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BONE MARROW, PERIPHERAL BLOOD AND PLASMA FOR QUANTITATION OF BCR-ABL TRANSCRIPT IN CHRONIC MYELOID LEUKEMIA

Nguyen Truong Son[1], Hoang Thi Thuy Ha[2]*, Pham Van Loi[2], Le Hung Phong[2], Vy The Ha[2], Phan Cong Hoang[2], Nguyen Thi Lan Huong[2], Tran Thanh Tung[2]

1. *Ministry of Health, Vietnam, Ba Dinh, Ha Noi, 100000, Vietnam.*
2. *Department of Hematology, Cho Ray Hospital, Ho Chi Minh City, 700000, Vietnam.*

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ABSTRACT

Molecular diagnosis based on the quantitative monitoring of BCR-ABL transcript in Chronic Myeloid Leukemia (CML) using quantitative real-time PCR (qRT-PCR) has been performed in the bone marrow. Recently, the reliability of using the source of peripheral as well as plasma for BCR-ABL transcript quantitation has been questioned. We herein reported a study on 172 paired samples, partitioned into 3 groups, including before treatment (newly diagnosed CML), under 1 year, and more than 1 year after initiation of TKI therapy for BCR-ABL transcript quantitation as performed by qRT-PCR. Based on the results, quantitatively, we concluded that there was only agreement of BCR-ABL measurements among bone marrow, peripheral blood, and plasma in the group of the newly diagnosed CML patients based on the evaluation of %IS-NCN and the kappa-value. Our data suggested that the source sample of peripheral blood and plasma were suitable for the BCR-ABL transcripts quantitation for CML patients without undergoing TKI treatment. The plasma-based quantification assay was more sensitive in untreated patients compared to the bone marrow test.

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Introduction

Chronic Myeloid Leukemia (CML) is a type of myeloproliferative neoplasms, specified by the high number of granulocytes in the peripheral blood, and accounted for 15%-20% of all cases of leukemia in adults [1-4]. In 1960, the abnormally shortened chromosome 22 resulting from reciprocal translocation with chromosome 9, known as the Philadelphia chromosome, was reported [5-7]. The t(9;22), somatically acquired tumor-specific chromosomal alteration, was found in over 90% of CMLs [8-10]. In the 1980s, the transposition creates the *BCR–ABL* fusion oncogene resulting from the juxtaposition of sequences of *BCR* gene (located on chromosome 22) (breakpoint cluster area) next to sequences from the *ABL* gene (located con chromosome 9) has been reported [1, 9-11]. As the results of transcription and translation of BCR-ABL fusion gene, based on the accurate location of the Ph transposition breakpoint, it encoded the 210 kDa (p210) BCR-ABL fusion protein or a 190 kDa (p190) BCR-ABL fusion protein within deregulated tyrosine kinase activity [9, 10]. It has been reported that the significant association between the p190 BCR-ABL with Ph-positive acute lymphoblastic leukemia and seldom emerges in CML diseased persons. Meanwhile, the p210 BCR-ABL was observed in more than ninety percent of CMLs patients. Hence, the attainment of the chimeric BCR-ABL oncogene, particularly the p210 BCR-ABL, was considered as the beginning stage in the pathogenesis of CMLs [10].

The early stage of CML is triggered by the tyrosine kinase activity of BCR-ABL [12-14]. Imatinib sesylate, the first FDA-approved Tyrosine Kinase Inhibitor (TKI), was successful in targeted treatment in the Ph+ CMLs patients, leading to a huge improvement in treatment efficacy [9, 15, 16]. The goal of TKI treatment is to achieve the major molecular reaction, which was described as a significant reduction of BCR-ABL transcript, eventually significantly reduced the risk of disease progression [9, 17]. Conversely, increasing the level of BCR-ABL transcript in the diseased persons, who did not obtain the major molecular response after therapy, conveyed a significantly increased risk of disease progression [9, 18]. Therefore, quantitative measurement of the level of BCR-ABL transcript in bone marrow may allow many advantages in designing the

Corresponding Author: Hoang Thi Thuy Ha; Department of Hematology, Cho Ray Hospital, Ho Chi Minh City, 700000, Vietnam. E-mail: thuyhado@yahoo.com.vn.

therapeutic strategy, including initial diagnosis as well as TKI-based treatment of patients with CMLs. The question is the practice to use peripheral blood as the source instead of bone marrow to evaluate the level of BCR-ABL transcript has been queried. However, it is finally not answered due to the controversy among studies. Therefore, here, this manuscript will report the application of real-time quantitative reverse transcription PCR (RQ-PCR) in the evaluation of the level of BCR-ABL transcript in bone marrow, peripheral blood, plasma in the Ph+ CMLs patients within/without the TKI-treatment in the Vietnamese population to find out their diagnostic values.

