Polycythemia Vera Evolution to Chronic Myelomocytic Leukemia: The Prognostic Value of Next Generation Sequencing

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Polycythemia vera (PV) is a myeloproliferative neoplasm (MPN) that usually evolves to a myelofibrotic phase and is characterized by the presence of cytopenias. There is also a small incidence of progression to acute leukemia, preceded or not by a myelodysplastic syndrome. However, the evolution of PV to a myelodysplastic/myeloproliferative disorder, such as chronic myelomonocytic leukemia (CMML), has been scarcely described. We report a case of *JAK2*V617F-positive PV which, 6 years after diagnosis, presented monocytosis along with dysplasia and genetic abnormalities indicating disease progression to CMML. This case also shows the crucial role of next generation sequencing (NGS) techniques in the study of MPNs to identify patients at high risk.

A 58-year-old Caucasian man was diagnosed with PV in 2013. The *JAK2*V617F mutation was present with allele burden of 34.13%. A bone marrow biopsy was performed showing absence of reticulinic fibrosis. The karyotype was normal, and no further molecular studies were performed at that time. The patient was diagnosed with PV, and therapeutic phlebotomies were started. In May 2016, treatment with hydroxyurea was initiated because of the appearance of leucocytosis and thrombocytosis. The blood counts remained within normal values under cytoreductive treatment for the following years.

In September 2019 the patient consulted due to general malaise. Blood counts showed pancytopenia with relative monocytosis. Bone marrow aspiration and biopsy were performed showing significant dyserythropoiesis and dysgranulopoiesis, as well as megakaryocytes with hyposegmented nuclei. An increase in the number of mature monocytes was observed and blasts and promonocytes accounted for 11% of the nucleated bone marrow cells. Flow cytometry revealed 5% of myeloid blasts, which were positive for CD34, CD117, HLA-DR, CD33, CD13, CD4 and CD11b, and mature plus immature monocytes represented 30% of the cellularity. Cytogenetics showed a complex karyotype, and eventual fluorescence in situ hybridization (FISH) analysis confirmed *TP53* deletion (Fig. 1). FISH for *BCR-ABL1*, *PDGFR-alpha* and *PDGFR-beta* were negative. A targeted NGS myeloid panel (Sophia Genetics, Switzerland) detected the *JAK2*V617F mutation together with other mutations (Table 1). Cytoreductive treatment was stopped and close follow-up was decided. Thirty days after discontinuation of hydroxyurea, leucocytosis (WBC 28×10^9/L) with absolute and relative monocytosis (7.8×10^9/L and 28%, respectively) were observed, as well as the presence of 4% of blasts on the peripheral blood smear, and an increase of serum lactate dehydrogenase (747 U/L, normal up to 240). Flow cytometry performed in peripheral blood showed that 95.16% of all monocytes were CD14$^+$/CD16$^-$ (classical subtype), suggesting the diagnosis of CMML.[1] A bone marrow aspirate showed dyserythropoiesis in 72% of precursors, dysgranulopoiesis in 90% and dysmegakaryopoiesis in 30%. Moreover, monocytes were increased (9%), and the sum of blasts and promonocytes again represented 11% of bone marrow cellularity. Additionally, 65% of ring sideroblasts were observed on Perls stain, with decreased macrophage iron levels. The blast population presented the same myeloid phenotype described in the previous study. Trephine biopsy showed hypercellular bone marrow, with an increase of the erythroid precursors and dysplastic megakaryocytes. The CD34 population positive by immunohistochemistry represented 5% to 10% of the bone marrow cellularity.

