

69-year-old patient with anemia and massive splenomegaly

Hombre de 69 años con anemia y esplenomegalia gigante

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Abstract

69-year-old patient with anemia and massive splenomegaly diagnosed with primary myelofibrosis (PMF) 13 years earlier. He presented a calreticulin (CALR) mutation and had started treatment with ruxolitinib due to disease progression. Three years after starting treatment, following a prior phase of leukocytosis with basophilia, the patient progressed to acute leukemia with acquisition of a BCR::ABL1 rearrangement, maintaining the CALR mutation. Additionally, the patient acquired a t(12;18)(q21;q21). Overall survival from diagnosis of PMF was 17 years. The acquisition of a BCR::ABL1 rearrangement in CALR-mutated PMF is very rare and has uncertain clinical significance. The initial presentation of the BCR::ABL acquisition as a blast crisis is exceptional. In PMF, the appearance of progressive leukocytosis with basophilia has been associated with progression to acute leukemia and should suggest a possible acquisition of a BCR::ABL1 rearrangement. In this patient, the transformation to a blast crisis could be associated with the presence of a t(12;18) in addition to the t(9;22).

Keywords: Primary myelofibrosis. BCR::ABL1. CALR. Chronic myeloid leukemia. Blast crisis.

Resumen

Paciente de 69 años con anemia y esplenomegalia gigante diagnosticado de mielofibrosis primaria (MFP) 13 años antes. Se objetivó mutación en calreticulina (CALR) y ante la progresión de la sintomatología inicia tratamiento con ruxolitinib. A los tres años del inicio de tratamiento, tras una fase previa de leucocitosis con basofilia, progresa a leucemia aguda con adquisición de un reordenamiento BCR::ABL1 y una t(12;18)(q21;q21), manteniendo la mutación de CALR. La supervivencia global desde el diagnóstico de MFP fue de 17 años. La adquisición de un reordenamiento BCR::ABL1 en una MFP CALR mutado es muy infrecuente y tiene un significado clínico incierto. La presentación inicial como crisis blástica de la adquisición de BCR::ABL es excepcional. En la MFP, la aparición de leucocitosis con basofilia progresiva se ha asociado a transformación a leucemia aguda y debería hacer pensar en una posible adquisición de reordenamiento BCR::ABL1. En este paciente la transformación a crisis blástica podría estar influenciada por la existencia de una t(12;18) adicional a la t(9;22).

Palabras clave: Mielofibrosis primaria. BCR::ABL1. CALR. Leucemia mieloide crónica. Crisis blástica.

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Introduction

Reason for consultation

Patient referred from the cardiology department for anemia.

Clinical history

- Family history: father died of lung carcinoma at the age of 40, sister died from cancer of unknown origin at the age of 60.
- Personal history: no known drug allergies. Former smoker for 20 years. Occasional drinker. No cardiovascular risk factors. Hyperuricemia with several gout attacks. Chronic kidney disease (2017). Heart failure and atrial flutter (2017). Currently on apixaban (temporarily withdrawn), digoxin, furosemide, carvedilol, and allopurinol (100 mg/day).

Case report

Hematological disease

Year 2005

Primary myelofibrosis (PMF) diagnosed privately following a gout attack. Abdominal ultrasound revealed the presence of splenomegaly. Normal blood count and renal function. No treatment or hematological follow-up, maintaining good quality of life.

Year 2018: first evaluation at our center

The patient was referred to our center due to a 5-month history of progressive general deterioration, with dyspnea on minimal exertion, severe asthenia, 10 kg weight loss, and diarrhea. He had visited the ER for a respiratory infection finding anemia in the transfusion range (hemoglobin [Hb] 77 g/L).

- Physical examination: ECOG 1. Marked cutaneous-mucosal pallor. Abdomen with giant, stony splenomegaly. Lower limbs with trophic disorders.
- Lab test results:
 - Blood count: leukocytes $6.01 \times 10^9/L$, Hb 97 g/L, platelets $125 \times 10^9/L$, reticulocytes $105.6 \times 10^9/L$.
 - Biochemistry: creatinine, 2.50 mg/dL; urea, 153 mg/dL; uric acid, 8.8 mg/dL; lactate dehydrogenase (LDH), 302 U/L; potassium, 6 mEq/L; β_2 -microglobulin, 22.80 mg/L.
- Imaging modalities:

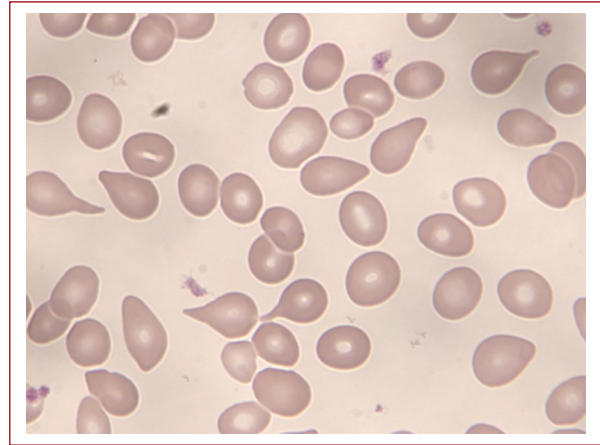


Figure 1. Peripheral blood (May Grünwald-Giemsa x1,000). Numerous dacryocytes, anisocytosis.

- Ultrasound: slight changes of chronic hepatopathy, with dilated portal vein at the hilum and marked splenomegaly of, at least, 20 cm.
- Thoracic and abdominal computed tomography: mediastinal lymphadenopathy. Predominant diffuse centrilobular emphysematous COPD pattern. Hepatosplenomegaly with splenoportal axis dilation. Focal splenic lesion. Upper retroperitoneal lymphadenopathy. Possible extramedullary hematopoiesis foci in right perirenal fat.
- Bone marrow cytology study:
 - Peripheral blood smear, differential count: basophils 6%, promyelocytes/myelocytes 2%, metamyelocytes 4%, bands 5%, segmented 64%, lymphocytes 11%, monocytes 8%; 6 erythroblasts per 100 leukocytes. Numerous dacryocytes observed (Fig. 1).
 - Bone marrow aspirate: hypocellular (+) without bone marrow clumps. Erythroid series (54.5%): well-represented at all stages with moderate maturation abnormalities. Granulocytic series (32.5%): well-represented, with slight dysmorphias. Monocytes 2.5%, lymphocytes 9.5%. Megakaryocytic series: numerous fragmented megakaryocytes in the tails of the smears. Occasionally small forms and even micromegakaryocytes are found.
- Pathology: hypercellular bone marrow with associated osteosclerosis, marked fibrosis reaching collagenous (grade MF-3), without accompanying siderosis, notable increase in megakaryocytes with atypical forms and intrasinusoidal location (Figs. 2-4).
- Cytogenetic study: 47,XY,+21[8]/46,XY[12].
- Molecular study:

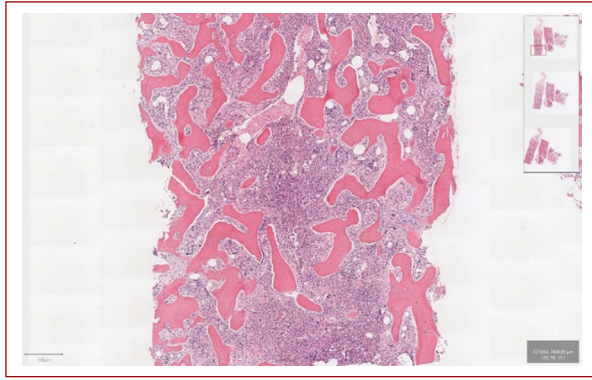


Figure 2. Bone marrow (hematoxylin-eosin x40). Increased cellularity, osteosclerosis.

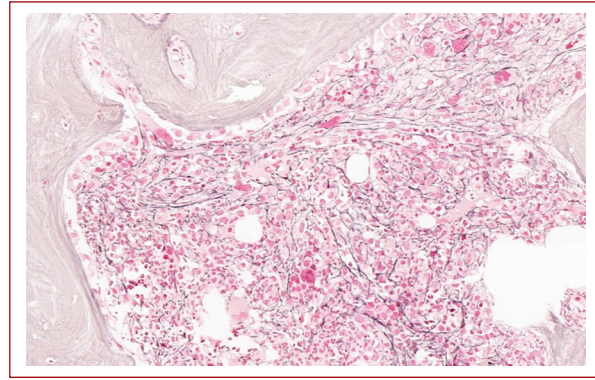


Figure 4. Bone marrow (reticulin x200). Interwoven reticulin fibers.

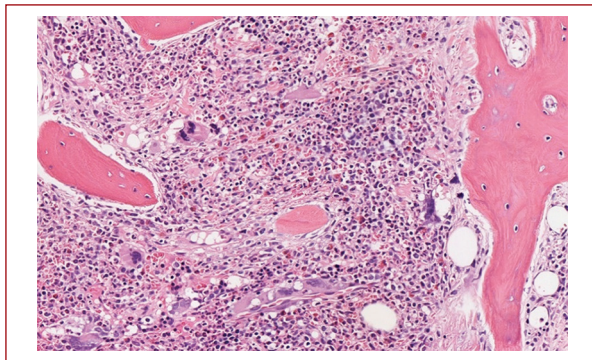


Figure 3. Bone marrow (hematoxylin-eosin x200). Intrasinusoidal atypical megakaryocytes.

