Advantages of digital PCR in the detection of low abundance *BCR-ABL1* gene in patients with chronic myeloid leukemia

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Abstract. Quantitative monitoring of *BCR-ABL1*[IS] gene using reverse transcription quantitative-PCR (RT-qPCR) is an important method for evaluating the treatment effects in patients with chronic myeloid leukemia (CML). Digital-PCR (dPCR) can be applied to detect the *BCR-ABL1* gene with high sensitivity. In the present study, the results of the Clarity™ dPCR system were compared with those of the RT-qPCR in order to determine whether dPCR can be applied in the clinical setting. A total of 83 patients were included in the present study, and they were divided into two groups according to the results of *BCR-ABL1*[IS] during ongoing monitoring. A total of 43 patients with undetectable *BCR-ABL1*[IS] where enrolled in group A. *BCR-ABL1* testing was performed using the dPCR system on the same peripheral blood samples of patients from group A, and the association between dPCR results and relapse was analyzed. The RT-qPCR platform and dPCR system were used simultaneously to detect the *BCR-ABL1* gene of another 40 patients who achieved either partial cytogenetic response (PCyR) or further response. Among patients with undetectable *BCR-ABL1*[IS], patients with dPCR-positive disease (*BCR-ABL1* >0.1%) were more likely to undergo molecular relapse (P=0.018). The results of dPCR detection of *BCR-ABL1*% were consistent with the RT-qPCR results (R^2=0.9510) in patients who achieved PCyR or further response. For samples with *BCR-ABL1*[IS] <1.0%, the consistency of the dPCR and RT-qPCR results was better than that of *BCR-ABL1*[IS] >1.0% (R^2=0.9488 vs. R^2=0.9264 for *BCR-ABL1*[IS]). The detection results of the *BCR-ABL1* gene in patients with CML using dPCR matched well with those from the RT-qPCR. To conclude, the results of the dPCR system can be applied as a supplement to the RT-qPCR platform, particularly for those with *BCR-ABL1*[IS] <1.0%.

Introduction

The *BCR-ABL1* gene is a molecular marker of chronic myeloid leukemia (CML), and its transcript level can accurately reflect tumor burden (1). Molecular monitoring refers to the detection of *BCR-ABL1* transcripts in the peripheral blood through the use of reverse transcription quantitative-PCR (RT-qPCR) (2,3). In October 2005, an International Standard was proposed for molecular testing, which included changing *BCR-ABL1* gene detection in every laboratory via a conversion factor (4). Therefore, *BCR-ABL1* gene detection was also converted to the international standard value *BCR-ABL1*[IS] in the present study (5,6).

Quantitative monitoring of *BCR-ABL1*[IS] via RT-qPCR is currently the gold standard method of evaluating patient response to tyrosine kinase inhibitors (TKIs) and subsequent classification into prognostic subgroups (7). However, RT-qPCR has a number of shortcomings: The difference in amplification efficiency between the reference gene and the target gene, and the difference between platforms and personnel skill level among different laboratories leads to errors in amplification efficiency (8). Therefore, a consistent conversion factor must be established and verified on a regular basis (9).

Digital PCR (dPCR) is used in the detection and quantification of nucleic acids (10). First, dPCR evenly distributes the reaction system into a large number of reaction units, and the number of nucleic acid sequences of interest conforms to the Poisson distribution (7). PCR amplification is then performed independently in each reaction unit. Following the end of the amplification, the fluorescence signal of each reaction unit is detected, and finally the copy number of the nucleic acid sequence of interest is calculated based on the ratio of the Poisson distribution and the reaction unit that is positive for the fluorescent signal in all the reaction units (7). The primary advantage of dPCR over RT-qPCR is that it can be performed without the requirement for a calibration curve,

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therefore offering more straightforward means of ensuring interlaboratory reproducibility (9,11).

