Prevalence of JAK2 V617F, CALR, and MPL W515L Gene Mutations in Patients with Essential Thrombocythemia in Kurdistan Region of Iraq

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ABSTRACT

Essential thrombocythemia (ET) is a clonal bone marrow stem cell disorder, primarily involving the megakaryocytic lineage. The WHO 2016 guidelines include the molecular detection of JAK2, MPL, and CALR mutations as a major diagnostic criterion for ET. This study aimed to determine the frequency of JAK2 V617F, MPL W515L, and CALR mutations in Iraqi Kurdish patients affected with ET, and to analyze their clinical and hematological features. A total of 73 Iraqi Kurdish patients with ET were enrolled as subjects, and analysis was achieved utilizing real-time PCR. The frequency of JAK2 V617F, CALR, and MPL W515L mutations was determined to be 50.7%, 22%, and 16.4%, respectively. No statistically significant difference was obtained when considering the age and gender among different genotypes. The JAK2 V617F mutated patients had significantly higher white blood cell counts and hemoglobin levels than the CALR-positive patients (P-value=0.000, 0.007, respectively), MPL W515L-positive patients (P-value=0.000, 0.000, respectively), and triple negative patients (P-value=0.000, 0.000, respectively). Also, the JAK2 V617F mutated patients showed higher platelet count as compared to the MPL W515L-positive patients (P-value=0.02) and triple negative patients (P-value=0.04). Furthermore, significantly lower white blood cell count and hemoglobin levels were associated with CALR positivity (P-value=0.000, 0.01, respectively), MPL W515L-positivity (P-value=0.001, 0.000, respectively), and triple negativity (P-value=0.000, 0.000, respectively), as compared to patients with combined mutations. In conclusion, apart from a relatively high frequency of MPL W515L mutation, our data is comparable to earlier reports, and highlights the importance of genotyping the JAK2 V617F, MPL W515L, and CALR mutations for accurate diagnosis of patients with ET.

INTRODUCTION

Essential thrombocythemia (ET) is an acquired chronic myeloproliferative neoplasm (MPN) that basically involves the megakaryocytic lineage. It is characterized by constant elevation of peripheral blood platelet count and increased numbers of large, mature megakaryocytes in the bone marrow and clinically by the tendency to thrombosis or hemorrhage [1]. To diagnose patients with ET, other reactive causes of high platelet count must be excluded such as inflammatory or infectious diseases, hemorrhage, and other MPNs. However, a major insight into the genetic lesions for the enhanced myeloproliferation and clonal dominance that characterizes the Philadelphia-negative MPNs including polycythemia vera (PV), primary myelofibrosis (PMF), and ET has been reported [2]. Mutations that disrupt the protein tyrosine kinase signaling are found...
to be associated with these disorders [3]. The Janus Kinase 2 gene (JAK2) V617F mutation was first discovered in MPN patients in 2005 [4]. JAK2 is a cytoplasmic tyrosine kinase with a central role in signal transduction from multiple hemopoietic growth factor receptors [4]. JAK2 V617F is an acquired gain-of-function mutation in exon 14 of the gene, causing valine to phenylalanine substitutions at position 617 located in the JH2 pseudokinase domain, interrupts the domains autoinhibitory effect and results in constitutive tyrosine phosphorylation activity and enhances downstream JAK2-STAT signaling pathway leading to increase cellular proliferation [4]. This point mutation is frequently detected in the three clinically distinct MPN, being identified in ∼95% in PV, ∼50∼60% in ET, and ∼50∼60% in PMF [1, 3, 5]. Furthermore, 5∼10% of patients with ET carry a somatic activating mutation at exon 10, codon 515 of myeloproliferative leukemia (MPL) virus oncogene encoding the thrombopoietin receptor that is essential for megakaryogenesis, platelet production, and hematopoietic stem cell homeostasis [6-9]. The diagnosis of patients with findings suggestive of ET but negative for JAK2 and MPL mutations, usually based on the clinical exclusion of reactive thrombocytosis and morphological changes in megakaryocytes in the bone marrow. Histopathological assessment is inherently vulnerable to inter-observer variation, needs an experienced hematopathologist, and lacks standardization. Therefore, ET patients negative for JAK2 V617F and MPL mutations have been found to carry novel mutations of the calreticulin gene (CALR) [7]. CALR is a functionally complex Ca²⁺-binding protein and plays a substantial role in a variety of biological systems including calcium dynamic equilibrium regulation, cell proliferation, differentiation, and apoptosis [10]. The CALR mutations located in exon 9 are somatic insertions or deletions. There are two major variants: type 1 (L367fs*46), resulting from a 52-bp deletion, and type 2 (K385fs*47), from a 5-bp (TTGTC) insertion [11]. This mutation has been reported in 25∼30% of cases of ET [12]. The aim of this study was to determine the prevalence, and clinical correlations of these three driver mutations JAK2 V617F, MPL W515L, and CALR in Iraqi Kurdish patients with ET.

