RESEARCH ARTICLE

Clinical Significance of *BCR-ABL* Fusion Gene Subtypes in Chronic Myelogenous and Acute Lymphoblastic Leukemias

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Abstract

Background: Some reports have suggested that chronic myeloid leukemia (CML) patients have a higher prevalence of *M-bcr* than acute lymphoblastic leukemia (ALL) patients, which show a higher prevalence of *m-bcr*. However, the relationship between *BCR-ABL* subtypes and progression of CML and ALL remains unclear. Materials and Methods: 354 CML chronic phase (CML-CP) patients, 26 CML blastic phase (CML-BP) patients and 72 ALL patients before treatment with *BCR-ABL* positive were recruited for blood routine examination and bone marrow smear cytology. Some 80 CML-CP and 32 ALL patients after imatinib (IM) treatment were followed-up for *BCR-ABL* relative concentrations detected after treatment for 3, 6 and 9 months and 1 year. Results: Before treatment, CML-CP patients showed lower *BCR-ABL* relative concentrations with a higher proportion of *M-bcr* (42.7%) compared to CML-BP and ALL patients while ALL patients had a higher *BCR-ABL* relative concentration with high expression of *m-bcr* (51.4%). Patients with *M-bcr* demonstrated higher WBC counts than those with *m-bcr* and the mixed group and higher PLT counts were noted in the CML-CP and ALL groups. After imatinib (IM) treatment, patients with *m-bcr* showed higher *BCR-ABL* relative concentrations in both CML-CP and ALL groups. Conclusions: This study identified the *BCR-ABL* gene as an important factor in CML and ALL cases. The *M-bcr* subtype was associated more with CML while the *m-bcr* subtype was associated more with ALL. Patients with *m-bcr* seem to have a poorer response to IM in either CML or ALL patients compared to *M-bcr* patients.

Keywords: *BCR-ABL* fusion gene - chronic myeloid leukemia - acute lymphoblastic leukemia

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Introduction

The Philadelphia chromosome (Ph), t (9; 22) (q34; q11.2) is transcribed into a fusion gene, *BCR-ABL* (Awan et al., 2012; Sabir et al., 2012). This translocation is one of the most common genetic abnormalities detected in leukemia. The site of the breakpoint in the *BCR* gene may influence the phenotype of diseases. In the vast majority of patients, the breakpoints in the *BCR* gene are clustered within three well-defined regions: *(I)* a 55 kb sequence of the first intron, called the minor breakpoint cluster region (m-bcr); *(II)* a 5.8 kb region spanning exons 12-16, called the major breakpoint cluster region (*M-bcr*), and finally; *(III)* intron 19, called *μ-bcr*. The resultant fusion transcript (e1-a2) (m-bcr) encodes a 190 kDa chimeric protein (p190), and in cases of *M-bcr*, codes a 210 kDa chimeric protein (p210) and in cases of *μ-bcr*, a 230 kDa protein (p230) (Fausel, 2007). Large studies indicated that *BCR-ABL* fusion gene is a highly useful diagnostic tool that controls the effectiveness of the chronic myelogenous leukemia (CML) treatment and indicates at an early stage resistance development or disease progression (Ohsaka et al., 2002; Hehlmann et al., 2007; Hunger, 2011; Ohanian et al., 2012; Sabir et al., 2012; Soheila et al., 2013). Recent research showed that *BCR-ABL* fusion gene can also be detected in 25-30% of adult and 2-5% of childhood cases of acute lymphoblastic leukemia (ALL), and less frequently, in acute myelogenous leukemia (AML) (Den Boer et al., 2009; Uchida et al., 2009; Yeung et al., 2012). In ALL, this genetic lesion is known to confer a very poor prognosis (Hanfstein et al., 2012), which made its detection important in planning aggressive therapies including allogeneic bone marrow transplant. For different *BCR-ABL* subtype, some reports indicated that *M-bcr* is sufficient to course CML and few CML cases with *m-bcr* were reported (Moorman et al., 2007; Yanada et al., 2008). In CML, the prevalence of *M-bcr* is higher than *m-bcr* (Press et al., 2007). In contrary, ALL patients showed a higher prevalence of *m-bcr* (Chomel et al., 2012). Although the distribution of *BCR-ABL* subtype have been

