

# Emergence role of nucleated red blood cells in molecular response evaluation for chronic myeloid leukemia

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**Purpose:** To investigate and evaluate the role of nucleated red blood cells (NRBCs) and other markers in predicting remission failure in chronic myeloid leukemia (CML) patients treated with imatinib.

**Methods:** Seventy-one CML patients with *BCR-ABL*(+) in bone marrow cells were selected for this study. Molecular response evaluations were done every three months according to the recommendations of European LeukemiaNet (ELN). Patients were defined as remission failure if *BCR-ABL* transcripts >10% after 6 months (T6), >1% after 12 months (T12), and >0.1% after 18 (T18) months of treatment. The logistic regression was used to determine the optimal cut-off point of each marker and test the association of marker level with remission failure.

**Results:** The median NRBC, white blood cells, blast cells, basophils, and platelets were declined parallel with the decreases of *BCR-ABL* transcripts in bone marrow cells after 6 months of treatment ( $P<0.001$ ). In addition, NRBC was almost not found in the blood of patients who archived good response at T6, T12, and T18 time-points. Interestingly, patients with a high level of NRBC (cut-off:  $0.003 \times 10^9/L$ ) have higher *BCR-ABL* transcripts compared to others. The elevated NRBC at T6 (OR=6.49,  $P=0.042$ ), T12 (OR=6.73,  $P=0.007$ ), and T18 (OR=5.96,  $P=0.009$ ) time-points was identified as an independent factor for the remission failure.

**Conclusion:** The results of this study showed that a high number of NRBC in peripheral blood of CML patients is associated with higher *BCR-ABL* transcripts in bone marrow cells. The elevated NRBC might serve as an independent marker for molecular remission failure in CML.

**Keywords:** NRBC, BCR-ABL, CML

## Introduction

Chronic myeloid leukemia (CML) is a hematological disease which has been characterized by the malignant proliferation of hematopoietic stem cells carried the translocation  $t(9;22)(q34;q11)$ .<sup>1</sup> This translocation leads to the formation of the *BCR-ABL* fusion gene which encodes for the similar name protein with hyper tyrosine kinase activity in cancer cells. The *BCR-ABL* rearrangement occurs in  $\approx 95\%$  CML patients with two major (b3a2 and b2a2; or p210 variant), one minor (e1a2 or p190), and some rare variants.<sup>1</sup> Before the imatinib era, CML patients were treated with chemotherapy agents in classical regimens such as hydroxyurea, cytarabine, and interferon-alpha. Imatinib therapy (a tyrosine kinase inhibitor, TKI) is the breakthrough in treatment for CML patients which help to improve the response rate, survival, and quality of life for

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patients compared to the classical chemotherapy.<sup>2</sup> The drug acts as the competitor with adenosine triphosphate to block the BCR-ABL fusion protein, hence inhibits the transductions of intracellular signaling pathways, and inhibits the uncontrolled proliferation of CML cells. After the success of imatinib, dasatinib and nilotinib were approved as the first-line agents in CML treatment recently.<sup>1,3,4</sup>

On TKI treatment monitoring, the *BCR-ABL* transcripts were recommended to be measured every three months to determine whether patients reach the molecular remission or not.<sup>1</sup> This diagnostic test was suggested to perform on the real-time quantitative polymerase chain reaction which is the current standard technique. Although the peripheral blood cells were approved recently as a sample source for *BCR-ABL* quantification, the diagnostic test performed on bone marrow cells still remains as the gold standard method.<sup>1</sup> Besides the *BCR-ABL* transcripts, the restore of hematopoiesis to normal, and some related immune cells such as neutrophils, blast cells, and basophils have been used in response evaluation for CML.<sup>1,5-7</sup>

One of the well-known cells of the hematopoietic system, the nucleated red blood cell (NRBC) is a promising marker for clinical application. The presence of NRBC in peripheral blood has been shown to be associated with a variety of serious conditions including solid cancer, hematological diseases, cardiovascular diseases, hemorrhage, infections, and others.<sup>8,9</sup> Besides, this cell plays an

important role as a powerfully predictive factor of survival for both hospitalized adult patients and infants.<sup>8,10-25</sup> However, a little information of NRBC in CML has been shown, especially in patients treated with TKI.<sup>9</sup> This study aims to investigate and evaluate the role of NRBC and other routine tests in predicting remission failure with imatinib in CML, as the simple, cost-effective markers.