MATERIALS AND METHODS

1. Patients and samples

This cross-sectional study was conducted following approval by the local institutional ethical committee (approval No. 59/6.2019). Informed consent was obtained from all enrolled subjects. A total of 73 patients diagnosed with ET at Hiwa Hemato-Oncology Hospital, Sulaymaniyah Province, Kurdistan Region of Iraq were included in this study between June to November 2019. The patients were identified as ET based on the 2016 WHO diagnostic criteria. Briefly, the patients were diagnosed as ET when they had unexplained thrombocytosis, megakaryocytic proliferation, and did not meet WHO criteria for other MPNs. Bone marrow aspiration and biopsy was performed for all patients through posterior iliac spine and the aspirate smears were stained with Leishman stain (Merck/ Darmstadt, Germany). A Trephine biopsy specimen of 1.5 cm in length placed in 10% buffered neutral formalin and sent to histopathology laboratory. Following overnight fixation, biopsy specimens were slowly decalcified with ethylene diamine tetra acetate (EDTA). Three micrometer thick sections were cut from the paraffin wax blocks with the rotary microtomes and stained with H&E. Figure 1 through 4 show the characteristic megakaryocyte in patients with ET. Clinical and hematological information were collected by reviewing the medical records. Genomic DNA for the analysis of the three driver mutations was isolated from peripheral blood samples using a fully automated magnetic bead nucleic acid extraction system (Zinexts, Taiwan). The Zinexts DNA extraction kit was used for the separation of genomic DNA by using 400 μL of whole blood with a final 100 μL DNA elute according to the manufacturer’s instruction. The isolated DNA was stored at −20°C until the time of analysis by real-time PCR with
LineGene9600PLUS (Bioer, China). Five different fluorescence dyes attached to the DNA probe running in four different channels were used to detect wild type alleles, mutant type alleles for the three examined genes, and the internal control in each patient. For the JAK2 V617F mutation Hex-BHQ dye was used, for the MPL W515L mutation Cy5-BHQ was used, for the CALR mutations Texas Red-BHQ and FAM-BHQ were used, while for the internal control Cy5.5-BHQ was used. The extracted DNA samples were quality and quantity checked by the spectrophotometer (Eppendorf Bio Photometer, Germany).

2. Mutation analysis

Newly designed primers were used in the current study. All 73 patients were examined for JAK2 V617F, MPL W515L, and CALR [type 1 (52-bp deletion) and type 2 (5-bp insertion)] mutations. All genomic DNA was amplified in a 40-cycle PCR reaction at an annealing
temperature of 59°C, 58°C, and 60°C for JAK2 V617F, MPL W515L, and CALR mutations, respectively as shown in Table 1. The specific primers and probes used to detect the corresponding mutations are shown in Tables 2∼4. The internal control template, probe, and primer were designed on the Homo sapiens ribonuclease P/MRP subunit p30 (RPP30), transcript variant 2, mRNA as shown in Table 5. Amplification was carried out in a 20 μL final volume containing 25~50 ng of genomic DNA in 1× TaqMan universal PCR master mix, 1× internal control mix, and 0.2 μM of each corresponding forward primers, reverse primers and probes. Furthermore, samples from positive (obtained from Tehran Medical Genetic Laboratory, Iran) and negative controls for each driver mutation were included in the experiment.

### 3. Statistical analysis

The statistical package SPSS version 25 (IBM Corp., Armonk, NY, USA) was used to analyze the data: The continuous data are summarized as the means (SD) and ranges. The Chi-Square test was applied to compare the differences in the categorical data. An independent
t-test was used for differences in continuous data. $P$ values <0.05 were regarded as statistically significant.