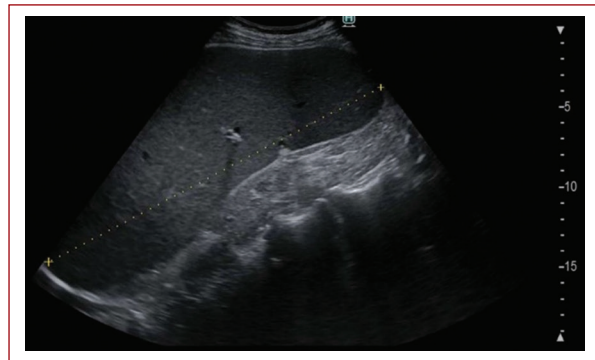


Figure 5. Splenomegaly with a maximum craniocaudal axis of 26 cm.

- Mutations in exon 9 of CALR: mutated. Amplification consistent with detection of a type 1 mutation: p.L367fs46.
- JAK2 V617F mutation: not mutated.
- Diagnosis: PMF (in osteosclerotic phase) CALR mutated. IPSS-2.
- Disease progression: started treatment with ruxolitinib, 15 mg every 12 hours, with clinical improvement and transfusion independence 17 months after initiating treatment. Three months later, leukocytosis with basophilia around 10% up to 15% without blasts appeared. Leukocytosis was progressive over a year, at the end of which the patient was hospitalized with significant clinical and analytical deterioration.

Year 2021: evaluation at admission

- Reason for admission: dyspnea.
- Physical examination: ECOG 1. Mild tachypnea. Oxygen saturation 87% without supplementation. Normal

cardiopulmonary auscultation. Abdomen with giant, stony splenomegaly, non-tender, no hepatomegaly.

– Lab test results:

- Blood count: leukocytes $180.93 \times 10^9/L$, Hb 80 g/L, platelets $175 \times 10^9/L$, reticulocytes $115.50 \times 10^9/L$.
- Biochemistry: creatinine 3.62 mg/dL, urea 141 mg/dL, uric acid 9.3 mg/dL, potassium 6 mEq/L, LDH 924 U/L, gamma-glutamyl transferase 197 U/L, C-reactive protein 10 mg/L.

- Imaging modalities: abdominal ultrasound with massive splenomegaly of, at least, 26 cm in craniocaudal diameter and marked dilation of the splenic and portal veins, the latter reaching a diameter of 15 mm (Fig. 5).

– Bone marrow cytology study:

- Peripheral blood smear: blasts 23%, basophils 31%, eosinophils 5%, promyelocytes 3%, myelocytes 3%, metamyelocytes 4%, bands 7%, segmented 20%, lymphocytes 2%, monocytes 2%. Erythroblasts 7 per 100 leukocytes. Anisopoikilocytosis and anisocytosis. Highly dysmorphic platelets,

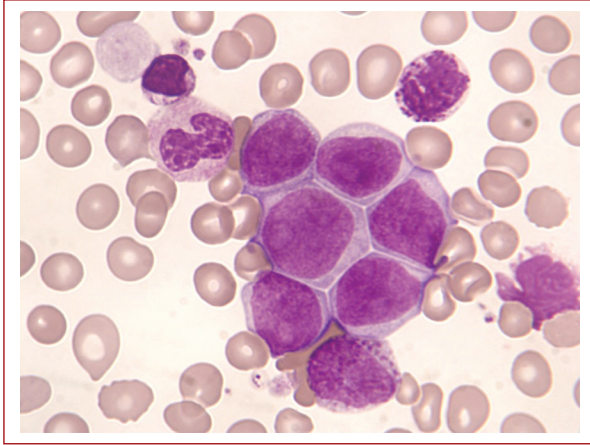


Figure 6. Peripheral blood (bone marrow aspirate x1000). Predominance of large blasts with a high nucleus/cytoplasm ratio, loose chromatin, at least, 1 large prominent nucleolus, and weakly basophilic agranular cytoplasm.

