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Original Article

Study on the detective value of qPCR for BCR-ABL fusion gene expression in patients with chronic myeloid leukemia

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Abstract: Objective: To explore the detection value of BCR-ABL fusion gene expression levels by qPCR in evaluating the disease severity and treatment effects of chronic myeloid leukemia (CML). Methods: From February 2016 to December 2017, 60 CML patients were included in this research. BCR-ABL fusion gene expression levels were detected by qPCR. The levels of BCR-ABL fusion gene expression in different CML stages and the relationship between BCR-ABL fusion gene expression and CML stages were analyzed. Before and after imatinib treatment, the levels of BCR-ABL fusion gene expression were also detected. Moreover, the disease-free survival time and rates of curative effect satisfaction were compared among different BCR-ABL fusion gene expression levels groups. The Receiver Operating Characteristic (ROC) curve was used to evaluate the value of BCR-ABL fusion gene expression predicting clinical effect of imatinib. Results: The level of BCR-ABL fusion gene expression was lowest in chronic CML patients and highest in acute CML patients. Pearson's correlation analysis indicated that the levels of BCR-ABL fusion gene expression were positively associated with disease progression of CML ($r=0.617$, $P=0.009$). With prolonged treatment, the BCR-ABL fusion gene expression levels were decreased. Compared with that before imatinib treatment, the levels of BCR-ABL fusion gene expression were significantly decreased ($P<0.001$). CML patients in the high-level of BCR-ABL fusion gene expression group had the shortest disease-free survival time ($P<0.05$) and lowest satisfaction rate ($P<0.001$). ROC curve indicated that BCR-ABL fusion gene expression had good value for prediction of clinical efficacy of imatinib ($P<0.05$). Conclusion: The expression level of BCR-ABL fusion gene by qPCR detection is helpful to evaluate disease progression and curative efficacy of imatinib in CML patients.

Keywords: BCR-ABL fusion gene, chronic myeloid leukemia, quantitative polymerase chain reaction, detective value

Introduction

Chronic myelogenous leukemia (CML) is the most common leukemia disease, which accounts for appropriately 15% to 20% of adult leukemia [1, 2]. Previous studies reported that CML is characterized by the presence of BCR-ABL fusion gene, which encodes a chimeric BCR-ABL protein that constitutively activates tyrosine kinase [3, 4]. Activation of tyrosine kinase could ultimately lead to uncontrolled cell growth and differentiation [5, 6]. Some studies showed that abnormal cell load in CML patients was associated with BCR-ABL fusion gene expression [7]. It is generally known that Imatinib is a first-line, molecule-targeted drug for CML treatment. It was reported that CML patients with elevated levels of BCR-ABL fusion gene expression were resistant to Imatinib [8]. Thus, it can be seen that effective monitoring of BCR-ABL fusion gene expression levels is of great clinical significance to assess the disease severity and treatment effect of Imatinib in CML patients.

Fluorescence in situ hybridization, cytogenetics, common polymerase chain reaction and other methods were used to evaluate the prognosis of CML patients. However, there are some limitations such as provision of only negative or

Table 1. The primer and probe sequences of ABL and BCR-ABL

Targeted genes		Primers (sequences 5'-3')
ABL	forward	5'-TGGAGATAACACTCTAAGCATAACTAAAGGT-3'
	Reverse	5'-GATGTAGTTGCTTGGGACCCA-3'
	TaqMan probe	5'-CCATTTTTGGTTTGGGCTTCACACCATT-3'
BCR-ABL	forward	5'-TCCGCTGACCATCAATAAGGA-3'
	Reverse	5'-CACTCAGACCCTGAGGCTCAA-3'
	TaqMan probe	5'-CCCTTCAGCGGCCAGTAGC ATCTGA-3'

positive results, long periods of detection, failure to reflect the severity or dynamic changes of CML, and low sensitivity and so on [9, 10]. Real-time quantitative PCR has recently been regarded as a tool for rapid and sensitive detection of BCR-ABL fusion gene expression. This technique shows the advantages of easy operation, low cost, high specificity and sensitivity, and short reaction period [11, 12]. There is little known about the role of qPCR detecting BCR-ABL fusion gene expression levels in assessing prognosis of CML patients, so more studies are needed for further confirmation. In this research, qPCR was applied to examine BCR-ABL fusion gene expression in 60 CML patients for investigating the role of BCR-ABL fusion gene expression in evaluating the disease severity and treatment effect of CML, in hope of providing experimental foundation for guiding treatment.