Materials and Methods

Ethics Statement and Sample Collection

The decision number of 602/CN-HĐĐĐ was obtained from the Institutional Ethics Board approval of the Medical Ethics Committee of the Cho Ray Hospital, HCMC, Vietnam. The enrolled patients were required to complete consent forms.

A total of 172 paired samples, comprised of both genders, spanning all adult ages, including bone marrow and peripheral blood with a confirmed diagnosis of Ph+ by Fluorescent *in situ* hybridization (FISH), were collected from December 2018 to July 2020 at the Department of Hematology, Cho Ray Hospital. Out of the 172 samples, there were 56 samples before treatment (newly diagnosed CML) (counting for 32.56%), 34 samples of under 1 year after commencement of TKI treatment (counting for 19.77%), and 82 samples of more than 1 year after commencement of TKI treatment (counting for 47.67%). All samples were available for further qRT-PCR analysis.

RNA Isolation, cDNA Synthesis, Quantitative Real-Time RT-PCR (qRT-PCR)

Total RNA was isolated from either bone marrow or peripheral blood according to the recommendation of the RNA Blood Mini kit (#Cat: 52304, QIAgen). Total RNA was isolated from plasma according to the guideline of DSP Virus/Pathogen Midi (#Cat: 937055, QIAgen). RNAs were synthesized into cDNA according to the guideline of the RT kit (#Cat: 679913, QIAgen). cDNA was stored at 4 ºC for further analysis.

The resulting cDNA were used for qRT-PCR analyses to analyze the BCR-ABL transcript presented in bone marrow, peripheral blood, or plasma according to the guideline of the Ipsogen BCR-ABL Mbcr RGQ RT-PCR kit (#Cat: 670913, QIAgen), and Ipsogen BCR-ABL mbcr kit (#Cat: 670013, QIAgen). The result of qRT-PCR was deemed positive for BCR-ABL transcript in the case of IS-NCN (international standard scale –normalize copy number) more than 0.003%

Statistical Considerations

Data was analyzed by using STATA 14.0 statistical software. All the p-values were two-sided, and p-value < 0.05 was considered a significant difference. The concordance rate of BCR-ABL results between two types of source samples was evaluated by Kappa test. The correlation between results was evaluated by Spearman of Pearson correlation test.

Results and Discussion

The Characteristic of 172 paired samples, divided into three groups, were summarized in **Table 1**.

Table 1. Clinical Data

	Group 1	**Group 2**	**Group 3**	**Total**
No. patients (%)	56 (32.56)	34 (19.77)	82 (47.67)	172 (100.00)
Sex				
No. male (%)	17 (30.36)	18 (52.94)	47 (57.32)	68 (39.53)
No. female (%)	39 (69.64)	16 (47.06)	35 (42.68)	104 (60.47)
Age, median	43.23	47.47	45.95	45.37

Note Group 1: Before treatment (newly diagnosed CML); Group 2: Under 1 year after commencement of TKI treatment; Group 3: More than 1 year after commencement of TKI treatment.

Fifty-six paired before treatment, thirty-four paired on under-12-month treated samples, eighty-two paired on over-12-month treated samples were evaluated using qRT-PCR. The positive cases of BCR-ABL transcript of each group from bone marrow, peripheral blood, or plasma were shown in **Table 2**. As the result, the BCR-ABL transcript was detected by qRT-PCR have shown that the source of bone marrow, peripheral blood and plasma are equally effective at detecting the number of BCR-ABL transcript positive case in the group of patient with recently detected CML. In the group of under-12-month treated samples, only equally effective at detecting the number of BCR-ABL transcript positive cases was found in the source of bone marrow and peripheral blood. In the group of more than 12 months treated samples, the high percentage of BCR-ABL transcript positive case was observed in the source of bone marrow (67 of 82 cases, counting for 81.71%), followed by the source of peripheral blood (61 of 82 cases, counting for 74.39%), and lowest in the source of plasma (33 of 82 cases, counting for 40.24%).

Table 2. Data Analysis of BCR-ABL Transcript

Bone marrow		**Peripheral blood**		**Plasma**	
P (n, %)	N (n, %)	P (n, %)	N (n, %)	P (n, %)	N (n, %)
Group 1 (n = 56)					
56 (100.00)	0 (0.00)	56 (100.00)	0 (0.00)	56 (100.00)	0 (0.00)
Group 2 (n = 34)					
34 (100.00)	0 (0.00)	34 (100.00)	0 (0.00)	28 (82.35)	6 (17.65)
Group 3 (n =82)					
67 (81.71)	15 (12.29)	61 (74.39)	21 (25.61)	33 (40.24)	49 (59.76)

Note Group 1: Before treatment (newly diagnosed CML); Group 2: Under 1 year after commencement of TKI treatment; Group 3: More than 1 year after commencement of TKI treatment.