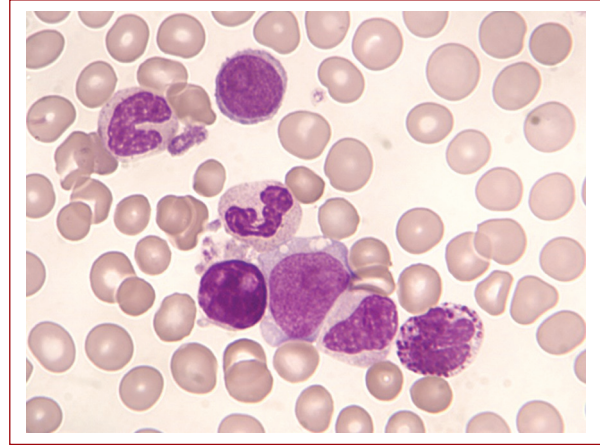


Figure 8. Peripheral blood (bone marrow aspirate x1000). A circulating micromegakaryocyte and young granulocytes can be observed.

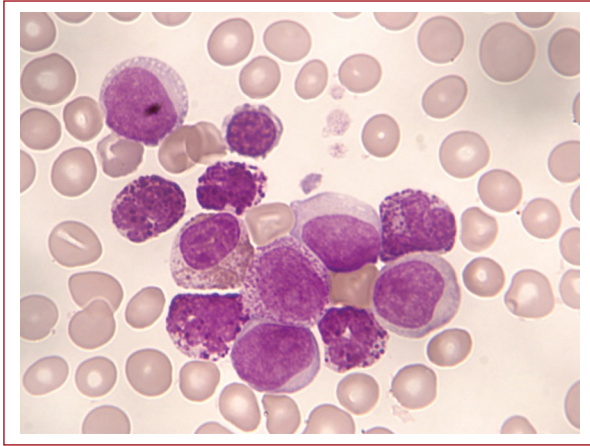


Figure 7. Peripheral blood (bone marrow aspirate x1000). Numerous basophils. The image also shows a hypogranular eosinophil, some blasts, and an erythroblast.

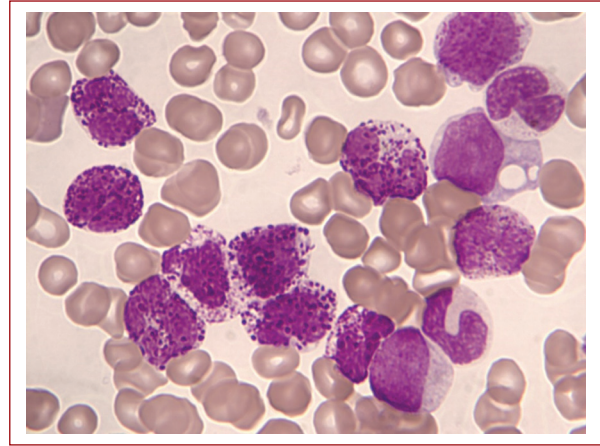


Figure 9. Bone marrow (bone marrow aspirate x1000). Intense basophilia is observed.

macrothrombocytes, some megakaryocyte nuclei, and micromegakaryocytes. Granulocytes with hypogranularity. Eosinophils with pre-eosinophilic granulation. Atypical basophils. Blasts described in the images (Figs. 6-8).

- Bone marrow aspirate: hypercellular ++. Megakaryocytic series: increased with severe dysmegakaryopoiesis, frequent micromegakaryocytes. Erythroid series 10.4% (dysplastic: macrocytic traits, megaloblastic chromatin, nuclear constrictions, some binucleated forms, hemoglobinization changes). Granulocytic series 33.7%, generalized hypogranularity, maturation disharmonies. Eosinophils 4%, basophils 26%, monocytes 1%,

lymphocytes 1.2%, plasmocytes 0.2%, blasts 23.5%. Blasts described in the images. Cytochemistry: periodic acid-Schiff (PAS) 28% (medium and fine variable granular pattern and some clumps [in the blast population]), MPO < 3%, acid phosphatases 90%, toluidine blue 31% with metachromasia (Figs. 9-19).

