**Name of Journal:** *World Journal of Clinical Cases*

**Manuscript NO:** 55851

**Manuscript Type:** CASE REPORT

**Primary myelofibrosis with concurrent *CALR* and *MPL* mutations: A case report**

Zhou FP *et al*. Co-mutation of *CALR* and *MPL* genes

Feng-Ping Zhou, Cheng-Cheng Wang, Hua-Ping Du, Shan-Bo Cao, Jin Zhang

**Feng-Ping Zhou, Hua-Ping Du, Jin Zhang,** Department of Hematology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou 310016, Zhejiang Province, China

**Cheng-Cheng Wang, Shan-Bo Cao,** Acornmed Biotechnology Co., Ltd., Beijing 100176, China

**Author contributions:** All authors contributed to the study conception and design; Zhou FP and Wang CC prepared the material and collected and analyzed the data; Du HP diagnosed and treated the patient; Cao SB analyzed the NGS data; Zhang J revised the manuscript for important intellectual content; all authors approved the final version of the manuscript.

**Corresponding author: Jin Zhang, MD, Attending Doctor,** Department of Hematology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, No. 3 East Qingchun Road, Hangzhou 310016, Zhejiang Province, China. zfpingzd@zju.edu.cn

**Received:** April 5, 2020

**Revised:** October 6, 2020

**Accepted:** October 19, 2020

**Published online:**

**Abstract**

BACKGROUND

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm (MPN) characterized by recurrent mutations in the *JAK2*, *CALR*, and *MPL* genes. The *CALR* and *MPL* co-mutation is very rare. To our knowledge, no more than five cases have been reported. Here, we report a case of PMF in which a *CALR* and *MPL* co-mutation was detected by next-generation sequencing (NGS) technology, and a literature review was performed.

CASE SUMMARY

A 73-year-old woman was admitted to our hospital in 2018 due to abdominal distension. The patient had splenomegaly, lymphadenopathy, leukopenia, anemia, and immature granulocytes in peripheral blood. There were dacrocytes and atypical megakaryocytes in bone marrow, and megakaryocytic proliferation was very active, accompanied by reticulin fibrosis grade 2. By NGS analysis of the bone marrow sample, we detected mutations in *CALR*, *MPL,* and *PIK3RI*, while *JAK2* *V617F* and *BCR-ABL* were negative. Therefore, the patient was diagnosed with PMF and received oral ruxolitinib. However, the spleen and hematologic responses were poor. We review the literature, analyze previous reports of the mutation sites in our patient and differences between our patient and other reported cases of co-mutated *CALR* and *MPL* genes, and discuss the reason why the *CALR* and *MPL* co-mutations are rare and possible mechanisms and their impact on the prognosis of patients.

CONCLUSION

*CALR* and *MPL* mutations can be concurrent in MPN, but they are rare. The use of NGS may help to identify more patients with co-mutated *CALR* and *MPL* genes. This will help to further explore the mechanism and its impact on these patients to develop appropriate treatment strategies.

**Key Words:** Primary myelofibrosis; *CALR*; *MPL*; Co-mutation; Next-generation sequencing; Case report

Zhou FP, Wang CC, Du HP, Cao SB, Zhang J. Primary myelofibrosis with concurrent *CALR* and *MPL* mutations: A case report. *World J Clin Cases* 2020; In press

**Core Tip:** We report a rare case of primary myelofibrosis in which a *CALR* and *MPL* co-mutation was detected by next-generation sequencing technology. It demonstrated that *CALR* and *MPL* mutations can be concurrent in myeloproliferative neoplasm. We review the literature, analyze previous reports of the mutation sites in our patient and differences between our patient and other reported cases of co-mutated *CALR* and *MPL* genes, and discuss the reason why the *CALR* and *MPL* co-mutations are rare and possible mechanisms and their impact on the prognosis of patients.

**INTRODUCTION**

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm (MPN) characterized by recurrent mutations in the *JAK2*, *CALR*, and *MPL* genes. Although these mutations were initially reported to be mutually exclusive in MPN, several studies have reported the presence of *JAK2-CALR* or *JAK2-MPL* co-mutations in PMF patients[1-4], and *CALR* and *MPL* co-mutation cases are still very rarely reported. With the increasingly wide application of next-generation sequencing (NGS) technology, rare myelofibrosis cases with co-mutated *CALR* and *MPL* genes are increasingly being detected. Here, we report a patient with PMF, in which co-mutated *CALR* and *MPL* genes were detected.