## RESULTS

As demonstrated in Table 6, out of 73 patients with ET, 37 (50.7%) patients carried JAK2 V617F mutation, 10 (13.7%) had CALR type 1 and 2 mutations, while 7 (9.6%) patients had MPL W515L mutation. Nineteen (26%) of the patients showed negative results for the three driver mutations (triple negative). Further, 10 patients carried more than one driver mutation, five patients had combined JAK2 V617F+ CALR, four cases had combined JAK2 V617F + MPL W515L, and only one patient exhibit triple positivity for the three mutations. Age, gender, and main hematological features of the 73 patients with ET are shown in Table 7. The correlation of the patients’ main clinical and hematological characteristics with their mutational status are reported in Table 8. Concerning age and gender, in the current study, there was no statistically significant difference between different genotypes. Patients with mutated JAK2 were associated with higher white blood cell count and hemoglobin level compared to patients with mutated CALR, mutated MPL, and triple-negative patients. Further, patients with mutated CALR and patients with mutated MPL were associated with significantly lower white blood cell count and hemoglobin level compared to patients with combined mutations. Also, triple-negative patients showed lower

### Table 6. Frequency of the three driver mutations in 73 patients with ET

| Driver Mutations | No. | %  |
|------------------|-----|----|
| JAK2 V617F<sup>a</sup> | 37  | 50.7% |
| CALR type 1 & 2 | 10  | 13.7% |
| MPL W515L | 7  | 9.6% |
| Triple negative | 19  | 26.0% |
| Total | 73  | 100.0% |

<sup>a</sup>Including the combined cases: JAK2 V617F+ CALR Type 1 & 2= 5 cases, JAK2 V617F+ MPL W515L=4 cases, JAK2 V617F+ CALR Type 1 & 2+ MPL W515L=1 case.

### Table 7. Main clinical and hematological features of the 73 patients with ET

| Parameters                           | Value               |
|--------------------------------------|---------------------|
| Gender No. (%)                       |                     |
| Male                                 | 37 (60.7)           |
| Female                               | 36 (49.3)           |
| Age (years) Mean (SD)                | 55 (17)             |
| Range                                | 12~89               |
| Leukocytes (×10<sup>9</sup>/L) Mean (SD) | 9.5 (3.2)         |
| Range                                | 4.0~15.3            |
| Hemoglobin (g/dL) Mean (SD)          | 13.2 (1.3)          |
| Range                                | 11.0~15.6           |
| Platelets (×10<sup>9</sup>/L) Mean (SD) | 949 (387)         |
| Range                                | 443~1900            |

### Table 8. Correlation of the three mutational status with the main clinical and hematological characteristics in 73 Iraqi Kurdish patients with ET

| Characteristics | JAK2 | CALR | MPL | Com. Mut | Trip-ve | $P$ 1 v 2 | $P$ 1 v 3 | $P$ 1 v 4 | $P$ 1 v 5 | $P$ 2 v 3 | $P$ 2 v 4 | $P$ 2 v 5 | $P$ 3 v 4 | $P$ 3 v 5 | $P$ 4 v 5 |
|-----------------|-----|------|-----|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| No. of Pt       | 27  | 10   | 7   | 10       | 19      | 0.32    | 0.27    | 0.31    | 0.09    | 1.00    | 0.09    | 0.09    | 0.67    | 0.08    |
| Sex M/F         | 14/13 | 7/3  | 2/5 | 7/3      | 7/12    | 0.09    | 0.76    | 0.63    | 0.15    | 0.39    | 0.72    | 0.06    | 0.51    | 0.51    | 0.09    |
| Age, years (range) | (27~89) | (36~80) | (12~70) | (40~72) | (18~79) | 0.000  | 0.000  | 0.99    | 0.000  | 0.04    | 0.08    | 0.001  | 0.39    | 0.000  |
| WBC, ×10<sup>9</sup>/L (range) | (8.0~15.3) | (4.0~10.0) | (5.0~10.0) | (7.0~15.0) | (5.0~11.2) | 0.007  | 0.000  | 0.000  | 0.75    | 0.000  | 0.09    | 0.01    | 0.13    | 0.000  | 0.28    | 0.000  |
| Hb, g/dL (range) | (12.0~15.6) | (11.0~15.0) | (11.0~13.0) | (13.0~15.0) | (11.0~14.0) | 0.11   | 0.02   | 0.81    | 0.04    | 0.23    | 0.22    | 0.86    | 0.06    | 0.40    | 0.19    |
| Platelets, ×10<sup>9</sup>/L (range) | (600~1820) | (550~1271) | (464~1357) | (600~1900) | (443~1723) | 0.11   | 0.02   | 0.81    | 0.04    | 0.23    | 0.22    | 0.86    | 0.06    | 0.40    | 0.19    |

