Case Report

Can Polycythemia Vera Evolve from Acute Myeloid Leukemia? Report of a Case Showing a Simultaneous Minor JAK2 V617F Mutated Clone

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Abstract. The evolution of myeloproliferative neoplasms (MPN) to acute myeloid leukemia (AML) occurs in 2-10% of patients, depending on the MPN subtype, treatment, and follow-up length. The reverse-path from AML to MPN has been rarely reported.

We herein present a 75 years old woman with AML, in whom a JAK2-V617F positive polycythemia vera (PV) emerged during follow-up, 19 months from the end of consolidation treatment. JAK2-V617F mutation screening retrospectively performed by Next Generation Sequencing (NGS) and JAK2 MutaScreen was negative on the bone marrow sample collected at AML diagnosis. However, using digital droplet PCR (ddPCR), we detected a minor JAK2 V617F mutated clone at AML onset. In addition, a TET2 R550 mutated clone persisted at stable levels throughout the disease course.

This case shows that a very small MPN clone masked at AML diagnosis may expand after treatment end and be erroneously interpreted as MPN evolving from AML. Very sensitive techniques such as ddPCR may help to unravel the true disease history in these cases.

Keywords: Polycythemia vera, Acute myeloid leukemia, JAK2 -V617F, TET2, NGS.

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Introduction. Myeloproliferative neoplasms (MPNs) are acquired clonal disorders characterized by aberrant hematopoietic proliferation and the increased tendency towards leukemic transformation.1,2 The risk of leukemic progression varies depending on the MPN subtype, with primary myelofibrosis (PMF) associated with the highest transformation risk (10-year risk: 10-20%), followed by polycythemia vera (PV) and essential thrombocythemia (TE) (10-year risk: 2-4% and about 1%, respectively).3,5 Activating mutations of the JAK/STAT pathway, primarily in the JAK2 gene (mainly JAK2 V617F), are frequently found in patients with MPNs and rarely occur...
in de novo Acute Myeloid Leukemia (AML) with a frequency of about 1%.1,6-7 This highlights the crucial role of JAK2 mutations as phenotypic drivers in MPN, particularly in PV, where JAK2 V617F mutation is found in 95% of patients.8 Here, we used this molecular marker to trace the origin of the disease in a case of PV manifested two years after AML diagnosis.

PV is a MPN characterized by abnormal red blood precursor cells proliferation and erythrocytosis, often associated with thrombocytosis and leukocytosis. Despite progression to AML is a possible evolution of MPN, only a few cases of JAK2 V617F-positive PV developing while in long-term remission from AML have been previously described.8-12 Here, we reported on a patient diagnosed with AML, who was treated with conventional 7+3 based-chemotherapy, achieved complete remission, and developed a JAK2-mutated PV two years after the end of consolidation treatment. We were interested in the biological features of the two diseases to define better the onset of the MPN clone and its kinetics.

A 73-year-old fit female came to our observation in June 2019 due to mild anemia (Hb 10.6 g/dl), monocytosis (1.760 x10^9/L), and neutropenia (0.700 x10^9/L). Personal and familiar history was negative, and blood counts assessed two years before were normal. The peripheral blood smear showed 25% myeloid blasts, and the bone marrow aspirate contained 80% CD34+/CD33+/CD7+, CD117+, CD13+, DR+, MPO+ blasts, consistent with the diagnosis of AML. Cytogenetics showed monosomy of chromosome X in 6 of 20 metaphases, while the molecular profile was negative for recurrent mutations, including mutations in FLT3, NPM1, IDH1, and IDH2 genes. Next-generation sequencing (NGS) was performed on DNA extracted from bone marrow mononuclear cells (BM-MNC) and highlighted the presence of TET2 p.Y1560* (variant allele frequency, VAF: 39.3%) and TET2 p.R550* (VAF: 46%) mutations. According to ELN 2017 risk stratification, the disease was classified as intermediate risk.13 She started the "7+3" chemotherapy regimen, obtaining complete remission (CR) and minimal residual disease negativity (MRD) by flow cytometry (sensitivity ≤ 0.035%). She then underwent two high-dose cytarabine consolidation courses. Treatment was complicated by one episode of pulmonary embolism during induction and later on by a catheter-related thrombosis during consolidation treatment. Thrombophilia tests, including antithrombin deficiency, protein C and S deficiency, factor V Leiden, prothrombin mutation, dysfibrinogenemia, anticaldiolin antibodies, anti-beta2 glycoprotein I antibodies, hyperhomocysteinemia and lupus anticoagulant, were negative. She was then monitored through sequential complete blood counts and BM aspirates, performed every three months. After two years, while still in CR, the patient's blood counts showed a progressive increase in hemoglobin and hematocrit (Figure 1). Erythropoietin levels were within normal lower limits (3.7 mU/mL, normal range: 3.7-31.5), and the JAK2-V617F mutation was positive in peripheral blood. The bone marrow biopsy showed increased cellularity (30%), normal myeloid maturation, less than 3% CD34+ blast cells, and no signs of fibrosis (MF-0, according to 2017 WHO classification),14 thus confirming the diagnosis of PV. The NGS analysis showed the following mutations: TET2 p.R550* (VAF: 46%), and JAK2 p.V617F (VAF: 43.4%), while cytogenetics was normal (46, XX). Figure 2 shows the expansion of the JAK2 V617F mutated clone. Due to the increased hematocrit, the patient started therapeutic phlebotomies and hydroxyurea, according to the "high" thrombotic risk category.15