Materials and methods

Subjects

This study was approved by the ethics committee and obtained written informed consent was generated from patients and their families. From February 2016 to December 2017, 60 patients with CML admitted to our hospital were included in this research. All the patients were diagnosed as having CML through pathological examination. The inclusion criteria was as follows: (1) An age of more than 18 old years; (2) Patients met the diagnostic criteria for CML by the National Comprehensive Cancer Network (NCCN) in 2015 [13]; (3) Neither contraindications to chemotherapy nor previous targeted drugs; (4) An expected life time of more than one year. The exclusion criteria was as follows: (1) Pregnant or lactating women; (2) Major organ dysfunction such as heart, lung, liver, kidney and so on; (3) Other types of leukemia and malignant tumors with brain or bone metastasis; (4) Patients who were allergic to imatinib; (5) Mental disorders; (6) Patients did not comply with this research or the clinical data was incomplete. According to the methods reported by Fava [14], patients were divided into three groups as follows: the low level group (BCR-ABL fusion gene expression less than 10%), the medium level group (BCR-ABL fusion gene expression between 10% and 50%), and the high level group (BCR-ABL fusion gene expression more than 50%). According to the staging of disease, Patients were assigned to either chronic stage, accelerated stage or acute stage. All the patients were followed up for one year. Patients were examined once every 3 months with telephone calls and clinic appointments.

Examination of BCR-ABL fusion gene expression by Real-time PCR

Five mL of blood was drawn from the median cubital vein of patients in a fasting state in the morning. All the blood was placed in an EDTA anticoagulation tube for detection. Leukocytes were extracted from the blood using human lymphocyte separation medium (Sigma, USA) after mixing. The Trizol reagent (Invitrogen, USA) was used for extracting the total RNA. Then, total RNA was synthesized into cDNA by reverse transcription polymerase chain reaction. The ABL gene expression was used as the internal reference. The primer and probe sequences of ABL and BCR-ABL are shown in **Table 1**. According to the instructions of PCR kits (Thermo Fisher Scientific, USA), Real-time PCR was performed. The reaction system was as follows: 1.8 μL of cDNA, 10 μL of Premix Ex Taq™ (2×), 0.6 μL of fluorescent probe solution, 0.4 μL of forward or reserve primer, and 6.8 μL of ddH_2O. PCR amplification was conducted in Applied Biosystems 7500 PCR System. The reaction conditions were as follows: re-denaturation at 95°C for 35 s, followed by 50 cycles of denaturation at 95°C for 4 s, renaturation at 57°C for 20 s, and extension at 55°C for 20 s. The relative BCR-ABL expression levels were obtained by the standard curve and Ct values. The computational formula was as follows: The expression of BCR-ABL fusion gene(%) = copy number of BCR-ABL fusion gene/copy number of ABL gene ×100%.

