Retrospective analysis of the clinical features of 172 patients with BCR-ABL1-negative chronic myeloproliferative neoplasms

Xiaolan Lin, Huifang Huang and Ping Chen*

Abstract

Background: To explore the clinical features of the patients with BCR-ABL1-negative chronic myeloproliferative neoplasms (MPNs) in our hospital and to reveal the unique features of BCR-ABL1-negative MPNs patients in our center.

Methods: Retrospective analysis of routine karyotype analysis results, driver gene mutations and other related clinical parameters of 172 patients with newly diagnosed BCR-ABL1-negative MPNs who were admitted to our hospital between October 2013 and June 2018.

Results: (1) The rate of karyotypic abnormalities were 25, 6.3 and 2.9% in primary myelofibrosis (PMF), polycythemia vera (PV) and essential thrombocythemia (ET) patients, respectively. (2) The mutation rate of JAK2-V617F was 62.5%, and that of the CALR, MPL and EZH2 genes was 4.2% in PMF. The mutation rates of JAK2-V617F and JAK2-12exon were 91.3 and 1.3% in PV, respectively. The mutation rates of JAK2-V617F and CALR were 69.1 and 11.8% in ET, respectively. (3) Patients with JAK2-V617F mutation than with the wild-type gene were more often female in PMF (P = 0.027); had higher peripheral blood white blood cell (WBC) counts (P = 0.006), platelet (PLT) count (P = 0.001) and splenomegaly (P < 0.05) in PV; and had higher WBC (P = 0.001), hemoglobin concentrations (P = 0.001), lower PLT (P = 0.037), splenomegaly and endogenous coagulopathy (P < 0.05) in ET. (4) Among the PV and ET patients, those with thrombus were older than those in the nonthrombotic group.

Conclusion: PMF patients have more chromosomal abnormalities than PV and ET patients, and the effect of driver mutations on the clinical features of patients with MPNs differs among the three subtypes.

Keywords: MPNs, Cytogenetics, Gene mutation, JAK2-V617F

Background

Chronic myeloproliferative neoplasms (MPNs) consist of a group of clonal hematopoietic stem cell diseases characterized by sustained proliferation of myeloid or multilineage myeloid cells. Among MPNs, polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are classified as BCR-ABL1-negative myeloproliferative neoplasms. Clinical manifestations include cytopenia of one or more lineages, thrombosis, myelofibrosis, varying degrees of extramedullary hematopoiesis and risk of leukemia conversion [1]. In recent years, with the advances of research in genetics and molecular biology, the pathogenesis, diagnosis, prognostic evaluation and treatment of such diseases have also progressed [2]. In the PMF patients, the international prognostic scoring system DIPSS-plus including cytogenetic indicators has been established, in which complex karyotype and karyotype abnormalities involving rearrangement of +8, −7/7q+, i(17q), −5/5q−, 12p+, inv(3) or 1q23 have been assigned to the poor prognosis group [3]. However, whether cytogenetic abnormalities should be included in...
In 172 patients, 135 cases JAK2-V617F negative MPNs were detected; the total mutation rate was 78.5%. The detection rate of JAK2-V617F gene mutation in PMF patients was 62.5% (15/24); 1 patient was negative for mutation, 1 patient had MPL gene mutation, 1 patient had CALR mutation, and 1 patient had EZH2 gene mutation; the overall mutation rate was 4.2%. The detection rate of JAK2-V617F gene mutation in PV patients was 91.3% (73/80). One of the 7 patients who was negative for mutations had a JAK2-12exon gene mutation with a mutation rate of 1.3%. The detection rate of the JAK2-V617F gene mutation in ET patients was 69.1% (47/68). Eight of the 21 patients with mutations had CALR mutation, and the mutation rate was 11.8% (8/68) (Table 2). The JAK2-V617F mutation rate in PV was significantly higher than that in PMF and ET (P < 0.01); the difference between the PMF and ET groups was not statistically significant (Table 3).

