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## Frequency of BCR-ABL 1 Fusion Transcripts Variants in Chronic Myeloid Leukemia Algerian patients

Fréquence des transcrits de fusion BCR-ABL 1 chez les patients Algériens atteints de leucémie myéloïde chronique

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### MOTS CLÉS

Leucémie Myéloïde Chronique, RT-PCR multiplex, Transcrit BCR-ABL1, transcrits de fusion rares, Co-expression b3a2/b2a2

### Résumé

**Objectif** - Dans le contexte de la leucémie myéloïde chronique (LMC), la recherche du transcrit de fusion BCR-ABL1 est devenue indispensable pour confirmer le diagnostic moléculaire. Dans cette étude, nous décrivons les différents types de transcrits de fusion BCR-ABL1 retrouvés chez des patients de l'Ouest algérien atteints de leucémie muéloïde chronique (LMC).

**Méthodes** - Au total 167 patients suspects de LMC ont été inclus dans cette étude. La recherche qualitative des transcrits de fusion a été réalisée au service de biochimie de l'Etablissement hospitalier et universitaire d'Oran, par la technique d'amplification en chaîne par polymérase après rétro-transcription (RT-PCR).

**Résultats** - Les deux types de transcrits de fusion BCR-ABL1 de type majeur Mb3a2 et Mb2a2 étaient présents respectivement dans 59,8 et 36.4 % des cas. Deux patients (1,8%) avaient un transcrit atypique rare de type e19a2 et deux autres (1,8%), ont co-exprimé les deux types b3a2 et b2a2.

**Conclusion** - Notre étude a confirmé les données de la littérature en montrant une incidence plus élevée du transcrit majeur.

## KEY WORDS

Chronic Myeloid Leukemia, multiplex RT-PCR, BCR-ABL1 Transcript, rare fusion transcripts, Co-expression b3a2/b2a2

## Abstract

**Aim** - In chronic myeloid leukemia (CML), the research of BCR-ABL1 fusion transcript became essential to confirm the molecular diagnosis. In this study, we describe the different types of BCR-ABL1 fusion transcripts found in CML patients in West Algerian patients.

**Methods** - A total of 167 CML suspected patients were included in our study. A qualitative research on fusion transcripts was carried out by reverse transcription polymerase chain reaction (RT-PCR) technique in the biochemistry laboratory of Oran's university hospital establishment.

**Results.** The two types of major BCR-ABL1 fusion transcript Mb3a2 and Mb2a2 were present in 59,8 % and 36,4% of cases respectively. Two patients (1,8%) had an atypical and rare transcript type e19a2 and two others (1,8%) co-expressed b3a2 and b2a2 transcript types.

**Conclusion** - Our study confirmed the data in the literature with a higher incidence of the major transcript.

## Introduction

The chronic myeloid leukemia (CML) is a rare chronic malignant hemopathy belonging to the group of myeloproliferative syndromes [1]. It represents approximately 7 to 15% of adult leukemia with worldwide incidence varying from 0,6 to 2,8 cases per 100.000 inhabitants [2].

In Algeria, the incidence is lower but it increased from 0,19 per 100.000 inhabitants in 1994 to 0,53/100.000 inhabitants in 2014[3]. On the cytogenetic exam, CML is characterized by presence of an abnormal chromosome in more than 95% of cases; the Philadelphia chromosome was discovered in the 60's [4]. It is the result of reciprocal translocation between the long arms of chromosomes 9 and 22: t (9-22) (q34; q11), which fuses the gene coding for the tyrosine kinase c-abl (cellular homologue of the viral oncogene Abelson) located on chromosome 9 to the «breakpoint cluster region (BCR)» gene located on chromosome 22. The resulting BCR-ABL1 fusion gene codes for an abnormal chimeric protein BCR-ABL1 of 210 kDa (p210) having a high constitutional tyrosine kinase activity responsible for the leukemic mechanism of CML [5, 6].

Depending on the position of the breakpoint on BCR and ABL genes, several chimeric mRNAs can be transcribed. In the context of CML, b2a2 (e13a2) or b3a2 (e14a2) transcripts (cut in Mbcr) are found in more than 95% of cases. Rarely, the break points interrupting the BCR and ABL1 genes can be in alternative regions leading to very rare and atypical transcripts: e8a2, e6a2, e19a2, e1a3, b2a3, b3a3 and e1a2 [7]. The e1a2 transcript is found especially in acute lymphoblastic leukemias with Philadelphia chromosome (LAL phi+).

The introduction in the 2000's of tyrosine kinase inhibitors (TKI), with imatinib as leader, revolutionized the prognosis of the disease and improved the overall survival of patients [8].