## Materials and methods

### Patients and sample

A total of 71 CML patients with *BCR-ABL*(+) in bone marrow cells who were treated with imatinib from Jan-2016 to May-2019 at Cho Ray Hospital were selected for this study (approval number 602-2017-CN-HDDD) (Figure 1). The molecular response evaluations were done every three months according to the recommendations of ELN.<sup>1</sup> Patients reach response milestones if the quantitative *BCR-ABL* transcripts  $\leq 10\%$  after 6 months,  $\leq 1\%$  after 12 months, and  $\leq 0.1\%$  after 18 months of treatment. Conversely, patients were defined as remission failure if *BCR-ABL* transcripts above these values. About 2mL bone marrow sample was used for *BCR-ABL* transcripts quantification at diagnosis time-point and serial treatments. The NRBC with other immune cells and biochemical markers were recalled from the laboratory database. These blood tests were performed on the Alinity-hq hematology

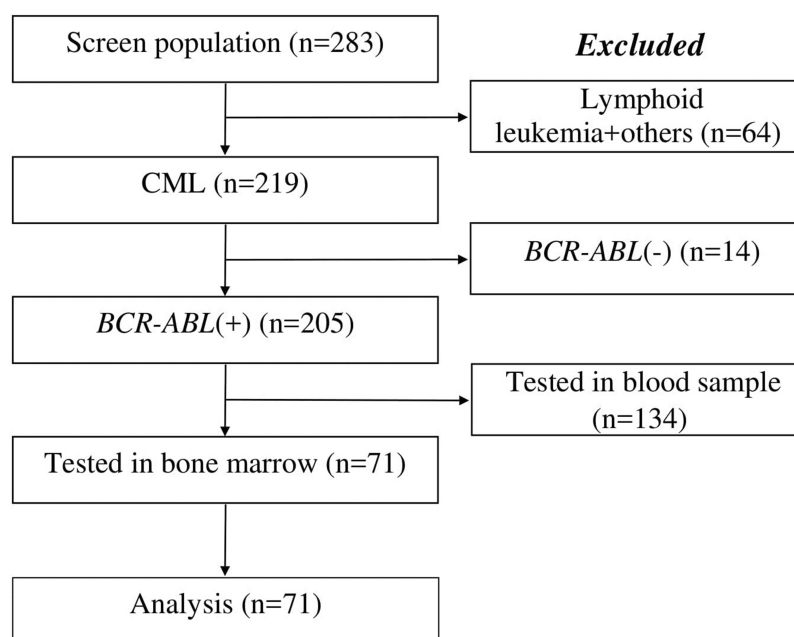


Figure 1 Patient selection.

analyzer and Accelerator a3600 automation system (Abbott Laboratories, Illinois, USA). Other diagnostic results of bone marrow cytology and *t(9;22)* translocation were collected from the medical records. Authors were permitted to collect the data with the responsibilities of personal information security. Because of the retrospective study, patients were not requested to write consent form.

## Total RNA extraction

The total RNA was extracted from  $10^7$  leukocytes from bone marrow sample using kit QIAamp RNA Blood Mini (Cat No./ID: 52304) according to the instructions of the manufacturer (Qiagen, Hilden, Germany). Briefly, samples were mixed with 10 mL EL buffer and incubated in 4 °C/15 mins to lysis red blood cells, then centrifuged at  $400\times g$ /4 °C/10 mins to remove supernatant. The collected leukocytes were lysed by 600 µL RLT buffer added 10 µL  $\beta$ 2-mercapthoethanol 100%. After that, the entire lysate was transferred to the QIAshredder column and centrifuged for 2 mins at  $14,000\times g$  to homogenize the samples. The RNA in the homogenized lysate was precipitated by 600 µL ethanol 70%, then captured on the silica membrane of QIAamp spin column in the centrifuge step ( $10,000\times g$ /4 °C/1 min). Approx of 700 µL RW1 and 700 µL RPE buffers were added to wash silica membrane, respectively. The total RNA was eluted in 30 µL RNase-free water and checked the concentration with purity by the BioDrop µLITE machine (BioDrop Ltd, UK) before storing in -80 °C until uses.

## BCR-ABL transcripts quantification

The *BCR-ABL* p210 b2a2 or b3a2, and p190 e1a2 transcripts in patient's sample were quantified by using kit Ipsogen BCR-ABL1 Mbc IS-MMR DX (Cat No./ID: 670823) and Ipsogen BCR-ABL1 mbcr (Cat No./ID: 670023), performed on the RotorGene Q 5Plex HRM machine according to the instructions of the manufacturer (Qiagen, Hilden, Germany). In the first step, reverse transcription reaction was prepared by mixing 1 µg heated RNA (10 µL) with 15 µL of RT buffer, then cycled at 25 °C for 15 mins, 50 °C for 60 mins, and 85 °C for 5 mins. In the second step, the quantitative polymerase chain reactions (qPCR) were prepared by mixing 12.5 µL qPCR master mix with 6.5 µL nuclease-free water, 1 µL primers and probe mix, and 5 µL PCR product from step one. PCR temperatures were set up as follows: 95 °C/10 seconds; 50 cycles of 95 °C/5 seconds and 60 °C/1 min; and then at 36 °C for 1 min. The standard curve was constructed using a standard series of  $10^1$ – $10^6$  copies *BCR-ABL*/

5 µL which were run within one-batch with the patient's sample. PCR reactions were analyzed by the Rotor-Gene Q Series Software V.2.3.1. *BCR-ABL* transcripts were reported as normalized copy number on the international scale.