Materials and methods

Patients and samples. A total of 83 patients were included in the present study, and these were divided into two groups: Groups A and B. All patients were diagnosed in the Department of Hematology of The Affiliated Hospital of Xuzhou Medical University between September 10, 2016 and March 4, 2017. A total of 43 patients with undetectable *BCR-ABL1*[IS] result from peripheral blood were selected as the group A. The median age of patients in group A was 45 years (age range, 13-72 years), including 22 men and 21 women. Only 25 patients from group A had scokal scores (1). The same blood samples of Group A patients were tested using the Clarity™ dPCR system (JN Medsys). Group B comprised of 40 patients who achieved either cytogenetic response or further response between January 3, 2017 and May 3, 2017. The median age of patients in group B was 49 years (age range, 10-68 years), including 24 men and 16 women. Only 24 patients from groups B had sokal scores. A RT-qPCR platform (Roche Diagnostics) and Clarity™ dPCR system was used to detect the *BCR-ABL1* fusion gene within the same peripheral blood sample, simultaneously. There was no BCR-ABL1 kinase domain mutation detected in patients enrolled in the present study. The study protocol was approved by the Ethics Committee of the Affiliated Hospital of Xuzhou Medical University, and all patients included in the present study provided written informed consent.

Quantification of the human BCR-ABL1 fusion gene using the Clarity™ dPCR system. DNA was diluted 10- or 100-fold prior to quantifying the human *BCR-ABL1* fusion gene using the Clarity™ dPCR system to achieve the expected target concentration range of 0.38 to 2,240 copies/μl reaction mixture. The samples were diluted using sterile water for injection. The probe and primers used were supplied by JN Medsys. The sequences of the primers and probes are listed in Table I. Each sample was a total of 15 μl, with a mixture of 0.25 μM forward and reverse primers, 0.25 μM probe, 1xMaster Mix, 1xClarity™ JN solution, 3 μl DNA sample and PCR grade water. Using the Clarity™ autoloader, the resultant mixture was then delivered onto the chip where it was subdivided into 10,000 partitions. The partitions were then sealed using Clarity™ Sealing Enhancer and 230 μl Clarity™ Sealing Fluid with the following thermocycling conditions: Initial cycle of 95°C for 10 min, 40 cycles of 95°C for 50 sec and 58°C for 90 sec (ramp rate, 1°C/sec). Following amplification via PCR, the tube strips were transferred to the Clarity™ Reader, which detects fluorescent signals from each partition simultaneously. The data were analyzed using Clarity™ software (version 1.0; JN Medsys), and a proprietary algorithm was used for setting each threshold based on fluorescent intensities to determine the proportion of positive partitions out of the total. Based on this information, the software determines the DNA copies/μl of the dPCR mix using the Poisson statistics. The mean partition volume of 1.336 nl was used to calculate the copy number. Each dPCR test was performed twice, and the average value was taken as the final result.

Statistical analysis. The results of *BCR-ABL1* transcripts were statistically analyzed, with descriptions of the data including the calculation of mediums, ranges, standard deviations (SDs), coefficients of variation (CVs). The association between age and relapse was evaluated using the χ^2 test. Age and dPCR outcome were assessed via Kaplan-Meier analysis and log-rank test. Linear regression analysis was used to analyze the results of dPCR and RT-qPCR, and R^2 represents the coefficient of determination. P<0.05 was considered to indicate a statistically significant difference.

Results

Baseline characteristics of patients. In the present study, 39 patients were MR4.5 (*BCR-ABL1*[IS] ≤0.003 2% or undetectable disease in cDNA over 32, 000 *ABL1* transcripts) when enrolled in Group A, and 4 patients were MR5. Table II presents the clinical features of Group A patients. The median age of the patients at diagnosis was 45 years (range, 13-72 years). The disease state of patients in group A was chronic phrase, no patients were in accelerated phase or blast phase, and 68.0% of these patients had a low Sokal Score (1). The median white blood cell (WBC) count at the time of initial diagnosis was 62.7x10^9/l (17.8x10^9/l-263x10^9/l), median hemoglobin (HB) level was 94 g/l (53 g/l-145 g/l), median platelet (PLT) count was 414x10^9/l (153x10^9/l-2886x10^9/l). Of the total patients, 37 received hydroxyurea; 41 received TKIs; 3 patients had been treated with interferon; and four had received a hematopoietic stem cell transplantation (HSCT). The median duration of TKI treatment of the patients in Group A was 46.5 months (range, 14.1-149.0 months) prior to enrollment in the present study. When *BCR-ABL1* gene was undetectable by RT-qPCR, the maximum of the *ABL1* control transcripts was 228,110 copies. dPCR was used to detect peripheral blood samples from patients at the time that their *BCR-ABL1*[IS] results were undetectable. The % of *BCR-ABL1* was between 0.0030 and 9.2390% and the median was 0.0517%. The *BCR-ABL1%* was <0.1% in 24 patients, and was >0.1% in other 19 patients.

