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THE MAIN *BCR-ABL* mRNA TRANSCRIPT TYPES AND HEMATOLOGICAL FEATURES OF NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN BURKINA FASO

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Abstract – *Objective:* *Chronic myeloid leukemia (CML) is characterized by the Philadelphia chromosome with an abnormally shortened chromosome 22, which is the result of a reciprocal translocation of chromosomes 9 and 22 that creates* BCR-ABL *fusion transcripts. CML accounts for approximately 15% of all leukemia cases and 24.3% of hematological disorders in Burkina Faso. The present study identified the main* BCR-ABL *fusion transcript variants using multiplex PCR in CML patients and investigated the hematological features at the time of diagnosis.*

Patients and Methods: *Total cellular RNA was extracted from 107 blood leukocytes using methods adapted from Chomczynsky and Sacchi (1987). A reverse transcription reaction was performed using a high capacity cDNA reverse transcription kit from Applied Biosystem (Ref 4368814) following the manufacturer's instructions.* BCR-ABL *transcript types were investigated using a homemade PCR method that was adapted and optimized from published protocols. A single reaction with multiple primers was used in multiplex PCR to detect and investigate the type and frequency of CML in 41 enrolled patients.*

Results: *The average age of patients was 39 years and ranged between 12 and 65 years. Two main transcript types were identified in 38 of the 41 patients included in the study. The most common transcripts were b2a2 (47.4%) and b3a2 (34.2%). Eight samples (18.4%) presented both types of transcripts. During the diagnosis, the average hemoglobin level, average white blood cell number and platelets in newly diagnosed CML patients were 8.3 g/dL; 270.1 G/L and 350.2 G/L, respectively.*

Conclusions: *Multiplex-PCR allowed for the rapid, specific and simultaneous detection of the most frequent* BCR-ABL *variant transcripts. The present study showed a higher frequency of b2a2 than b3a2 transcripts in Burkina Faso CML patients. These findings will guide us in the choice of specific* BCR-ABL *variant primers for the monitoring of patients undergoing Imatinib treatment.*

KEYWORDS: *Chronic myeloid leukemia,* BCR-ABL *variants, Multiplex PCR, Hematological features, Burkina Faso.*

Chronic myeloid leukemia (CML) is a clonal disorder of hematopoietic stem cells that results in increased myeloid cells, erythroid cells and platelets in peripheral blood. This myeloproliferative disorder is characterized by a shortened chromosome 22 named Philadelphia (Ph) chromosome, which results from

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a reciprocal translocation between the long arms of chromosomes 9 and 22, t(9;22)(q34;q11). The molecular consequence of the Ph chromosome is the fusion of the BCR (Breakpoint Cluster Region) gene on chromosome 22 to the ABL (Ableson Leukemia Virus) gene on chromosome 9. The translocation is found in over 90% of CML patients[1], and results in the generation of the *BCR-ABL* oncogene that encodes the chimeric BCR-ABL oncoprotein, which has a constitutive tyrosine kinase activity that promotes the growth advantage of leukemic cells[2]. The *BCR-ABL* gene encodes different fusion proteins that vary in size depending on the breakpoint in the *BCR* gene. Three breakpoint cluster regions in the *BCR* gene were described: major (M-BCR), minor (m-BCR), and micro (µ-BCR) breakpoint, which correspond to the three fusion proteins. Breakpoints that occur in M-BCR join exon 13 (also known as b2) or 14 (also known as b3) with exon 2 of *ABL* (a2), which results in the fusion transcripts b2a2 and b3a2, respectively[3,4].

The clinical path in CML is generally divided into a chronic phase, accelerated phase, and blast phase[5]. Common features in the chronic phase are fatigue, weight loss, abdominal fullness, bleeding, purpura, splenomegaly, leukocytosis, anemia, and thrombocytosis. The definition of the accelerated phase is vague, but the blast phase is defined by the presence of 30% or more leukemic cells in peripheral blood or the presence of extramedullary infiltrated blast cells[6]. The distinction of the different phases is important because patients whose leukemia is in the lymphoid blast phase respond to treatment regimens that are active against acute lymphoid leukemia[7].