These results demonstrated that the disease had undergone several changes after 6 years of evolution, such as remarkable dysplasia involving the three myeloid lineages, appearance of atypical monocytosis and the presence of an immature population (promonocytes and blasts) representing less than 20% of the bone marrow cellularity. These findings led to the diagnosis of CMML. These cytologic changes were associated with the acquisition of clonal genetic abnormalities. The karyotype switched from normal to complex with the acquisition of
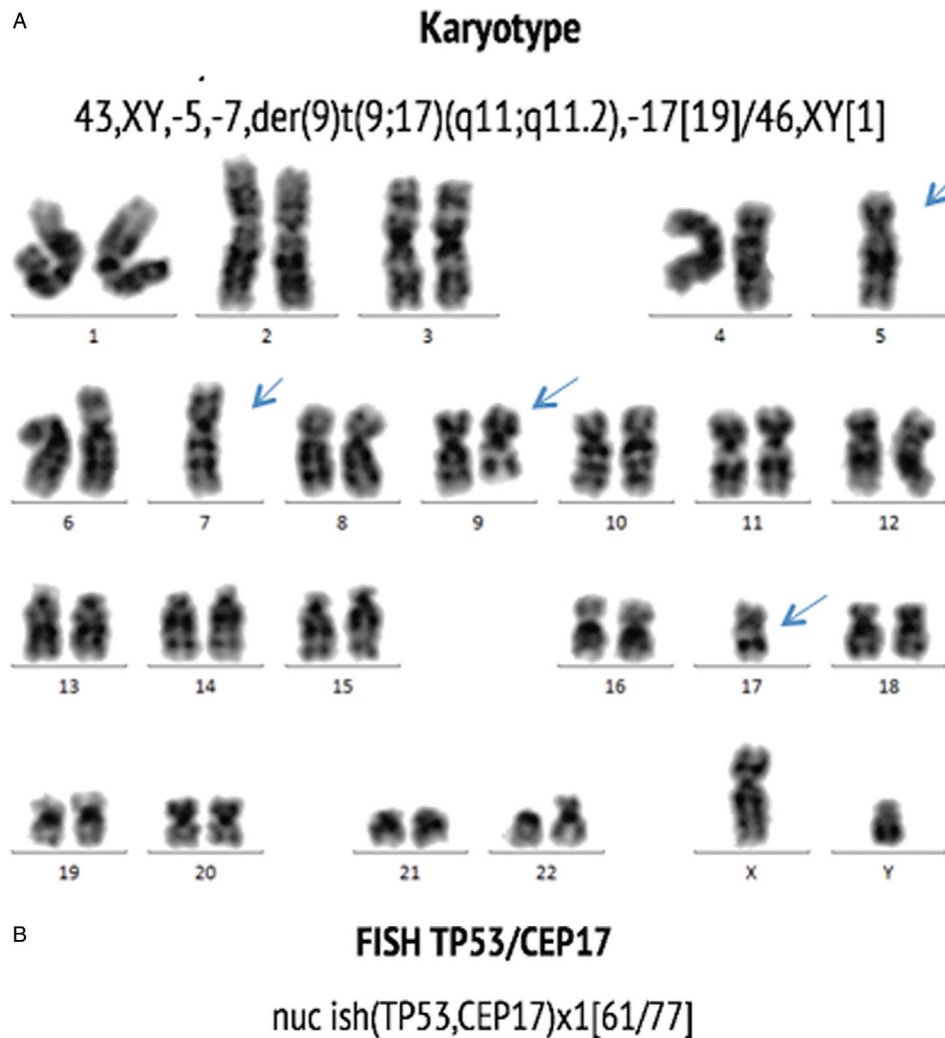
Figure 1. Karyotype and FISH analysis performed at disease progression. (A) Karyotype in the bone marrow sample: 20 metaphases were analyzed. Nineteen metaphases presented a hypodiploid, complex and monosomic karyotype. The following abnormalities were observed: chromosomes 5, 7, 17 monosomies and an unbalanced translocation between 9q11 and 17q11.2. (B) FISH TP53/CEP17 study in interphase nucleus (XL TP53/17cen probe, MetaSystems). The TP53 gene is located at 17p13 (red signal) and the CEP17 probe is located at the centromere of chromosome 17 (green signal). Only one red signal for the 17p13 region is observed, confirming a TP53 deletion. The presence of a single green signal can be explained by the unbalanced translocation between 9q11 and 17q11, and the subsequent loss of one chromosome 17 centromere.

TP53 deletion (the absence of TP53 deletion at PV diagnosis was confirmed by FISH). Furthermore, the NGS study showed the presence of additional mutations (Table 1). To confirm whether these mutations were already present at the time of PV diagnosis, an NGS study was retrospectively performed. Strikingly, the same mutations along with a JAK2V617F mutation were already

Table 1

Variants detected in next generation sequencing (NGS) targeted gene panel, performed at PV diagnosis (2013) and progression (2019).

Classification*	Gene	Chr.	Exon	c.DNA	Protein	Total readings	VAF† in 2019 (%)	VAF† in 2013 (%)
Class 1	*JAK2*	9	14	c.1849G>T	p.(Val617Phe)	5233	**91.71**	43.68
Class 1	*ASXL1*	20	13	c.1934dupG	p.(Gly646Trpfs*12)	6563	**35.91**	10.44
Class 1	*TP53*	17	5	c.523C>G	p.(Arg175Gly)	3325	**82.41**	13.18
Class 3B(‡)	*TET2*	4	3	c.1285G>A	p.(Gly429Arg)	5582	**50.21**	46.96

* Classification of the variants:

Class 1: relevant in the clinical management of myeloid hemopathies. It has been established as a pathogenic variant in myeloid hemopathies and alters an actionable gene.

Class 2: it has been established as a pathogenic variant in solid tumors or non-myeloid hemopathies and alters an actionable gene.

Class 3: variant not previously described, affects an actionable gene and in silico predictors or classifies mutations as:

● Class 3A: likely pathogenic.

● Class 3B: uncertain significance (VUS).

● Class 3C: likely benign.

† VAF: variant allele frequency.

‡ This variant has not been previously described in public databases (either as somatic or germline) and in silico predictors classified it, some as benign and others as pathogenic. In this situation, NGS recommendations suggest to classify these variants as of uncertain significance.

Chr = chromosome.

present 6 years ago, but with lower variable allele frequencies (VAFs) (Table 1). All the changes observed suggested that we were in front of the same disease but evolving into a pre-leukemic phase.