Abbreviations: Pt, Patients; Com Mut, combined mutations; Trip-ve, triple-negative; v, versus.
white blood cell count and hemoglobin levels in comparison to patients with combined mutations. Concerning the platelet count, apart from patients with mutated JAK2 that were associated with higher platelet count as to patients with mutated MPL and triple-negative patients, there was no statistically significant difference between the other genotypes.

**DISCUSSION**

This is the first study to characterize the genotype profile of Iraqi Kurdish patients with ET. Using real-time PCR with specific primers and probes for the three driver mutations, we found that 54/73 (74%) of the ET patients carried JAK2 V617F, CALR, or MPL W515L mutations, highlighting the value of combined genetic testing for the diagnosis of ET patients. The JAK2 V617F was the most common mutation, being detected as a single driver mutation or in combination with the other two mutations in 37/73 patients (50.7%). Although CALR and MPL W515L mutations were detected (as single mutation) in only 10/73 (13.7%) and (7/73) 9.6%, respectively, taking into consideration the double mutational positivity and the triple mutational positivity, the CALR and MPL W515L mutations were identified in 16/73 (22%) and 12/73 (16.4%), respectively. The frequency of JAK2 V617F mutation in our study is in agreement with previous reports from China (57.79%) [13], (56.3) [14], Korea (57.1%) [15], and Italy (57%) [5]. While other studies reported a higher frequency such as studies from Argentine (61.2%) [16], India (92.3%) [17], and Korea (63.3%) [8]. Also, some researchers observed a lower frequency of JAK2 V617F mutation (31%) [18] and (35.7%) [19]. In the current series, the frequency of CALR (22%) is consistent with earlier reports [15, 20-24] but, higher than previous reports from Korea (17.7 %) [8], and Italy (14%) [25]. In concern to MPL W515L mutation, our observation (16.4%) is higher than earlier records that ranged from 1% to 14% [18, 25–30]. This variation in the frequency of the driver mutation could be related to different characteristics of the studies cases including sample size, disparate sensitivities of the methods used, and ethnicity-based diversity in the genetic backgrounds.

In the current study, no statistically significant difference in age and gender was observed among different genotypes. This finding is in agreement with earlier reports [4, 5, 7, 8, 10, 16, 20]. Similar to previous reports, patients with mutated JAK2 V617F showed significantly higher hemoglobin levels, white blood cell count, and platelet count compared to patients with other driver mutations and triple negative patients [6–8, 10, 16, 20]. Further, patients with mutated CALR, patients with mutated MPL W515L, and triple negative patients had lower hemoglobin levels and white blood cell count compared to patients with combined mutations comparable to earlier records [7, 16].

In conclusion, our data reveal that the mutation profile in ET patients in our locality is in line with earlier reports. Further our findings demonstrate similarities with these records in concern to hematological features of patients with ET. However, we notice a rather higher frequency of MPL W515L mutation in our cohort. Lastly, our study accentuates the influence of JAK2 V617F, MPL W515L, and CALR genotyping for a precise diagnosis of ET patients because, in certain cases, the determination of disease-specific mutations may justify the use of mutation analysis for concluding the diagnosis, prognosis, and assessment of response to treatment.

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REFERENCES

1. Lee KK, Cho H, Chi H, Kim DY, Chae SL, Huh HJ. A Case of post-essential thrombocytopenia myelofibrosis with severe osteoarthritis. Korean J Lab Med. 2010;30:122-125. https://doi.org/10.3343/kjlm.2010.30.2.122

2. Kausansky K. On the molecular origins of the chronic myeloproliferative disorders: it all makes sense. Blood. 2005;105:4187-4190. https://doi.org/10.1182/blood-2005-03-1287

3. Ma W, Kantarjian H, Zhang X, Yeh C, Zhang Z, Verstoskis S, et al. Mutation profile of JAK2 transcripts in patients with chronic myeloproliferative neoplasias. J Mol Diagn. 2009;11:49-53. https://doi.org/10.2353/jmoldx.2009.080114

4. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton C. JAK2, MPL, and CALR mutations in Chinese Han patients with essential thrombocytopenia. Hematology. 2017;22:145-148. https://doi.org/10.1080/20553253.2016.1252003

5. Kim SY, Im K, Park SN, Kwon J, Kim J, Lee DS. CALR, JAK2, and MPL mutations in Korean patients with Philadelphia chromosome-negative myeloproliferative neoplasms. Korean J Clin Lab Sci. 2017;53, No. 1, March 2021: 47

6. Wang J, Zhang B, Chen B, Zhou R, Zhang Q, Li J, et al. JAK2, MPL, and CALR mutations in Chinese Han patients with essential thrombocytopenia. Hematology. 2017;22:145-148. https://doi.org/10.1080/20553253.2016.1252003

7. Kim BH, Cho YU, Bae MH, Jang S, Seo EJ, Chi HS, et al. JAK2 mutation analysis between JAK2, MPL, and CALR mutations in patients with myeloproliferative neoplasms primary myelofibrosis, essential thrombocythemia, polycythemia vera, and myeloproliferative neoplasm, unclassifiable. Am J Clin Pathol. 2015;143:635-644. https://doi.org/10.1093/ajcp/aqv078

8. Baehr HU. JAK2: An emergent and very flexible kinase. Oncogene. 2007;26:5011-5021. https://doi.org/10.1038/sj.onc.1210697

9. Lin Y, Liu E, Sun Q, Ma J, Li Q, Cao Z, et al. The prevalence of JAK2, MPL and CALR mutations in Chinese patients with BCR-ABL1-negative myeloproliferative neoplasms. Am J Clin Pathol. 2015;144:161-171. https://doi.org/10.1369/ajcp.2015.07.004

10. Lang T, Nie Y, Wang Z, Huang Q, An L, Wang Y, et al. Correlation analysis between JAK2, MPL and CALR mutations in patients with myeloproliferative neoplasms of Chinese Uygur and Han nationality and their clinical characteristics. J Int Med Res. 2018;46:4650-4659. https://doi.org/10.1177/0300060517787719

11. Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013;369:2379-2390. https://doi.org/10.1056/NEJMoa1311347

12. Vainschenker W, Constantinescu SN. Plt 1. Recent advances in understanding myelofibrosis and essential thrombocytopenia. F1000 Research. 2016;5:1-13. https://doi.org/10.12688/f1000research.8081.1

13. Ji L, Qian M, Wu N, Wu J. Significance of combined detection of JAK2V617F, MPL and CALR gene mutations in patients with essential thrombocytopenia. Exp Ther Med. 2017;13:947-951. https://doi.org/10.3892/etm.2017.4077

14. Wu Z, Zhang X, Xu X, Chen Y, Hu T, Kang Z, et al. The mutation profile of JAK2 and CALR in Chinese Han patients with Philadelphia chromosome-negative myeloproliferative neoplasms. J Hematol Oncol. 2014;7:1-10.

15. Kim HR, Choi HJ, Kim YK, Kim HJ, Shin JH, Suh SP, et al. Allelic expression imbalance of JAK2 V617F mutation in BCR-ABL−negative myeloproliferative neoplasms. PLoS ONE. 2013;8:e52518. https://doi.org/10.1371/journal.pone.0052518

16. Oteda M, Bragou JM, Calvo KL, Williams GM, Carbonell MM, Pratti AF, CALR, JAK2 and MPL mutation status in argentinean patients with BCR-ABL1−negative myeloproliferative neoplasms. Hematology. 2018;23:208-211. https://doi.org/10.1080/10553321.2017.1385981

17. Syeed N, JAK2 and Beyond: JAK2V617F mutational study of myeloproliferative disorders and haematological malignancies. Asian Pac J Cancer Prev. 2015;24:107-3615. https://doi.org/10.1355/JPACVP.2015.19.12.3611

18. Jaradat SA, Khasawneh R, Kamal N, Matalka I, Al-Blushawi M, Al-Sweidan S, et al. Analysis of JAK2V617F mutation in Jordanian patients with myeloproliferative neoplasms. Hematol Oncol Stem Cell Ther. 2015;8:161-166. https://doi.org/10.1016/j.hemonc.2015.07.004

