JAK2 or CALR Mutations Status and Bone Marrow Histology in Patients with Essential Thrombocythemia in Chinese

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Research

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Abstract

Objectives: To characterize the clinical, and bone marrow histopathological features of essential thrombocythemia (ET) with different gene mutations status of CALR and JAK2.

Methods: 159 patients of ET were centrally analyzed from January 2016 to December 2019, including 59 cases with CALR mutation, 96 JAK2 mutation, 2 MPL mutation, and 2 cases were triple-negative (TN). Bone marrow pathology observation and determination were performed by 2 immobilized experienced morphological specialists.

Results: Compared to ET with JAK2 mutation, patients with CALR mutation were younger (p=0.000), showed lower count of white blood cell (WBC) and level of hemoglobin (p=0.001, p=0.001), and higher count of platelet (p=0.001). In the bone marrow (BM) biopsy, the median number of megakaryocyte and clusters of megakaryocytes in each high power field (HPF) of vision in patients with CALR mutations were lower than JAK2 mutations patients (p=0.001, p=0.001), thrombotic events in two group was different (5% vs 11.5%) (p=0.03).

Conclusion: In Chinese ET patients, patients with CALR mutations were younger, and had lower levels of Hb, and count of WBC, the lower thrombotic evens although with higher platelet counts than those with JAK2 mutation. Patients with JAK2 mutations had a higher median number of megakaryocytes and median number of clusters of megakaryocytes, the clinical significance is worth exploring.

Background

Essential thrombocythemia (ET) is a sub-type of myeloproliferative neoplasm (MPN) that characterized by colonel disease of myeloid stem cells. Driving gene study demonstrated that patients with ET show a mutation of the $JAK2^{V617F}$ gene in approximately 60 ~ 65% of cases, of the CALR gene in about 20 ~ 25%, and of the gene encoding the MPL in 3 ~ 8%; a small group of patients with ET (5 ~ 10%) do not carry any of these abovely mentioned somatic mutations and are therefore regarded as “triple-negative” (TN) [1]. In patients with ET, there have exist the definite correlation between gene mutations and clinical features and prognosis: patients with the $JAK2^{V617F}$ mutation showed with the highest Hb level and higher risk of thrombosis; those with the CALR mutation experience the lowest risk of thrombosis, despite significantly higher platelet (PLT) levels. MPL mutated patients had the lowest Hb levels, and significantly higher rates of transformation to myofibrils (MF) and acute myeloid leukemia (AML) [2, 3]. The Korea study reported that patients with CALR mutations had lower hemoglobin levels and leukocyte and granulocyte counts, tended to have higher platelet counts, more frequently progressed to accelerated or blast phase disease compared with patients with a JAK2 mutation, although the number of patients was small[4]. So the question is asked that whether there existed some difference in clinical characteristics between Asian or Europe and America patients with ET. Is there have the association with the clinical characteristics of different driver mutants and blood hematopathological changes of the Chinese patients with ET? We investigated a retrospective analysis that these 159 patients with diagnosed ET

from January 2016 to December 2019, compare the clinical and bone marrow (BM) biopsy characteristics of patients with different sub-types of ET.

1. Materials And Methods

Patients

159 patients with ET, admitted to the Department of Hematology-oncology Jiangsu Province Hospital/ The First Affiliated Hospital of Nanjing Medical University, Ningxia People's Hospital/The first affiliated hospital of northwest university for nationalities from January 2016 to December 2019.

Diagnostic Criteria

2017 WHO diagnostic criteria[5], major criteria: ①platelets ≥ 450 × 10^9/L; ②BM morphological features: high BM cellularity the highest number of dysmorphic mature megakaryocytes, atypical hyperplasia of erythroid and granulocytic lineages or shift to left, and very few number of reticular fiber; ③not meeting $BCR\text{-}ABL^+$ chronic myelogenous leukemia, polycythemia vera (PV), primary myelofibrosis (PMF), myelodysplastic syndrome and WHO criteria for other myloid neoplasms; ④JAK2, CALR or MPL mutated. Minor criteria: other clonal marker present for myeloid neoplasms or no evidence of reactive thrombocytosis. Diagnosis can be made by meeting 4 main criteria or front 3 main and minor criteria.

