The JAK2 V617F Mutation in Chronic Myeloid Leukaemia within a BCR-ABL Positive Cohort of Beninese Patients

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Authors’ contributions

This work was carried out in collaboration between all authors. Authors SA and IA designed the study, performed the literature searches, statistical analysis wrote the protocol and wrote the first draft of the manuscript. Authors SA, IA, MA and AA managed the DNA extraction and PCR protocol. Authors BH, RM and LA handled the clinical management of patients. Authors BA, RD, FG and AL provided the scientific coordination of this study. All authors read and approved the final manuscript.

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ABSTRACT

Chronic myelogenous leukaemia (CML) is an acquired myeloproliferative disorder (MPD) characterized by a chromosomal abnormality (the Philadelphia chromosome) that causes the chimeric BCR-ABL oncogene. An acquired genetic mutation in exon 12 of the JAK2 tyrosine kinase gene leading to a substitution of a valine for a phenylalanine (V617F) has been described as the most common form of CML for those who test negative for the Philadelphia (Ph) chromosome. According to World Health Organization (WHO) classifications (2008), the JAK2 V617F mutation

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and the BCR-ABL translocation are mutually exclusive for Ph(-) and Ph (+) MP, respectively. We studied the JAK2 V617F mutation in Ph+ myeloid leukaemia in a cohort of 27 Beninese patients. The ARMS multiplex PCR technique was used to identify the JAK2 V617F mutation in all patients. Most of the patients were diagnosed as in the chronic phase (88.9%) of the disease, and all of them were carriers of the Philadelphia chromosome and considered Ph (+). No patients with the BCR/ABL translocation carried the JAK2 V617F mutation. JAK2 V617F is specific to Philadelphia gene negative MP.

Keywords: JAK2 V617F; BCR-ABL; Chronic Myelogenous Leukaemia (CML).

1. INTRODUCTION

Chronic myelogenous leukaemia (CML) is a myeloproliferative disorder (MP), a malignant disease of hematopoietic stem cells, characterized by the proliferation of progenitors of myeloid cells [1]. The course of the disease inevitably takes three phases, and in the absence of adequate treatment, it leads to an acute phase of leukaemia in three to five years that is particularly resistant to therapy and most often fatal. The Philadelphia chromosome (Ph), the most important chromosomal abnormality for CML, is a reciprocal and balanced translocation between chromosomes 9 and 22, termed as t(9;22)(q34; q11). This translocation induces the formation of a chimeric gene, the BCR-ABL gene, that codes for an oncoprotein with constitutive tyrosine kinase activity that disrupts several cell signalling pathways [2,3].

The Janus Kinase 2 (JAK2) gene is located on the short arm of chromosome 9 at position 9p24. The JAK protein, which has tyrosine kinase activity, mediates between membrane receptors and signalling molecules. In 2005, four teams working independently identified an acquired clonal and somatic mutation that interplays in the gene coding for the JAK2 cytoplasmic protein (JAK2 V617F) present in the majority of patients with polycythaemia vera, essential thrombocythaemia and primary myelofibrosis [4-7]. The JAK2 V617F mutation corresponds to the substitution of a guanine at position 1849 by a thymine in exon 14 of the JAK2 gene [8]. This substitution results in the replacement of a valine with a phenylalanine at codon 617 within the JH2 domain of JAK2 gene [8]. The JAK2 V617F mutation induces a decrease in the regulatory activity of the JH2 domain on the JH1 domain. In 2008, according to WHO classifications, the JAK2 V617F mutation and the BCR-ABL translocation were mutually exclusive of MP Ph (+) and MP Ph (-), respectively [9]. However, some cases indicating the presence of these two abnormalities have been reported [10-13]. In these cases, there were several clones of carriers of different abnormalities, and therefore, there was a significant risk for resistance to treatments based on tyrosine kinase inhibitors. Here, we investigated JAK2 V617F within a cohort of Beninese patients with chronic myeloid leukaemia.

2. PATIENTS AND METHODS

2.1 Patients

This study included 27 patients with CML. The diagnosis of CML was made on the basis of a haemogram and myelogram and confirmed by cytogenetic analysis for the presence of t(9;22)(q34;q11) (see Fig. 1).