- Flow cytometry: the sample shows 23.7% basophils of the total cells, 6.3% immature blasts, increase in CD117+CD34– precursors (4.2%), most differentiating to basophils (70.9%), presence of micromegakaryocytes (4.5%), and 8.7% of an unidentified pathological population.
- Cytogenetic study:

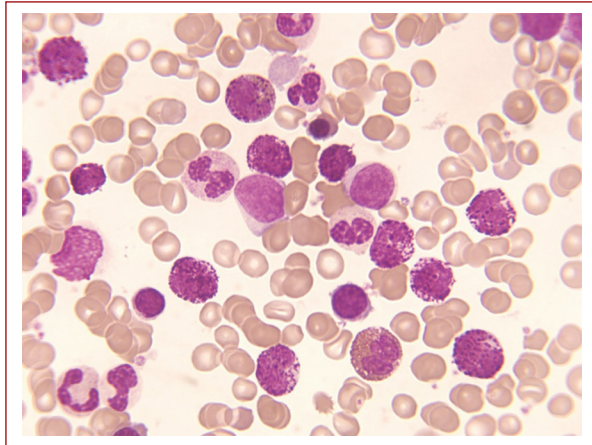


Figure 10. Bone marrow (bone marrow aspirate x630). Marked basophilia and presence of blasts (1 in the central area) are noted.

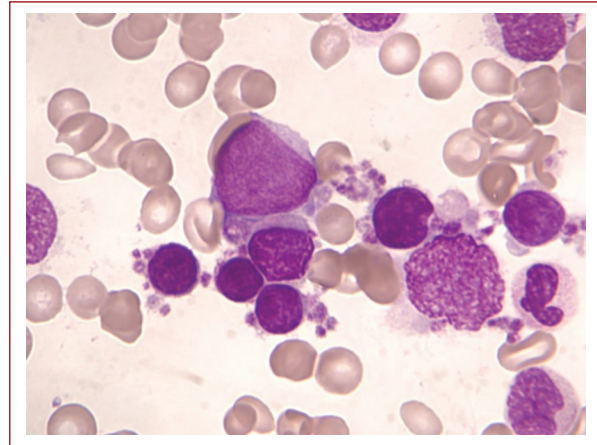


Figure 13. Bone marrow (bone marrow aspirate x1000). Several micromegakaryocytes next to a large blast.

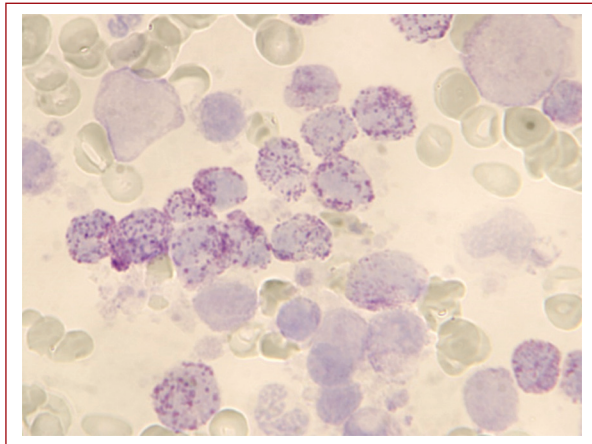


Figure 11. Bone marrow (toluidine blue x1000). Group of basophils showing metachromasia.

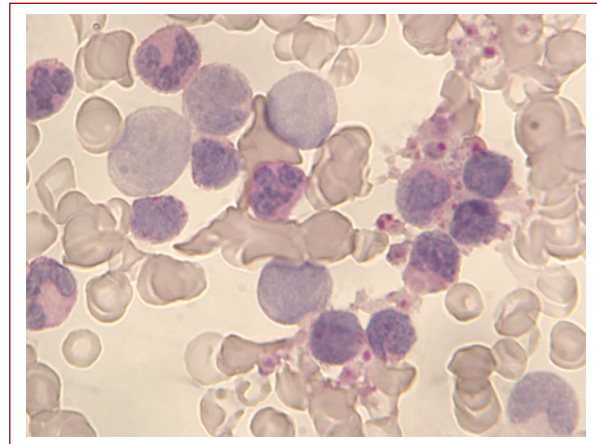


Figure 14. Bone marrow (PAS reaction x1000). Group of PAS-positive platelet-generating micromegakaryocytes. PAS: periodic acid-Schiff.

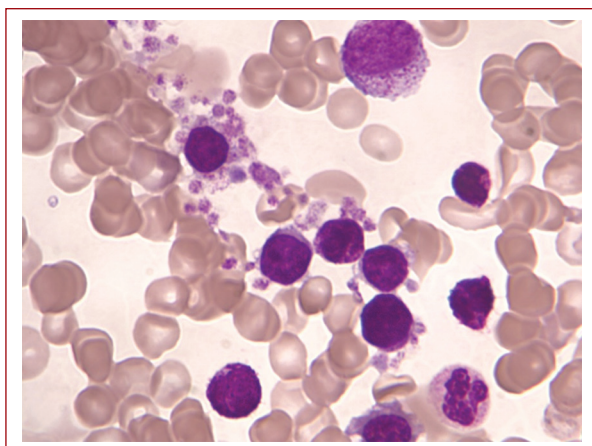


Figure 12. Bone marrow (bone marrow aspirate x1000). Cluster of platelet-generating micromegakaryocytes.