There were two patients already at PCyR when they were enrolled in Group B; 32 patients were CCyR; and 6 patients were MMR. Table II presents the clinical features of Group B patients. The median age at diagnosis was 47 years (range, 10-68 years). The median WBC count at the time of initial diagnosis was 126.7x10^9/l (10.8x10^9/l-700.7x10^9/l); HB level was 97 g/l (70 g/l-140 g/l); PLT count was 445x10^9/l (184x10^9/l-1,536x10^9/l). Of the total patients, 39 received hydroxyurea; 34 received TKIs; three received both TKIs and interferon; 2 patients received a HSCT; and 1 patient was diagnosed in the accelerated phase of disease at the initial diagnosis. The median duration of TKI treatment of patients in Group B was 24.7 months (3.4-127.9 months) prior to enrollment in the present study. When the *BCR-ABL1%* results detected via dPCR achieved PCyR, the PCyR or MMR values were between 0.0260 and 22.5714%, and the median was 0.6237%. The *BCR-ABL1%* results detected by dPCR were <1.0% in 25 patients, and was >1.0% in other 15 patients.

Molecular relapse of Group A patients. RT-qPCR was used to detect *BCR-ABL1* level in the peripheral blood of patients

Table I. Oligonucleotides used for *BCR-ABL1* amplification.

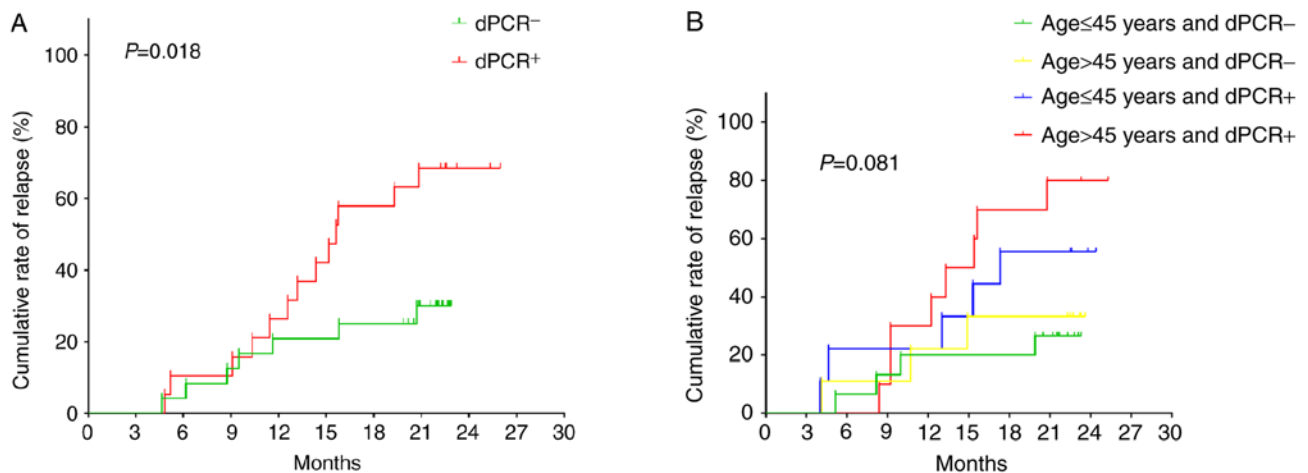| Oligonucleotide	Sequence (5'-3')
BCR-ABL1 forward	TCCGCTGACCATCAATAAGGA
BCR-ABL1 reverse	CACTCAGACCCTGAGGCTCAA
ABL forward	TGGAGATAACACTCTAAGCATAACTAAAGGT
ABL reverse	GATGTAGTTGCTTGGGACCCA
BCR-ABL probe	CCCTTCAGCGGCCAGTAGCATCTGA
ABL probe	CCATTTTTGGTTTGGGCTTCACACCATT