## Statistical analysis

The Chi-square or Fisher's exact tests were used to compare the relative frequencies while the Kruskal-Wallis rank was used to test the difference of median diagnostic values between groups. To determine the optimal cut-off point of each marker for the treatment failure (failure to reach response milestones), the logistic regression was used to construct the receiver operating characteristic (ROC) curve and calculate the Youden's index. Association of marker levels with treatment failure were assessed by the odds ratio (OR) with 95% confident interval (95% CI). All data of the study were analyzed by R statistical software v.3.5.1 (R foundation, 1020 Vienna, Austria).  $P < 0.05$  was considered statistically significant.

## Results

### Patient characteristics

A total of 71 CML (39 males and 32 female) patients with *BCR-ABL* (+) in bone marrow cells were selected for this study (Figure 1). Patients were categorized into three groups based on the leukemic phenotype and NRBC cut-off ( $0.003\times 10^9/L$ ): 7 cases of accelerated phase with  $NRBC \geq 0.003\times 10^9/L$ ; 64 cases of chronic phase including 31 cases with  $NRBC \geq 0.003\times 10^9/L$  and 33 cases with  $NRBC < 0.003\times 10^9/L$  (Table 1). The median age of all patients was 39 (from 31 to 47) years old.

Most of the patients had been diagnosed with the hypercellular bone marrow (56 cases, 78.9%), myeloid-to-erythroid ratio  $>5:1$  (58 cases, 81.7%) and peripheral white blood cells  $>50\times 10^9/L$  (63 cases, 88.7%) (Table 1). The increased blast cells ( $\geq 10\%$ ; in the bone marrow and peripheral blood) was observed in 7 cases (accelerated phase), of which one patient had basophils number  $>20\%$ . In the cytogenetic assessment, the *t(9;22)* translocation was found in bone marrow cells with a median value of 56.5% (95% CI: 49.2–62.7%). Sixty-one cases (85.9%) have the translocation value  $>35\%$ . The PCR analyses found the *BCR-ABL* p210 transcripts in bone marrow cells with a median value of 63.81% (95% CI: 53.44–74.37%). Almost patients (64 cases, 90.1%) have the quantitative *BCR-ABL* p210

**Table 1** Baseline characteristics of the patients

Variable	Total (n=71)	Accelerated phase, n=7	Chronic phase, n=64		P-value
			High-NRBC, n=31	Low-NRBC, n=33	
<b>Age, years (95% CI)</b>	39 (31–47)	45 (26–59)	39 (28–46)	39 (29–49)	-
<b>Gender</b>					0.832
Female	32	4	14	14	
Male	39	3	17	19	
<b>Bone marrow cellularity</b>					0.443
Normal	15	0	8	7	
Hyper	56	7	23	26	
<b>Myeloid:Erythroid ratio</b>					0.952
≤5:1	13	1	6	6	
>5:1	58	6	25	27	
<b>Peripheral white blood cells</b>					0.192
≤50×10 <sup>9</sup> /L	8	0	6	2	
>50×10 <sup>9</sup> /L	63	7	25	31	
<b>Blast cells</b>					<0.001
<10%	64	0	31	33	
≥10%	7	7	0	0	
<b>Basophils</b>					0.099
≤20%	70	6	31	33	
>20%	1	1	0	0	
<b>Platelets</b>					0.545
≤1000×10 <sup>9</sup> /L	63	7	26	30	
>1000×10 <sup>9</sup> /L	8	0	5	3	
<b>FISH: t (9;22) translocation</b>					0.996
≤35%	10	1	4	5	
>35%	61	6	27	28	
<b>BCR-ABL transcripts</b>					0.805
≤35%	7	0	4	3	
>35%	64	7	27	30	
<b>Treatment</b>					0.046
Imatinib	67	5	30	32	
Imatinib+Nilotinib	4	2	1	1	

**Notes:** High-NRBC: NRBC≥0.003×10<sup>9</sup>/L; Low-NRBC: NRBC<0.003×10<sup>9</sup>/L.

**Abbreviations:** 95% CI, 95% confident interval; FISH, fluorescent in situ hybridization; NRBC, nucleated red blood cell.

transcripts >35% at the diagnosis. *BCR-ABL* p190 transcript was not found in the study subjects.