The fundamental criterion for diagnostic confirmation of CML remains the demonstration of the translocation t (9; 22) (q34; q11) in cytogenetics, and molecular characterization of the BCR-ABL1 fusion gene. The fusion of the BCR and ABL1 genes is objectified by PCR amplification on a total RNA extract obtained after reverse transcription (RT-PCR). A qualitative reverse transcriptase PCR on peripheral blood cells is mandatory to identify the type of BCR-ABL1 transcripts that can be appropriately followed when assessing response to TKI therapy [9].

The major problem with molecular diagnosis is that some patients can express variant transcripts. It is highly recommended to use multiplex amplification strategies able to amplify most of these transcripts [10, 11]. In this study, we present the molecular profile of western Algerian patients with CML.

## 2. Material and methods

This is a descriptive monocentric study with prospective collection involving 167 patients over a period of 6 years (2013-2019). Patients were mainly from Oran and from the other cities of the west Algeria (Mostaganem, Mascara, Sidi Bel Abbes, Ain Temouchent, Relizane, Tiaret and El Bayedh). The study was carried out at the biochemistry department of Oran's university hospital establishment. The study involved all patients over of 18 years old, regardless of sex, admitted for confirmation of diagnosis of CML.

The samples used for the study are whole blood collected

in tubes with ethylene diamine tetra tripotassium acetate (K3-EDTA) as an anticoagulant. The extraction of ribonucleic acids (total RNA) was carried out on a Maxwell 16® automatic extractor (Promega kit). To appreciate the purity of the extracted samples, RNA is quantified by spectrophotometer after a double reading absorbance at 260nm and 280nm.

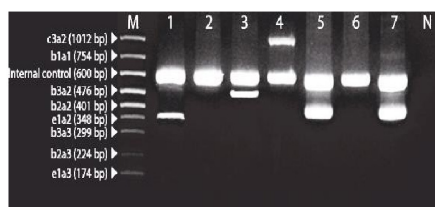
The qualitative research of the BCR-ABL1 transcript was carried out by reverse transcriptase polymerase chain reaction (RT-PCR) Multiplex technique using the seeplex™ Leukemia BCR/ABL kit (Seegene, Seoul, Korea). For each amplification, negative and positive controls were tested. The amplified products were separated according to their size by electrophoretic migration on 2% agarose gel and revealed by ethidium bromide.

The interpretation of the results was made after checking for the presence of ABL1 control gene bands (600 bp) showing good RNA quality, negative and positive control and, in the case of positive samples, a band corresponding to the expected size in the case of positive samples, a corresponding band to the expected size by comparing to the size marker used: Mb3a2 (476pb), Mb2a2 (401pb) e1a2 (348pb) and c3a2 or e19a2 (micro: 1012 bp) (Figure 1).

## Results

A total of 167 patients were included in our study. The molecular diagnosis of CML was confirmed in 107 patients (64%) with middle age 47, 60 ± 13, 76 years and extremes between 19 and 78 years. The sex ratio (m/w) is 1,05. However, the molecular study allowed us to exclude the diagnosis of CML in 60 patients (36%). The molecular study by multiplex RT-PCR has revealed that 103 patients (96%) had the major type of molecular rearrangement. Two patients expressed a rare and atypical transcript e19a2 (1,8%) and two others had a co-expression of b3a2 / b2a2 (1,8%) (Figure 2). The clinical and epidemiological characteristics and the results of the multiplex RT-PCR of our study group are shown in Table 1.

Fig1. Electrophoretic separation on agarose gel "Seeplex®" according to the size of the fragments.

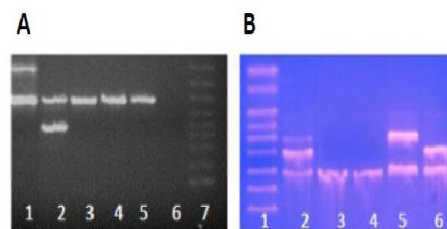


**N** : Negative control  
**1-7** :Samples : 1(e1a2), 2 and 6 negatives. 3 (b3a2), 4 (c3a2 ou e19a2), 5 et 7 (b2a2).  
**M** : Size marker

Table 1. Baseline characteristics of 107 patients with chronic myeloid leukemia

Variable	
<b>Gender, N (%)</b>	
• Men	55(51%)
• Women	52 (49%)
<b>Age</b>	
Median age (range)	48 [19-78]
<b>Disease phase, N (%)</b>	
• Chronic	100 (93)
• Accelerated	05 (05)
• Blastic	02 (02)
<b>BCR-ABL1 fusion transcripts, N (%)</b>	
• Mb3a2	64 (59.8)
• Mb2a2	39 (36.4)
• e19a2	02 (1.8)
• b3a2/b2a2	02 (1.8)
<b>Sokal score, N (%)</b>	
• Low	30 (28)
• Intermediate	51 (48)
• High	26 (24)
<b>EUTOS score, N (%)</b>	
• Low	85 (79)
• High	22 (21)
<b>WBC count, 10<sup>9</sup>/L median (range)</b>	141.9 [3.4-2210]
<b>Hb , g/dL median (range)</b>	10 [5.3-14]
<b>PLT count, g/L median (range)</b>	419 [90-710000]
<b>Myelemia, median (range)</b>	33.5 [0-80]
<b>Spleen, cm median (range)</b>	9.5 [0-40]

Fig 2. Multiplex RT-PCR products of patient samples.