19. Nancy LM, Samantha GB, Javier GE, Perla CP, Valeria GO, Virginia RN, et al. The mutation profile of JAK2, MPL and CALR in Mexican patients with Philadelphia chromosome-negative myeloproliferative neoplasms. Hematol Oncol Stem Cell Ther. 2015;8:161-21. https://doi.org/10.1016/j.hemonc.2014.12.002

20. Lu MY, Chao HY, Sun AN, Qiu HY, Hien ZM, Tan XW, et al. Clinical significance of JAK2, CALR, and MPL gene mutations in 1648 Philadelphia chromosome-negative myeloproliferative neoplasms patients from a single center. Chinese Journal of Hematology. 2017;38:295-300. https://doi.org/10.3760/cma.j.issn.0253-2727.2017.04.007

21. Misawa K, Yasuda H, Araki M, Ochiai T, Morishita S, Shirane S, et al. Clinical hematological relevance of JAK2V617F, CALR, and MPL mutations in Vietnamese patients with essential thrombocytopenia. Asian Pac J Cancer Prev. 2019;20:2775-2780. https://doi.org/10.7314/APJCP.2019.20.9.2775

22. Li MY, Chao HY, Sun AN, Qiu HY, Hien ZM, Tan XW, et al. Clinical significance of the JAK2 V617F mutational study of myeloproliferative disorders and haematological malignancies. J Int Med Res. 2017;45:150-156. https://doi.org/10.1177/0300060517787719

23. Misawa K, Yasuda H, Araki M, Ochiai T, Morishita S, Shirane S, et al. Clinical hematological relevance of JAK2V617F, CALR, and MPL mutations in Vietnamese patients with essential thrombocytopenia. Asian Pac J Cancer Prev. 2019;20:2775-2780. https://doi.org/10.7314/APJCP.2019.20.9.2775

24. Gedlik A, Arengo G, Prati AF, CALR, JAK2 and MPL mutation status in argentinean patients with BCR-ABL1−negative myeloproliferative neoplasms: an immunohistochemical study. Clin Lymphoma Myeloma Leuk. 2015;15:785-789. https://doi.org/10.1016/j.clml.2015.08.084

25. Gardner JA, Peterson JD, Turner SA, Soares BL, Lancer CR, Dos Santos LL, et al. Detection of CALR mutation in clonal and non clonal hematologic disorders using fragment analysis and next-generation sequencing. Am J Clin Pathol. 2016;146:448-455. https://doi.org/10.1093/ajcp/aqw129

26. Lussana F, Carobbio A, Salmogiraghi S, Giglielmelli P, Vannucchi AM, Bottazzi B, et al. Driver mutations (JAK2V617F, MPLW515L/K or CALR), pentraxin-3 and C-reactive protein in essential thrombocytopenia and polycythemia vera. J Hematol Oncol. 2017;10.3343/kjcls.org
26. Xu W, Li JY, Xia J, Zhang SJ, Fan L, Qiao C. MPL W515L mutation in Chinese patients with myeloproliferative diseases. Leuk Lymphoma. 2008;49:955-958. https://doi.org/10.1080/10428190802035966

27. Chen X, Qi X, Tan Y, Xu Z, Xu A, Zhang L, et al. Detection of MPL exon10 mutations in 103 Chinese patients with JAK2V617F-negative myeloproliferative neoplasms. Blood Cells Mol Dis. 2011;47:67-71. https://doi.org/10.1016/j.bcmd.2011.04.004

28. Toyama K, Karasawa M, Yokohama A, Mitsui T, Uchitani H, Saitoh T, et al. Differences in the JAK2 and MPL mutation status in the cell lineages of the bcr/abl-negative chronic myeloproliferative neoplasm subtypes. Intern Med. 2011;50:2557-2561. https://doi.org/10.2169/internalmedicine.50.5429

29. Small W, Doubaj Y, Laarabi FZ, Ilahyai J, Kerbout M, Mikdame M, et al. CALR gene mutational profile in myeloproliferative neoplasms with nonmutated JAK2 in Moroccan patients: a case series and germline in-frame deletion. Curr Res Transl Med. 2017;65:15-29. https://doi.org/10.1016/j.retram.2016.08.002

30. Schnittger S, Bacher U, Eder C, Dicker F, Alpermann T, Grossmann V, et al. Molecular analyses of 15,542 patients with suspected BCR-ABL1-negative myeloproliferative disorders allow to develop a stepwise diagnostic workflow. Haematologica. 2012;97:1582-1585.