### Changes of *BCR-ABL* transcripts and other diagnostic values during treatment

All of 71 patients were treated with a standard dose of imatinib (400 mg/day), in which 4 cases were switched to the treatment with Nilotinib (300 mg/day) (Novartis, Basel, Switzerland). The median time of the treatment

follow-up was 39 months. We noted that median values of *BCR-ABL* transcripts, white blood cells, blast cells, basophils, platelets, and NRBC were declined after 6 months of treatment compared to the initial values (at T0 time-point) ( $P<0.001$ ) (Table 2). After 12 and 18 months of treatment, the *BCR-ABL* transcripts were continued to be decreased considerably compared to the value at T6 time-point ( $P=0.008$ ) while white blood cells ( $P=0.582$ ) and basophils ( $P=0.070$ ) were not

**Table 2** Laboratory data at serial treatment time-points

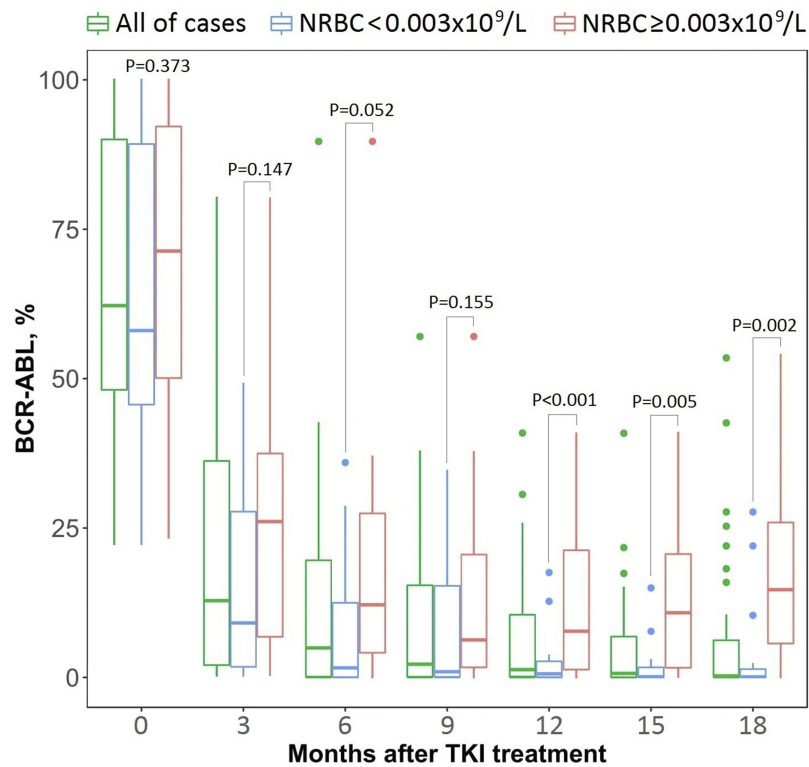
Variable	T0 (n=71)	T6 (n=71)	T12 (n=59)	T18 (n=58)	P-value
<b>BCR-ABL, % (All patients)</b>	63.81 (53.44–74.37)	4.83 (1.73–10.82)	1.07 (0.28–2.98)	0.51 (0.09–1.81)	<0.001 <sup>a</sup> 0.008 <sup>b</sup>
<b>BCR-ABL, % (Low-NRBC)</b>	58.04 (48.61–74.54)	1.59 (0.06–9.92)	0.60 (0.04–2.66)	0.09 (0.04–0.67)	<0.001 <sup>a</sup> 0.069 <sup>b</sup>
<b>BCR-ABL, % (High-NRBC)</b>	71.35 (52.47–80.93)	12.15 (4.09–26.17)	7.73 (3.47–25.07)	17.04 (2.10–38.86)	<0.001 <sup>a</sup> 0.724 <sup>b</sup>
<b>RBC, ×10<sup>12</sup>/L</b>	3.26 (3.08–3.41)	3.73 (3.57–4.02)	3.71 (3.46–3.94)	3.70 (3.51–3.86)	<0.001 <sup>a</sup> 0.754 <sup>b</sup>
<b>NRBC, ×10<sup>9</sup>/L</b>	0.043 (0.000–0.253)	0.000 (0.000–0.001)	0.000 (0.000–0.002)	0.000 (0.000–0.000)	<0.001 <sup>a</sup> 0.766 <sup>b</sup>
<b>WBC, ×10<sup>9</sup>/L</b>	160.7 (138.4–185.7)	5.5 (4.8–6.2)	5.5 (4.3–6.1)	5.5 (4.8–6.3)	<0.001 <sup>a</sup> 0.582 <sup>b</sup>
<b>Blast cells, %</b>	1.6 (1.3–1.9)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	<0.001 <sup>a</sup> 0.998 <sup>b</sup>
<b>BASO, %</b>	7.5 (6.7–8.9)	0.5 (0.4–0.5)	0.5 (0.4–0.7)	0.6 (0.5–0.6)	<0.001 <sup>a</sup> 0.070 <sup>b</sup>
<b>PLT, ×10<sup>9</sup>/L</b>	402 (352–487)	157 (126–162)	157 (135–176)	183 (163–204)	<0.001 <sup>a</sup> 0.004 <sup>b</sup>
<b>Uric acid, mg/dL</b>	5.52 (4.92–6.32)	4.74 (4.12–5.80)	4.95 (4.71–5.59)	5.11 (4.69–5.46)	0.776 <sup>a</sup> 0.968 <sup>b</sup>
<b>Bilirubin, mg/dL</b>	0.59 (0.53–0.67)	0.60 (0.55–0.70)	0.60 (0.51–0.71)	0.60 (0.52–0.67)	0.802 <sup>a</sup> 0.618 <sup>b</sup>
<b>Creatinine, mg/dL</b>	0.75 (0.70–0.81)	0.71 (0.70–0.81)	0.75 (0.70–0.80)	0.78 (0.70–0.80)	0.284 <sup>a</sup> 0.104 <sup>b</sup>
<b>Sodium, mmol/L</b>	138.0 (138.0–139.0)	139.5 (138.9–140.1)	139.2 (138.6–139.7)	139.2 (138.7–139.7)	0.091 <sup>a</sup> 0.480 <sup>b</sup>
<b>Potassium, mmol/L</b>	3.89 (3.70–3.99)	3.62 (3.57–3.70)	3.64 (3.60–3.75)	3.67 (3.61–3.83)	0.001 <sup>a</sup> 0.252 <sup>b</sup>
<b>Chloride, mmol/L</b>	104.8 (104.0–105.4)	107.1 (106.2–108.0)	107.2 (106.6–107.9)	107.0 (106.4–107.6)	0.003 <sup>a</sup> 0.962 <sup>b</sup>
<b>Calcium, mmol/L</b>	2.2 (2.1–2.2)	2.2 (2.1–2.2)	2.2 (2.2–2.2)	2.2 (2.2–2.2)	0.673 <sup>a</sup> 0.577 <sup>b</sup>