- Karyotype: 46,XY,t(9;22)(q34;q11.2), t(12;18)(q21;q21) [20] (Fig. 20).
- Fluorescence in situ hybridization (FISH): BCR::ABL rearrangement (94%).
- Molecular study:
 - BCR-ABL p210 [t(9;22)]: mutated. Quantification: 100%.
 - Mutation study detected by massive sequencing on the Ion S5 platform with the Oncomine Myeloid Research Assay panel covering a total of 40 genes: Calreticulin: c.1099_1150delCTTAAGGAGGAGGAA GAAGACAAGAAACGCAAAGAGGAGGA GGAGGCAGAGG (exon 9), p.Leu367fs, VAF: 48.88%.
- Diagnosis: myeloid blast crisis as a progression of CALR-mutated PMF with acquisition of BCR::ABL

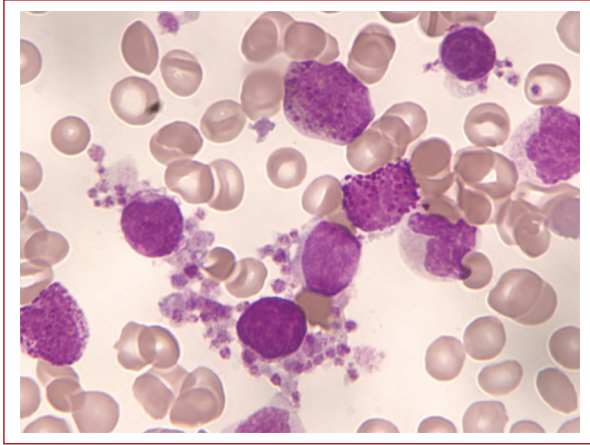


Figure 15. Bone marrow (bone marrow aspirate x1000). Micromegakaryocytes, basophils, and an eosinophil with pre-eosinophilic granulation.

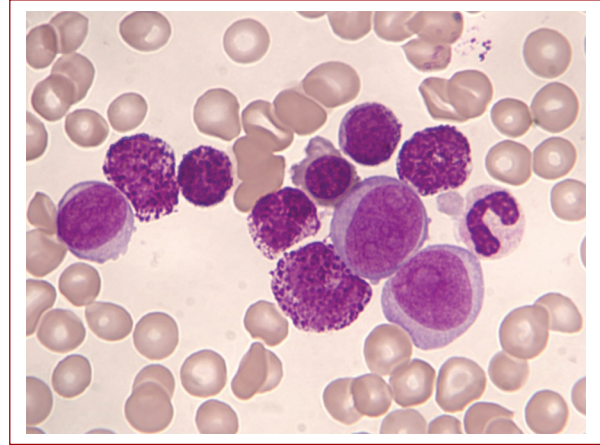


Figure 17. Bone marrow (bone marrow aspirate x1000). Three blasts are observed alongside marked basophilia.

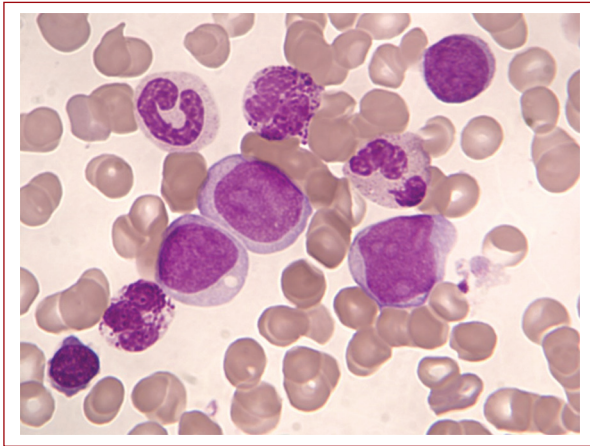


Figure 16. Bone marrow (bone marrow aspirate x1000). Three large blasts in the center, with loose chromatin, nucleoli, and weakly basophilic agranular cytoplasm.