Concomitant disease and disease outcome
There were 27 cases of current or previous thrombosis in 172 patients, including 22 cases of cerebral infarction, 1 case of cerebral infarction with iliac vein thrombosis, 2 cases of myocardial infarction, 1 case of splenic vein thrombosis, and 1 case of pulmonary artery and calf vein thrombosis; the thrombosis rates of PMF, PV and ET were 8.3, 16.3, and 17.6%, respectively. Additionally, there were 7 cases of bleeding, including 3 cases of gingival bleeding, 3 cases of cerebral hemorrhage, and 1 case of gastrointestinal bleeding. No significant

**Table 1** The karyotypes of the 13 cases with abnormal chromosome

| Disease | Karyotypes |
|---------|------------|
| PMF     | 46,XY,del(11)(q21)[20] |
|         | 46,XY,-20,−mar[11] |
|         | 44−46,XY(t(8;17)(q13;q11)[CP3]/46,XY[7] |
|         | 46,XY(t(8;9)(p22;p22)[5] |
|         | der(15) |
|         | 46,XY(t(11;22)(q25;q11)add(18)p11.3)[20] |
| PV      | 46,XY,add(6)(q25),del(9)(q13)[8]/46,XY,del(6)(p23)[2]/46,XY[10] |
|         | 46,XY,del(20)[q13.1q13.3][10] |
|         | 46,XX,t(3:13)(q29q12)[13] |
|         | 46,XX,t(6;8)(p22;q21)[3] |
|         | 46,XY,del(5)(q14q23)[13] |
| ET      | 46,XY,add(15)(q26)[11]/46,XY[9] |
|         | 46,XX(dic(1:1))[q44p11][20] |

**Table 2** Distribution of driven gene mutations (n(%) )

| Disease | JAK2-V617F mutation | Non-JAK2-V617F mutation | JAK2-12exon | EZH2 |
|---------|---------------------|-------------------------|-------------|------|
| PMF     | 15(62.5)            | 1(4.2)                  | 1(4.2)      | 0    |
|         |                     |                         | 6(25)       |      |
| PV      | 73(91.3)            | 0                       | 0           | 1(1.3) |
|         |                     |                         | 6(7.5)      |      |
| ET      | 47(69.1)            | 8(11.8)                 | 0           | 0    |
|         |                     |                         | 13(19.1)    |      |
differences were detected with regards to thrombosis, bleeding events, leukemia, and progression of bone marrow fibrosis among the three groups. The level of spleen enlargement was higher than that in PV, which was greater than that in ET. The difference among these groups was statistically significant ($P < 0.001$). The level of LDH in PMF was greater than that in PV and ET ($P \leq 0.002$); the difference between PV and ET was not statistically significant. (Table 3).

**Comparison of the clinical parameters of MPN patients with mutant and wild-type JAK2-V617F**

The clinical parameters included gender, age, peripheral blood WBC count, Hb concentration, PLT count,
spleenomegaly, thrombotic events, LDH, coagulation function and chromosomal abnormalities. The results are shown in Table 4. Compared with patients with wild-type genes, more patients with PMFs were female ($P = 0.027$). PV patients carrying a JAK2 V617F mutation had higher peripheral blood WBC counts ($P = 0.006$) and PLT counts ($P = 0.001$) and were more prone to splenomegaly ($P < 0.05$). ET patients carrying a JAK2 V617F mutation had higher peripheral blood WBC counts ($P = 0.001$), higher Hb concentrations ($P = 0.001$), and lower PLT counts ($P = 0.037$) and were more prone to splenomegaly and abnormal endogenous coagulation ($P < 0.05$).

Comparison of the laboratory and clinical indicators of the thrombotic and nonthrombotic groups
Color Doppler Ultrasound or angiography were used to assess the clinical data patient to verify the thrombotic condition. Compared with the non-thrombotic group, the thrombotic group of PV and ET patients had higher age characteristics ($P < 0.05$). However, there were no statistically significant differences between the other observed clinical indicators or in the indicators between the two groups of PMF patients.

