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CASE REPORT

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# Bone marrow fibrosis, sequence variant of asxl1, and Sjögren syndrome: A case report

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**Abstract**

Only proven pathogenic mutations associated with myeloid neoplasms are key to establish the clonal nature of the bone marrow fibrosis. In cases with genetic variants of uncertain meaning, the clinical picture may be required to rule out secondary causes.

**KEYWORDS**

ASXL1, autoimmune, bone marrow fibrosis, molecular, Sjögren syndrome

## 1 | INTRODUCTION

The distinction between primary myelofibrosis (PMF) and autoimmune myelofibrosis (AIMF) can be a diagnostic challenge because of the overlapping findings. We report a case of difficult differential diagnosis between PMF and AIMF in whom we simultaneously detected a variant sequence of ASXL1, which was interpreted as a clonal marker, and a diagnosis of Sjögren syndrome (SS) was established.

Bone marrow fibrosis (BMF) is a histopathological finding presents in several diseases (malignant tumors, endocrine, and autoimmune disorders and infections).[1,2]

Autoimmune myelofibrosis (AIMF) is an uncommon cause of nonmalignant BMF, it can occur in the presence of a systemic autoimmune disease, or it can be the primary finding in the absence of a clinically diagnosed autoimmune disease if serologic evidence of autoantibodies is detected.[3] AIMF is characterized by cytopenias and autoantibodies with nonclonal BM fibrosis, and it is distinguished from primary myelofibrosis by the absence of splenomegaly and leukoerythroblastic reaction.[4-6]

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm (MPN) characterized by BM fibrosis, ineffective hematopoiesis, and a bad prognosis. In 90% of cases, we can identify a clonal mutation that confers a proliferative advantage, being the most common JAK2, MPL, and CALR. The remaining 10% of cases without any of these three clonal markers harbor a wide range but uncommon activating mutations and are considered as being "triple-negative". One of these additional mutations detected in PMF involves the gene ASXL1 ("additional sex combs 1 like"), and it has been associated with a bad outcome.[7] The detection of a clonal marker is one of the criteria for the diagnosis of the PMF (WHO 2008 and 2016).

Differential diagnosis between PMF and nonneoplastic BMF (as AIMF) can be a diagnostic challenge, because of the overlapping pathologic findings in the two entities and the important differences in prognostic and therapeutic options.

## 2 | CLINICAL CASE

A 48-year-old woman with a history of hypertension and two cesarean deliveries presented to the emergency room in October 2013 with a marked anemic syndrome. The blood tests showed hemoglobin (Hb) of 4 g/dL with normal platelet and leukocyte counts, without signs of hemolysis. It was oriented as iron deficiency anemia, and two red blood cell units were transfused.

She remained clinically stable during a year, with Hb >10 g/dL, until November 2014, when she came again to the Hospital with an anemic syndrome. The blood test showed Hb 7.6 g/dL with reticulocytopenia and without signs of hemolysis. No teardrop cells were observed in the peripheral blood. To study this nonregenerative anemia, a bone marrow study was requested. No material was obtained in the bone marrow aspiration, and the bone marrow biopsy was suggestive of MPN (increased hematopoietic cellularity with tissue displacement, presence of dysmorphic megakaryocytes, and extense reticulin fibrosis, grade 3) (Figure 1). BCR-ABL1, JAK2, CALR, and MPL were negative, and no cytogenetic study was performed. It did not meet all the criteria for the diagnosis of PMF according to the WHO 2008 classification. The patient was asymptomatic at follow-up with chronic anemia but without transfusion requirements (Hb > 10 g/dL).

One year later, in November 2015, she had a new episode of anemia of the same characteristics without leucoerythroblastic reaction nor splenomegaly. A new bone marrow study was done with similar findings to the previous biopsy (Figure 2). Cytogenetics: 46, XX. Molecular: SETBP1 and CSF3R were negative, and a mutation was detected in exon12 of ASXL1c2400T > G/p.D800E (VAF 55.4%).

During the follow-up, the patient showed salivary gland adenitis, a dry eye with Raynaud's syndrome, so she was referred to the rheumatology unit and was diagnosed with SS without specific treatment requirements.

Despite presenting an autoimmune disease, which could be a cause of BMF, we classified the patient as having a PMF in accordance with the 2016 WHO classification, due to the presence of a high-risk clonal marker (ASXL1). A donor search for hematopoietic stem cell transplantation (HSCT) was started.