Based on the percentage of detecting positive cases, it could be concluded that there were equal effectiveness in all sources of samples in a group of patients with recently detected CML, and also in the source of bone marrow and peripheral blood in a group of under-12-month treated patients. However, to answer the question is the reliability of these sources for BCR-ABL1 detection for further diagnostic application, the quantification of whether or not there is a quantitatively disparate among them should be verified as performed by determination of IS-NCN.

The results of %IS-NCN of BCR-ABL transcript of all sources of samples in a group of patients with recently detected CML were shown in **Figure 1**. As shown in **Figure 1**, the value of %IS-NCN were 62.34%, 59.88%, and 70.58% in the bone marrow, peripheral blood, and plasma, respectively. No significant difference was found between the %IS-NCN in the bone marrow and peripheral blood ($p = 0.32 > 0.05$), thus, it indicated that equal effectiveness was observed in the detection of BCR-ABL transcript in the bone marrow and peripheral blood. In the source of plasma, the value of %IS-NCN was significantly more than bone marrow ($p = 0.02 < 0.05$), thus, it indicated that the efficiency of BCR-ABL transcript detection in plasma was better than in bone marrow, which was considered as the standard source for detecting BCR-ABL transcript. Additionally, the agreement between bone marrow and peripheral blood, bone marrow, and plasma was quantified by the kappa test. As the results show, $\kappa = 1.0$, suggesting that the performance of BCR-ABL transcript detection in peripheral blood, plasma was equivalent to that of the detection in the bone marrow.

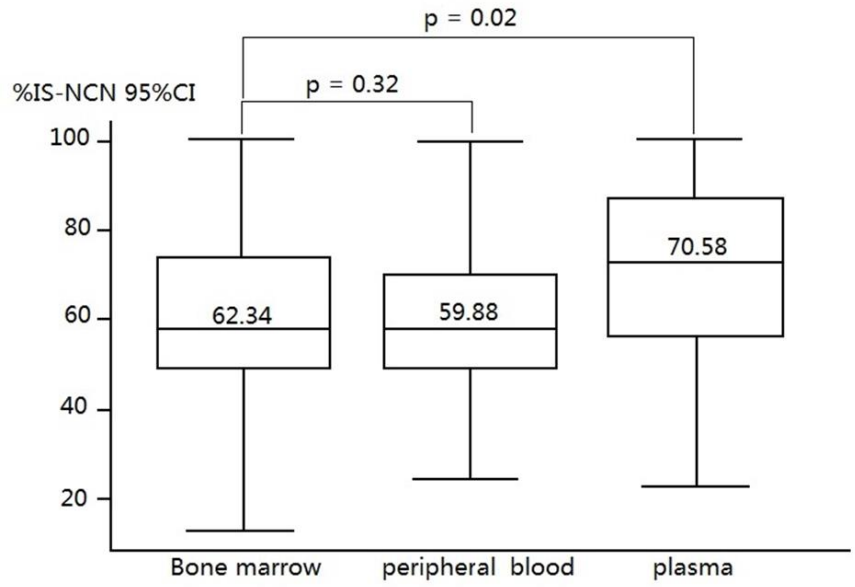

Figure 1. The Value of %IS-NCN in All Sources of Sample in the Group of Patient with Recently Detected CML

Concerning the group of patients within under 1 year after commencement of TKI treatment, the results of IS-NCN of BCR-ABL transcript detection in the bone marrow and peripheral blood were shown in **Figure 2**. Even though the accordance between bone marrow and peripheral blood was observed ($\kappa = 1.0$), as shown in **Figure 2**, the value of %IS-NCN in bone marrow was significantly more than peripheral blood ($p = 0.04 < 0.05$), suggesting that qRT-PCR monitoring of BCR-ABL transcripts in the patient under 1 year after commencement of TKI therapy showed lack of agreement in the bone marrow and peripheral blood samples.