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studied in CML and ALL separately (Hanfstein et al., 2012; Marin et al., 2012), few studies have investigated the *BCR-ABL* subtype distribution in CML-CP, CML-BP and ALL patient simultaneity and there is still lack of data on the impact of *BCR-ABL* subtypes in progression of CML and ALL. It is also a matter of contention if *BCR-ABL* subtype may be significantly predictive for the long-term outcome of CML and ALL patients treated with imatinib (IM) first line. In this study, we focused on analyzing the distribution difference of *M-bcr* and *m-bcr* in CML-CP, CML-BP and ALL patients with bone marrow smear cytology, blood routine examination results and *BCR-ABL* concentration before treatment in order to make a preliminary investigation on the relationship between *BCR-ABL* subtype and patient feature in different disease entities. Moreover, *BCR-ABL* concentration after treatment for 3, 6, 9 months and 1 year were also followed-up to investigate the association between long-term outcome of CML and ALL patients treated with IM first line and *BCR-ABL* subtype elementarily. These data confirm and further strengthen that *M-bcr* and *m-bcr* play differently in leading to leukemia and may influence the prognosis. *BCR-ABL* subtype should be considered while making treatment protocols.

Materials and Methods

Study population

During June 2007 to December 2009, a total of 452 *BCR-ABL* positive patients diagnosed strictly according to WHO diagnostic criteria as CML-CP, CML-BP and ALL before treatment in the West China Hospital were enrolled into the present study. All patients enrolled were *BCR-ABL* positive and clinical data such as WBC, RBC, PLT, and primitive cell ratio for all patients were collected and assessed. 80 CML-CP patients and 32 ALL patients under imatinib (IM) treatment (400mg qd for remaining life) followed-up for at least 1 year with *BCR-ABL* relative concentration detected after 3 months, 6 months, 9 months and 1 year were enrolled in prognosis analysis. Written Informed consents were obtained from all included individuals and this study was approved by the ethical committee of West China Hospital, Sichuan University.

RNA extraction

Bone marrow samples were collected from these patients and subjected to cytogenetic analysis and RNA isolation isolated within 24 hours using QIAamp RNA Blood Mini-Kit (QIAGEN, Hilden, Germany).

Reverse transcription (RT) and QPCR for BCR-ABL relative quantification

Reverse transcription was carried out with a cDNA Synthesis Kit (Roche Diagnostics) according to the manufacturer's instructions. The resulted cDNA was used as the template of real-time PCR reaction, which was performed in a LightCycler 2.0 real-time fluorescence PCR detection system, according to the manufacture's instruction .The concentration of *BCR-ABL* fusion gene in patient sample was calculated on the basis of the standard curve established with K562 cell standard samples and normalizing their amplification to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). The relative gene expression level was calculated on the basis of the ΔCt.

Reverse transcription (RT) PCR and electrophoresis for BCR-ABL subtyping

According to F Gabert (14), the ENF501 (BCR3173-3193: 5'-TCCGCTGACCATCAATAAGGA-3') was selected as the forward primer for the RT-PCR detection of *M-bcr* transcripts and ENF402 (BCR1727-1744: 5'-CTGGCCCAACGATGGCGA-3') was selected as the forward primer for the RT-PCR detection of *m-bcr* transcripts. A reverse primer, ENR 561 (ABL277-257: 5'-CACTCAGACCCT GAGGCTCAA-3') was used as reverse prime for both *M-bcr* and m-bcr. One-step RT-PCR was carried out with one-step RT-PCR Kit (QIAamp) according to the manufacturer's instructions. The thermal conditions consisted of reverse transcriptase at 50°C for 30 min, Denature at 95°C for 15min, and amplification at 95°C 30s; 54°C 1 min, 72°C 1 min for 42 cycles; and extend at 72°C for 10min. K562 cell RNA was used as *M-bcr* positive control and TOM-1 cell RNA as *m-bcr* positive control. For subtype *M-bcr*, a special band of 149bp was amplified and as for m-bcr, a band of 92bp was amplified. The electrophoresis condition was 150V for 30min.