**CASE PRESENTATION**

***Chief complaints***

Abdominal distension for 2 mo.

***History of present illness***

A 73-year-old woman was admitted to Sir Run Run Shaw Hospital, Zhejiang University School of Medicine in 2018 due to abdominal distension for 2 mo, especially after eating with no complaints of abdominal pain, diarrhea, vomiting, or reduced anal exhaust and defecation.

***History of past illness***

The patient had a history of a left kidney cyst.

***Personal and family history***

She denied any other specific personal or family history of other diseases.

***Physical examination***

The patient’s conjunctiva and skin were pale, and the sclera was not yellow. Slightly swollen lymph nodes could be felt in the neck and armpit. The abdomen looked a little distended and felt soft, with no tenderness; the spleen under the ribs could be felt, and the texture was slightly hard. The rest of the abdomen did not have any obvious masses. The bowels sounded normal.

***Laboratory examinations***

Peripheral blood tests demonstrated a leukocyte count of 2.9 × 109/L, platelet count of 126 × 109/L, and hemoglobin of 87 g/L, and a smear revealed immature granulocytes of 4%. Liver function tests showed normal levels of transaminases, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, direct bilirubin, indirect bilirubin, serum creatinine, and potassium. Lactate dehydrogenase was 570 IU/L, and total cholesterol was 2.6 mmol/L.

***Imaging examinations***

An abdominal computed tomography scan showed that the spleen was enlarged and uniform (Figure 1), with a left kidney cyst. Abdominal ultrasound showed that the spleen was enlarged, with a thickness of 5.6 cm and a length of 16.1 cm.

***Pathological examination***

Bone marrow aspiration revealed erythroid and megakaryocytic hyperplasia; dacrocytes were easily observed, and megakaryocytes had atypia (Figure 2). Bone marrow biopsy showed megakaryocytic proliferation and atypia, accompanied by reticulin fibrosis grade 2 (Figure 3).

***Examination for gene mutations***

The bone marrow samples were sequenced by NGS, which showed mutations in *CALR*, *MPL,* and *PIK3RI* (Table 1), while *JAK2* *V617F* and *BCR-ABL* were negative.

**FINAL DIAGNOSIS**

The patient was ultimately diagnosed with PMF (International Prognostic Scoring System score = 3, high risk).

**TREATMENT**

The patient received oral ruxolitinib 15 mg twice per day.

**OUTCOME AND FOLLOW-UP**

Half a year later, the spleen response was poor, and the size of the spleen increased from 16.1 × 5.6 cm to 17.7 × 6.6 cm. Anemia was progressively aggravated in the meantime and developed to blood transfusion dependence, indicating that PMF with co-mutated *MPL* and *CALR* genes cannot benefit from ruxolitinib.

**DISCUSSION**

Most PMF patients carry *JAK2*, *MPL,* or *CALR* driver mutations[5]. Mutations in *MPL* and *CALR* are drivers in the pathogenesis of MPNs, and mutant *CALR* can bind with *MPL* to activate the JAK-STAT signaling pathway. Previous studies have shown that *JAK2*, *MPL,* and *CALR* are mutually exclusively mutated in MPN patients[6,7]. However, studies have also reported the presence of *JAK2-CALR* or *JAK2-MPL* co-mutations in PMF patients[1-4], and cases of *CALR* and *MPL* co-mutations are still very rarely reported. Here, we report the case of a PMF patient with *CALR*-p.364fsand *MPL*-p.X636W mutations. The *CALR*-p.364fsmutation is usually detected in essential thrombocytosis (ET) and PMF patients, while the *MPL*-p.X636Wmutation is not a common mutation and has only been reported in refractory anemia with ring sideroblast patients[8,9]. Furthermore, we also detected a low-burden *PIK3R1* mutation in this case. The *PIK3R1* gene belongs to the Akt signaling pathway and plays a direct role in regulating cell survival, growth, and differentiation. Mutated *PIK3R1* can cause aberrant PI3K signaling, and the p.R228fs mutation site has already been reported in acute lymphocytic leukemia patients[10,11]. To the best of our knowledge, this is the first report of a *CALR* and *MPL* double mutant PMF patient from East Asia.