CML accounts for approximately 15% of all worldwide leukemia cases[8]. In Burkina Faso and Africa in general, few data exist and are very fragmented. CML represents 24.52% of hematological malignancies in Togo[9], and it represents 24.3% of hematological malignances in Burkina Faso[10]. CML is a health problem with diagnostic difficulties, and patients face problems in developing countries. Rapid detection of the Ph chromosome or the precise identification of the *BCR-ABL* oncogene is essential in diagnosis and patient monitoring. CML diagnosis in Burkina Faso is essentially cytological and often occurs at the late stage of the disease. *BCR-ABL* mRNA transcript type is not routinely identified, in contrast to the European Leukemia Net recommendations. With the discovery of antityrosine kinases, such as Imatinib, which are used as therapeutics in CML, the rapid detection of the Philadelphia chromosome or the precise identification of the *BCR-ABL* oncogene becomes essential.

The present study identified the main *BCR-ABL* variants in Burkina Faso via the optimization of simple, reliable and reproducible multiplex PCR to allow an early and accurate diagnosis of CML and improve the monitoring of patients.

PATIENTS AND METHODS

Setting and population

This prospective study was performed in the Charles De Gaulle University Center Laboratory of Ouagadougou in Burkina Faso. Patients referred for a CML diagnostic were recruited between June 2018 and October 2019 from three university hospitals.

Sampling

The samples consisted of the withdrawing of 5 mL venous blood from each patient into an ethylenediaminetetraacetic acid (EDTA) tube. A laboratory processed the samples within 72 hours (h) of collection. Lysates were stored at -80 °C before extraction.

Hematological analysis and ribonucleic acid (RNA) extraction

Blood cell counts were performed using the hematology analyzer XN-1000™ from SYSMEX Corporation (Kobe, Tokyo, Japan) following the manufacturer's instructions. For RNA extraction, we used a method adapted from Chomczynsky and Sacchi[11] and total cellular RNA was extracted from 10^7 leukocytes. The amount of RNA was controlled using the Qubit™ fluorometer Q3287 from Invitrogen (Carlsbad, CA, USA) according to the manufacturer's instructions.

Reverse Transcription Polymerase Chain Reaction (RT-PCR) analysis

For the reverse transcription reaction, we used a high capacity complementary deoxyribonucleic acid (cDNA) reverse transcription kit from Applied Biosystems (Foster City, CA, USA, Ref 4368814), following the manufacturer's instructions. Briefly, 1 µg of RNA was incubated with the RT master mix in a final volume of 20 µL at 37°C for 2 h. The reaction was stopped by heating at 85°C for 5 minutes (min), and the resulting cDNA was stored at –20°C. A negative control (water instead of RNA) was included in all reactions. A homemade multiplex PCR method was optimized to investigate *BCR-ABL* transcript types, including various primer combinations as listed in Table 1. PCR was performed in a total volume of 20 µL reaction mixture containing 0.6 µM of each primer, 2.5 mM magnesium chloride ($MgCl_2$), 200 µM of deoxy-

TABLE 1. Primer sequences used in one step multiplex RT-PCR.

Primer name	*Sequences*
BCR-c	5' ACC GCATGTTCCGGGACA AAAG 3'
B2B	5' ACAGAATTCGCTGACCATCAATAAG 3'
C5e	5' ATAGGATCCTTTGCAACCGGGTCTGAA 3'
CA3	5'TGTTGACTGGCGTGATGTAGTTGCTTGG3'

ribonucleotides triphosphates (dNTP), and 1 U/µL Taq polymerase FIREPol^{R} from Solis Biodyne (Tartu, Estonia). Thermal cycling was initiated in a 2720 Cycler of Applied Biosystems (Foster City, CA, USA) under the following conditions: an initial denaturation step at 95°C for 10 min, followed by 40 cycles of denaturing at 95°C for 30 seconds (s), primer annealing at 55°C for 30 s, extension at 72°C for 90 s, and a final extension step at 72°C for 10 min. Optimal reaction conditions were adopted to investigate *BCR-ABL* transcript types in our cDNA samples. PCR products were separated using electrophoresis in a 2% agarose gel. The gel was stained with ethidium bromide. The expected bands were as follows: 808 base pairs (bp), normal BCR; 481 bp, e1a2; 385 bp, b3a2; and 310 bp, b2a2.

Statistical analysis

The data were analyzed using the Epi Info version 7 software developed by Centers for Disease Control and Prevention (CDC) Atlanta, Georgia, USA. The chi-squared test was used for comparisons, and each value was considered statistically significant for $p \leq 0.05$.

Ethical considerations

The hospital Ethical Committee approved our study. Written informed consent was obtained from all the participants, and the confidentiality of all patients was respected.

RESULTS

Characteristics of patients

Forty-one patients with suspected CML at three University Hospitals were enrolled in this study from June 2018 to October 2019. The average age of patients was 39 years and ranged between 12 and 65 years old. Hepatosplenomegaly was observed in 55% patients with suspected CML at diagnosis.