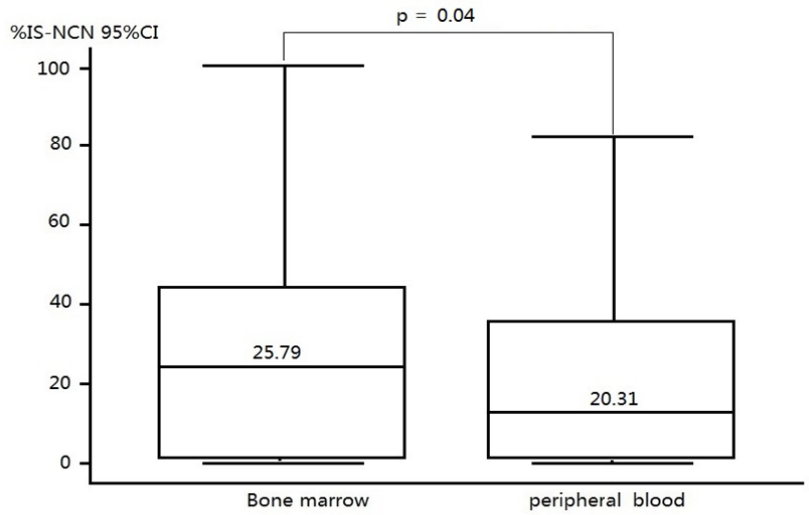

Figure 2. The value of %IS-NCN in the source of bone marrow and peripheral blood in the group of a patient under 1 year after commencement of TKI therapy

Therefore, based on this statistical analysis, it is concluded that, quantitatively, bone marrow, peripheral blood and plasma measurements of BCR-ABL are similar, suggesting that using the source of peripheral blood, plasma as the substitute for bone marrow in controlling BCR-ABL transcript levels in the patient with newly diagnosed CML.

It has been reported that the precisely quantifying BCR-ABL transcripts as a surrogate indicator of illness that emerges to have independent prognostic importance for CML diseased persons [15, 19]. Due to its specificity and sensitivity, qRT-PCR has been considered a powerful tool in detecting the BCR-ABL transcript in CML patients [9, 20]. qRT-PCR analysis in bone marrow was considered as the golden test for monitoring the quantitative definition of the levels of BCR-ABL transcript in CML patients [4, 19]. Besides the advantage of bone marrow test is that of its hypercellularity with marked granulocytic proliferation and significantly increased myelocytes, the bone marrow sampling still provides several distinct disadvantages, such as costly, pain, bruising, increasing the risk of infection or tingling in the legs. Therefore, finding the different less invasive source samples as the substitute for bone marrow in the monitoring of BCR-ABL transcript levels in the patient with newly diagnosed CML was a necessity. Recent studies showed that the quantitative analysis could be performed in the peripheral blood as well as plasma of patients, who were either newly diagnosed CML or following the period of TKI treatment. However, there are still controversies among these studies. To determine whether quantitatively controlling of BCR-ABL transcript of CML is comparable using peripheral blood, plasma to bone marrow samples, we performed a prospective study using qRT-PCR for paired samples, including collecting from bone marrow, peripheral blood, and plasma from a total of 172 individuals, divided into three categories as described above.

In our study, **Table 2** displayed the percentage of BCR-ABL transcript positive case in all groups, suggesting that there were no disparate sources of sample in the group of newly diagnosed CML samples, as well as between bone marrow and peripheral blood in a group of under-12-month treated samples. The differences of percentage BCR-ABL transcript positive case between bone marrow and plasma in a group of under-12-month treated samples, as well as all sources in the more than 12 months after initiation of TKI therapy samples, indicated that the bone marrow was the optimal source of samples for monitoring the BCR-ABL transcript for CML patients. For the newly diagnosed CML data, as shown in **Figure 1**, there were an agreement between bone marrow and peripheral blood within the p-value < 0.05, and the kappa value of 1.0. Especially, the %IS-NCN of plasma was significantly higher than bone marrow ($p < 0.05$), suggesting that an agreement and better efficiency of BCR-ABL transcript detection were achieved in the plasma test compared to the bone marrow test. It was similar to the study of the previous study was that the peripheral blood cells are the proper source of RNA for the quantitative detection of BCR-ABL transcript [21]. According to the TKI treatment, such as imatinib, the results suggested that low levels of BCR-ABL transcript in peripheral blood, as well as plasma, were perhaps the side effects of TKI medicines in removing monocytes, macrophages in patients of CML. As a result, an insufficient number of macrophages among patients receiving tyrosine kinase treatment would delay the clear-up procedure of dead BCR-ABL positive cells [21]. Therefore, based on our results, the peripheral blood, as well as plasma, were the substitutable material for bone marrow for quantifying BCR-ABL transcript condensation in newly diagnosed CML diseased persons.

Conclusion

The source sample of peripheral blood and plasma were appropriate for the quantification of BCR-ABL transcripts as the surrogate marker for CML patients without underwent TKI treatment. The plasma was more sensitive for quantification assay of BCR-ABL transcripts in untreated patients compared to bone marrow test.

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Conflict of interest: None

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