JAK2-mutated acute myeloid leukemia: comparison of next-generation sequencing (NGS) and single nucleotide polymorphism array (SNPa) findings between two cases

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ABSTRACT

JAK2 mutations are rare in *de novo* acute myeloid leukemia (AML), and *JAK2*-mutated acute myeloid leukemia (AML) patients usually have a previous history of myeloproliferative neoplasms (MPNs). Current advances in laboratory techniques, such as single nucleotide polymorphism array (SNPa) and next-generation sequencing (NGS), have facilitated new insight into the molecular basis of hematologic diseases. Herein, we present two cases of *JAK2*-mutated AML in which both SNPa and NGS methods added valuable information. Both cases had leukemogenic collaboration, namely, copy-neutral loss of heterozygosity (CN-LOH), detected on chromosome 9. One of the cases exhibited both *JAK2* and *IDH2* mutations, most likely having originated as an MPN with leukemic transformation, while the other case was classified as a *de novo* AML with *JAK2*, *CEBPA,* and *FLT3* mutations.

Keywords
Cytogenetics; Leukemia, Myeloid, Acute; Polymorphism, Single Nucleotide; Sequence Analysis, DNA

INTRODUCTION

Myeloproliferative neoplasms (MPNs) are clonal disorders of hematopoietic stem cells with increased proliferation of myeloid cells and effective maturation, leading to peripheral blood leukocytosis and excess erythrocytes or platelets.[1] According to the World Health Organization (WHO) 2016 revision of the classification of myeloid neoplasms and acute leukemia, MPNs are subdivided into (i) chronic myeloid leukemia (*BCR-ABL1*–positive); (ii) chronic neutrophilic leukemia; (iii) polycythemia vera; (iv) primary myelofibrosis; (v) essential thrombocythemia; (vi) chronic eosinophilic leukemia; and (vii) unclassifiable myeloproliferative neoplasm.[2] The final evolution of MPNs involves progression to fibrosis or leukemic transformation. Acute myeloid leukemia (AML) following MPNs is characterized by 20% or more blasts being present in the bone marrow (BM) or peripheral blood (PB).[3] MPN patients who develop AML have a poor prognosis, with a median survival of fewer than 6 months.[4] Leukemic transformation occurs in 1%, 4% and 20% of patients with essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PM), respectively, over a 10-year period.[5]

Mutations in *JAK2* have been identified in the majority of patients with PV, ET, and PM, underscoring

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the importance of constitutive activation of *JAK*2 signaling caused by mutations.[6] Genetic studies of paired samples before and after AML transformation suggest that there are at least two distinct routes for leukemic transformation. Patients who exhibit the *JAK2* mutation in MPNs progress to AML, suggesting that this mutation might be associated with the acquisition of additional genetic alterations. In contrast, patients who harbor the *JAK2* mutation in their MPNs have no evidence of the mutation in leukemic blasts post-MPN AML.[6,7] It is important to highlight that, despite the cooccurrence of *JAK2* mutations with other genetic alterations, *JAK2* mutations have rarely been reported in *de novo* AML (<5%).[8,9]

Cytogenetic and molecular cytogenetic techniques combined may provide a comprehensive analysis of chromosomal aberrations. Advances in laboratory techniques, such as single nucleotide polymorphism array (SNPa), a molecular cytogenetic method, and high-throughput sequencing, have facilitated new insight into the molecular basis of hematologic diseases. SNPa is a sensitive technique used to perform high-resolution genome-wide DNA copy number analysis and to detect copy-neutral loss of heterozygosity (CN-LOH). Next-generation sequencing (NGS) is capable of detecting single nucleotide variants (SNVs) or small insertions and deletions that have recently been shown as important molecular phenomena in AML.[10] To substantiate this idea, we describe two *JAK2*-mutated AML cases for which both SNPa and NGS added valuable information.

METHODS

Patients

The patients described in this report were seen for follow up at the Hospital São Paulo of the Federal University of São Paulo, São Paulo, Brazil. The Ethics Committee of the institution approved the study, and written informed consent was obtained from all patients (CAAE 00547512.5.0000.5505).