BCR-ABL mRNA transcript types identification

Multiplex-PCR is useful for the simultaneous detection of different transcripts that are expressed in the same patient. From the 41 samples tested in this study using multiplex RT-PCR, 38 patients were positive for *BCR/ABL* fusion gene, i.e., 92% of the total concerned population (Figure 1). This multiplex PCR enabled the detection of M-BCR/ABL and m-BCR/ABL fusion transcripts in one PCR. The sensitivity allows for the fast screening and detection of therapy-relevant rearrangements. This technique is useful for initial diagnosis to guide the clinical management of CML.

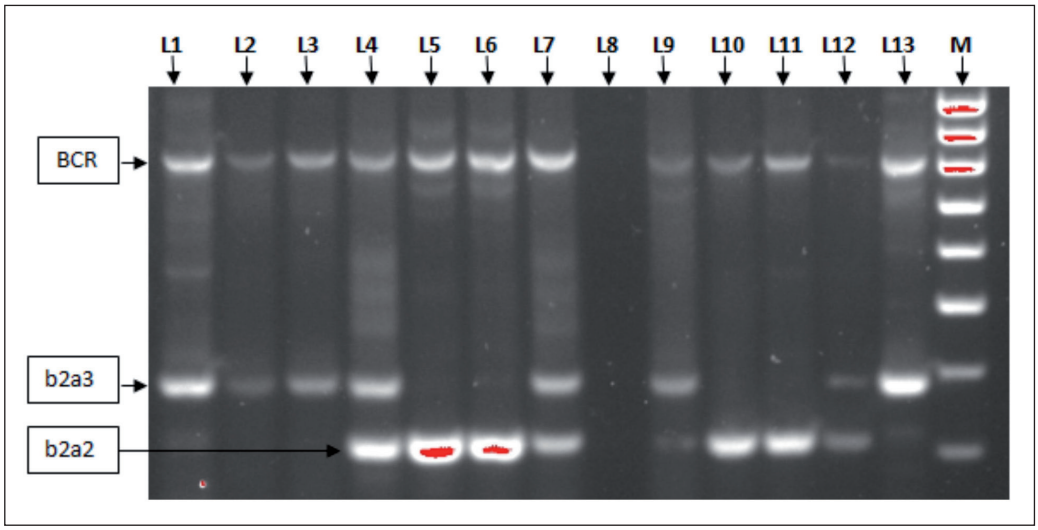

Fig. 1. Agarose gel electrophoresis of multiplex-PCR products from patients' samples. M: 100 bp DNA marker; Lane 8: Negative control; Lanes 5, 6, 10, and 11: 310 bp PCR products of the b2a2 BCR/ABL transcript; Lanes 1, 2, 3, and 13: 385 bp PCR products of the b3a2 BCR/ABL transcript. Lanes 4, 7, 9, and 12: 385 and 310 bp, dual PCR products of the b3a2 and b2a2 BCR/ABL transcripts.

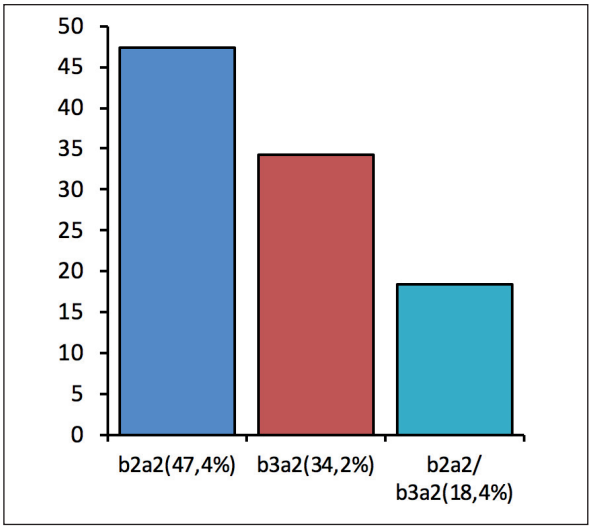

Fig. 2. Frequencies of main *BCR-ABL* transcript types in Burkina Faso.

The most commonly encountered transcripts in the present study were b2a2 and b3a2 in 18 (47.4%) and 13 (34.2%) of the 38 patients, respectively. Seven (18.4%) of the 38 samples contained dual transcripts of the combination of b2a2/b3a2 (Figure 2).

The main type of BCR-ABL transcription found in our study was b2a2 with frequency of 47.4%. The b3a2 type was found at a lower frequency of 34.2%, and the combination of b2a2 and b3a2 were found at 18.4%.