**Notes:** Results were presented as median with 95% confident interval (95% CI); High-NRBC: NRBC $\geq 0.003 \times 10^9/L$ ; Low-NRBC: NRBC $< 0.003 \times 10^9/L$ ; <sup>a</sup>T6, T12, T18 versus T0. <sup>b</sup>T6 versus T12 versus T18.

**Abbreviations:** RBC, red blood cells; WBC, white blood cells; BASO, basophils; NRBC, nucleated red blood cells; PLT, platelets; T0, at diagnosis; T6, after 6 months of treatment; T12, after 12 months of treatment; T18, after 18 months of treatment.

changed. We also noted that median blast cells and circulating NRBC dropped down to zero from the 6th months to beyond (Table 2). Interestingly, we found that patients with a high level of circulating NRBC ( $\geq 0.003 \times 10^9/L$ ) have higher *BCR-ABL* transcripts compared to others (Figure 2).

The median red blood cells, electrolyte sodium, and chloride values were increased after 6 months of treatment (*P*-values were <0.001, 0.091, and 0.003, respectively), then stable for the following months (*P*=0.754, 0.480, and 0.962, respectively) (Table 2). Conversely, the potassium level was decreased after 6 months (*P*=0.001), and



**Figure 2** Changes of quantitative *BCR-ABL* transcripts during tyrosine kinase inhibitor (TKI) therapy.

unchanging for the later ( $P=0.252$ ). Other biochemical values were not changed during treatment ( $P>0.05$ ).

**Associated factors for the response failure**

By the end of 6 months of treatment, forty-four (62.0%) patients reached good response ( $BCR-ABL\leq 10\%$ ), in which optimal response ( $BCR-ABL<1\%$ ) was noted in 27 (38.0%) cases. Twenty-seven (38.0%) cases failed to reach response milestone at T6 time-point (Table 3). At T12 time-point, the molecular data of 59 cases were available for response evaluation. Of which, thirty patients (50.8%) attained while 29 (49.2%) cases failed to reach the

response target ( $BCR-ABL\leq 1\%$ ). These numbers at T18 time-point were 24 (41.4%) and 34 (58.6%) cases, respectively.

The optimal cut-off point and the area under the ROC curve (AUC) value of each marker for the treatment failure at T6, T12, and T18 time-points were shown in Table 4 (only markers with  $AUC\geq 0.55$  have been shown). At the first milestone (T6), patients with high levels of circulating NRBC ( $\geq 0.003\times 10^9/L$ ,  $P=0.009$ ), basophils ( $\geq 0.6\%$ ,  $P=0.029$ ), and low levels of peripheral red blood cells ( $\leq 4.29\times 10^{12}/L$ ,  $P=0.057$ ), electrolyte sodium ( $\leq 138.9$  mmol/L,  $P=0.051$ ), and chloride ( $\leq 108.1$  mmol/L,  $P=0.018$ ) have the higher incidence of treatment failure ( $BCR-ABL>10\%$ ) compared to others. The multivariable analysis has shown that elevated

**Table 3** Molecular response of the study subjects

Response targets	Yes, n (%)		No, n (%)	
	High-NRBC	Low-NRBC	High-NRBC	Low-NRBC
<b><i>BCR-ABL</i>≤10% at T6 (n=71)</b>	2 (2.8)	42 (59.2)	8 (11.3)	19 (26.7)
<b><i>BCR-ABL</i>≤1% at T12 (n=59)</b>	3 (5.0)	27 (45.8)	10 (17.0)	19 (32.2)
<b><i>BCR-ABL</i>≤0.1% at T18 (n=58)</b>	2 (3.5)	22 (37.9)	12 (20.7)	22 (37.9)

**Notes:** High-NRBC:  $NRBC\geq 0.003\times 10^9/L$ ; Low-NRBC:  $NRBC<0.003\times 10^9/L$ .  
**Abbreviations:** T6, after 6 months of treatment; T12, after 12 months of treatment; T18, after 18 months of treatment.