The clinical evolution was not typical of PMF: she had episodes of severe anemia needing a transfusion but responsive corticosteroid therapy; she did not have splenomegaly nor leucoerythroblastic reaction. Erythropoietin was also administered without clinical benefits. We decided to perform a new bone marrow study to reassess the disease. A bone marrow biopsy was performed after corticosteroid treatment, and it showed polymorphic hematopoiesis with normal megakaryocytes and the absence of BMF (Figure 3). Because of a reversible BMF in the context of an autoimmune disease, she was diagnosed with AIMF.

We repeated the molecular study in a peripheral blood sample, detecting the same mutation in ASXL1 (exon 12: c2400T > G/p.D800E; VAF 53.5%). This sequence variation has not been described as polymorphism, and its pathogenic effect, if any, is currently unknown. The presence of a mutation of an uncertain significance, even in a heterozygote state without changes in the VAF, suggested us to hold the follow-up in the hematology department. The patient is currently free of symptoms.

## 3 | DISCUSSION

Bone marrow fibrosis is an uncommon finding associated with different clinical disorders. The histologic traits include

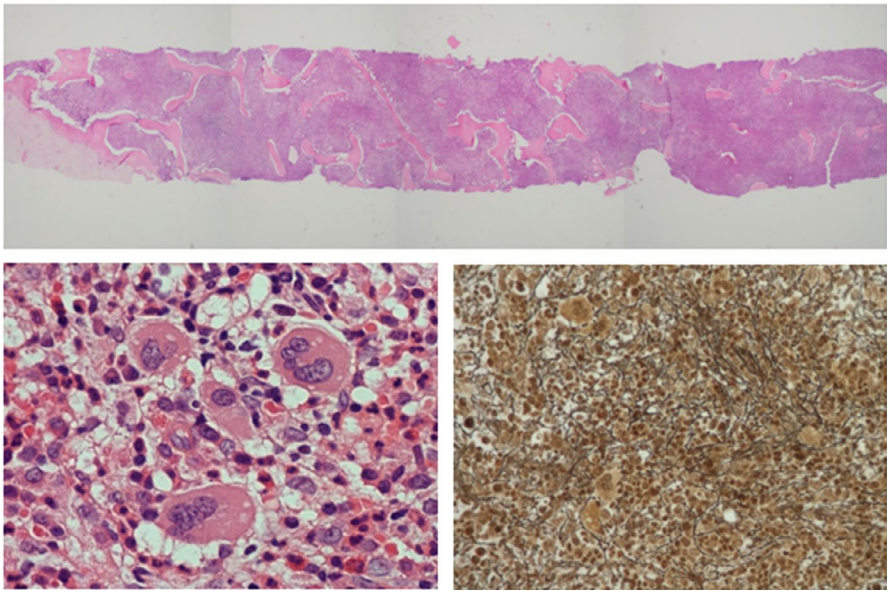

**FIGURE 1** First bone marrow biopsy (November 2014): increased hematopoietic cellularity with tissue displacement, presence of dysmorphic megakaryocytes, and extensive reticulin fibrosis, grade 3