To support the hypothesis that the CMML had evolved from *JAK2*V617F-positive PV, we purified CD14⁺/CD16⁻ cells (classical subtype monocytes, which are increased in CMML) from peripheral blood by cell sorting (BD FACSAria II), and Sanger sequencing confirmed the presence of the *JAK2*V617F and *ASXL1* mutations (already present at PV diagnosis) in this CMML cell subtype. With these results, the patient was diagnosed with PV that had transformed into CMML type 2. Due to the poor prognosis conferred by the cytogenetic and molecular alterations, treatment with intensive chemotherapy followed by an allogeneic bone marrow transplant was considered. Before the treatment was started and only six weeks after the diagnosis of CMML, the disease progressed to acute myeloid leukemia. Finally, the patient was treated with Venetoclax and Azacytidine and died of respiratory failure one month later without an identified infectious agent.

Polycythemia vera is a myeloproliferative neoplasm characterized by polyglobulia, bone marrow erythroid hyperplasia and the presence of a *JAK2* mutation.[2] The median survival of patients with PV is approximately 14 years, being up to 24 years in younger patients.[2] The main PV-related complications with an impact on overall survival (OS) are thrombotic events and progression to myelofibrosis or acute myeloid leukemia.[3] At 10 years, the probability of progression to acute leukemia is 2.3% to 14.4%, and it is often preceded by myelodysplasia.[4] In fact, it has been observed that dyserythropoiesis acquisition in general, and especially the presence of ring sideroblasts, are markers of progression in PV.[5]

Although only 20% of patients with PV present cytogenetic abnormalities at diagnosis,[4] patients may acquire karyotype alterations during the course of the disease.[4] Multiple studies have associated the acquisition of a complex karyotype with an increased risk of progression to blast phase,[3,6] as occurred in the present case.

Regarding molecular findings, recent retrospective studies have reported that approximately 30% of *JAK2*-positive PV cases present concomitant mutations other than *JAK2* (most frequently *TET2* and *ASXL1*).[7] The presence of *ASXL1*, *SRSF2*, and *IDH2* mutations has been associated with a lower OS and an increased risk of progression to fibrosis or blast phase.[2,7,8] On the other hand, although the *TP53* mutation is observed in only 1% of PV

patients in chronic phase,[6,7] it is detected in approximately 45% cases of acute leukemia evolved from PV.[7,8]

Although acute leukemia, preceded or not by a myelodysplastic phase, is widely described as a complication of PV, evolution to CMML seems to be uncommon and has been scarcely reported. In an article published by Holcombe in 1991, 2 cases similar to the case described herein were reported, but genetic analyses were not available in those patients.[9] Recently, Andrei et al published a case report describing a CMML transformation from PV with acquired mutations other than *JAK2*.[10] In this case they confirmed that the CD14⁺ population carried the *JAK2*V617F mutation, and they concluded that the CMML was originated from the PV clone. On the other hand, the *JAK2*V617F mutation is reported to be present in 7.8% of de novo CMML cases,[10] especially in proliferative subtypes.

Absolute monocytosis can be seen in myeloid neoplasms other than CMML.[11] In the case of primary myelofibrosis, monocytosis has been associated with progression to an accelerated phase.[12] Regarding PV, monocytosis is a frequent finding with an incidence of nearly 21%.[13] The Barraco study suggested that PV patients with monocytosis present a specific phenotype consisting in older age (>60 years) and a higher frequency of other mutations (*TET2* and *SRSF2*).[13] Furthermore, monocytosis in PV cases seems to have a negative impact on OS, identifying a subgroup of patients with a biologically more aggressive disease that share some genetic features with CMML.[13]

In the case described here, additional mutations were detected at PV diagnosis, and two (*ASXL1*, *TP53*) have been reported to confer poor prognosis in several myeloid neoplasms.[6,7] Strikingly, the evolution to CMML -with the subsequent progression to acute leukemia- was associated with a significant increase of the VAF of all the mutations, including the two with poor prognosis. However, the *TET2* VAF detected in the 2013 and 2019 studies would suggest that it may be a typical polymorphism for this patient, but since the patient died before germline tissue could be obtained, we were unable to verify the true origin of this variant. These findings highlight the crucial role that NGS techniques currently have in the diagnosis and follow-up of MPN, being able to identify patients with higher risk of evolution to a leukemic phase and, consequently, the use of NGS should be considered and extended to routine practice.[7,14] In fact, a recently study by Tefferi et al demonstrated the utility of integrating genetic information in the new survival scores for MPN.[14]

In summary, we present an uncommon case of transformation of PV to CMML with the same genetic alterations in the cells of

the 2 populations of the disease (indicating a common origin). The aggressive character of the disease and the fatal outcomes reported suggest that the appearance of monocytosis in PV cases, as described in primary myelofibrosis, might be considered as a sign of evolution to an accelerated or preleukemic phase. This case also highlights the usefulness of performing NGS studies at diagnosis of MPN and considering some genetic alterations in upcoming prognostic scores for PV, as suggested in recent studies.[14]

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