Table 2. Basic data of patients

Parameter	Values
Number of cases	60
Sex (cases)	
Male	36
Female	24
Average age (years)	43.1±3.8
BMI (kg/m^2)	22.3±1.2
Course of disease (years)	2.4±1.1
Disease stages (cases)	
Chronic stage	36
Acute stage	11
Accelerated stage	13

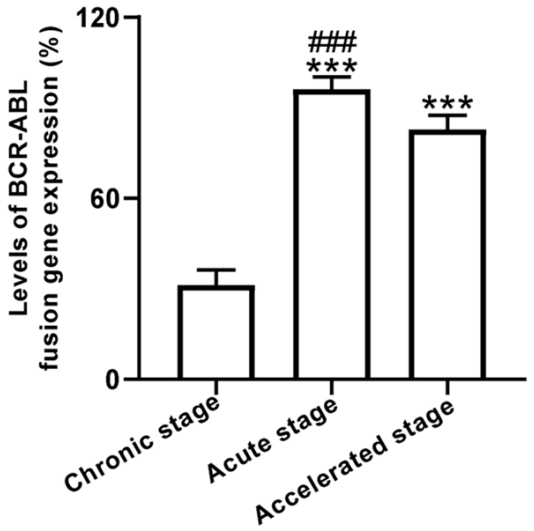

Figure 1. The levels of BCR-ABL fusion gene expression in CML patients with different stages. Compared with chronic stage, *** indicated P<0.001; Compared with accelerated stage, ### indicated P<0.001.

Outcome measures

Primary outcome measures: Before treatment, The BCR-ABL fusion gene expression was statistically analyzed in patients with different disease stages. All patients received targeted therapy with imatinib. BCR-ABL fusion gene expression was detected before treatment and at different time points (3 months, 6 months, 9 months and 12 months) after treatment.

Secondary outcome measures: Disease free survival was compared among patients with different levels of BCR-ABL fusion genes. The satisfaction of therapeutic effect was compared at one year after treatment among patients with different levels of the BCR-ABL fusion gene. The evaluation criteria for satisfaction of therapeutic effects were as follows [15]: patients achieve partial remission at cytogenetical and hematological examination in 3 months treatment; pateints achieve complete remission at cytogenetical examination in 6 months treatment; patients achieve complete remission at molecular examination in 12 months treatment.

Statistical analysis

The whole data were analyzed using SPSS statistical software version 22.0 (IBM, USA). The measurement data were calculated as the mean. Independent sample t test was applied for comparison between two groups, while one-way Anova and post hoc Bonferroni test was applied for comparison among more than three groups. The enumeration data were expressed as number/percentage (n/%); comparison was conducted with chi-square test. Pearson's correlation analysis was used to detect the correlation between BCR-ABL fusion gene expression and disease stages. Receiver operating characteristic curve (ROC) was applied to predict the role of BCR-ABL fusion gene expression in imatinib treatment according to the area under the curve. The difference was considered statistically significant if the *P* value was less than 0.05.

Results

Basic data for patients

The patients' information was summarized in **Table 2**. There were 60 CML patients in this study including 36 cases with a chronic stage, 11 cases with an acute stage and 13 cases with an accelerated stage. The average age of CML patients was 43.1±3.8 years. Among them, there were 36 males and 24 female. The disease course was 2.4±1.1 years and the body mass index (BMI) was 22.3±1.2 kg/m^2.

Detection of BCR-ABL fusion gene expression in CML patients with different stages

BCR-ABL fusion gene expression in patients with chronic, acute and accelerated stages was 31.24±4.97%, 96.12±4.17% and 82.73±4.69%, respectively. As shown in **Figure 1**, acute CML patients had the highest level of

Table 3. Correlation analysis between BCR-ABL fusion gene expression levels and disease progress of CML

Parameter	R	P
Levels of BCR-ABL fusion gene expression	0.617	0.009

BCR-ABL fusion gene expression and chronic CML patients had the lowest level. Statistical significance was found among patients with different stages (P<0.001). As seen in **Table 3**, Pearson's correlation analysis showed that the levels of BCR-ABL fusion gene expression were positively associated with disease progress of CML.

Detection of BCR-ABL fusion gene expression in CML patients before and after imatinib treatment

As seen in **Table 4**, the levels of BCR-ABL fusion gene expression in CML patients after imatinib treatment were lower than that before treatment, and there was a significant statistical difference (P<0.001). Furthermore, with the treatment prolonged, the BCR-ABL fusion gene expression levels were decreased.