Detection of Mutation

Genomic DNA was extracted from BM or peripheral blood samples at diagnosis, and were performed on $JAK2^{V617F}$, CALR, MPL gene. The following primers were used for polymerase chain reaction(PCR) amplification of exon 9 of the CALR gene: CALR forward(CALR-F): ACAACTTCCT CATCACCAACG; CALR reverse(CALR-R): GGCCTCAGTCCAGCCCTG(Sangon company, Shanghai, China). JAK2 exon 14 forward,5'-TCC TCA GAA CGT TGA TGG CA-3'; JAK2 exon 14 reverse, 5'-ATT GCTTTC CTT TTT CAC AA-3'; JAK2 exon 12 forward, 5'-CTC CTC TTT GGA GCA ATT CA-3'; JAK2 exon 12 forward, 5'-CTC CTC TTT GGA GCA ATT CA-3'; JAK2 exon 12 reverse, 5'-CCA ATG TCA CAT GAA TGT AA-3'; MPL forward: 5'-TGG GCC GAA GTC TGA CCC TTT-3'; MPL reverse, 5'-ACA GAG CGA ACC AAG AAT GCC TGT-3'. PCR was performed using 100 ng template DNA in 25 μL PCR solution, included 10 pmol of each primer，15 nmol of dNTPs，1 U of Taq DNA polymerase, provided buffer solution and Mg^{2+}. The following reaction condition: an initial 5-minute denaturation step at 95°C followed by 35 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 1 minute, with a final 7-minute extension at 72 °C, saved at 4°C. The PCR products were evaluated on 15 g/L of agarose gel electrophoresis (220V, 35 minutes). In approximately 306 bp of objective band were obtained on 15 g/L of agarose gel electrophoresis (220V, 35 minutes) form the PCR products that were performed on ultraviolet ray after stained with EB [6, 7].

BM Morphological Assessment

BM biopsies (the size no less than 2mm × 10 ~ 15 mm) were taken from the posterior-superior iliac crest during the investigation, using a domestic B65-01-needle. Serial section (3-μm-thick) from 10% of AF

solution fixed for 4 hours, after ethonal gradient dehydration, paraffin-embedded BM biopsies were placed into the center of the embedded frame, and then poured into paraffin, after solidification, biopsies were stained with hematoxylin-eosin (HE). Immunohistochemistry were the following: CD34, MPO, CD235a, CD42b, CD68, CD3, CD20. All total bone morrow biopsies were commonly reviewed and performed by a seasoned of two hematopathologists.

Megakaryocytes Classification

Morrow cellularity were observed on the high power field according to the classification criteria, the number of clusters of megakaryocytes ≥ 3 dense clusters of megakaryocytes.

Data Measurement

Histological section was evaluated on the microscope of Nikon clips. An extensive quantification and description of the megakaryocytes was performed. For such purpose, the median number of megakaryocytes in 10 different high-power fields was assessed in each section; and the median number of clusters of 10 different megakaryocytes were assessed in each high-power fields of vision.

Routine Examination

The examination assessed for a total of patients were the following: routine blood examination, biochemistry, blood smear, thrombin, tumor marker; chest radiography, abdominal ultrasonography, and cranial computed (CT).

Statistical Analyses

Statistical analyses were performed using SPSS 21.0 software. The parameters between patient groups were statistically assessed by one-way analysis of variance (ANOVA), and measurement data used in ($x \pm SD$); multiple comparisons was estimated by SNK-q test, enumeration data were used in percentage (%), and estimated by χ^2 test. As a level of significance $\alpha = 0.05$, P□0.05 was considered statistically significant.

2. Results

2.1 Clinical Characteristics of Enrolled Patients

The $JAK2^{V617F}$ mutation was identified in 96 of 159 cases (60.3%), the CALR mutation in 59 cases (37.1%), and the MPL mutation in 2 cases (1.3%); 2 cases (1.3%) were TN. Due to low number of MPL and TN mutated cases, they were not used in cohort study, and only input the data. Age of presentation was lower in CALR mutated patients (52.25 ± 13.79 years) than $JAK2^{V617F}$mutated patients (63.93 ± 10.95 years) ($t = 5.82$, $p = 0.000$), there were no significant difference in gender.