**Table 4** Associated factors for the treatment failure

Variable	Cut-off	AUC	Univariable analysis		Multivariable analysis	
			OR (95% CI)	P-value	OR (95% CI)	P-value
BCR-ABL>10% after 6 months of treatment						
RBC, ×10 <sup>12</sup> /L	≤4.29	0.6166	3.73 (0.96–14.51)	0.057	–	–
NRBC, ×10 <sup>9</sup> /L	≥0.003	0.6006	8.84 (1.71–45.65)	0.009	6.49 (1.09–39.33)	0.042
BASO, %	≥0.6	0.5816	3.16 (1.12–8.87)	0.029	–	–
Sodium, mmol/L	≤138.9	0.5913	2.42 (0.98–6.45)	0.051	–	–
Chloride, mmol/L	≤108.1	0.6481	4.37 (1.29–14.77)	0.018	–	–
BCR-ABL>1% after 12 months of treatment						
RBC, ×10 <sup>12</sup> /L	≤4.01	0.6517	5.78 (1.42–23.44)	0.014	7.09 (1.29–38.84)	0.024
NRBC, ×10 <sup>9</sup> /L	≥0.003	0.6977	4.92 (1.55–15.64)	0.007	6.73 (1.69–26.76)	0.007
Potassium, mmol/L	≥3.62	0.5989	2.54 (0.87–7.36)	0.086	–	–
Chloride, mmol/L	≤104.7	0.5672	3.43 (0.81–14.53)	0.095	–	–
BCR-ABL>0.1% after 18 months of treatment						
RBC, ×10 <sup>12</sup> /L	≤4.01	0.6294	3.10 (1.17–8.24)	0.023	4.11 (1.28–13.20)	0.018
NRBC, ×10 <sup>9</sup> /L	≥0.003	0.6690	6.00 (1.59–18.10)	0.007	5.96 (1.54–21.71)	0.009
BASO, %	≥0.8	0.6183	3.08 (0.99–9.66)	0.051	–	–
Sodium, mmol/L	≤139.8	0.6218	3.67 (1.31–10.24)	0.013	–	–

**Abbreviations:** RBC, red blood cells; WBC, white blood cells; BASO, basophils; NRBC, nucleated red blood cells; PLT, platelets; AUC, area under the ROC curve; OR, odds ratio.

NRBC is an independent factor for the treatment failure at T6 (adjusted OR=6.49, 95% CI: 1.09–39.33,  $P=0.042$ ).

At the second and third milestones (T12 and T18), we noted that red blood cells and circulating NRBC are independent factors for the failure of response targets (adjusted OR: 7.09 and 4.11 for RBC; 6.73 and 5.96 for NRBC, respectively). Remarkably, at all three evaluation time-points, the elevated NRBC is the worse indicator in treatment with imatinib ( $P=0.042$ , 0.007, and 0.009, respectively).

## Discussion

NRBC is a part of the hematopoietic system which appears in peripheral blood of neonates for several days after birth, but not in the blood of healthy adults.<sup>10,26</sup> The mechanism in which NRBC is released into the blood of critical illness such as CML is unclear to date. It might be because of the hypoxia and inflammation in the tumor microenvironment which causes hematopoietic stress, or the increased level of erythropoietin, and cytokines such as interleukin 3 and interleukin 6.<sup>10,19,27–30</sup> Previous studies have shown that the presence of NRBC in peripheral blood is associated with high mortality in many severe diseases including leukemia.<sup>8,10–25</sup> Some studies even showed the role of NRBC as a supplementing factor

which helps to improve the prognostic power of the current used clinical standard.<sup>14,16</sup> In spite of that, a few studies described the role of this cell in CML, especially in patients treated with targeted therapy.<sup>9</sup>

We investigated and showed that the number of NRBC and other immune cells such as white blood cells, blast cells, and basophils dropped quickly parallel with the decreases of *BCR-ABL* transcripts in CML bone marrow cells after 6 months of treatment with imatinib ( $P<0.001$ ). Besides, the NRBC has not been found in peripheral blood of almost patients who archived good response (Table 3). This is consistent with results of Danise et al, in which 100% of patients at diagnosis decreased to 0% of cases at remission time-point have the presence of NRBC in peripheral blood.<sup>9</sup> Notably, we found that the maintenance of high NRBC number in the blood is associated with high *BCR-ABL* transcripts. This might be explained by the existence of a high number of leukemic cells with *BCR-ABL* arrangement which causes the extramedullary hematopoiesis leads to the penetration of NRBC to the bloodstream.<sup>10</sup>