Hematological features of the newly diagnosed chronic myeloid leukemia

Analyses of the mean hemoglobin (HGB) level, the average number of white blood cells (WBC) and platelets (PLT) in newly diagnosed CML patients are shown in Table 2. Our results showed that 81.6% of patients had WBC higher than 50 G/L at diagnosis. The distribution according to the clinical phases of CML showed 35 patients (92.1%) were in a chronic phase. Three patients (7.9%) were in an accelerated phase of CML. For patients in an accelerated phase, the mean value of hemoglobin, the average number of WBC and the average number of platelets were 5.8 (3.7-7.6), 210.9 (97.8-399.6) and 92.6 (19-201), respectively. Our study also showed that 23 (60.5%) of the patients had a normal platelet count at diagnosis.

TABLE 2. Global hematological features of new CML patients at diagnosis between June 2018 and October 2019.

Parameters	*Mean*	*Median*
WBC (G/L)	270.1	290.1
HGB (g/dL)	8.3	7.8
PLT (G/L)	350.2	244.5

We analyzed the hematological features according to the *BCR-ABL* transcript type of newly diagnosed CML patients (Table 3). There was no significant difference between hemoglobin and platelets. However, the WBC of b2a2/b3a2 patients were higher than patients with b2a2 or b3a2 transcripts.

DISCUSSION

The present study identified the main *BCR-ABL* fusion transcript variants in CML patients in Burkina Faso and investigated the hematological features at diagnosis. CML is a pluripotent myeloproliferative stem cell disorder that is consistently associated with the *BCR-ABL* fusion gene. *BCR-ABL* mRNA transcript type detection in research is relatively expensive. This expense led us to investigate a more economic and comprehensive homemade conventional PCR method for its integration into routine monitoring protocols.

The average age of patients at diagnosis was 39 years and ranged between 12 and 65 years. Similar studies in Niger[12] and Togo[9] reported mean ages at diagnosis of 46.7, and 42.32 years, respectively. These findings show that CML is more frequent in young adults. The results of our study showed a higher frequency of b2a2 than b3a2 in CML patients in Burkina Faso (47.4% *vs.* 34.2%). Osman et al[13] reported that the frequencies of b2a2 and b3a2 were 53.5% and 41.9% of patients in Sudan. Paz-y-Miño et al[14] showed that up to 94.6% of patients had b2a2 fusion transcripts in Ecuador, and only 5.4% had b3a2 transcripts. Arana-Trejo et al[15] found b2a2 transcripts in 48% of cases in Mexico, b3a2 in 35% of cases, and b3a2/b2a2 in 7% of cases. These findings are concordant with our study. However, Essohana et al[9] detected b2a2 BCR-ABL in 38.24% of cases in Togo, b3a2 in 55.8% of cases, and the combination of b3a2 and b2a2 transcripts in 5.8%. Another result with a predominance of b3a2 transcripts was found in German and Italian patients. Hanfstein et al[16] reported b3a2 transcripts in 45% of German cases, b2a2 BCR-ABL transcripts in 41% of patients and 14% with both transcripts. Castagnetti et al[17] also showed a predominance of b3a2, with b3a2 in 52% of cases, b2a2 in 36% of cases and the combination of b3a2 and b2a2 in 11% of Italian patients.

Our results indicated that the coexpression of b2a2 and b3a2 transcripts in patients with CML was approximately 18.4%. This value is similar

TABLE 3. Hematological features of new CML patients at diagnosis according to the *BCR-ABL* transcript type between June 2018 and October 2019.

Parameters	*Median*			*p-value*
	b2a2	*b3a2*	*b2a2/b3a2*	
WBC (G/L)	231.2	248	381.5	0.04
HGB (g/dL)	7.7	7.8	8.4	0.86
PLT (G/L)	201	235.5	309	0.7

to Marcelle et al[18]. The author reported up to 20% for the simultaneous presence of both transcripts. Their study electrophoresed the transcripts, stained the gels with ethidium bromide, and visualized and blotted the bands using the Southern technique. To further confirm the identity of the bands, fragments were eluted from the gel, cloned into pUC 19, and sequenced. However, other authors reported a lower incidence, as described in Figure 3. Hanfstein et al[16] measured *BCR-ABL* transcripts in Germany using multiplex RT-PCR. Osman et al[13] detected transcripts using PCR and allele-specific primers in Sudan, and Arana-Trejo et al[15] performed nested and RT-PCR in Mexico. These differences in frequencies may be due to the sensitivity of the technique used, but the genetic background of the populations cannot be disregarded.