Compared to those in patients with JAK2 mutations, leukocyte count ($9.70 \pm 4.57 \times 10^9$/L versus $12.86 \pm 6.91 \times 10^9$/L; $t = 3.11$, $p = 0.002$) and Hb levels (130.17 ± 12.25 g/L versus 149.22 ± 19.30 g/L, $t = 6.78$, $p = 0.000$) and RBC count ($4.37 \pm 0.51 \times 10^{12}$/L versus $4.97 \pm 0.83 \times 10^{12}$/L; $t = 4.97$, $p = 0.000$) were lower in

patients with CALR mutation. Plt count was higher in patients who had CALR mutations, (942.98 ± 376.10 × 10^9/L versus 824.01 ± 263.10 × 10^9/L; t=-2.31, p = 0.022). The serum level of LDH was similar (335.06 ± 154.43 U/L versus 378.07 ± 164.81 U/L; t=-2.31, p = 0.096).

Among the 14 patients with thrombotic events, three (5%) had a CALR mutation, other 11 patients with JAK2 mutation (11.5%)(p = 0.03)(Table 1).

Among the CALR frame shift mutations, 27 patients had typical type 1 deletions (c.1099-1150del52; p.L367fs*46), and 32 patients had type 2 insertions (c.1154-1155insTTGTC;p.K385fs*47). There were no significant differences regarding age, count of leukocytes, RBC, PLT, levels of Hb, the serum levels of D-dimer and LDH between patients with type 1 or type 2 mutations (P□0.05).

Compared with patients with $JAK2^{V617F}$ (Table 1), patients with CALR1 or CARL2 mutations were younger (t=-3.814, P = 0.000; t=-5.579, P = 0.000), and presented lower RBC count (t=-3.274, P = 0.000; t=-4.107, P = 0.000), Hb level (t=-4.397, P = 0.001; t=-5.693, P = 0.000), respectively. But lower WBC counts (t=-2.996, P = 0.003) was found in patients with CARL2 mutation. And higher PLT count was only found in patients with CALR1 mutation (t = 3.138, P = 0.002).

No statistical differences were observed for the serum levels of LDH, and D-dimer between patients with CALR1 or CARL2, and $JAK2^{V617F}$ mutations (P□0.05).

Table 1
Clinical and Laboratory characteristics of 155 Patients with essential thrombocythaemia

Variable	CALR		$JAK2^{V617F}$
	CALR1	CALR2	
Male/Female	11/16	13/19	50/46
Age (years)	54.37 ± 13.31	50.47 ± 14.14	63.93 ± 10.95
WBC count (× 10^9/L)	10.55 ± 5.08	8.99 ± 4.04	12.86 ± 6.91
RBC count (× 10^{12}/L)	4.41 ± 0.63	4.34 ± 0.39	4.97 ± 0.83
HB(g/L)	131.48 ± 15.32	129.06 ± 9.01	149.22 ± 19.30
PLT count (× 10^9/L)	1022.52 ± 373.47	875.88 ± 370.81	824.01 ± 263.10
LDH(IU/L)	369.89 ± 92.16	386.63 ± 208.72	335.06 ± 154.433
D-dimer	0.31 ± 0.27	0.25 ± 0.10	0.42 ± 0.47
Thrombotic events	1	2	11

2.2 BM Biopsy Histopathological Section

BM Biopsy of the JAK2-mutated cases with ET showed active proliferation of granulocytic, erythroid and megakaryocytic lineages, a total of 15,714 megakaryocytes were observed in the study, the largest diameter of megakaryocyte was 49.77 ± 11.22 um, median 48.39 um (range 35.34 ~ 86.48 um). BM Biopsy of the CALR mutated cases were only showed active proliferation of megakaryocytic lineages, total number of megakaryocyte were 4750, and the largest diameter of megakaryocyte was 46.65 ± 8.02um, median 47um (range 30.36 ~ 70.76 um), there were no significant differences among these two genotypes (p = 0.16) (Table 2). In addition, patients with JAK2 mutations had a higher median number of megakaryocytes (16.65 ± 5.11/HPF vs 8.05 ± 2.77 /HPF, P = 0.001) and median number of clusters of megakaryocytes (2.83 ± 0.60 vs 0.48 ± 0.64/HPF, P = 0.001)(Fig. 1,Fig. 2). There were no significant differences among fibrosis 1(10% vs 14.4%,P = 0.14).