Blood routine examination

Blood routine examination including red blood cell (RBC) counts, white blood cell (WBC) counts, platelet counts was examined using the fully automated hematology analyzer XE-5000™ (Sysmex, Kobe, Japan).

Bone marrow smear cytology

The bone marrow smears were stained with Wright's-Giemsa, and examined by light microscopy. Two hundred nucleated cells were classified and the percentages of all types of cells were calculated. Primitive cell ratio (%) represented the percentage of Primitive cell in total nucleated cells.

Statistical analysis

All measurements were performed in at least triplicate. Results were expressed as a range with median. SPSS software (version 15.0, SPSS Inc., USA) was used for statistical analysis. Chi-square test was used for *BCR-ABL* subtype distribution analysis and Kruskal Wallis test was used for quantitative data analysis. Level of statistical significance $p<0.05$ was used in all analyses.

Results

Basic characteristics of studied patients

After adjustment was made for smoking, age and other general cancer-related factors, 452 patients enrolled in the current study. The average age of the patients was 39.6 years, and M/F was 270/182. All the patients were diagnosed in the light of the clinical manifestations and bone marrow cytogenetic examination report in accordance with the WHO's diagnostic criteria and were divided into three groups: group1: CML-CP patients with

BCR-ABL positive. (n=354); group2: CML myeloid BP patients with *BCR-ABL* positive. (n=26); group3: ALL patients with *BCR-ABL* positive. (n=72). The average age of the patients and M/F ratio in different group were shown in Table 1 and there showed no significant difference in age and M/F among CML-CP, CML-BP and ALL groups.

Detection of BCR-ABL fusion gene relative concentration in CML-CP, CML-BP and ALL patients before treatment

ALL patients have the highest relative concentrations of *BCR-ABL* fusion gene (median=65.80%, P5-P95: 2.26% to 470.18%) among three groups, followed by CML-BP group (median=21.60%, P5-P95: 9.55% to 255.7%) and CML-CP patients (median=12.54% P5-P95: 3.26% to 32.63%), as shown in Figure 1. With Kruskal Wallis test, there was significant difference between CML-CP, CML-BP and ALL patient, $p<0.0001$ (Table1).

Distribution of BCR-ABL subtype in CML-CP, CML-BP and ALL patients

In CML-CP patients, 151/354 (42.66%) showed subtypes of *M-bcr*, 25/354 (7.06%) showed subtypes of m-bcr, 173/354 (48.87%) showed subtypes associated with both *M-bcr* and *m-bcr* and 5/354 (1.41%) showed subtypes of *μ-bcr*. In CML-BP patents, 7/26 (26.9%) showed subtypes associated with *M-bcr*, 8/26 (30.7%) showed subtypes associated with m-bcr, 11/26 (42.3%) showed subtypes associated with both *M-bcr* and m-bcr. For 72 ALL patient, 8/72 (11.1%) showed subtypes

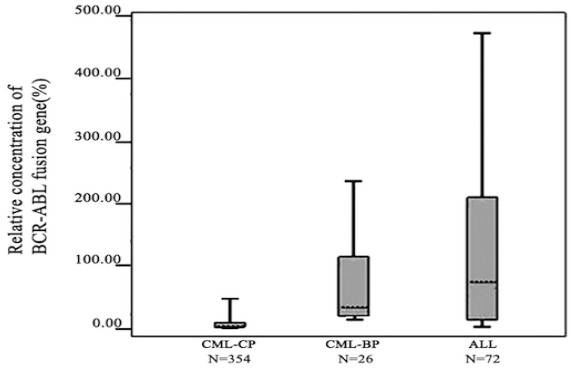

Figure 1. Relative Concentration (%) of BCR-ABL in Three Patient Groups

Table 1. Basic Characteristic of the CML-CP, CML-BP and ALL Patients

	Number	Age(years), median(P5-P95)	Gender (Male/Female)
All patients	452	41(14, 64)	270/182
CML-CP patients	354	40(17, 62)	212/142
CML-BP patients	26	42(15,57)	15/11
ALL patients	72	40(16,63)	43/29
p value	/	0.432	0.354