Importantly, patients with elevated NRBC have a higher likelihood of remission failure at different milestones (T6, T12, and T18) according to the ELN definitions ( $P=0.042$ , 0.007, 0.009, respectively). To our

knowledge, this is the first time that NRBC is identified as an independent factor for the treatment failure in CML. In clinical practice, the appearance with a high number of NRBC suggests the clinicians about the loss of remission, thereby lead to the next decision makings such as drug interaction evaluation and mutational analysis.<sup>1</sup> Besides, NRBC is a simple marker integrated with the complete blood count, therefore the uses of this parameter in treatment evaluation do not require additional costs.

In this study, the *BCR-ABL* transcripts in bone marrow cells (gold standard sample) were quantified and reported according to the international scale which was highly recommended in targeted treatment monitoring for CML.<sup>1</sup> However, this study has limitations of a single-center and retrospective study. In addition, the sample size in the study is limited. Further research with a large cohort should be conducted to confirm our findings.

In conclusion, the results of this study showed that patients with a high number of NRBC in peripheral blood have higher *BCR-ABL* transcripts in bone marrow cells compared to others. The elevated NRBC might serve as an independent marker for remission failure in CML treated with imatinib.

## Ethics approval and informed consent

This study was considered and approved by the Ethics Committees of Cho Ray Hospital (approval number: 602-2017-CN-HDDD). Patient consent was not required.

## Data availability

The data of this study are available from the corresponding author with a reasonable request.

## Author contributions

Son Truong Nguyen is the senior author who contributed to study design; Thang Thanh Phan, Ha The Vy, and Toan Trong Ho collected the clinical data; Tuyen Thi Bich Pham, Thao Thi Le, Suong Phuoc Pho, Vinh Thanh Tran, and Tung Thanh Tran collected the laboratory data; Thang Thanh Phan and Son Truong Nguyen performed the data analysis and wrote the manuscript. All authors contributed toward data analysis, drafting and revising the paper, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest in this work.

## References

- Hochhaus A, Saussele S, Rosti G, et al. Chronic myeloid leukaemia: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2017;28(Suppl.4):iv41–iv51. doi:10.1093/annonc/mdx075
- O'Brien SG, Guilhot F, Larson RA, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Eng J Med*. 2003;348(11):994–1004. doi:10.1056/NEJMoa022457
- Cortes JE, Saglio G, Kantarjian HM, et al. Final 5-year study results of DASISION: the dasatinib versus imatinib study in treatment-naïve chronic myeloid leukemia patients trial. *J Clin Oncol*. 2016;34:2333–2340. doi:10.1200/JCO.2015.64.8899
- Hochhaus A, Saglio G, Hughes TP, et al. Long-term benefits and risks of frontline nilotinib vs imatinib for chronic myeloid leukemia in chronic phase: 5-year update of the randomized ENESTnd trial. *Leukemia*. 2016;30:1044–1054. doi:10.1038/leu.2016.5
- Brück O, Blom S, Dufva O, et al. Immune cell contexture in the bone marrow tumor microenvironment impacts therapy response in CML. *Leukemia*. 2018;32:1643–1656. doi:10.1038/s41375-018-0175-0
- Lekovic D, Gotic M, Milic N, et al. Predictive parameters for imatinib failure in patients with chronic myeloid leukemia. *Hematology*. 2017;22(8):460–466. doi:10.1080/10245332.2017.1302179
- Chikkodi SV, Malhotra P, Naseem S, et al. Factors affecting early molecular response in chronic myeloid leukemia. *Clin Lymphoma Myeloma Leuk*. 2015;15(Suppl):S114–S119. doi:10.1016/j.clml.2015.03.014
- Schwartz SO, Stansbury F. Significance of nucleated red blood cells in peripheral blood: analysis of 1,496 cases. *JAMA*. 1954;154(16):1339–1340. doi:10.1001/jama.1954.02940500019007
- Danise P, Maconi M, Barrella F, et al. Evaluation of nucleated red blood cells in the peripheral blood of hematological diseases. *Clin Chem Lab Med*. 2012;50(2):357–360. doi:10.1515/cclm.2011.766
- Otsubo H, Kaito K, Asai O, et al. Persistent nucleated red blood cells in peripheral blood is a poor prognostic factor in patients undergoing stem cell transplantation. *Clin Lab Haem*. 2005;27:242–246. doi:10.1111/j.1365-2257.2005.00687.x
- Kho AN, Hui S, Kesterson JG, McDonald CJ. Which observations from the complete blood cell count predict mortality for hospitalized patients? *J Hosp Med*. 2007;2(1):5–12. doi:10.1002/(ISSN)1553-5606
- Stachon A, Holland-Letz T, Krieg M. High in-hospital mortality of intensive care patients with nucleated red blood cells in blood. *Clin Chem Lab Med*. 2004;42(8):933–938. doi:10.1515/CCLM.2004.151
- Stachon A, Segbers E, Holland-Letz T, et al. Nucleated red blood cells in the blood of medical intensive care patients indicate increased mortality risk: a prospective cohort study. *Crit Care*. 2007;11:R62. doi:10.1186/cc5932
- Stachon A, Becker A, Kempf R, et al. Re-evaluation of established risk scores by measurement of nucleated red blood cells in blood of surgical intensive care patients. *J Trauma*. 2008;65:666–673. doi:10.1097/TA.0b013e318181e524
- Desai S, Jones SL, Turner KL, et al. Nucleated red blood cells are associated with a higher mortality rate in patients with surgical sepsis. *Surg Infect*. 2012;13(6):360–365. doi:10.1089/sur.2011.089
- Monteiro Junior JG, Torres Dde O, Da Silva MC, et al. Nucleated red blood cells as predictors of all cause mortality in cardiac intensive care unit patients: a prospective cohort study. *PLoS One*. 2015;10(12):e0144259. doi:10.1371/journal.pone.0144259
- Menk M, Giebelhäuser L, Vorderwülbecke G, et al. Nucleated red blood cells as predictors of mortality in patients with acute respiratory distress syndrome (ARDS): an observational study. *Ann Intensive Care*. 2018;8:42. doi:10.1186/s13613-018-0387-5