Table 2
Number and histological features in patients with essential thrombocythemia according to genotype

Variable	CALR	$JAK2^{V617F}$	P Value
Largest diameter of megakaryocyte	46.65 ± 8.02	49.77 ± 11.22	0.16
Median number of megakaryocytes per HPF in BM	8.05 ± 2.77	16.65 ± 5.11	0.001
Median number of clusters of megakaryocytes per HPF in BM	0.48 ± 0.64	2.83 ± 0.60	0.001
Fibrosis M1	4	11	0.14

3.Discussion

It is well known that the type of gene mutation can identify different sub-types of ET.

Previous study [3, 8, 9] showed that the age of presentation was the highest in JAK2-mutated patients, it was same that the majority of patients are older than 60 in JAK2 mutated patients in this study. It was reported that the patients with ET who had CALR mutation showed a preference for males, the highest Plt count, the lowest leukocytosis, Hb level, LDH level, the lowest number of thrombotic events and cardiovascular events, low risk of transformation to myelofibrosis and leukemia, and long-term survival rates and better prognosis[3, 10].

In our study, the CALR mutated patients was younger than the JAK2 mutated patients. The ET patients with CALR mutation were also associated with normal WBC count and RBC count, but higher Plt count.

In addition, patients with CALR1 mutation or CALR2 mutation with lower leukocyte counts and hemoglobin levels than those with JAK2 mutation, in accordance with previous reports [10]. The patients with CALR1 and CALR2 mutations displayed the similar leukocyte counts, hemoglobin levels, but patients

with CALR1 mutation revealed higher platelet counts than those with JAK2 mutation. These findings were not consistent with the report by Guo et al [10], it was reported patients with CALR2 mutation exhibited lower WBC counts and higher platelet counts than patients with JAK2 mutation. These observations indicate that CALR mutation and JAK2 mutation exert different effect on leukocyte and erythroid cell. Patients with CALR1 mutation displayed higher platelet counts than those with JAK2 mutation. These findings indicate that it is worth further bone marrow pathology to see the count and distribution of megakaryocytes.

The most overt abnormalities in BM biopsy was increased quantity and volume of megakaryocytes (more than 13/HPF), $JAK2^{V617F}$- mutated had high BM cellularity, the highest number of dysmorphic megakaryocytes, and few “staghorn” megakaryocytes [11]. CALR-mutated ET showed a reduced BM cellularity, many clusters of large megakaryocytes and only a few dysmorphic megakaryocytes[12];

Megakaryocytes exhibited specific morphological features in the various categories of MPNs. ET exhibited giant megakaryocytes, abundant mature large cytoplasm, staghorn, hyperlobated nuclei in dense clusters[13, 14].In this study, the most prominent feature of megakaryocytes in bone marrow biopsy is the hyperproliferation of megakaryocytes, which is characterized by a large number of large cell bodies with abundant cytoplasm, multi-lobulated nuclei and different sizes. The majority of megakaryocytes are mature megakaryocytes, and megakaryocytes are pleomorphic. in accordance with previous reports [13, 14]. No statistical differences were observed for diameter range of megakaryocytes between ET patients with CALR and JAK2 mutations. CALR mutations group presented loose and rare clusters of megakaryocytes, JAK2 mutations group showed dense clusters of megakaryocytes, and the median number of megakaryocytes and median number of clusters of megakaryocytes per HPF in JAK2-mutated ET were significantly higher than that CALR-mutated ET. and no statistical differences were observed for diameter range, the median number of megakaryocytes and median number of clusters of megakaryocytes per HPF of megakaryocytes between ET patients with CALR1 and CALR2 mutations. However, these findings were not consistent with the report by Achille Pich [12], this difference in genetic background is probably associated with the different population, these should be certainly verified in large clinical study.

Patients with ET who had CALR mutations had slightly higher ratios of progression to post -ET myelofibrosis than did patients with JAK2 mutations, but this difference was not statistically significant [4, 15]. In this study, none of the patients had fibrosis or leukemia transformation during follow-up. In our data, no correlation analysis was performed because of the small number of patients with MPL-mutated and TN ET.