Table 2. Constituent Ratio of BCR/ABL Subtypes in Different Groups

	M-bcr (%N)	*m-bcr* (%N)	Mixed (%N)
CML CP	42.66 (151)	7.06 (25)	48.87 (173)
CML BP	26.9 (7)	30.7 (8)	42.3 (11)
ALL	11.1 (8)	51.39 (37)	36.11 (26)
p value	0		

associated with *M-bcr*, 37 /72 (51.39%) showed subtypes associated with m-bcr, 26/72 (36.11%) showed subtypes associated with mixed group. 1/72 (1.39%) showed *μ-bcr* subtype. The result showed that CML-CP patients have a higher expression of *M-bcr* compare to CML-BP and ALL patients while ALL patients have a higher expression of m-bcr. With Chi-square test, there was significant difference between CML-CP, CML-BP and ALL patients, $p<0.001$. The results are shown in Table 2.

Association analysis of BCR-ABL subtype with clinical feature in CML and ALL patients

Patients with *M-bcr*, *m-bcr* and mixed subtype were enrolled with blood routine examination results and bone marrow smear cytology results collected while patients with *μ-bcr* were excluded because of case number. In CML-CP group, 349 Patients were categorized into three groups by *BCR-ABL* subtype: *M-bcr*, *m-bcr* and mixed subtype. Patients with *M-bcr* have significantly higher WBC counts (184.76×10^9/L)and higher PLT counts (392×10^9/L) than *m-bcr* (WBC:11.64×10^9/L, PLT:206×10^9/L)and mixed group (WBC:81×10^9/L, PLT:293×10^9/L). Statistical analysis with Kruskal Wallis test showed that there were significantly differences in WBC and PLT counts among three groups, $p<0.001$ (Table 3). However, there were no significant differences in RBC counts (median 4.2, 3.8, 3.9×10^{12}/L in *M-bcr*, *m-bcr* and mixed group), *BCR-ABL* relative concentration (median 13.14, 2.41, 11.95%) and primitive cell ratio (median 1.0, 1.5, 1.5%). In CML-BP group, 26 cases were classified into three groups according to *BCR-ABL* subtypes. Patients with *M-bcr* have higher WBC counts (78.03×10^9/L) than *m-bcr* (26.41×10^9/L) and mixed group (29.71×10^9/L)with statistically significant $p=0.007$ (Table 3). However, there showed no significant differences among three groups in RBC counts (median 2.7, 2.5, 2.9, $\times10^{12}$/L in *M-bcr*, *m-bcr* and mixed group), PLT count (median 204, 153, 118×10^9/L) and *BCR-ABL* relative concentration (median 51.99, 16.08, 10.48 respectively), and primitive cell ratio (53.5%, 68.5%, 61.5%). In ALL group, 71 cases were also classified into *M-bcr*, *m-bcr* and mixed groups. The Statistical analysis showed that there were clear difference in WBC counts ($p<0.001$) and PLT counts ($p=0.016$) among three groups (Table 4). Patients with *M-bcr* have notable higher WBC counts (92.72×10^9/L) and PLT counts (189×10^9/L) than *m-bcr* (WBC:15.92×10^9/L, PLT:72×10^9/L) and mixed group (WBC:12.65×10^9/L, PLT:92×10^9/L). However, there showed no significant difference among three groups in RBC counts (median 2.3, 2.1, 2.9, 2.4 $\times10^{12}$/L in *M-bcr*, *m-bcr* and mixed group), *BCR-ABL* relative concentration (40.95, 28.92, 19.82 respectively) and primitive cell ratio (75.5%, 79.5%, 72.5 respectively).