Figure 1. Risk of relapse associated with the dPCR results in Group A patients (P=0.018). (A) The association between the risk of relapse and age and the dPCR results of Group A patients (P=0.081). (B) Relapse was defined as *BCR-ABL1*[IS] >0.1%. dPCR+ indicates BCR-ABL1 >0.1%, dPCR- indicates *BCR-ABL1* <0.1%. ⊥ indicates censored observations. dPCR, digital PCR.

in Group A every 1-3 months starting from their initial enrollment date in the present study. Molecular relapse was defined as a *BCR-ABL1* level >0.1%. Relapsed patients ended their follow-up at the time of recurrence. Patients who did not relapse were followed up until October 31, 2018. During the follow-up period, 37 patients received TKIs according to the original protocol; two received both TKIs and interferon; and 4 patients who received HSCT did not take TKIs after the transplantation. At the end of the follow-up, depth of remission in two patients maintained MR5, 21 patients maintained MR4.5, and 20 patients had a molecular relapse. None of the patients included in the present study succumbed. No mutations in the *BCR-ABL1* kinase domain were detected. The median time to molecular relapse was 11.0 months (range, 3.6-18.4 months). The median follow-up time for all patients was 20.1 months (range, 3.6-25.3 months). Of the 24 patients with *BCR-ABL1* level <0.1% detected by dPCR, seven relapsed. Of the 19 patients with *BCR-ABL1* >0.1%, 13 experienced recurrence. (Fig. 1A; χ^2=5.560; P=0.018). The median *BCR-ABL1* level detected by dPCR system in the relapsed patients was 0.1860% (range, 0.0062-9.2390%), and the median *BCR-ABL1*% in patients without recurrence was 0.0537% (range, 0.0003-1.5323%) (P=0.080). Fig. 1B and Table III present relapse by age (≤45 years or >45 years) (χ^2=1.773; P=0.183). There was no statistically significant difference in the Kaplan-Meier survival curve (Fig. 1B; χ^2=6.731; P=0.081).

Comparison of BCR-ABL1 detected by RT-qPCR and dPCR in Group B patients. Table IV presents the SD and CV of the *BCR-ABL1* gene in Group B patients detected by two platforms. The SD and CV of dPCR detection results were lower than RT-qPCR in the two groups with *BCR-ABL1*[IS] <1.0% or >1.0%. Scatter plots demonstrate the linear relationship between the quantification of *BCR-ABL1* transcript copies (green plots), *ABL1* (blue plots) and *BCR-ABL1* (red plots) (Fig. 2). Quantification of the cDNA derived from clinical samples by RT-qPCR was compared with the dPCR platform. *BCR-ABL1* transcript copy numbers, *ABL1* transcript copy numbers and *BCR-ABL1*% measured by dPCR platform revealed a good association with RT-qPCR across all sample groups (Fig. 2). Fig. 3 presents *BCR-ABL1*% of the two disease levels (*BCR-ABL1*[IS] <1.0% or *BCR-ABL1*[IS] >1.0%) and the matched non-CML controls measured by dPCR and RT-qPCR. The samples for blank controls were generated with water instead of cDNA. The non-CML control groups also revealed a positive measurement for *BCR-ABL1* and can be distinguished from the test for patients with CML.

Discussion

At present, RT-qPCR is the preferred method for detecting BCR-ABL1 for the initial diagnosis and ongoing monitoring of CML (3,9). However, RT-qPCR can lead to errors in *BCR-ABL* detection. As such, appropriate correction factors

Table II. Baseline patient characteristics of groups A and B.