The disease-free survival in CML patients with different BCR-ABL fusion gene expression levels after treatment

As seen in **Figure 2**, the average disease-free survival time was 5.15±2.32 months in the high-level group, 14.28±5.96 months in the medium level group and 21.64±6.98 months in the low-level group. There were significant statistical differences among groups (F= 26.14, P<0.001). The ROC curve of predictive values of BCR-ABL fusion gene expression showed that the cutoff value was 65.78%, the specificity was 0.465, and sensitivity was 0.776 (AUC=0.658, 95% CI: 0.556-0.804). Significant differences were found (P=0.025), as seen in **Figure 3**.

The rate of curative effect satisfaction in CML patients with different BCR-ABL fusion gene expression levels

As seen in **Table 5**, the *rate of curative effect satisfaction* was 63.16% (12/19) in the high-level group, 40.74% (11/27) in the medium-level group and 14.29% (2/14) in the low-level group. There were significant statistical differences among groups (χ^2=5.936, P=0.041).

Discussion

With an increase in age, the occurrence rate of CML increases remarkably, and there is even a trend of younger patients. CML disease causes a serious threat to human health and quality of life [16]. It is reported that BCR-ABL fusion gene expression plays an important role in the development of CML disease, and seriously affects the prognosis of patients [17].

So far, qPCR has been widely applied to accurately detect BCR-ABL fusion gene expression. In this research, BCR-ABL fusion gene expression in 60 CML patients was examined by the method of qPCR. The results showed that BCR-ABL fusion gene expression level in CML patients with an acute stage was significantly higher than that in the accelerated stage. BCR-ABL fusion gene expression level in CML patients with an accelerated stage was markedly higher than that in CML patients with a chronic stage. Moreover, the results of Pearson's correlation analysis indicate that BCR-ABL fusion gene expression levels were significantly associated with the disease stages. Thus it can be seen that the stages of CML patients can be accurately evaluated according to the BCR-ABL fusion gene expression levels. This result is similar with the findings reported by Kang et al [18]. Previous studies reported that in comparison with that in the control group, the positive rates of BCR-ABL fusion gene were significantly higher and the statistical differences were markedly found, which indicates that the monitoring of BCR-ABL fusion gene expression is helpful for diagnosis of CML [19]. Other studies also showed that the dynamic monitoring of BCR-ABL fusion gene expression levels detected by qPCR was helpful to screen patients with relapsed leukemia and it contributed to early intervention and improvement of survival time in CML patients [20].

Furthermore, the monitoring of BCR-ABL fusion gene expression plays an important role in evaluating therapeutic effects [21]. It was reported that post-transplant intervention for CML patients receiving hematopoietic stem cell transplantation was based on BCR-ABL fusion gene expression and immunomodulation treatment had the advantages of the high-

Table 4. The changes of BCR-ABL fusion gene expression levels in CML patients during imatinib treatment

Parameter	Treatment time				
	Before treatment	3 months	6 months	9 months	12 months
Levels of BCR-ABL fusion gene expression (%)	56.12±4.31	11.25±3.28***	3.04±1.34***	1.14±0.78***	0.041±0.003***

Note: Compared with before treatment, ***indicated P<0.001.

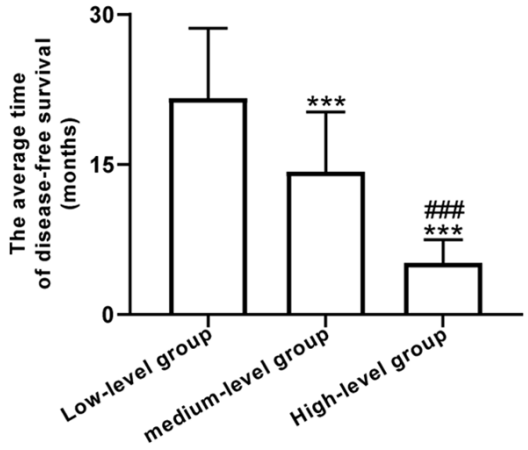

Figure 2. Comparison of disease-free survival time among CML patients with different BCR-ABL fusion gene expression levels. Compared with low-level group, *** indicated P<0.001; Compared with medium-level group, ### indicated P<0.001.