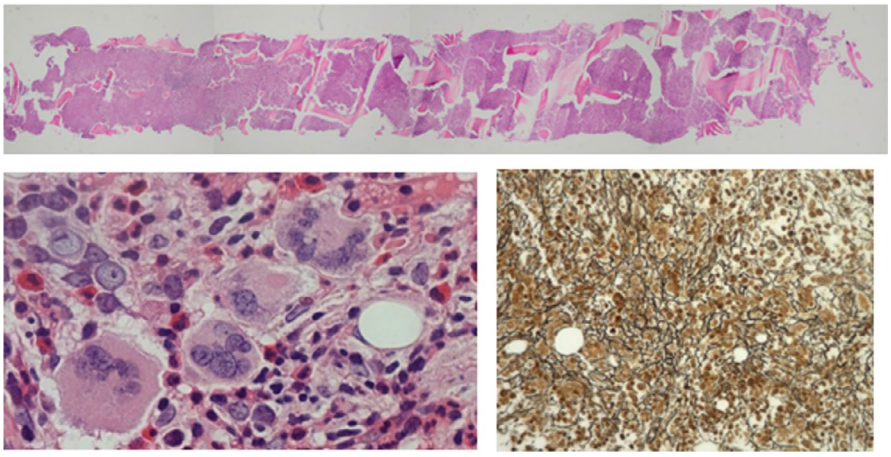

**FIGURE 2** Second bone marrow biopsy (November 2015)

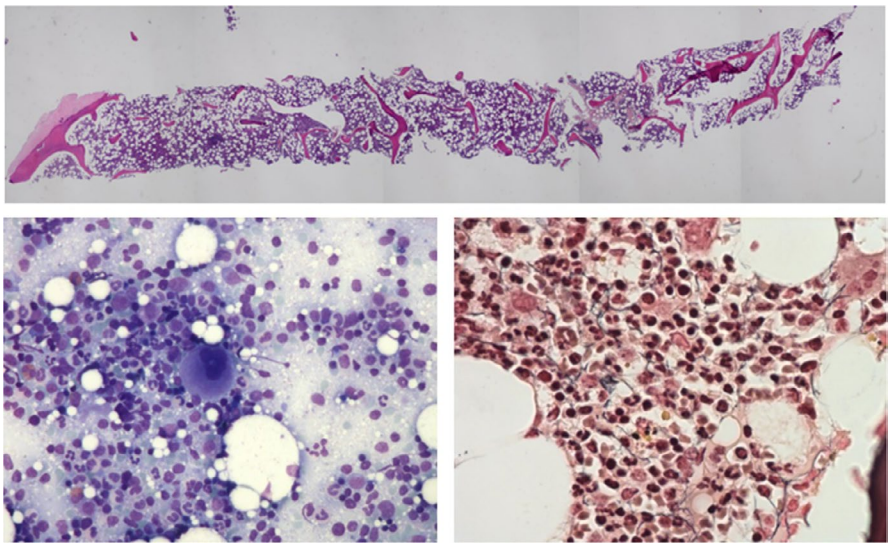

**FIGURE 3** Bone marrow study after corticosteroid therapy (September 2018): polymorphic hematopoiesis, normal megakaryocytes, and no bone marrow fibrosis

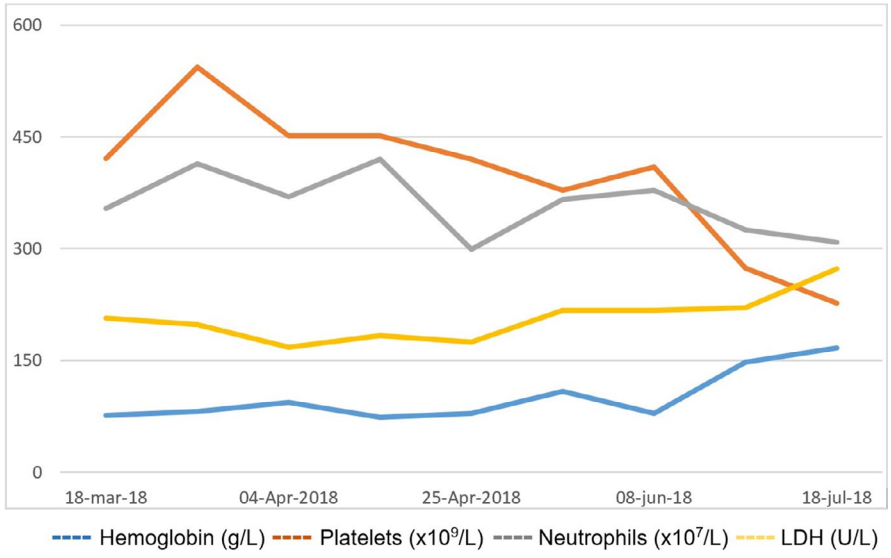


**FIGURE 4** Evolution of blood counts after the start of corticosteroids (day 1:3/18/18)

excessive deposits of extracellular matrix proteins (reticulin), with hypercellularity, neoangiogenesis, and fibroblast proliferation. In primary hematologic disorders, fibrosis is due to the dysregulation of cytokines which arise in association with a malignant clone so the fibrosis could sometimes be reversed if the neoplastic cells are eradicated (as in the setting of HSCT). Marrow fibrosis could also occur in nonhematologic disorders such as chronic infectious diseases in which the infection-mediated chronic inflammation may lead to enhanced production of cytokines. The pathogenesis of AIMF

is not well understood but the immune cells appear to drive the process of fibrosis.[1-2,8]

AIMF is an infrequent cause of nonmalignant BMF, it can occur in the presence of systemic autoimmune disease, or it can be primary. There are certain criteria for establishing the diagnosis (all must be met): (a) grade 3-4 reticulin fibrosis, (b) absence of grouped or atypical megakaryocytes, (c) absence of erythroid dysplasia, basophilia, or eosinophilia, (d) mature lymphocytes in the bone marrow, (e) absence of osteosclerosis, (f) absence or mild splenomegaly, (g) serologic evidence of autoantibodies, and (h) absence of other causes of BMF.[3]