Association analysis of BCR-ABL subtype with BCR-ABL relative concentration after imatinib treatment

Patients with IM-treatment were followed up for at least one year and 80 CML-CP patients and 30 ALL patients were enrolled with *BCR-ABL* relative concentration detected after 3 months, 6 months, 9 months and 1 year. No CML-BP patients were enrolled as only 3 patients were followed long enough. 80 CML-

Table 3. Clinical Characteristic of CML-CP, CML-BP and ALL Patients

	M-bcr	m-bcr	Mixed	*p*
CML-CP patients	151	25	173	
AGE median(P5-P95)	44(19, 66)	36(22, 43)	37(16, 62)	0.000**
RBC (×1012/L), median(P5-P95)	4.2(2.6-6.7)	3.8(2.4-6.2)	3.9(2.4-5.9)	0.0523
WBC (×109/L), median(P5-P95)	184.76(14.96, 487.15)	11.64(4.14, 27.51)	81(10.56, 393.20)	0.000**
PLT (×109/L), median(P5-P95)	392(130, 537)	206(69, 300)	293(1097, 917)	0.000**
Primitive cell ratio (%)	1.0 (0.5, 4.5)	1.5 (0.5, 4.5)	1.5 (0, 4.5)	0.431
BCR-ABL relative concentration at diagnosis (%) median(P5-P95)	13.14(3.42, 24.06)	13.14(3.43, 26.14)	11.95(3.14, 31.56)	0.752
CML-BP patients	7	8	11	
AGE median(P5-P95)	28(12, 56)	39(35,68)	43(26,48)	0.349
RBC (×1012/L), median(P5-P95)	2.7(2.0-4.9)	2.5(1.8-3.8)	2.9(1.7-3.9)	0.143
WBC (×109/L), median(P5-P95)	78.03(39.98, 226.55)	26.41(14.15, 65.14)	29.71(1.79, 75.6)	0.007**
PLT (×109/L), median(P5-P95)	204(93, 831)	153(41, 325)	118(45, 1146)	0.349
Primitive cell ratio (%)	53.5(32.5, 93.5)	68.5(38.5, 94.5)	61.5(42.5, 93.5)	0.137
BCR-ABL relative concentration At diagnosis (%) median(P5-P95)	51.99(5.27, 242.94)	16.08(0.08, 255.72)	10.48(5.04, 26.48)	0.131
ALL patients	8	37	21	
AGE median(P5-P95)	40(34, 50)	35(15, 59)	40(16, 64)	0.432
RBC (×109/L), median(P5-P95)	2.3(1.7,3.2)	2.1(1.8-3.4)	2.4(1.9-3.6)	0.324
WBC (×109/L), median(P5-P95)	97.72(34.22, 249.32)	15.92(4.57, 85.03)	12.65(2.2, 54.23)	0.000**
PLT (×109/L), median(P5-P95)	189(84, 839)	72(16, 293)	92(15, 395)	0.016*
Primitive cell ratio (%)	75.5(54.5, 98.5)	79.5(58.5, 96.5)	72.5(60.5, 98.0)	0.452
BCR-ABL relative concentration at diagnosis (%) median(P5-P95)	80.95(6.03, 659.75)	28.92(8.21, 213.74)	19.82(2.49, 309.74)	0.12

*p<0.01; **p<0.05

Table 4. *BCR-ABL* Relative Concentration of CML-CP and ALL Patients after Imatinib Treatment

	M-bcr	m-bcr	MIX	*p*
CML-CP patients (n)	40	14	26	
BCR-ABL relative concentration after 3 months (%) median(P5-P95)	1.6(0, 8.87)	5.1(0, 16.8)	3.2(0.0, 11.5)	0.040*
BCR-ABL relative concentration after 6 months (%) median(P5-P95)	0.05(0.0, 7.2)	2.41(0.0, 13.8)	0.9(0.0, 9.0)	0.049*
BCR-ABL relative concentration after 9 months (%) median(P5-P95)	0.0(0.0, 4.5)	1.20(0, 8.9)	0.6 (0.0, 4.32)	0.020*
BCR-ABL relative concentration after 1 year (%) median(P5-P95)	0.0(0.0,0.5)	0.3(0.0, 9.3)	0.2(0.0, 5.76)	0.007**
Replase after treatment	0	2/12	0	
ALL patients(n)	4	20	8	
BCR-ABL relative concentration After 3 months (%)	0.32(0.0, 9.12)	1.29(0.0, 7.8)	3.26(0.0, 9.92)	0.59
BCR-ABL relative concentration After 6 months (%)	0(0.0, 4.62)	1.02(0.0, 6.12)	0.28(0.0, 6.12)	0.244
BCR-ABL relative concentration After 9 months	0(0.0, 1.72)	0.52(0.0, 5.31)	0.1(0.0, 5.01)	0.139
BCR-ABL relative concentration (%) After 1 year (%)	0(0.0, 0.92)	0.98(0.0,4.32)	0(0.0, 3.32)	0.012*
Replase after treatment	0	1/20	0	