Conventional Cytogenetics

Karyotypes were made from BM samples using standard methods, and aberrations were described according to the International System for Human Cytogenetic Nomenclature (ISCN) 2016.[11]

Single Nucleotide Polymorphism Array

SNPa was performed on total BM (cases 1 and 2) and buccal cells (only case 1) samples. Two hundred and fifty nanograms of DNA were extracted and digested, amplified, purified, fragmented, labeled and hybridized using the Affymetrix CytoScan® HD Array. CEL files were created using the GeneChip® System 3000 7G according to the manufacturer's instructions. CEL files were analyzed using Chromosome Analysis Suite v3.0 (ChAS) software. Regions of copy number variations (CNVs) larger than 1 Mb and CN-LOH larger than 10 Mb identified by the ChAS Software or detected by visual inspection, regardless of gene content, are considered true aberrations, with the exception of those known to be normal genomic variants (present in the Genomic Variants Database [http://projects.tcag.ca/variation]) or identified in constitutional (buccal cells) SNPa analysis.[10] Aberrations identified by SNPa were described according to the ISCN 2016.

Next-Generation Sequencing

Total BM was used for DNA extraction. A 19-gene panel was screened using the Ion AmpliSeq AML Research Panel (Thermo Fisher). Reads were mapped to the HG19 reference human genome, and alignment results were analyzed using CLC Genomics Workbench software.[10] *FLT3-ITD* was tested in addition to the gene panel by PCR and capillary electrophoresis using the following primers: forward 5'–6FAM- tgcctattcctaactgactcatc-3' and reverse 5'- tctttgttgctgtccttccac-3'.

CASE REPORTS

Case 1: A 66-year-old female patient was referred to the Hematology service for investigation of polyglobulia (hemoglobin 17.0 g/dL and hematocrit 53.4%) associated with weight loss (18 kg over the last six months). However, admission lab tests showed hemoglobin of 9.4 g/dL, hematocrit of 33.4%, WBC count of 47.8 × 10⁹/L (with 22% blasts) and platelet counts of 119.0 × 10⁹/L. Bone marrow aspirate smear revealed 20% of blasts, and multiparametric flow cytometry immunophenotyping was compatible with AML (Positive: CD45 moderate, CD34, HLA-DR,

CD117, CD33 heterogeneous, CD13 heterogeneous, CD11b partial, CD64 partial heterogeneous, CD36 partial, CD7 partial. Negative: CD14, CD65, CD41, CD2, CD4, CD56, CD19, CD10). The bone marrow karyotype is as follows: 48,XX,+8,+21[11]/46,XX[1]. SNPa results are as follows: arr[hg19] (8)x2-3; arr[hg19] (21)x2-3; arr[hg19] 9p24.3p13.3(192128_35098008) hmz (Figure 1). NGS analysis revealed *IDH2* R140Q and *JAK2* V617F mutations. The patient was on chronic osteomyelitis treatment prior to initiating chemotherapy and died one month after admission due to infection.

Case 2: A 77-year-old female patient was admitted to hematology service with complaints of fatigue and easy bruising. Lab tests on admission showed hemoglobin of 10.3 g/dL, WBC count of 99.9 × 10^9/L (with 45% blasts) and platelet counts of 23.0 × 10^9/L. Bone marrow aspirate, showing 40% blasts and multiparametric flow cytometry immunophenotyping, was compatible with AML (Positive: CD34, CD117, CD13, CD45 moderate, CD33, HLA-DR, CD64, CD7. Negative: CD11b, CD10, CD19, CD15, CD36, CD14, CD65, CD2, CD56). The bone marrow karyotype did not present metaphases. SNPa results are as follows: arr[hg19] 8q23.1q24.12(107621735_119398978)×1; arr[hg19] 9p24.3p13.1(192128_40087758) hmz; arr[hg19] 11p15.5p11.2(198509_47404314) hmz

(mosaic) (Figure 2). NGS results revealed mutations in *FLT3-ITD*, *CEBPA* E309_T310insE and *JAK2* V617F. The patient was on conventional chemotherapy treatment and died three months after admission due to infection.

DISCUSSION

The two cases presented herein were classified as AML, one of which had a previous history of polyglobulia and weight loss (case 1), while the other had become recently symptomatic (case 2). Based on the clinical history, one may interpret the first case as leukemic transformation of a myeloproliferative neoplasm and the second as *de novo* AML.

Which tests could confirm the above-mentioned hypotheses: Conventional cytogenetics (karyotype), molecular cytogenetics (SNPa) or molecular tests (NGS)?