The dual expression of b2a2 and b3a2 may be the result of an alternative splicing mechanism rather than the presence of two different clones. Only one of the transcripts would prevail as the disease progresses[20].

One-step multiplex RT-PCR has its own advantages because it discriminated b2a2 and b3a2 in one single procedure. The benefit of the multiplex approach is the simplification and shortening of the RT-PCR procedure for the detection of b2a2 and b3a2 in a single reaction. Second, the amount of RNA used is minimized. Multiplex PCR assay is a clinically useful, efficient and fast procedure for the detection of fusion gene products, and it saves time and cost[21]. This technique is useful for the initial diagnosis to guide the clinical management of CML. Determination of the type of fusion transcript is crucial for primer choice and quantitative monitoring of the course of the disease during treatment. Our study performed a multiplex RT-PCR technique in all patients diagnosed with CML, and the *BCR/ABL* fusion gene was found in 92.6% of patients. Another study reported that more than 95% of patients with the diagnosis of CML were positive for the *BCR/ABL* fusion gene[22]. The variant types diagnosed in CML patients were essentially M-BCR (b2a2, b3a2). These results are consistent with the findings that b2a2 and b3a2 are expressed in 97–98% CML patients[23]. This study did not reveal significant differences between sex or all *BCR-ABL* variant transcripts ($p = 0.09$).

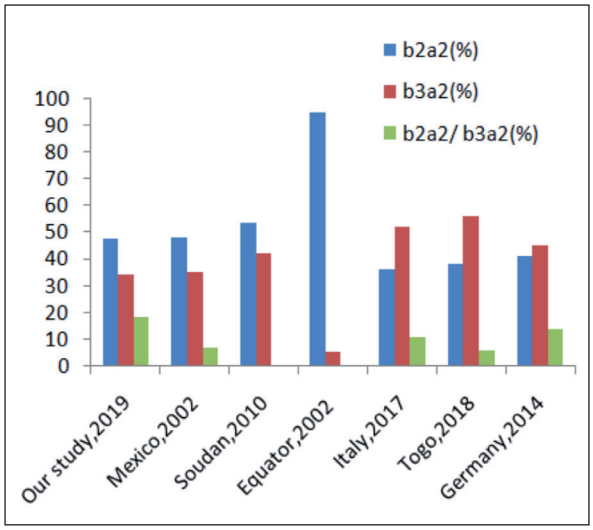

Fig. 3. Comparison of the frequencies of b3a2 and b2a2 *BCR-ABL* transcripts found in our study to other studies in the world (adapted from Mir et al[18]).

Our results did not show significant differences in hemoglobin level between b2a2 and b3a2 ($p = 0.86$). No difference in platelets absolute count was found between b2a2 and b3a2 ($p = 0.7$). However, our results showed significant differences in absolute leukocytes count in the b3a2, b2a2 and combination b2a2/b3a2 variants ($p = 0.04$). We showed that 81.6% (31/38) of patients had an absolute count of leukocytes higher than 50 G/L at diagnosis, and significant leukocytosis at diagnosis was 270 G/L on average. Segbena et al[24] reported an average leukocytosis of 188.71 G/L in Togo in 2012. Generally, leukocytosis and anemia are almost constant in CML patients at diagnosis. Hematological parameter degradation is a consequence of a delay in consultation. Delays in consultation, lack of specialists, patients' unfavorable economic conditions and technical difficulties contribute to the late diagnosis of the disease. Essohana et al[9] reported similar observations in 2018 in Togo. During 15 months of recruitment in the 3 major university hospitals in Burkina Faso,

we reported 38 new cases of CML, with an annual incidence of 31 cases. Koulidiati et al[10] reported hospital incidences of 5.8 cases/year in Burkina Faso in 2015. Essohana et al[9] in Togo and N'dhatz et al[25] in the Ivory Coast reported hospital incidences of 3.4 cases/year and 5.82 cases/year of CML, respectively. These data were mostly from retrospective studies because of the difficulties of keeping medical records and the lack of centralization of medical data in these countries. Our findings may reveal an underestimation of CML in Burkina and the subregion countries in general.

CONCLUSIONS

Multiplex-PCR allows for the rapid, specific and simultaneous detection of the most frequent *BCR-ABL* variant transcripts in patients with CML. The present study showed a higher frequency of b2a2 transcripts than b3a2 transcripts in CML patients in Burkina Faso. These findings will help guide the choice of specific primers for *BCR-ABL* variant identification, quantification and the molecular follow-up of patients undergoing Imatinib treatment.

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Conflict of Interests:
The authors declare no potential conflicts of interests.

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