**p<0.01; *p<0.05

CP patients followed-up were also divided into three groups according to the *BCR-ABL* subtypes. In 40 *M-bcr* patients, the median *BCR-ABL* relative concentration after 3 months, 6 months, 9 months and 1 year treatment were 1.6%, 0.05%, 0.0%, and 0.0%. In 14 *m-bcr* patients, the median *BCR-ABL* relative concentration were 5.1%, 2.41%, 1.2% and 0.3% and in 26 mixed patients, 3.2%, 0.9%, 0.6% and 0.2% respectively. Patients with *m-bcr* showed notable higher *BCR-ABL* relative concentration than *M-bcr* and mixed group with significantly differences and the differences enlarged with further treatment as shown in Table 4. Two patients showed *m-bcr* got relapse. In 32 ALL patients followed-up, 4 patients were *M-bcr* subtype, with median *BCR-ABL* relative concentration of 0.32%, 0.0%, 0.0%, 0.0% respectively. 20 were *m-bcr* subtype with median *BCR-ABL* relative concentration of 1.29%, 1.02%, 0.52%, 0.98% and 8 were mixed with median *BCR-ABL* relative concentration of 3.26%, 0.28%, 0.1%, 0.0% respectively. Patients with *m-bcr* showed higher *BCR-ABL* relative concentration after treatment for 1 year with significant difference (p=0.012). One patient showed *m-bcr* got relapse.

Discussion

BCR-ABL fusion gene is one of the most commonly detected abnormal genes in leukemia. Though the *BCR-ABL* subtype is clearly defined, the distribution and expression levels of *BCR-ABL* subtypes, their relationships with clinical manifestations and prognosis in CML and ALL have not been well established yet. It has been reported that the expression of *BCR-ABL* is associated with the disease severity. In our previous researches (Lu et al., 2011), we have confirmed the significance of Real-time fluorescent PCR in monitoring treatment responses, and identification of relapse in CML patients. Some pervious showed (Gabert et al., 2003; Tripathi et al., 2011) that in patients who receive interferon treatment that does not induce molecular and cytogenetic remission, the increased expression of *BCR-ABL* suggests that the disease may