Earlier research reports show that *CALR* and *MPL* mutations are mutually exclusive in MPN patients. This case demonstrated that *CALR* and *MPL* mutations can coexist in MPN patients. Three MPN cases with co-mutated *CALR* and *MPL* genes have already been reported since 2017[12-14]. Partouche *et al*[12] reported that a post-ET PMF patient carried type I *CALR* and *MPL* *W515R* mutations, with an variant allele frequency (VAF) of 59.1% and 31.7%, respectively. Tashkandi *et al*[13] reported a post-ET PMF patient carrying a *CALR* (p.L367Tfs\*46) mutation at an allele frequency of 47% and two *MPL* (p.S505C and p.W515L) mutations at a lower allele frequency of 4%. Bernal *et al*[14] detected a *CALR* (p.L367fs\*48) mutation at an allele frequency of 30%and a *MPL* (p.Trip515L) mutation at an allele frequency of 11.75% in an ET patient. In contrast to these reported cases, we identified an uncommon mutation in *MPL* (p.X636W) in PMF patients, and its VAF was higher than that of the *CALR* mutation in our patient.

Patients with concurrent *CALR* and *MPL* mutations are truly rare in the clinic. In PMF patients, the frequency of *CALR* (20%-25%) and *MPL* (7%) gene mutations is low[1,6,7]. Before 2017, there were no case reports about *CALR* and *MPL* co-mutations. Currently, a few MPN patients have been detected, which is inseparable from the progress of molecular testing technology. From previous cases, we can see that in MPN patients, a *MPL* mutation VAF is usually low[2,3,15], and some uncommon mutations exist. Traditional Sanger sequencing may miss these mutations because of its test range and sensitivity[7,13-15]. High-throughput sequencing may cover the full loci, and the detection VAF limit was lowered to 1%. Therefore, MPN patients with concurrent *CALR* and *MPL* mutations can be accurately detected.

The *MPL* mutation may have been a molecular event independent of the *CALR* deletion or a secondary event in the *CALR*-containing clone. Some researchers have proven that the *CALR* gene mutation is an early event during disease development, and *MPL* is a passenger event[2,13]. It is speculated that the mutated *CALR* gene can trigger genome instability, which then causes mutations in the *MPL* gene[13]. This is a possible explanation for why *CALR* and *MPL* can co-mutate. However, in our case, the *MPL* mutation was improbable as a secondary event because its VAF was more than 50%, indicating the possibility of a germline mutation. Some research has already proven that MPN patients can carry germline MPN mutations[16-18]. The co-mutation mechanism is not clear and still needs more investigations.

Gene mutations can predict prognosis in PMF patients. *CALR* mutations indicate a good prognosis in ET and PMF patients[19,20]. Compared to *JAK2* or triple-negative patients, *CALR* mutation-positive patients have a longer overall survival and low risk of thrombogenesis[1,7,19,21]. Patients who harbor the *MPL* *W515L/K* mutation have a moderate prognosis and higher risk of [thrombogenesis](file:///C:\Users\acorndx_1005\AppData\Local\youdao\dict\Application\7.5.0.0\resultui\dict\?keyword=thrombogenesis) than *CALR* mutation-positive patients[19]. The prognosis of patients with *MPL* and *CALR* co-mutations is still unknown due to the limited number of patients, but it seems that these patients have more aggressive progression based on our patient and other reported cases[12,13]. Our patient’s disease progression was rapid. In Partouche’s and Tashkandi’s patients, the *CALR* mutation was a pre-existing clone in the ET stage, and the *MPL* mutation was acquired when the disease developed into PMF. Disease acceleration may be associated with *MPL*-mutated clones. Because of the lack of patients with coexisting mutations, further investigations in large cohorts are needed to better quantify the presence and clinical significance.

**CONCLUSION**

In conclusion, we have described a rare case of PMF with concurrent *CALR* and *MPL* mutations. This demonstrates that *CALR* and *MPL* mutations are not mutually exclusive in MPN. The use of NGS may help to identify more patients with *CALR* and *MPL* co-mutations. This will help to further explore the mechanism and its impact on these patients to develop appropriate treatment strategies.