BMF of unknown etiology was classified as PMF, but since 2008 WHO classification, there are some specific criteria for its diagnosis. These criteria were modified in the 2016 WHO classification.[9,10] For its diagnosis, 3 major and 1 minor criteria must be met. Major criteria are the following: (a) megakaryocyte proliferation and atypia (small-to-large megakaryocytes with aberrant nuclear/cytoplasmatic ratio and hyperchromatic and irregularly folded nuclei and dense clustering), (b) not meeting WHO criteria for BCR-ABL1 chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), myelodysplastic syndrome (MDS), or other myeloid neoplasm, (c) presence of JAK2, CALR, MPL mutation or, in the absence of any of these mutations, the presence of another clonal marker (the search for the most frequent accompanying mutations—ASXL1, EZH2, TET2, IDH1/IDH2, SRSF2, SF3B1—are thus very helpful in determining the clonal nature of the disease) or absence of evidence for reactive BMF. Minor criteria are the presence of one or more of the following confirmed in two consecutive determinations: anemia (not attributed to a comorbid condition), leukocytosis $\geq 11 \times 10^9$/L, palpable splenomegaly, LDH level above the upper limit of the institutional reference range (Figure 4), and/or leukoerythroblastosis.

Distinguishing between PMF and nonneoplastic BMF (as AIMF) can be a practical diagnostic challenge, because of the overlapping findings in the two entities and the important differences in terms of prognostic and therapeutic options. PMF is a BCR-ABL1-negative MPN associated with a poor prognosis, and HSCT is the only curative option. AIMF has a favorable prognosis, and it is reversible with a short course of corticosteroids.

Loss-of-function mutations and deletions in ASXL1 are observed in patients with myeloid malignancies (more frequently in myelodysplastic syndromes) and are associated with poor outcomes.[11] The ASXL1 gene is located in the chromosomal region 20q11, and the mutations related to myeloid neoplasms are mostly heterozygous, frameshifts, and located in exon 12, affecting the C-terminal plant homeodomain (PHD domain). The predicted truncated protein would lack its PHD domain and compromise its function, playing a role in leukemogenesis.[12-14] Our patient presents a mutation in the exon 12 of ASXL1 (c2400T > G/p.D800E). This change has been reported 3 times in the COSMIC database (one pancreas, one bone, and one hematopoietic sample). FATHMM prediction categorizes this change as neutral (nonpathogenic) with a 0.15 score. Furthermore, T to G changes are the less common ASXL1 mutations (2% of the reported cases). Bearing all this information in mind and with a stable VAF around 50% on two occasions, we think that this ASXL1 change is nonpathogenic. Provided that ASXL1 mutations are considered clonal markers in PMF, we suggest that its pathogenicity should be tested using available databases before establishing a PMF diagnosis.

## 4 | CONCLUSIONS

At presentation, we did not consider AIMF diagnosis given the absence of known autoimmune disease. The changes in 2016 WHO classification (where only one minor criterion—anemia—was needed for the PMF diagnosis) suggested that a PMF diagnosis could be established despite the atypical clinical evolution (absence of systemic symptoms, splenomegaly…), absence of other cytopenias, raised LDH or leucoerythroblastic reaction. The finding of a bona fide clonal mutation (ASXL1) can be very helpful in controversial cases, where the morphological findings and clinical presentation are ambiguous, but ASXL1 changes of uncertain meaning can also be a misleading factor (as it was in our case). We must distinguish between PMF, with a compatible bone marrow study and a clonal marker, but atypical clinical evolution, and AIMF, with typical evolution but the presence of an ASXL1 sequence variant. In this scenario, it is very important to consider the whole clinical picture in context; in the case under discussion, the bone marrow study after corticosteroid therapy confirmed the diagnosis.

### CONFLICT OF INTEREST

None declared.

### AUTHOR CONTRIBUTIONS

MS: has made substantial contributions to conception and design, and analysis and interpretation of data, and wrote the manuscript; EB: performed molecular analysis; MP: performed molecular analysis; AM-R: obtained morphologic and molecular data; SS: served as clinician and patient-reported in charge; AM: involved in pathology data; EB, MP, AM-R, SS, and AM: contributed to comments and suggestions; CM: involved in morphologic assessment; JFN: involved in design and interpretation of data. Corresponding authors—CM and JFN: have made substantial contributions to conception and

design, and have been involved in drafting the manuscript or revising it critically for important intellectual content.

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