The limitations of this study are the retrospective nature of the study, but the differential diagnosis of this disease can be confirmed by observing and analyzing morphological characteristics and distribution of the megakaryocytes in Bone morrow (BM)biopsy.

Conclusions

In conclusion, our results indicate that patients with CALR-mutated ET had slightly higher Plt levels, normal leukocytes and Hb levels in BM and tended to be more younger in comparison with JAK2-mutated ET, significant differences were not found in level of LDH and D-dimer between these two mutation groups; distinct morphological patterns of ET are associated with different gene mutations, supporting the classification of ET into different subtypes. Histological characteristics and distribution of megakaryocytes at differential diagnosis have a significant value for the ET patients with different gene mutations. Hence, it is helpful to distinguish it from different mutated ET by observing and analyzing the morphological changes and distribution of megakaryocytes in BM biopsy, and also provide definite help for clinical diagnosis and treatment.

Declarations

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Authors’ disclosures of potential conflicts of interest

The author(s) indicated no conflicts of interest.

Ethics approval and consent to participate

The study was approved by an ethics committee with the informed consent of all participants.

Consent for publication

The authors declare there is no conflicts of interest regarding the publication of this paper.

Authors' contributions

He GS and Bao SH designed the study, Ye XP colleted and analyzed data and wrote the manuscript; Muhtar Y collected and interpreted data and helped to write the manuscript; Li YQ, Ma J collected and analyzed data.

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Figures

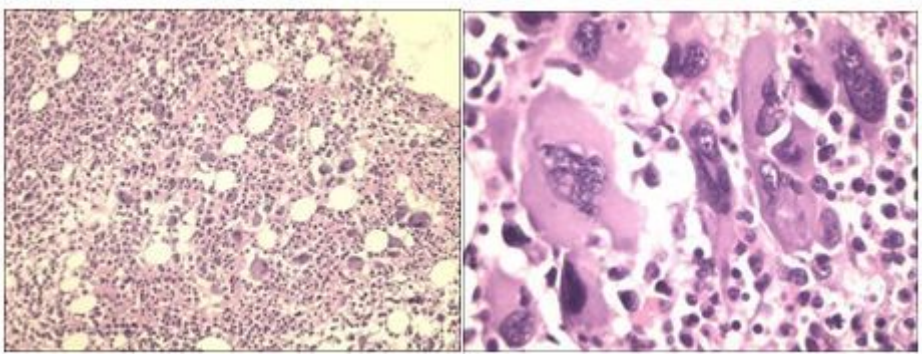

Figure 1

Bone morrow(BM)biopsy of JAK2-mutated ET with marked megakaryocytic proliferation showing overt clusters of pleomorphic megakaryocytes（HE,x100； x400）

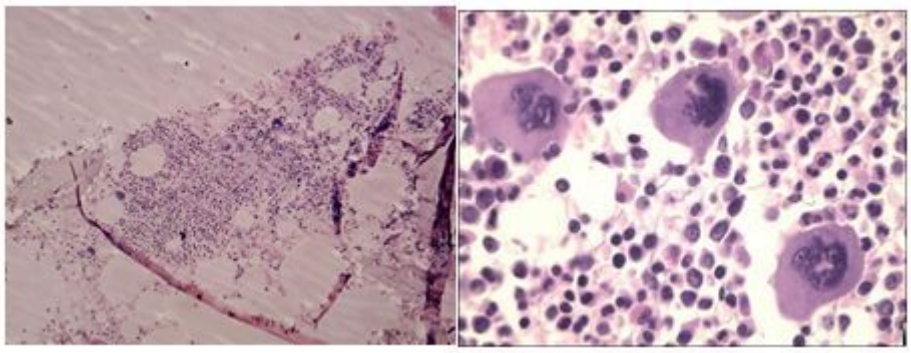

Figure 2

Bone morrow(BM)biopsy of CALR-mutated ET with megakaryocytic proliferation showing loose clusters of pleomorphic megakaryocytes（HE,x100； x400）

Supplementary Files

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- CALR.xls
- JAK2.xlsx