![Fusion gene BCR/ABL shown by FISH with MetaSystems probes](image)

Fig. 1. Fusion gene BCR/ABL shown by FISH with MetaSystems probes

2.2 Sampling

In all patients, DNA extracted by the phenol-chloroform method was used for PCR-ARMS to search for the JAK2 V617F mutation. This technique was adapted from Jones et al. and was used to identify point mutations [14]. It uses four types of primers: a pair of external primers, a
specific sense primer of the wild-type allele and a specific antisense primer of the mutated allele known in our case as mutation JAK2G1849T. The sequence of primers used is shown in Table 1.

Table 1. Primer sequence used for PCR

| FO JAK2 | FAM-TCC TCA GAA CGT TGA TGG CAG |
| FWT JAK2 | FAM-GCA TTT GGT TTT AAA TTA TGG AGT ATa T |
| RO JAK2 | ATT GCT TTC TTT CAC AAG AT |
| RMT JAK2 | GTT TTACTT ACT CTC GTC TCC ACA Aa |

(FO = Forward outer, RO = Reverse outer, FWT = Forward wild type (normal), RMT = Reverse mutated)

The PCR mix was reconstituted from buffer, magnesium chloride, a mixture of deoxyribonucleotides (deoxynucleotides) and Taq Gold polymerase. A thermocycler (SimplAmpR, Lifes Sciences, USA) was used for amplification according to the following program: 95°C, 10 minutes (94°C, 45”; 62°C, 90”, 65°C, 25”) x 29 cycles and 65°C for a continuous period of time. The PCR products were separated on 1.5% agarose gel. Then, these products were visualized with a LOMBART R UV lamp transilluminator and photographed.

This study, carried out as part of academic work (was done as part of a master’s thesis), was conducted in strict compliance with Good Clinical Practice (GCP) rules. Informed consent was obtained from all patients. Confidentiality was rigorously respected during data collection. The information was processed anonymously. The results of these analyses were communicated to their physicians for the benefit of patients.

3. RESULTS

The mean age of the patients in our study was 46.51 ± 12.26 years with extremes ranging from 22 years to 70 years. There was predominance of males, with 20 men and 7 women, and a sex ratio of 2.87 to 1. At diagnosis, leukocyte count was greater than 50 10^9/L and present in all patients with an average of 197.03 ± 154.73 10^9/L and extremes of 3.3 at 544.7 10^9/L. An analysis of the bone marrow of all 27 patients confirmed a chronic stage of the disease in 88.9% of patients. The Philadelphia chromosome was searched for with fluorescence in situ hybridation (FISH) with MetaSystemsR Probes. All 27 patients included in our study carried the Philadelphia chromosome, and they were all under treatment based upon tyrosine kinase inhibitor. For all 27 patients, the search for the JAK2 V617F mutation by ARMS PCR using the four pairs of primers was negative. Fig. 2 illustrates the results for some patients after amplification and separation on agarose gel. With regard to this gel, patients 1 to 13 had a band at 463 bp and a single band at 229 bp. Therefore, these patients carried the non-mutated normal form of the JAK2 gene. They did not have a 279 bp band, which is the size of the JAK2 V617F mutation.

Fig. 2. Photo 1: 1.5% agarose gel separation after ARMS PCR amplification (Patients 1 to 13)

L: Size marker; T+: Positive control; T−: Negative control; To: Water control; 1-13: Patients 1 to 13
4. DISCUSSION

In our series of patients, CML was seen in young adults and in the elderly. The diagnosis of CML was based on clinical signs, almost constant leucocytosis and a myelogram that showed hyperplastic marrow. This diagnosis was confirmed by the presence of the BCR-ABL translocation, which is a pathognomonic abnormality in CML. This translocation is involved in 95% of cases detected by FISH and in 100% of cases detected by Polymerase chain reaction (PCR). The Philadelphia chromosome results from a reciprocal and balanced translocation between the long arm of chromosome 22 and the short arm of chromosome 9, termed as t(9;22)(q34.1,q11.2). This chromosomal translocation induces a genetic rearrangement between the Abelson gene (ABL) that is normally present on chromosome 9 and the breakpoint cluster region (BCR) gene that is located on chromosome 22. The resulting BCR-ABL fusion gene has a strong tyrosine kinase activity, and it is responsible for the leukemic transformation [2,3].