**REFERENCES**

1 **Tefferi A**, Lasho TL, Finke CM, Knudson RA, Ketterling R, Hanson CH, Maffioli M, Caramazza D, Passamonti F, Pardanani A. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. *Leukemia* 2014; **28**: 1472-1477 [PMID: 24402162 DOI: 10.1038/leu.2014.3]

2 **Lundberg P**, Karow A, Nienhold R, Looser R, Hao-Shen H, Nissen I, Girsberger S, Lehmann T, Passweg J, Stern M, Beisel C, Kralovics R, Skoda RC. Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. *Blood* 2014; **123**: 2220-2228 [PMID: 24478400 DOI: 10.1182/blood-2013-11-537167]

3 **McGaffin G**, Harper K, Stirling D, McLintock L. JAK2 V617F and CALR mutations are not mutually exclusive; findings from retrospective analysis of a small patient cohort. *Br J Haematol* 2014; **167**: 276-278 [PMID: 24935260 DOI: 10.1111/bjh.12969]

4 **Pardanani A**, Guglielmelli P, Lasho TL, Pancrazzi A, Finke CM, Vannucchi AM, Tefferi A. Primary myelofibrosis with or without mutant MPL: comparison of survival and clinical features involving 603 patients. *Leukemia* 2011; **25**: 1834-1839 [PMID: 21691276 DOI: 10.1038/leu.2011.161]

5 **Takenaka K**, Shimoda K, Akashi K. Recent advances in the diagnosis and management of primary myelofibrosis. *Korean J Intern Med* 2018; **33**: 679-690 [PMID: 29665657 DOI: 10.3904/kjim.2018.033]

6 **Klampfl T**, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD, Them NC, Berg T, Gisslinger B, Pietra D, Chen D, Vladimer GI, Bagienski K, Milanesi C, Casetti IC, Sant'Antonio E, Ferretti V, Elena C, Schischlik F, Cleary C, Six M, Schalling M, Schönegger A, Bock C, Malcovati L, Pascutto C, Superti-Furga G, Cazzola M, Kralovics R. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med* 2013; **369**: 2379-2390 [PMID: 24325356 DOI: 10.1056/NEJMoa1311347]

7 **Nangalia J**, Massie CE, Baxter EJ, Nice FL, Gundem G, Wedge DC, Avezov E, Li J, Kollmann K, Kent DG, Aziz A, Godfrey AL, Hinton J, Martincorena I, Van Loo P, Jones AV, Guglielmelli P, Tarpey P, Harding HP, Fitzpatrick JD, Goudie CT, Ortmann CA, Loughran SJ, Raine K, Jones DR, Butler AP, Teague JW, O'Meara S, McLaren S, Bianchi M, Silber Y, Dimitropoulou D, Bloxham D, Mudie L, Maddison M, Robinson B, Keohane C, Maclean C, Hill K, Orchard K, Tauro S, Du MQ, Greaves M, Bowen D, Huntly BJP, Harrison CN, Cross NCP, Ron D, Vannucchi AM, Papaemmanuil E, Campbell PJ, Green AR. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *N Engl J Med* 2013; **369**: 2391-2405 [PMID: 24325359 DOI: 10.1056/NEJMoa1312542]

8 **Pardanani AD**, Levine RL, Lasho T, Pikman Y, Mesa RA, Wadleigh M, Steensma DP, Elliott MA, Wolanskyj AP, Hogan WJ, McClure RF, Litzow MR, Gilliland DG, Tefferi A. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood* 2006; **108**: 3472-3476 [PMID: 16868251 DOI: 10.1182/blood-2006-04-018879]

9 **Beer PA**, Campbell PJ, Scott LM, Bench AJ, Erber WN, Bareford D, Wilkins BS, Reilly JT, Hasselbalch HC, Bowman R, Wheatley K, Buck G, Harrison CN, Green AR. MPL mutations in myeloproliferative disorders: analysis of the PT-1 cohort. *Blood* 2008; **112**: 141-149 [PMID: 18451306 DOI: 10.1182/blood-2008-01-131664]

10 **Gutierrez A**, Sanda T, Grebliunaite R, Carracedo A, Salmena L, Ahn Y, Dahlberg S, Neuberg D, Moreau LA, Winter SS, Larson R, Zhang J, Protopopov A, Chin L, Pandolfi PP, Silverman LB, Hunger SP, Sallan SE, Look AT. High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia. *Blood* 2009; **114**: 647-650 [PMID: 19458356 DOI: 10.1182/blood-2009-02-206722]