Cytogenetic abnormalities identified by karyotype allow the detection of clonal abnormalities, classifying cases into favorable, intermediate or unfavorable prognosis. Karyotype remains one of the most powerful predictors of outcome in AML.[12] However, approximately 50% of AML cases present a normal karyotype or present noninformative results, requiring other tests to better characterize the disease.[10]
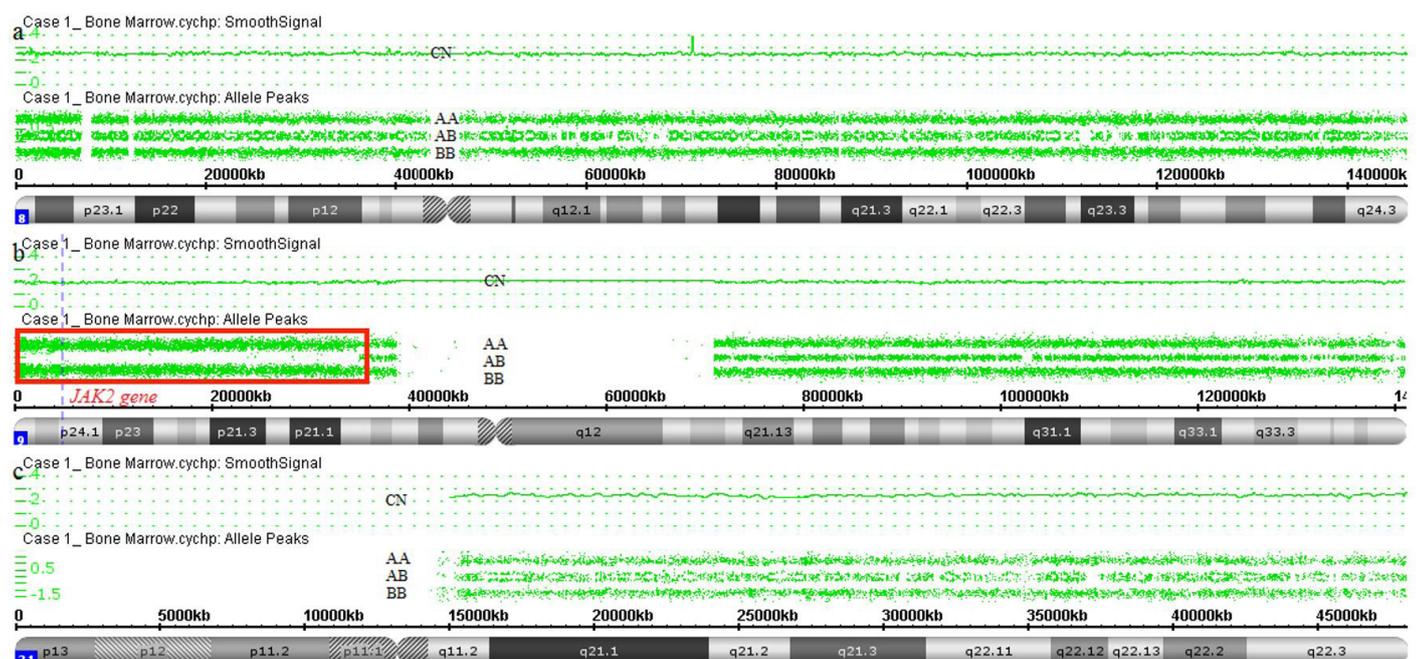

Figure 1. Genomic alterations detected by SNPa (Affymetrix CytoScan® HD array) in case 1. **a** – Mosaic gain of one entire chromosome 8 (CN: 2.57). **b** – Red box showing CN-LOH in 9p24.3p13.3. The JAK2 gene is located in this region. **c** – Mosaic gain of chromosome 21 (CN: 2.54).

Figure 2. Genomic alterations detected by SNPa (Affymetrix CytoScan® HD array) in case 2. **a** – Red box showing deletion in 8q23.1q24.12 (CN: 1.00). **b** – Red box showing CN-LOH in 9p24.3p13.1. The JAK2 gene is located in this region. **c** – Red box showing mosaic CN-LOH in 11p15.5p11.2.

Table 1. Technique comparison for identifying genetic abnormalities

Technique	Characteristics				
	Resolution	**Gain and Loss**	**Translocation**	**CN-LOH**	**SNVs/INDELs**
Karyotype	5Mb	Yes	Yes	No	No
SNPa	<1Mb	Yes	No	Yes	No
NGS	1Bp	Yes*	No	No	Yes

SNPa: Affymetrix CytoScan™ HD. NGS: Ion AmpliSeq™ AML Research Panel. *SNVs (Single Nucleotide Variants) / INDELs (Insertions and Deletions <50 base pairs).