**A-** 1: e19a2; 2 :b2a2; 3,4,5 : Negative patient; 6: Negative control;7: Size marke  
**B-** 1 : Size marke;2:b3a2/b2a2 ;3 and 4: Negative patient; 5:b2a2; 6:b3a2

### 3. Discussion

In the present study, we choose the Multiplex PCR with commercial kit to do a qualitative research of the different BCR-ABL1 transcripts. This choice was dictated by the great specificity and sensitivity of the kit using dual primer technology DPO™ (Dual oligo primer or DOP).

Conventional RT-PCR can be used to detect BCR-ABL1 transcripts of CML, however it can give a false negative result for atypical transcript and co-expression complexes, its protocol uses three distinct reactions: the first one for b3a2 and b2a2, second one for e1a2 and finally, the third one for c3a2 (or e19a2). On the other hand, the multiplex RT-PCR uses in a same reaction several pairs of specific primers, can identify the different variants of BCR-ABL1: Mb2a2, Mb3a2, m e1a2, b1a1, b3a3, e19a2, b2a3, c3a2 and e1a3, with 0.1% of detection limit. Indeed, several studies explained its interest in the determination of molecular isoforms. Moreover, the Korean study realized on 548 patients had compared the results of the two techniques and had found the same type of transcripts except for a patient detected as an atypical e1a3 transcript type by RT-PCR multiplex but negative by the conventional one [12].

Other studies on different ethnic groups identified atypical BCR-ABL1 transcripts on different parts of BCR and ABL1 exons such as e1a3, e2a1, e6a2, e8a2, e13a3 (or b2a3) and e14a3 (or b3a3) [13-15]. Other transcripts can be identified such as e12a3, e6a3, e19a3, e20a2 and e20a3 [7]. In the present study, the major transcript was present in 96% of cases with a higher incidence of the molecular isoform b3a2 than b2a2 (59,8 vs 36,4%). The variable frequencies were reported by several study groups about incidence of transcripts. Indeed, in a meta-analysis study of 26 articles, 22 (85%) had a higher incidence of the b3a2 transcript but in only 4 works, the incidence of b2a2 was higher [16]. In a series of 45503 newly diagnosed CML patients reported from 45 countries, it was found that the proportion of b2a2 and b3a2, including the cases co-expressing b3a2 and b2a2, was 37,9% and 62,1%, respectively. Other, rare transcripts were reported in 666/34561 patients (1,93%) [17].

In The Tunisian population study of Bennour et al, similar results were found, with a frequency of b3a2 (63,63%) twice more than of b2a2 (36,36%) [18]. Another Algerian study of 87 patients with CML, had found rates of 54; 44,60 and 1,20% respectively for the b3a2, b2a2 transcripts and the co-expression b3a2 / b2a2 [19].

In Far and middle Eastern countries (Korea, India, Iran, Syria and Irak population), the frequencies are almost similar with also a higher incidence of b3a2 than b2a2 [12, 20-23]. Similar frequencies had also been found in studies of European populations (Germany and Italy) [24, 25]. Paradoxically, in some populations, the incidence of b2a2 was higher than that of b3a2 with variable frequencies: Syrian population (57,1 vs 14,3%) [26], Mexican population (48 vs 35%) [27], Sudanese population (53,5

vs 41,9%) [28], USA population (42 vs 41%) [29], and Ecuadorian population (95 vs 5%, P= 0.01) [30]. This could be explicated by the difference of genetic profiles of these populations compared to the others.

The difference in frequency of b3a2 and b2a2 rearrangements observed in these different studies could be explained by different factors: geographic and ethnic origin, environmental factors, and the sensitivity detection of the method used. The co-expression b3a2/b2a2 found in our study could be explained by the alternative splicing process.

In Other studies; atypical complex co-expressions were found like those of the Mexican study (e1a2 / b3a2 / b2a2 and e1a2 / b2a2 / e19a2). These complex co-expressions reflect the existence of several leukemic clones [27].

### Conclusion

Our study allowed us, to make the molecular certainty diagnosis of CML. The diagnosis by multiplex gives more informations about variant transcripts, allowing a better choice of the specific primers for the subsequent following up of the molecular response under treatment.

At diagnosis, the determination of the molecular isoform and co-expressions is also clinically interesting to identify the relationships that may exist between the type of the hybrid protein and the phenotypic expression. Moreover, this study will offer possibility to compare, during molecular monitoring, the response to therapy with tyrosine kinase inhibitors function of BCR-ABL1 transcript type.

**Conflicts of interest:** None.

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