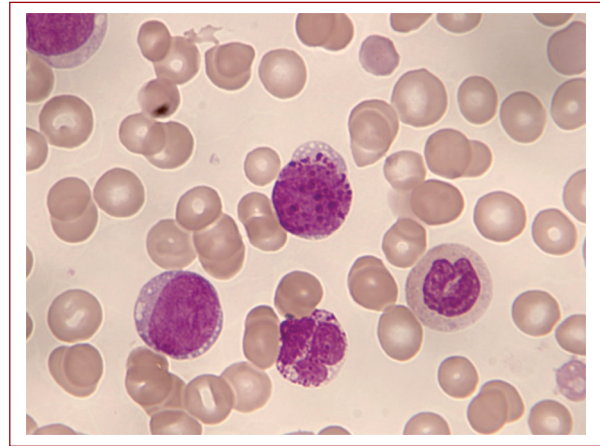


Figure 18. Bone marrow (bone marrow aspirate x1000). Blast-like cell with coarse basophilic granulation and fine vacuolization (at the center).

rearrangement, without ruling out prior transformation to chronic myeloid leukemia (CML) (chronic and/or accelerated phase).

- Disease progression: the patient received cytoreductive treatment with hydroxyurea and supportive care for tumor lysis syndrome with good response. Once stabilized, the patient's hematopathy was treated with ruxolitinib and imatinib, which was then replaced by dasatinib due to gastrointestinal intolerance. He maintained periodic transfusion requirements.
- Subsequent follow-up:
 - Molecular follow-up (blood): two months later, molecular biology detected BCR::ABL 75%, at 9 months 7.04%, and at 1 year 9.3%.

- Bone marrow control 3 months after acute leukemia diagnosis: hypocellular without significant blast infiltration, with flow cytometry showing 1.1% blasts and FISH showing 29% BCR::ABL rearrangement.

Finally, the patient passed away due to a septic episode 4 years after the beginning of follow-up at our center and 1 year after progression to acute leukemia.

Discussion

In conclusion, this is a patient diagnosed with PMF. Thirteen years after the diagnosis, the patient was re-evaluated due to clinical deterioration, revealing fibrotic phase PMF (osteosclerosis) with mutated calreticulin,

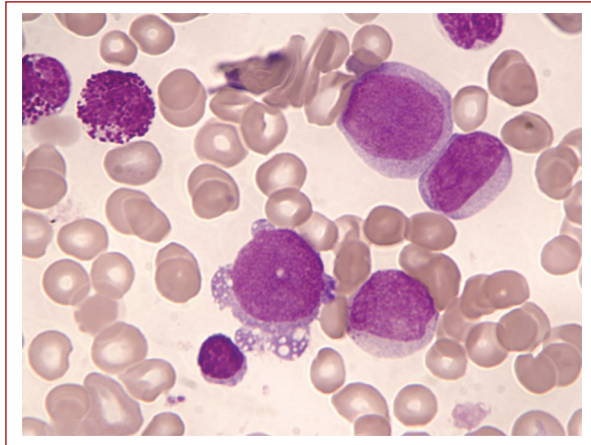


Figure 19. Bone marrow (bone marrow aspirate x1000). Atypical proerythroblast with numerous vacuoles in mottled cytoplasmic projections.

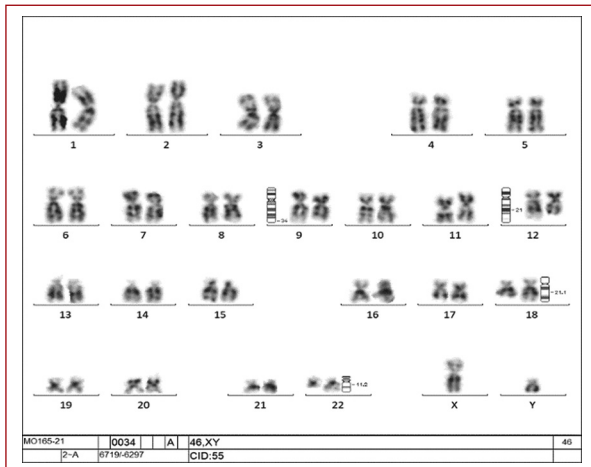


Figure 20. Karyotype 46,XY,t(9;22)(q34;q11.2),t(12;18)(q21;q21)[20].

and treatment with ruxolitinib was initiated. Three years after starting treatment, following a prior phase of leukocytosis with basophilia, disease progressed into acute leukemia with the acquisition of a BCR::ABL rearrangement while maintaining the calreticulin mutation, resulting in an overall survival of 17 years.

PMF is a chronic Philadelphia chromosome-negative myeloproliferative neoplasm characterized by predominant expansion of bone marrow megakaryopoiesis and granulopoiesis, along with reactive fibrous connective tissue deposition. It progresses from an initial prefibrotic stage, in which the bone marrow is hypercellular with minimal or absent reticulin fibrosis to a well-established fibrotic stage with marked reticulin and/or collagen

fibrosis, eventually leading to osteosclerosis, along with a leukoerythroblastic reaction and dacryocytes in peripheral blood, gradual depletion of bone marrow cellularity, and organomegaly due to extramedullary hematopoiesis¹.