Our first case presented a hyperdiploid karyotype, (trisomies 8 and 21), an aberration observed in 0.7% of AMLs,[13] allowing no additional clarification to the posed question. However, it allowed the classification of this case as intermediate risk by the European Leukemia Net genetic classification,[14] although this is a conceptually a high-risk disease as it fits into the "AML with myelodysplasia-related changes" (post-MPN AML). For case 2, the karyotype was noninformative, and in this condition, risk stratification could not be correctly interpreted.

SNPa is a complementary tool to karyotype, and may be applied for the evaluation of chromosomal losses, gains and CN-LOH. Combining SNPa with karyotype increases the detection rate of abnormalities

in 28%.[15] Advances in high-throughput sequencing techniques have facilitated new insights into the molecular basis of AML.[12,16] Indeed, each test has distinct performances characteristics, with variability in the detection rate being defined by the type of genomic alteration (Table 1).

SNPa confirmed the karyotype results in case 1 and added new information in both cases: CN-LOH on chromosome 9 (cases 1 and 2), CN-LOH on chromosome 11 (case 2) and partial deletion of chromosome 8 (case 2). CN-LOH indicates that an individual possesses two identical copies of one chromosome or chromosome region, i.e., this abnormality represents an important mechanism by which point mutations and other micro lesions can be

established in a homozygous state.[17] *JAK2* and *FLT3* mutations share a common mechanism that confers augmented reactive oxygen species, which induce DNA damage, resulting in homologous recombination events that initiate CN-LOH.[18]

NGS identified mutations in both cases, two in case 1 and three in case 2. The *JAK2* [V617F] mutation was identified in both cases. Case 1 presented 72.5% allelic frequency, and case 2 exhibited 94.3% allelic frequency. These values are compatible with a CN-LOH on chromosome 9 detected by SNPa, where the *JAK2* is gene located. In MPNs, the high *JAK2* [V617F] allele burden in PV represents a risk factor for progression to myelofibrosis. In contrast, high *JAK2* [V617F] allele burden is not significantly related to leukemic transformation.[19] The *JAK2* mutation constitutively activates the JAK/STAT signaling pathway, but the prognostic implications of this alteration remain unclear.[20]

Regarding the other mutations, *IDH2* (case 1) is involved in DNA methylation, and the relationship of this mutation with prognosis is still uncertain.[21] However, some groups have identified the *IDH2* mutation in a subset of patients with MPN who underwent leukemic transformation.[22,23] In addition, combined expression of *JAK2* [V617F] and *IDH2* [R140Q] induces MPN progression, alters stem/progenitor cell function and impairs differentiation in mice.[24]

The *CEBPA* mutation is involved in the development of myeloid progenitors into differentiated neutrophils. Previous studies suggest that monoallelic mutations in *CEBPA* are prognostically neutral, whereas biallelic mutations *in trans* are associated with a favorable prognosis in cytogenetically normal AML.[25] *FLT3-ITD* constitutively activate tyrosine kinase by interfering with the autoinhibitory function of the juxtamembrane domain, leading to enhanced RAS, MAPK, and STAT5 signaling. Mutations in this gene are associated with worse prognosis.[25]

JAK2 mutations are rare in *de novo* AML, and AML patients with *JAK2* mutations usually have a history of an antecedent MPN. The most common mutations observed in *de novo* AML, including *NPM1*, *DNMT3A*, and *FLT3*, are largely absent from AMLs that occur following an MPN.[6] Despite the infrequent incidence of *JAK2* mutation in AML, mutated *JAK2* could be therapeutically targeted in some cases with *JAK2* mutations.[8]

SNPa and NGS added valuable information to these analyses, as both cases exhibited leukemogenic collaboration of the detected mutations: one had *JAK2* and *IDH2* mutations, being most likely an MPN with leukemic transformation, while the other could be a *de novo* AML with *JAK2*, *CEBPA,* and *FLT3* mutations.

In summary, the present work supports the use of both SNPa and NGS as important tools for patients with AML, offering insights into the molecular pathogenesis and indicating that different mutation combinations and cytogenetic results could further stratify prognosis.

The authors retain an informed consent signed by the patients, and this manuscript is in accordance with the Institutional Ethics Committee.

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