene mutations have been described: TET2 (in 5% of cases), DNMT3A (in 1–5%), and ASXL1 (in 2–5%) [16]. Thirty-five other gene mutations have been reported [7]. Additional gene mutations (TET2, ASXL1, CBL, SH2B3, SF3B1, FLT3, ADAMTS1, TP53, EGFR, and EZH2) were found by next-generation sequencing [15, 17]. Interestingly, a number of new mutations (SH2B3, SF3B1, U2AF1, TP53, IDH2, and EZH2) have been found to have adverse prognostic significance in ET [18]. Mutations in other genes might be important for the development of myeloproliferative neoplasms. This has to be verified in larger studies, however, if additional gene screening (with the exception of SRSF2, ASXL1, TP53, and EZH2) is to offer more diagnostic and prognostic information.

In conclusion, our results indicate that distinct morphological patterns of ET are associated with different gene mutations, supporting the classification of ET into different subtypes.

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Statement of Ethics

All procedures followed were in accordance with the ethics standards of the local institutional committee on human experimentation and the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from the patient to be included in the study.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Table

Table 1.
Association between gene mutation, clinical and haematological features, and bone marrow histology in our essential thrombocythaemia patients

	Total ($n = 90$)	TN ($n = 7$)	*MPL* ($n = 4$)	*JAK2*^{V617F} ($n = 53$)	*CALR* ($n = 26$)	*p*
Age, years	60.8±15.4	63±10	80.3±5	57.8±15.8	63.2±14.4	0.02
Hct, %	42.1±5	37.5±1.7	39±2	43.5±5.3	40.8±4	0.02
Hb, g/dL	13.7±2	12.8±1	12.6±1	14.1±1.8	13.4±2	0.09
RBC count, $\times 10^{12}$/L	4.833±0.7	4.914±1.2	4.182±0.5	5.066±0.6	4.423±0.6	0.002
WBC count, $\times 10^{9}$/L	9.36±7	7.44±4.1	8.48±2.7	10.39±8.7	7.88±1.4	0.4
Plt count, $\times 10^{9}$/L	772.7±263	924.4±379	993±90	687.8±246	871.1±213	0.001
Spleen size, cm	12±2.9	10.4±0.8	10.7±1.2	12.4±3.5	12.2±2	0.3
LDH, IU/L[a]	451±173	416±162	368±150	444±138	525±266	0.5
Marrow cellularity, %	66.7±11.5	70±8.2	55±17.3	69.9±10	61.2±11.5	0.01
CD34+ blasts, %	2.4±1.3	2.5±1.2	1.5±0.7	2.45±1.3	2.3±1.3	0.8
Megakaryocytes[b]	85.8±29.8	89.3±20.2	111.3±39	78.2±25.6	96.6±34.1	0.02
Cloudy[b]	11.6±7	9±3	18.7±8	11.2±6.6	12.1±7.8	0.1
"Staghorn"[b]	6.5±6.2	10.8±4.9	14.7±10	2.5±2.9	12.2±4.2	0.000
Dysmorphic[b]	6.3±6.4	3.4±3.4	2.5±2.6	8.6±6.4	2.9±5.3	0.000
Clusters[c]	8.5±5.8	13±2.6	15.2±6.4	5.2±4	13.1±4.8	0.000
Fibrosis[d], *n* (yes/no)	13/77	2/5	1/3	6/47	4/22	0.5

Values are means ± SD, unless otherwise indicated. RBC, red blood cell; WBC, white blood cell; Plt, platelet; Hb, haemoglobin; Hct, haematocrit; LDH, lactate dehydrogenase.
[a] normal range 250–450 IU/L; [b] cell numbers/10 HPF; [c] number of clusters/10 HPF; [d] WHO MF-1.

Figure

Fig. 1.

a Bone marrow (BM) biopsy of JAK2V617F-mutated essential thrombocythaemia (ET) showing hypercellular (85%) marrow with erythroid hyperplasia. Dominici stain. ×200. b BM biopsy of JAK2V617F-mutated ET showing many dysmorphic megakaryocytes. HE. ×400. c BM biopsy of ET with JAK2V617F mutation showing 2 dysmorphic megakaryocytes. Dominici stain. ×400. d BM biopsy of JAK2V617F-mutated ET: dysmorphic megakaryocytes are strongly stained with anti-von Willebrand factor polyclonal antibody. von Willebrand Factor immunostaining. ×400. e BM biopsy of CALR-mutated ET with a 60% marrow cellularity. Dominici stain. ×200. f BM biopsy of CALR-mutated ET with a large cluster of megakaryocytes. HE. ×400. gBM biopsy of CALR-mutated ET with a cluster of typically large megakaryocytes. Dominici stain. ×200. hBM biopsy of MPL-mutated ET with a relatively low (50%) cellularity. Dominici stain. ×200. i BM biopsy of MPL-mutated ET with large megakaryocytes tightly clustered. Dominici stain. ×400. j BM biopsy of MPL-mutated ET showing a megakaryocyte with a deeply lobed and hypersegmented (staghorn-like) nucleus. Dominici stain. ×400.