18. Purtle SW, Horkan CM, Moromizato T, et al. Nucleated red blood cells, critical illness survivors and postdischarge outcomes: a cohort study. *Crit Care*. 2017;21(1):154. doi:10.1186/s13054-017-1724-z
19. Kuert S, Holland-Letz T, Friese J, Stachon A. Association of nucleated red blood cells in blood and arterial oxygen partial tension. *Clin Chem Lab Med*. 2011;49(2):257–263. doi:10.1515/CCLM.2011.041
20. Shah R, Reddy S, Horst HM, et al. Getting back to zero with nucleated red blood cells: following trends is not necessarily a bad thing. *Am J Surg*. 2012;203(3):343–345. doi:10.1016/j.amjsurg.2011.10.002
21. Boskabadi H, Maamouri G, Sadeghian MH, et al. Early diagnosis of perinatal asphyxia by nucleated red blood cell count: a case-control study. *Arch Iran Med*. 2010;13(4):275–281. doi:10.10134/AIM.005
22. Cremer M, Roll S, Gräf C, et al. Nucleated red blood cells as marker for an increased risk of unfavorable outcome and mortality in very low birth weight infants. *Early Hum Dev*. 2015;91(10):559–563. doi:10.1016/j.earlhumdev.2015.06.004
23. Kil TH, Han JY, Kim JB, et al. A study on the measurement of the nucleated red blood cell (nRBC) count based on birth weight and its correlation with perinatal prognosis in infants with very low birth weights. *Korean J Pediatr*. 2011;54(2):69–78. doi:10.3345/kjp.2011.54.2.69
24. Li J, Kobata K, Kamei Y, et al. Nucleated red blood cell counts: an early predictor of brain injury and 2-year outcome in neonates with hypoxic-ischemic encephalopathy in the era of cooling-based treatment. *Brain Dev*. 2014;36(6):472–478. doi:10.1016/j.braindev.2013.06.012
25. Walsh BH, Boylan GB, Murray DM. Nucleated red blood cells and early EEG: predicting Sarnat stage and two year outcome. *Early Hum Dev*. 2011;87(5):335–339. doi:10.1016/j.earlhumdev.2011.01.041
26. Christensen RD, Henry E, Andres RL, Bennett ST. Reference ranges for blood concentrations of nucleated red blood cells in neonates. *Neonatology*. 2011;99:289–294. doi:10.1159/000320148
27. Stachon A, Bolulu O, Holland-Letz T, Krieg M. Association between nucleated red blood cells in blood and the levels of erythropoietin, interleukin 3, interleukin 6, and interleukin 12p70. *Shock*. 2005;24(1):34–39.
28. Dulay AT, Buhimschi IA, Zhao G, et al. Nucleated red blood cells are a direct response to mediators of inflammation in newborns with early-onset neonatal sepsis. *Am J Obstet Gynecol*. 2008;198:426.e1–426.e9. doi:10.1016/j.ajog.2008.01.040
29. Romero R, Savasan ZA, Chaiworapongsa T, et al. Hematologic profile of the fetus with systemic inflammatory response syndrome. *J Perinat Med*. 2011;40(1):19–32. doi:10.1515/JPM.2011.100
30. Danise P, Amendola G, Concilio RD, et al. Nucleated red blood cells and soluble transferrin receptor in thalassemia syndromes: relationship with global and ineffective erythropoiesis. *Clin Chem Lab Med*. 2009;47(12):1539–1542. doi:10.1515/CCLM.2009.340

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