We then traced back the JAK2 V617F mutated clone at the time of AML diagnosis using the MutaScreen assay (Ipsogen, Luminy Biotech, Marseille, France), which provides 2% cut-off sample (COS) positivity, and NGS (2% sensitivity). Both were negative for JAK2

![Figure 1](image1.png) **Figure 1.** Increase in hemoglobin and hematocrit after AML chemotherapy, while the patient was in CR. Progressive increase of hemoglobin and hematocrit until August 2021, when the patient started to be treated with phlebotomies and hydroxyurea. AML: acute myeloid leukemia. CR: complete remission. HCT: hematocrit (%). HB: hemoglobin (g/dl).

![Figure 2](image2.png) **Figure 2.** Fish plot showing variation of mutations over time. Variant Allele Frequency by NGS (Percentage of sequence reads observed matching a specific DNA variant divided by the overall coverage at that locus); FA: Fractional abundance by ddPCR (Absolute quantification of mutant clone divided by the absolute quantification of mutant in addition to wild type clones); AML: Acute Myeloid Leukemia; PV: Polycythemia Vera.
Figure 3. Droplet digital PCR assay for JAK2 V617F mutation detection in BM-MNCs during the disease course. Profile of JAK2 V617F of the sequential samples at diagnosis and follow-up, analyzed by ddPCR. Representative 1-D plots of the ddPCR amplification of a JAK2 V617F mutant (left panel) and a JAK2 wild-type allele (right panel). The pink line indicates the threshold. NTC: no template control.

Discussion. We report on a patient erroneously diagnosed as MPN evolving from AML, in which a small mutated clone present at the time of AML onset expanded during AML follow-up and manifested as overt PV. Indeed, during AML follow-up, the high levels of hemoglobin and the persistently increased hematocrit provided the clues to research the JAK2 mutation in the peripheral blood, which resulted positive.

Tracking back the JAK2 mutation at the AML diagnosis, we found a minor subclone detectable by ddPCR only, confirming this approach’s ability to detect mutant cases early during the disease course. The small clone was probably suppressed by the overt AML blast infiltration at diagnosis. Now the question is whether the two diseases are independent, or can AML be considered an evolution of PV in this case?

The patient was found to carry two TET2 mutations at high VAF. One disappeared at the time of achievement of CR, indicating that it was probably related to the AML clone, while the other was present at high VAF both at AML and PV diagnosis, suggesting that this was a large mutated clone, generating genomic instability, hence the predisposition for the development of both diseases. MPN may have arisen independently, but it is impossible to exclude that the diseases might have evolved from a common precursor.

The second hypothesis would be that AML represented the evolution of MPN. JAK2-WT development of PV has been described in 2-4% of cases, and usually, in these cases, TET2-mutation occurs “first”, as in our patient. However, secondary AMLs are generally characterized by poor prognosis and unfavorable or complex karyotype. In contrast, in this case, the intermediate-risk and absence of adverse mutations like TP53 render the diagnosis of de novo AML more likely. Accordingly, complete remission was achieved after "7+3" induction chemotherapy followed by a prolonged disease-free survival, which has now reached 33 months.

The two diseases are most likely independent. Indeed, Hb, HCT, WBC, and PLTs measured two years before AML diagnosis were normal, indicating that MPN was not present then. The patient did not have any other blood tests performed until AML diagnosis; however, it is unlikely that a MPN could have evolved in AML during this time, given that the average evolution time is usually longer (incidence of leukemic transformation of PV and ET: 2%-5% at 15 years). The patient had a pulmonary embolism and a catheter-related thrombosis during AML chemotherapy. Thrombophilic and cardiovascular risk factors (obesity, hypercholesterolemia, hypertension,
smoking, and second tumor) were negative. Therefore, we can suppose that the thrombotic tendency could be favored by the presence of the small myeloproliferative clone present at AML diagnosis.

One additional hypothesis is that the initial low-level JAK2 mutation was part of clonal hematopoiesis of indeterminate potential (CHIP), where JAK2 is one of the most frequently mutated genes.21,22,12 The lack of sequential samples after AML diagnosis did not allow for the correlation of the kinetics of emergence of the JAK2 mutant clone. Previous studies reported in the literature speculated the possibility that intensive ablative therapy favoring the presence of the small myeloproliferative clone present at AML diagnosis.

In conclusion, we interpret the two diseases as simultaneous and not sequential, further supporting the idea of competing clones in myeloid malignancies.23 The availability of sensitive diagnostic techniques such as ddPCR may provide the necessary diagnostic support to unravel these situations.

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