Discussion
MPN is a group of heterogeneous diseases originating from hematopoietic stem cells. It is also a collective term for a group of neoplastic diseases characterized by clonal proliferation of one or more lineages of relatively mature bone marrow cells. No reliable epidemiological investigation in China has been reported. In our hospital, the age of incidence of BCR-ABL1-negative MPNs is 18–78 years old. The incidence rate of middle-aged and elderly people is the highest, the median age of onset is 59 years old, and the ratio of male to female is 1.4:1.

The total incidence of abnormal MPN karyotypes in this study was 7.6%, including 25% for PMF, 6.3% for PV, and 2.9% for ET; the PMF karyotype abnormality was the highest. However, in foreign research reports, the proportion of PMF karyotype abnormalities is 33–55% [4–7]. However, the data at our center were roughly consistent with the incidence of PMF chromosomal abnormalities reported by other centers in China [8]. The most common of the karyotype abnormalities was trisomy 8 (28%), followed by the complex anomaly (19%) and 20q- (15%). Six chromosomal abnormalities were observed in 24 PMF patients in our hospital, and none of them showed +8. The abnormalities seen were −20, +mar, del(11q), der(15), add(18p) and three equilibrium translocations: t(8;17), t(8;9), and t(11;22). One of the patients died of pulmonary infection (karyotype 46, XY, −20, +mar[11]) 22 days after the initial diagnosis. Another patient converted to acute monocytic leukemia at the initial diagnosis at 53 months (karyotype 46,XY, del(11)(q21) [20]). Many foreign studies have shown [9, 10] that the incidence of karyotype abnormalities in the initial diagnosis of PV was 15 to 25%; common abnormalities included del(20q), +8, +9, in addition to dup(1q), del(13q), and del(5q). The rate of chromosomal abnormality in PV patients in this study was lower than that reported in the foreign literature, but it was consistent with Linmin Duan et al. reports [11]. The abnormalities included del(20q), del(5q), add(6q), del(6p), and del(9q) and two equilibrium translocations t(3;13) and t(6;8). One patient developed bone marrow fibrosis 72 months after diagnosis (karyotype 46,XY,del(20)(q13.1q13.3)[10]). The rate of chromosomal abnormality of ET patients was the lowest in this study, consistent with Lan HF et al. reports [12]. Only 1 case with add(15q) and dic(1;1) was abnormal. In summary, the rate of karyotype abnormality in patients with MPNs in this study is different from that in other reports, may be related to the genetic background of different ethnic groups. It is also possible that karyotype analysis consisted mostly of normal cells rather than tumor cells due to the susceptibility to blood thinning in patients with MPN.

In this study, the JAK2-V617F mutation had the highest detection rate in PV (91.3%), relative to ET (69.1%) and PMF (62.5%), which was consistent with the literature [13–16], followed by the CALR gene mutation. The mutation rates in PMF and ET were 4.2 and 11.8%, respectively, but they were not detected in PV. The detection rate reported by Tefferi [17] and Haslam [18] was significantly lower than that detected by Rotunno [19], which may be related to the small number of specimens, the error rate of the results, the sensitivity of the experimental methods, and the lack of screenings for three driver genes in some patients. The MPL gene and JAK2-12 exon mutations were rare: only one MPL mutation was found in PMF (4.2%), and one JAK2 -12 exon mutation (4.2%) was found in PV, which was consistent with foreign reports [20, 21]. Chromatin-modifying genes (ASXL1 and EZH2) were associated with transformed acute leukemia, and Delic et al. [22] used second-generation gene sequencing to determine that ASXL1 and EZH2 mutations were frequently mutated in PMF patients but rarely mutated in ET and PV patients. Thus it may be the reason that higher rate of transformation of AML in PMF patients than ET and PV patients. In this study, one case of EZH2 mutation was also detected in patients with PMF. This patient has not been discharged after multiple treatments but has not converted to leukemia.