11 **Andersson AK**, Ma J, Wang J, Chen X, Gedman AL, Dang J, Nakitandwe J, Holmfeldt L, Parker M, Easton J, Huether R, Kriwacki R, Rusch M, Wu G, Li Y, Mulder H, Raimondi S, Pounds S, Kang G, Shi L, Becksfort J, Gupta P, Payne-Turner D, Vadodaria B, Boggs K, Yergeau D, Manne J, Song G, Edmonson M, Nagahawatte P, Wei L, Cheng C, Pei D, Sutton R, Venn NC, Chetcuti A, Rush A, Catchpoole D, Heldrup J, Fioretos T, Lu C, Ding L, Pui CH, Shurtleff S, Mullighan CG, Mardis ER, Wilson RK, Gruber TA, Zhang J, Downing JR; St. Jude Children's Research Hospital–Washington University Pediatric Cancer Genome Project. The landscape of somatic mutations in infant MLL-rearranged acute lymphoblastic leukemias. *Nat Genet* 2015; **47**: 330-337 [PMID: 25730765 DOI: 10.1038/ng.3230]

12 **Partouche N**, Conejero C, Barathon Q, Moroch J, Tulliez M, Cordonnier C, Giraudier S. Emergence of MPLW515 mutation in a patient with CALR deletion: Evidence of secondary acquisition of MPL mutation in the CALR clone. *Hematol Oncol* 2018; **36**: 336-339 [PMID: 28556926 DOI: 10.1002/hon.2431]

13 **Tashkandi H**, Moore EM, Tomlinson B, Goebel T, Sadri N. Co-occurrence of type I CALR and two MPL mutations in patient with primary myelofibrosis. *Ann Hematol* 2017; **96**: 1417-1418 [PMID: 28502030 DOI: 10.1007/s00277-017-3022-x]

14 **Bernal M**, Jiménez P, Puerta J, Ruíz-Cabello F, Jurado M. Co-mutated CALR and MPL driver genes in a patient with myeloproliferative neoplasm. *Ann Hematol* 2017; **96**: 1399-1401 [PMID: 28516193 DOI: 10.1007/s00277-017-3023-9]

15 **Boddu P**, Chihara D, Masarova L, Pemmaraju N, Patel KP, Verstovsek S. The co-occurrence of driver mutations in chronic myeloproliferative neoplasms. *Ann Hematol* 2018; **97**: 2071-2080 [PMID: 29951914 DOI: 10.1007/s00277-018-3402-x]

16 **Vainchenker W**, Kralovics R. Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. *Blood* 2017; **129**: 667-679 [PMID: 28028029 DOI: 10.1182/blood-2016-10-695940]

17 **Ding J**, Komatsu H, Iida S, Yano H, Kusumoto S, Inagaki A, Mori F, Ri M, Ito A, Wakita A, Ishida T, Nitta M, Ueda R. The Asn505 mutation of the c-MPL gene, which causes familial essential thrombocythemia, induces autonomous homodimerization of the c-Mpl protein due to strong amino acid polarity. *Blood* 2009; **114**: 3325-3328 [PMID: 19483125 DOI: 10.1182/blood-2008-04-149047]

18 **Lombardi AM**, Ferrari S, Barzon I, Navaglia F, Fabris F, Vianello F. A novel germ-line mutation of c-mpl gene in a sporadic case of essential thrombocythemia. *Blood Cells Mol Dis* 2017; **64**: 51-52 [PMID: 28391042 DOI: 10.1016/j.bcmd.2017.03.012]

19 **Rumi E**, Pietra D, Pascutto C, Guglielmelli P, Martínez-Trillos A, Casetti I, Colomer D, Pieri L, Pratcorona M, Rotunno G, Sant'Antonio E, Bellini M, Cavalloni C, Mannarelli C, Milanesi C, Boveri E, Ferretti V, Astori C, Rosti V, Cervantes F, Barosi G, Vannucchi AM, Cazzola M; Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative Investigators. Clinical effect of driver mutations of JAK2, CALR, or MPL in primary myelofibrosis. *Blood* 2014; **124**: 1062-1069 [PMID: 24986690 DOI: 10.1182/blood-2014-05-578435]

20 **Palandri F**, Latagliata R, Polverelli N, Tieghi A, Crugnola M, Martino B, Perricone M, Breccia M, Ottaviani E, Testoni N, Merli F, Aversa F, Alimena G, Cavo M, Martinelli G, Catani L, Baccarani M, Vianelli N. Mutations and long-term outcome of 217 young patients with essential thrombocythemia or early primary myelofibrosis. *Leukemia* 2015; **29**: 1344-1349 [PMID: 25801912 DOI: 10.1038/leu.2015.87]