The JAK2 V617F mutation was detected in 2005, and according to WHO classifications, this mutation is characteristic of BCR/ABL negative myeloproliferative syndromes. Therefore, the detection of the JAK2 V617F mutation is an indispensable tool for a diagnostic approach to MPD (WHO, 2008). While this mutation is very specific to Phi MPDs, rare cases of patients carrying both the BCR-ABL translocation and the JAK2 V617F mutation have been reported in the literature [10-13]. Recently documented cases of these manifestations being found in the same patient have been discovered particularly after the patient has been treated with tyrosine kinase inhibitors. This would indicate an earlier presence of BCR-ABL translocated cell clones with the JAK2 V617F mutation, or it could be a secondary appearance of the BCR-ABL translocation in a patient carrying the JAK2 V617F mutation. However, some patients have a simultaneous appearance of both abnormalities. Patients exhibiting these two abnormalities would be prone to relapses and/or failures with tyrosine kinase inhibitors [15,16].

In our series, the search for the JAK2 V617F mutation was negative for all 27 patients. The absence of this mutation leads us to conclude that the JAK2 V617F mutation is exclusively specific for BCR-ABL negative myeloproliferative syndromes. However, it should be noted that the JAK2V617F mutation is localized on exon 14, whereas other mutations on exon 12 have been described by some authors [17]. Was there a presence of other molecular abnormalities especially on exon 12 in our population?

Table 2. Demographic and clinical features of the patient at diagnosis

<table>
<thead>
<tr>
<th>Features</th>
<th>Number of patient (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splénomegaly</td>
<td>19/27 (70.4%)</td>
</tr>
<tr>
<td>Headache</td>
<td>16/27 (59.3%)</td>
</tr>
<tr>
<td>Asthenia</td>
<td>26/27 (96.3%)</td>
</tr>
<tr>
<td>Fever</td>
<td>19/27 (70.4%)</td>
</tr>
<tr>
<td>Loss of weight</td>
<td>25/27 (92.6%)</td>
</tr>
<tr>
<td>Vertigo</td>
<td>16/27 (59.3%)</td>
</tr>
<tr>
<td>Conjunctival Palor</td>
<td>21/27 (77.8%)</td>
</tr>
</tbody>
</table>

Moreover, it should be noted that the JAK2 V617F mutation can be detected at very low levels simultaneously with BCR-ABL during the first diagnosis of CML or during the evolution of the disease. In this case, two situations have been described: the development of CML in patients that already have an MP with mutation of JAK2 (MP, TE or PV) and the occurrence of an MP or a PV (JAK2 V617F) in patients with CML in remission from imatinib or another TKI. Based on these observations, it seems that the JAK2 V617F anomaly appears prior to the acquisition of the 9/22 translocation [10,18,19].

In our population, no patients presented the JAK2 V617F mutation. Benguerachi working in Algeria in 2014 and using PCR-RFLP also found no cases of the JAK2 V617F mutation among 21 chronic phase patients [20]. In our study, we used a PCR-ARMS technique that was also used by Marwah working in Iraq in 2013 with 89 with chronic phase CML patients and accelerated with imatinib mesylate [11]. They reported a case of a JAK2 V617F mutation in a 52-year-old woman with chronic phase CML and who had been using imatinib-mesylate for five years.

In our cohort, however, the search for the JAK2 V617F mutation was justified in the event of a...
transformation of the phenotype of the disease or secondary resistance to the imatinib-based treatment, which is the basic treatment for CML in Benin.

5. CONCLUSION

Our study assessed the possible involvement of the JAK2 V617F mutation in a cohort of 27 Beninese patients with chronic myeloid leukaemia. The search for the mutation was negative for all patients. Our study thus proved that the JAK2 V617F mutation and the BCR-ABL translocation are specific for Ph (-) and Ph (+) myeloproliferative syndromes, respectively.

CONSENT

As per international standard or university standard, patient’s written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international or university standards, written ethical approval has been obtained and is preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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