Tefferi and others [23, 24] found that patients with JAK2-V617F mutation-positive PMF had higher WBC counts and lower Plt counts in peripheral blood, but in this study, the JAK2-V617F mutant in PMF showed only
gender differences compared with wild-type genes. This result may be related to the fact that the bone marrow of PMF patients often failed to be cultured due to a dry tap, resulting in a small quantity of specimens. Patients with PV with JAK2-V617F mutation in this study were associated with higher WBC and Plt counts and larger spleens, similar to HE Zhipeng et al. reports [25]. In contrast, foreign reports of JAK2-V617F mutations in PV patients were associated with higher Hb levels and lower Plt counts [21], which may be related to ethnic differences. ET with JAK2-V617F mutation in this study was associated with higher WBC and Hb and lower Plt, consistent with previous reports [23, 24]. It was also found to be associated with a larger spleen and was more susceptible to endogenous coagulopathy than in a negative patient.

Bleeding and thrombosis are common complications of MPN and have an important impact on morbidity and mortality. This study showed that the incidence of thrombosis in PMF, PV, and ET was 8.3, 16.3, and 17.6%, respectively, which was consistent with reports by Falanga [26]. Some scholars have analyzed the clinical features and prognosis of patients with PMF and found that patients with JAK2-V617F mutation-positive PMF have an increased incidence of thrombosis. Those with homozygous mutations were more likely to develop leukemia [27]. The study of PV patients found that the age of onset in the JAK2-V617F-positive group was higher than that in the negative group, which was clinically more prone to complications of myelofibrosis, hemorrhage and thrombosis [28]. De Grand et al. [29] found that JAK2-V617F mutations in PV patients could increase the risk of thrombosis by activating Lu/BCAM-mediated red blood cell adhesion aggregation. Lu et al. [30] also found that JAK2-dependent signaling pathways played an important role in platelet activation in PV patients and increase the risk of thrombosis. No significant differences in the thrombosis and hemorrhagic events were observed between the three groups of diseases and the mutant and wild-type JAK2-V617F in this study, which may be related to the number of cases or ethnic differences. In PV and ET patients, the thrombotic group was older than the nonthrombotic group. Older patients had slower blood flow, increased viscosity, arteriosclerosis, and severe damage to vascular endothelial cells. At the same time, the incidence of thrombotic diseases such as diabetes and hypertension was high, which may lead to thrombosis. Therefore, anticoagulation intervention in elderly patients can help reduce the occurrence of thrombosis. Dunlap, J. et al. [31] reported that the frequency of cytogenetic abnormality in the JAK2+ group was higher than that in the JAK2-group (23/45 (51%) vs 14/52 (27%), respectively). In this study, considering the chromosomal abnormalities of the three groups, no statistically significant difference was found in the rates in the JAK2-V617F mutation group and the wild-type group, which may be related to the small number of enrolled cases.

LDH can reflect the rate of cell replication and apoptosis. Many studies have found that high serum LDH is found in PMF and progressive MF (post-PV and post-ET) and is included in the secondary diagnostic criteria [32]. Through multivariate analysis studies, Mohamad Cherry et al. [33] found that age and high levels of LDH were negatively correlated with overall survival in patients with ET and PV. In this study, the elevated level of LDH in PMF was significantly greater than that in ET and PV, but the difference between PV and ET was not statistically significant. This also indicated that the tumor burden of PMF was higher than that of PV and ET, while there was no significant difference in the elevated levels of LDH between mutant and wild-type JAK2-V617F in the three groups.

In summary, MPN is a heterogeneous group of diseases. This study describes the cytogenetics, gene mutations, and clinical features of each disease. With the advancement of gene detection technology, different types of CALR mutations, MPL gene mutations and triple-negative mutations may have further implications.