Variables	Group A (n=43)		Group B (n=40)	
	n	%	n	%
Sex				
Male	22	51.2	24	60.0
Female	21	48.8	16	40.0
Age (years)				
≤45	24	55.8	17	42.5
>45	19	44.2	23	57.5
Disease status				
Chronic phase	43	100.0	39	97.5
Accelerated phase	0	0.0	1	2.5
Blast crisis	0	0.0	0	0.0
ECOG PS				
0	39	90.7	37	92.5
1	4	9.3	3	7.5
Sokal score range				
<0.8	17	68.0[a]	11	45.8[b]
0.8-1.2	5	20.0	10	41.7
>1.2	3	12.0	3	12.5
Interferon				
No	40	93.0	37	92.5
Yes	3	7.0	3	7.5
Hydroxyurea				
No	6	14.0	1	2.5
Yes	37	86.0	39	97.5
Treatment response				
PCyR	0	0.0	2	5.0
CCyR	0	0.0	32	80.0
MMR	0	0.0	6	15.0
MR4.5	39	90.7	0	0.0
MR5	4	9.3	0	0.0

[a]Percentages based on 25 patients with available Sokal Score at diagnosis. [b]This percentages is based on 24 patients with available Sokal Score. ECOG, Eastern Cooperative Oncology Group; PS, performance status; PCyR, partial cytogenetic response; CCyR, complete cytogenetic response; MMR, major molecular response.

Table III. Correlation between age and relapse status in Group A patients.

Variable	Maintain MMR	Relapse	Total
Age ≤45 years	15	9	24
Age >45 years	8	11	19
Total	23	20	43

There is no statistically significant difference in the risk of relapse between those younger than 45 years and older than 45 years. (χ^2=1.773; P=0.183) MMR, Major molecular response.

Table IV. Comparison of SD and CV of BCR-ABL1% detected by RT-qPCR and dPCR in Group B patients.

BCR-ABL1[IS] level	SD	CV
BCR-ABL1[IS] <1.0%		
RT-qPCR	0.276	0.705
dPCR	0.273	0.703
BCR-ABL1[IS] >1.0%		
RT-qPCR	6.541	1.061
dPCR	4.645	0.930

RT-qPCR, reverse transcription quantitative-PCR; dPCR, digital PCR; SD, Standard deviation; CV, coefficient of variation.

need to be established and periodically verified (8,9). In the present study, only dPCR could be applied to the detection of *BCR-ABL1* gene, as dPCR is able detect a lower level of *BCR-ABL1* gene than RT-qPCR (8). dPCR has a higher sensitivity, which is an advantage when detecting very low levels of the *BCR-ABL1* gene (12). For molecular response monitoring of rare fusion transcripts associated with CML, dPCR is a very useful tool (13). Patients who intend to discontinue TKIs must achieve deep molecular remission, as when the RT-qPCR result is undetectable, a positive dPCR result may indicate a higher risk of relapse (14). The data from the present study demonstrated that there was no statistically

significant difference between the *BCR-ABL1* level detected by dPCR in relapsed and non-relapsed groups (P=0.080), but patients with *BCR-ABL1* >0.1% were more likely to experience molecular relapse (P=0.018), which is consistent with a previous study (14). The results from the present study also demonstrated that patients aged <45 years were more likely to relapse, but this was not statistically significant and therefore further larger scale studies are required (14). In the present study, the molecular relapse rate of patients in Group A was 46.5%, even if they achieved MR4.5 or MR5, suggesting it is necessary to closely monitor the *BCR-ABL1* gene for patients who achieved further MR (3,14). An ideal strategy would be to determine the *BCR-ABL1* level every 1-3 months, in order to expose early molecular relapse (3).