21 **Tefferi A**, Guglielmelli P, Larson DR, Finke C, Wassie EA, Pieri L, Gangat N, Fjerza R, Belachew AA, Lasho TL, Ketterling RP, Hanson CA, Rambaldi A, Finazzi G, Thiele J, Barbui T, Pardanani A, Vannucchi AM. Long-term survival and blast transformation in molecularly annotated essential thrombocythemia, polycythemia vera, and myelofibrosis. *Blood* 2014; **124**: 2507-2513 [PMID: 25037629 DOI: 10.1182/blood-2014-05-579136]

**Footnotes**

**Informed consent statement:** The patient provided informed written consent during the treatment.

**Conflict-of-interest statement:** The authors declare that they have no conflicts of interest to disclose.

**CARE Checklist (2016) statement:** The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Unsolicited manuscript

**Peer-review started:** April 5, 2020

**First decision:** September 24, 2020

**Article in press:**

**Specialty type:** Medicine, research and experimental

**Country/Territory of origin:** China

**Peer-review report’s scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): 0

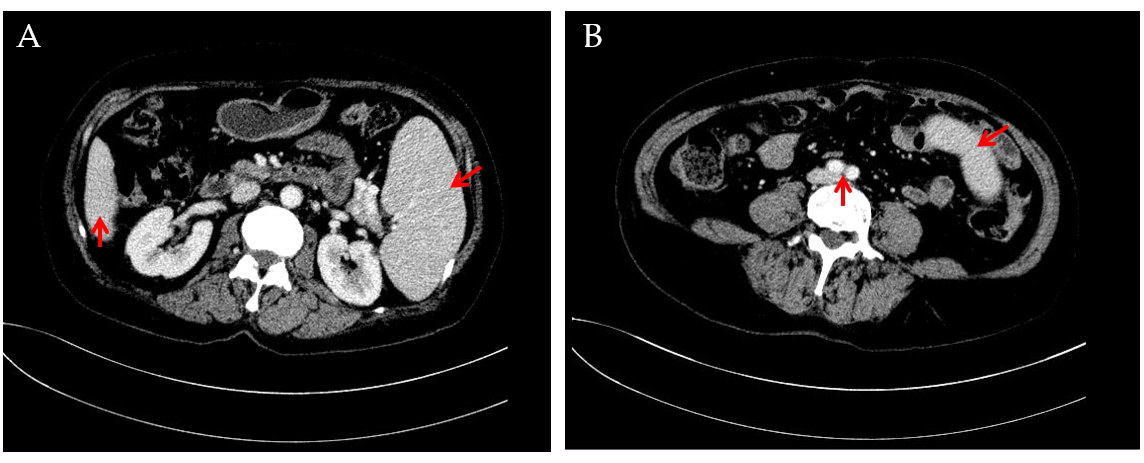
Grade C (Good): C

Grade D (Fair): D

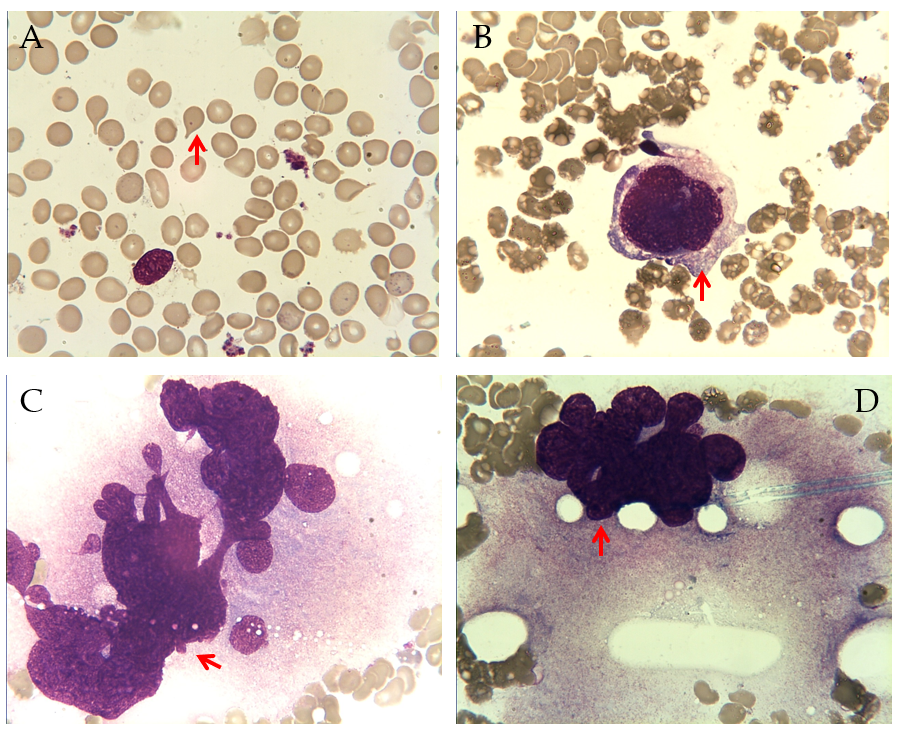
Grade E (Poor): 0

**P-Reviewer:** Barosi G, Tomizawa M **S-Editor:** Chen XF **L-Editor:** Wang TQ **P-Editor:**

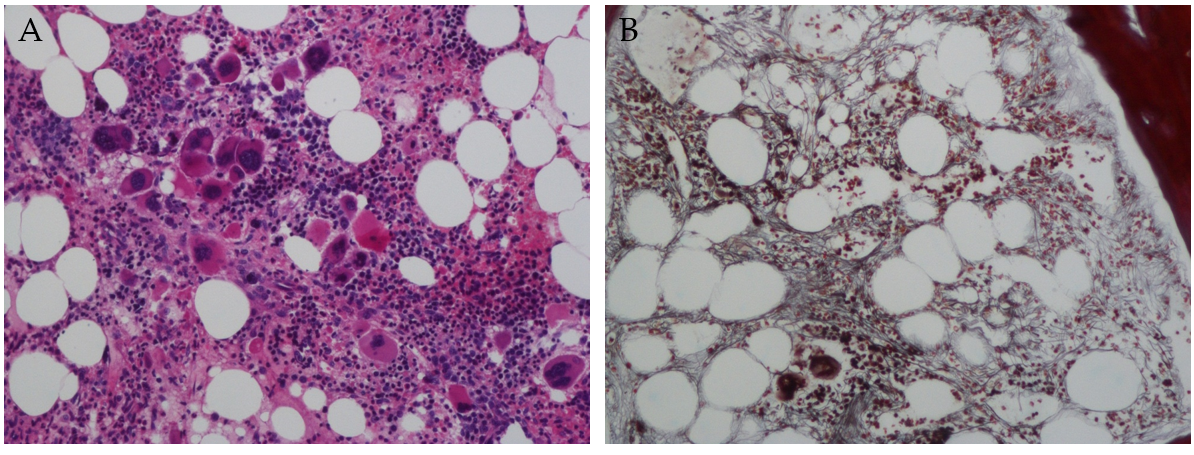