PMF-associated driver mutations occur in the JAK2 V617F (50-60%), CALR (30%), or MPL (8%) genes. Specifically, CALR mutations result from heterozygous insertions and/or deletions in exon 9 of the gene. Type 1 mutations are caused by a 52-bp deletion, and type 2 mutations by a 5-bp insertion. CALR mutations are associated with younger age, higher platelet counts, lower frequency of leukocytosis, anemia, and transfusion requirements, fewer spliceosome mutations, and lower prognostic scores (DIPSS-plus) vs. JAK2 mutations. Some studies attribute these characteristics solely to type 1 mutations, with type 2 mutations having similar characteristics to JAK2. Type 1 CALR mutations, like the one in this patient with long survival, have the best prognosis in PMF^{2,3}.

The disease significantly shortens patient survival, although heterogeneously, as some die within 1 to 2 years while others, fewer, survive more than 20 years. Longevity is impacted by the stage at diagnosis (prefibrotic vs. established fibrotic stage) and the possible progression to acute leukemia, which occurs in approximately 10% to 20% of cases within 10 years. Predictive markers for this transformation include peripheral blood monocytosis, increased blasts in blood and/or bone marrow, increased CD34+ cells in the bone marrow biopsy forming clusters with abnormal distribution, and the acquisition of adverse risk mutations (ASXL1, EZH2, SRSF2, or IDH1/2)^{1,4}.

Recently, persistent basophilia for more than three months, especially in CALR-mutated PMF, has been identified as a risk factor for developing acute leukemia⁵. In the case presented here, the patient developed leukocytosis with basophilia 1 year before progressing to acute leukemia. A notable aspect of this case is the acquisition of a BCR::ABL rearrangement during progression to myeloid blast crisis, while retaining the CALR mutation. Differential diagnosis in PMF progression is whether it started as a BCR::SABL blast crisis or a transformation to chronic and/or accelerated phase CML with subsequent blast crisis. In this case, persistent basophilia may indicate progression to AML and acquisition of BCR::ABL.

The acquisition of a BCR::ABL1 rearrangement in CALR-mutated PMF is very rare (26 patients described in the literature from 2014 through 2023, since the mutation was first described), and its clinical significance is uncertain⁶⁻⁸. Only one published case demonstrated temporality,

initially finding a CALR mutation that later acquired the BCR::ABL1 rearrangement. This alteration may phenotypically manifest as CML, PMF, or essential thrombocythemia. In this patient, the main morphological differences between osteosclerotic phase PMF and BCR::ABL blast crisis are evident in megakaryopoiesis morphology (morphological variability and dysplasia vs. massive predominance of micromegakaryocytes) and in the significant increase in blood and bone marrow basophilia in the blast crisis vs. the fibrotic phase. Initial presentation as a blast crisis of BCR::ABL acquisition is exceptional^{9,10}. In this patient, transformation to blast crisis may be impacted by the presence of an additional t(12;18) to t(9;22).

Regarding the therapeutic approach of PMF, hyperproliferative signs and splenomegaly improve with ruxolitinib, a JAK1 and JAK2 inhibitor, although the only potentially curative treatment is allogeneic hematopoietic stem cell transplantation. Published cases of CML with coexistence of a JAK2/MPL/CALR clone have shown a poorer response to tyrosine kinase inhibitors and worse disease progression, with development of blast crisis.

Conclusions

- The coexistence of CMN driver mutations and BCR::ABL rearrangement is rare but should be considered in cases with atypical clinical and cytological progression.
- In the follow-up of PMF, the appearance of leukocytosis with progressive basophilia should raise suspicion of transformation to acute leukemia and/or progression with acquisition of BCR::ABL rearrangement.
- The acquisition of chromosomal abnormalities in the karyotype, in addition to mutations already included in prognostic indices, could contribute to disease progression.
- With available evidence, the current treatment for CALR+ neoplasms with BCR::ABL rearrangement would include a combination of tyrosine kinase inhibitors and JAK2 inhibitors.

Funding

None declared.

Conflicts of interest

None declared.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article. Furthermore, they have acknowledged and followed the recommendations as per the SAGER guidelines depending on the type and nature of the study.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Use of artificial intelligence for generating text. The authors declare that they have not used any type of generative artificial intelligence for the writing of this manuscript, nor for the creation of images, graphics, tables, or their corresponding captions.

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