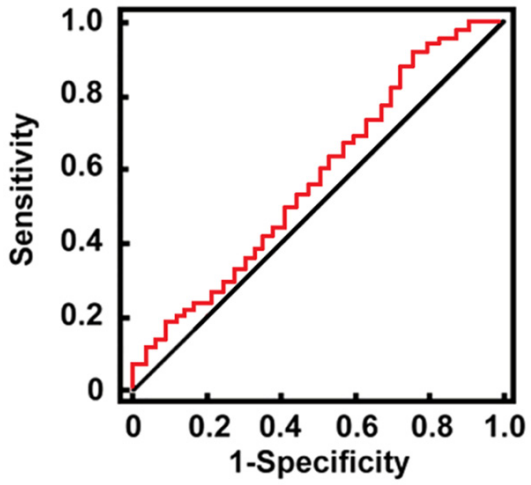

Figure 3. The ROC curve for BCR-ABL fusion gene expression predicting clinical effect of imatinib in CML patients.

est negative conversion rate of BCR-ABL fusion gene [22]. Additionally, a cytogenetic test revealed 5-10% of CML patients had negative Ph chromosome, but approximately 30-50% of them showed positive Ph chromosome by a

Table 5. Comparison of *satisfaction* rates among CML patients with different BCR-ABL fusion gene expression levels

Groups	Cases	Satisfaction rates (%)
low-level group	19	63.16
medium-level group	27	40.74*
high-level group	14	14.29*,#

Compared with the low-level group, *P<0.05; compared with the medium-levle group, #P<0.05.

BCR-ABL gene test, suggesting that detection of BCR-ABL fusion gene is favorable for evaluation of minimal residual disease [23]. In this research, the effect of dynamic detection of BCR-ABL fusion gene expression examined by qPCR in CML patients treated by imatinib was evaluated. It is suggested that with prolonged treatment time, the BCR-ABL fusion gene expression level was decreased. The most rapid decline occurred at 3 months after imatinib treatment, which is similar with the results of Murai et al's study [24]. This research also indicated that CML patients with low-level of BCR-ABL fusion gene expression had the longest disease-free survival time and highest satisfaction rate. And there were significantly statistical differences among different groups of BCR-ABL fusion gene expression levels in term of disease-free survival time and satisfaction rate. The ROC curve revealed that detection of BCR-ABL fusion gene expression was helpful to evaluate the efficacy of targeted therapy with imatinib in CML patients. Therefore, dynamic monitoring of BCR-ABL fusion gene expression contributes to evaluate poor response or resistance to imatinib at an earlier time. This helps us to adopt more appropriate regimens for early treatment, thereby realizing accurate treatment in patients with different stages of CML and improving survival time.

However, there are still some limitations in this study, such as having a small sample size, being a single-center study, unclear cut-off val-

ues for BCR-ABL fusion gene expression of recurrent CML patients by the molecular and biological detection, and no supply of dynamic changes of BCR-ABL fusion gene expression in CML patients with different stages, and so on. Prospective randomized controlled trials with larger sample size and multi-center studies are required in future research to further validate the value of qPCR detecting BCR-ABL fusion gene expression in CML patients.

In conclusion, the examination of BCR-ABL fusion gene expression by qPCR contributes to evaluating the disease stages. Dynamic monitoring of BCR-ABL fusion gene is helpful to assess the efficacy of imatinib treatment. It has a broad application prospect for detection of BCR-ABL fusion gene expression by qPCR in diagnosis and treatment of CML patients in the future.

Disclosure of conflict of interest

None.

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