**Figure legends**



**Figure 1 Computed tomography images showing splenomegaly.** A and B: Abdominal computed tomography images showing that the inferior margin of the spleen was lower than that of the liver (A) and reached the level of abdominal aortic bifurcation (B).



**Figure 2 Bone marrow aspiration findings.** A: Dacrocytes were easily observed; B-D: Images showing the presence of megakaryocyte atypia, including abnormal nuclear-cytoplasmic ratios, malformed nucleus (B), abnormal chromatin clumping with hyperchromatic nucleus (C), and extensive lobulation of the nucleus (D).



**Figure 3 Bone marrow biopsy findings.** A: Hematoxylin and eosin staining showed megakaryocytic proliferation and clustered distribution (magnification, 200 ×); B: Gomori staining showed diffuse and dense increase in reticulin fibres (magnification, 200 ×).

**Table 1 Gene mutation list in the** **primary myelofibrosis patient**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **VAF** | **HGVS** | **Exonic function** |
| *CALR* | 30.04% | NM\_004343: c.1092\_1143del (p.364fs) | Frameshift deletion |
| *MPL* | 50.11% | NM\_005373: c.1908A>G (p.X636W) | Nonsynonymous SNV |
| *PIK3R1* | 3.18% | NM\_00181523: c.683delG (p.R228fs) | Frameshift deletion |

VAF: Variant allele frequency; HGVS: Human Genome Variation Society; SNV: Single nucleotide variant.