Conclusion
We provided an important information of cytogenetics, gene mutation and clinical features of MPNs in our region. We found that PMF patients have more chromosomal abnormalities than PV and ET patients, and the types of driver mutations are closely related with the clinical features in BCR-ABL1-negative MPN patients.

Materials and methods
Study design
The study retrospectively analyzed 172 patients with BCR-ABL1-negative MPNs who were newly diagnosed in our hospital between June 2013 and June 2018. The patients’ diagnoses were established according to the 2016 WHO diagnostic criteria [2].

Observational index
Clinical data of the patients with PMF, PV and ET included age of onset, gender, white blood cell (WBC) count during blood routine examination on admission, hemoglobin (Hb) and platelet (PLT) counts, coagulation (PT, APTT, FIB, D-Di) and LDH, karyotype analysis and mutations such as JAK2-V617F, concomitant disease (splenomegaly, thrombosis) and disease outcome.

Bone marrow karyotype analysis
Five to six milliliters of bone marrow was collected from patients and anticoagulated with heparin. After counting,
the chromosomes were prepared by direct and short-term culture methods with \((1 \sim 2) \times 10^6/\text{mL}\), and karyotype analysis was performed by R Banding. Karyotype abnormalities were identified and described in accordance with the International System for Human Cytogenetics (ISCN, 2013) [34].

**Gene detection**

PCR or Sanger sequencing was used to detect mutations in the \(JAK2\), \(CALR\), and \(MPL\) genes. Patients who harbored no mutation in these three genes were further tested for the presence of \(TET2\), \(ASXL1\), \(EZH2\) and \(SRSF2\) mutations. A ratio of the copy number of the target gene to the copy number of the reference gene \(>1\) was considered a positive result [35].

A volume of 3 mL of the patient’s bone marrow anticoagulated with EDTA was subjected to DNA extraction (Sangon). The concentration and purity of the DNA product were measured by ultraviolet spectrophotometry (Thermo NanoDrop). The PCR primers were added to the PCR products were electrophorically isolated, purified, and subjected to bidirectional gene sequencing using an ABI 3730 sequencer. The sequencing results were compared with GenBank data to identify the type of gene mutation.

**Statistical analysis**

Statistical analyses were performed by using SPSS 17.0 statistical software. The mean values of the measured data were compared by analysis of variance (ANOVA) or the Kruskal-Wallis test. The count data were compared with the \(\chi^2\) test. Differences with \(P < 0.05\) were considered statistically significant.

**Abbreviations**

AML: Acute myeloid leukemia; \(ASXL1\): Additional sex combs like 1; \(CALR\): Calreticulin; \(DIPSS\)-plus: Dynamic International Prognostic Scoring System-plus; \(ET\): Essential thrombocythemia; \(EZH2\): Enhancer of zeste homolog 2; \(ISCN\): International System for Human Cytogenetics; \(JAK2\): Janus kinase 2; \(MPL\): Myeloproliferative leukemia virus; \(MPN\): Myeloproliferative neoplasms; \(PCR\): Polymerase chain reaction; \(PMF\): Primary myelofibrosis; \(PV\): Polycythemia vera; \(SRSF2\): Serine and arginine rich splicing factor 2; \(TET2\): Ten-eleven translocation oncogene family member 2

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**Authors’ contributions**

XLL and PC designed the study. XLL and HFH performed data acquisition. XLL carried out the majority of the data analysis work and drafted the manuscript. XLL and PC had full access to all study data and take responsibility for the integrity of the data, the accuracy of the data analysis, and interpretation of data. All authors were responsible for critical revisions, and all authors read and approved the final version of this work.

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**Availability of data and materials**

All data generated or analyzed during this study are included in this published article and its additional files.

**Ethics approval and consent to participate**

The study was approved by the Ethics Committee of Fujian Medical University Union Hospital, and bone marrow samples were collected after obtaining written informed consent from each patient.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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