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Case Report Open Access

A Difficult Diagnosis of a Myelodysplastic Syndrome/Myeloproliferative Neoplasm in its Limiting form and Interest of Next Generation Sequencing

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Introduction

Myelodysplastic/myeloproliferative neoplasms (MDS/MPN) as chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), atypical chronic myeloid leukemia (CML) *BCR-AB1L* negative, MDS/MPN-RS-T and MDS/MPN unclassifiable are defined in the classification WHO 2016. But in this classification, MDS/MPN unclassifiable is the less defined his category and is a diagnosis of exclusion.

These forms of MDS/MPN unclassifiable (MDS/MPN-U) have often a normal karyotype or show abnormalities in common with MDS. In absence of those cytogenetics abnormalities, this pathology is characterized by the presence of dysplasia over one or more lineage, cytopenia, no excess of blast in the bone marrow (<5%) and blood (<1%) and usually, there is a granulocytic or thrombocytic proliferation.

So, it is difficult to classify some patients on the simple clinical, cytological and cytogenetical presentation. Generally, the molecular biology is used to research a MPN molecular abnormally. Thus, without MPN molecular abnormally, it is important to realize a mutational profile of a suspected MDS/MPN-U.

Case and Methods

We report the case of a patient diagnosed in 2017 in CH Valenciennes. The diagnosis was established on the data of cytology (hemograms, myelogram), cytogenetics/FISH (medullary karyotype) and molecular biology.

A 65-year-old man was admitted in emergencies for a change of general state, asthenia, paleness and a loss of weight. At the entrance, the full blood count revealed a non-regenerative anemia (Hb: 6.9 g/dL, reticulocytes: 95 × 10^9/L), a hyperleukocytosis (WBC: 118×10^9/L) and a thrombopenia (platelets: 63 × 10^9/L). The peripheral blood smear demonstrated an important increased number of white blood cells. The formula shows 76% neutrophils (86×10^9/L), 20% immature granulocytes and 1% of blast with granulocytes hypogranulation. The following formulas do not still show the presence of blast.

A bone marrow biopsy showed a hypercellular. Megakaryocytes were normal. We noticed a hyperplasia of granulocyte lineage with a respected maturation and a moderate hypogranulation. Erythroblastic lineage was discreetly decreased. There was no excess of blasts (4%). The cytological aspect suggested a myeloproliferative neoplasm (MPN). Bone marrow conventional cytogenetic analysis revealed a male karyotype without abnormaly. The fluorescence in situ hybridization (FISH) was also negative.

Molecular biology was then essential to the malignant blood disease diagnosis. The research for *BCR-ABL1* was negative in PCR-RT. *JAK2* mutation by digital PCR, *CALR* mutation by analysis of fragment and Sanger, *MPL* mutation by HRM (High Resolution Melt) and Sanger were all negative. A research of atypical fusion transcript in MLPA-RT (Reverse Transcriptase Multiplex Ligation-dependant Probe Amplification) was also negative.

Finally, the final possibility was to establish a molecular profile of myeloid neoplasm, a high throughput sequencing targeting 36 genes was realized. Next generation sequencing showed the presence of six mutations: *ASXL1* (Variant allele frequency [VAF] 50%), *EZH2* (VAF 50%), *CBL* (VAF 3%), *NRAS* (VAF 46%), *RUNX1* (45%), *SMC3* (VAF 50%).

Discussion

Diagnosis is not possible with cytological, cytogenetically and basic molecular biology. Next generation sequencing allows to detect several mutations to give an indication to the malignant blood disease. In fact, each mutation is present in different pathology.

ASXL1 mutations, gene involved in the reshaping of the chromatine [1], are frequently detected in myeloid malignant blood disease (CMML, MDS, AML (acute myeloid leukemia)) [2] and present a poor prognosis [3].

CBL mutations, gene coding for an ubiquitine ligase allowing the ubiquitination of many substrats and then those substrate are deteriorate by the proteasome [4], are detected in MDS/MPN, CMML, JMML [5]. *EZH2* mutations, a repressor gene of transcription [6], are detected in MDS and MDS/MPN [7]. *NRAS* mutations, affect intracellular way RAS and play an important role in the regulation of proliferation, survival, differentiation and cellular migration [8], are detected in MDS with a higher risk of transformation into AML [9].

RUNX1 mutations, gene coding for sub-unit of the heterodimeric complex core binding factor (CBF) and conferring a high stability on the DNA, essential to the definitive hematopoiesis [10] , are detected in MDS, AML and CMML [11,12]. *SMC3* mutations, composant of cohesin complex which allows the sister chromatid stability during the mitotic division [13], are detected in MDS, CMML, MPN and AML [14]. Mutational profile of malignant blood disease gives an indication to the diagnosis of limiting forms of MDS/MPN.

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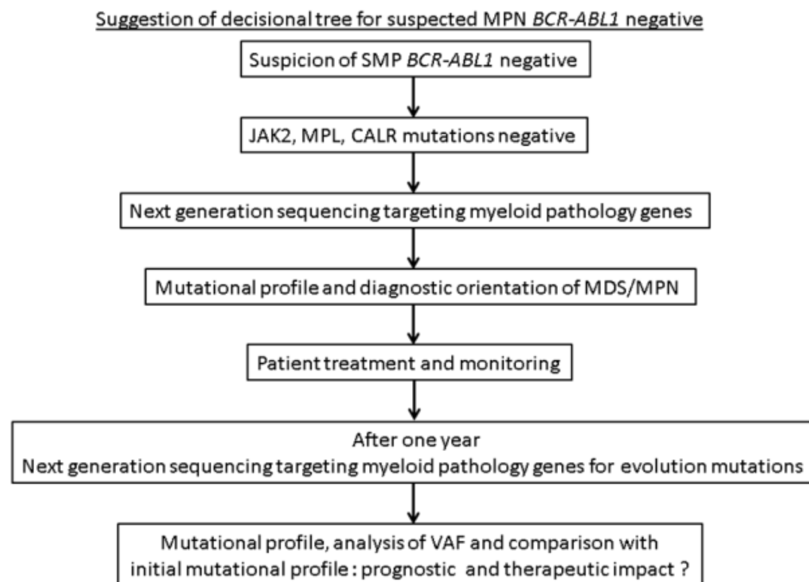

Figure 1: Suggestion of decisional tree for suspected MPN *BCR-ABL1* negative.

Conclusion

This case confirms the complexity the MDS/MPN-U diagnosis. This diagnosis requires cytological and cytogenetically analysis, and particularly a myeloid molecular analysis.

It's mandatory for a differential diagnosis to search the genes classically found in MPN BCR-ABL1 negative (*JAK2*, *MPL*, *CALR*). The high throughput sequencing of 36 genes allows us to obtain a mutational profile of the myeloid blood disease and give an indication for the diagnosis (Figure 1).

In our case report, the next generation sequencing allowed to classify the patient in a limiting form MDS/MPN. This patient presents a higher risk of transformation into AML. He is now treated by cytoreductive agent. It is necessary to follow the molecular and cytological evolution of this patient. The presence of new molecular alteration could impact the prognosis and change the treatment.

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