develop into an accelerated phase or a blastic phase, which is valuable for evaluating the prognosis of the patients (Cetin et al., 2012; Mu et al., 2012). In our present study, we observed significant different expression of *BCR-ABL* gene in CML-CP patients, CML-BP patients and ALL patients, which indicated that the expression of *BCR-ABL* was higher in CML-BP patients and Ph+ ALL patients than that in CML-CP patients. This result was not only consistent with other studies (Cetin et al., 2012; Ernst et al., 2012; Mu et al., 2012) but also a beneficial complement to other studies. It was reported in some early reports that *m-bcr* might be frequent in B lymphoid progenitor cells but uncommon in hematopoietic stem cells, explaining the rarity of *m-bcr* in CML but *M-bcr* and *m-bcr* per se can induce the myeloproliferative process, resulting in promotion of chronic phase CML (Bhatia et al., 2012; Gokbuget et al., 2012). These reports also showed that in patients showed a higher incidence of expression of *m-bcr* than *M-bcr* in ALL (Lee et al., 2005; Wang et al., 2011). In our study, we found distinct different distribution of *M-bcr*, *m-bcr* and mixed subtype among CML-CP, CML-BP and ALL patients. Our results confirmed that CML-CP patients have more *M-bcr* while ALL patients with more m-bcr, which was similar to other studies. In consistent with previous reports the occurrence of mixed subtype was higher than that reported (Li et al., 2010), this difference may result from the mutual distraction during PCR. In this study, we analyzed ages, bone marrow smear cytology results, blood routine examination results and *BCR-ABL* concentration before treatment in CML-CP CML-BP and ALL patients with different subtypes. In both CML-CP group and CML-BP group, patients with *M-bcr* showed older age with higher WBC and PLT counts. In contrast, *m-bcr* occurs more likely in younger CML patients, and atypical patients without markedly increased WBC counts. These results suggested that *M-bcr*, which encodes the p210, was associated with the typical CML with older age and markedly increased WBC counts. This finding agrees with the reports that over-expression of p210 leads to the occurrence of the CML (Killick et al., 1999). In ALL patients, our study showed that patients with *M-bcr* have a significantly higher WBC counts which was coincident with other reports that patients with Ph-positive ALL presented with higher WBC counts (Killick et al., 1999; Matutes et al., 2011). Overall, *M-bcr* occurs more frequently in typical CML patients and high-WBC ALL patients. Following the results of the IRIS multicenter trial (Druker et al., 2006), IM promptly became the standard frontline therapy of CML in CP and it is well-known that relative *BCR-ABL* concentration is an important prognosis factor (Mizuta et al., 2012), *BCR-ABL* relative concentration after treatment was chosen as an indicator of prognosis. The followed-up dates indicated that patients with *M-bcr* showed a lower relative *BCR-ABL* concentration after treatment in both CML-CP and ALL, which coincided with reports that (Al-Seraihy et al., 2009; Weinberg et al., 2010) these patients who show older ALL patients, exhibits a resistance to IM treatment more likely with a poor prognosis. These results support our predetermination that patients with *M-bcr* have a better prognosis than with *m-bcr* subtype. No CML-BP patient was followed long enough, because most of them were under chemotherapy or allogenetic bone marrow transplantation therapy. It has been reported that the *BCR-ABL* protein shows an increased tyrosine kinase activity and it seems to deregulate the normal cytokine-dependent signal transduction pathways leading to the inhibition of apoptosis. From our research, patients with different *BCR-ABL* subtype showed clear difference in clinical feature and prognosis, which gave us a hint that different *BCR-ABL* subtypes may play different role in leukemogenesis. Early reports indicated that the Rac-family kinases have been implicated in *BCR-ABL* subtype signaling (Mulloy et al., 2010) and in the progression of CML and Ph+ ALL (Cho et al., 2005). All these prompted us a potential direction to further study the mechanism of leukemogenesis in different *BCR-ABL* subtypes. From the present study, we obtain some important information regarding. Firstly, we find that the expression of *BCR-ABL* is significantly different between CML-CP and CML-BP patients, suggesting that an abrupt increase in *BCR-ABL* expression may imply that the disease may start to enter an accelerated phase or a blastic crisis phase. Secondly, we confirm that *BCR-ABL* subtypes occur differently between the CML patients and ALL patients. Thirdly, our study suggests that patients with the *m-bcr* subtype may have a worse prognosis than patients with the *M-bcr* subtype. But there are still some limitations in our study. First, the statistical difference may be affected by the small sample sizes in CML-BP patients and ALL patients. Second, the mechanisms of *M-bcr* and *m-bcr* and their associated prognosis in different diseases need further study in a large sample of patients with a long-term follow-up.

In summary, we investigate the expression of *BCR-ABL* subtypes in CML-CP patients, CML-BP patients and Ph+ ALL patients, and find that *M-bcr* subtype and *m-bcr* subtype occur differently among these patients. *M-bcr* occurs more frequently in classical CML patients and younger ALL patients, who show a good response to imatinib. In contrast, *m-bcr* occurs more frequently in atypical CML patients and older ALL patients, who exhibit a resistance to imatinib treatment with a poor prognosis.

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