The main advantage of dPCR is that it can be performed without the need for a calibration curve, therefore offering a simpler method of ensuring reproducibility between different laboratories (11). In addition, dPCR can provide greater confidence in detecting low *BCR-ABL1* copy number concentrations at the limits of current RT-qPCR technology (12). Goh *et al* (15) compared dPCR and RT-qPCR to detect *BCR-ABL1* fusion gene in patients with CML and revealed that its sensitivity was 3 times higher than RT-qPCR. The data from the present study revealed that the results of dPCR for *BCR-ABL1* transcription, ABL1 transcription and *BCR-ABL1*% were in accordance with those of RT-qPCR, and the coherence at *BCR-ABL1*[IS] <1.0% was better than that at >1.0%. Normalization using the *ABL1* gene appeared to lead to error in the results, as there was
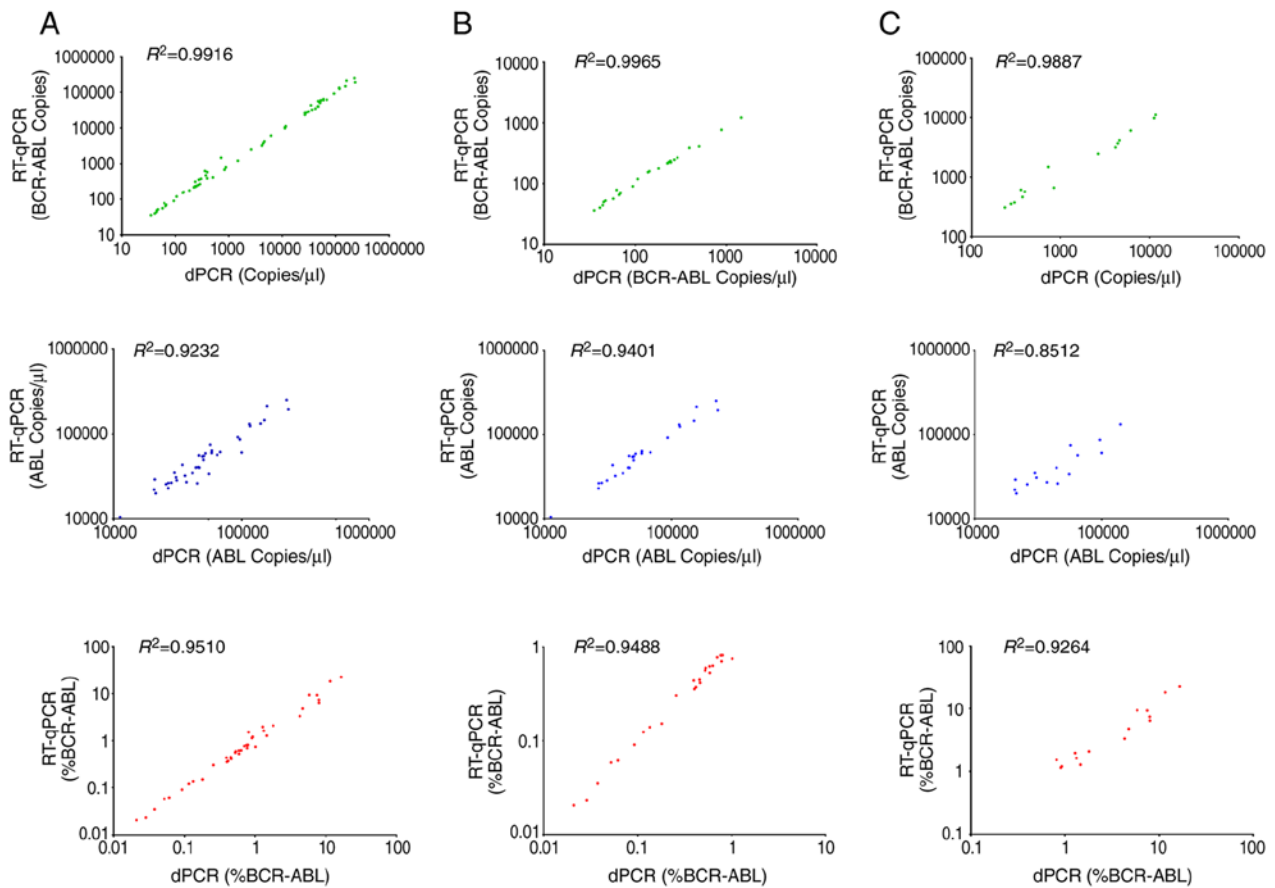
Figure 2. Comparison of RT-qPCR with dPCR for the quantification of all patients. (A) *BCR-ABL1*[IS] <1.0% patients (B) and *BCR-ABL1*[IS] >1.0% patients (C) in Group B. (B) The slope of these lines are 0.912, 1.013 and 1.150 for *BCR-ABL* Copies, *ABL* Copies and *BCR-ABL%*; (C) 0.901, 0.814 and 1.128 for *BCR-ABL* Copies, *ABL* Copies and *BCR-ABL%*, respectively. Each data point represents the value derived from the two PCR methods. Each sample was detected twice, and the average value was taken as the final result. RT-qPCR, reverse transcription quantitative-PCR; dPCR, digital PCR.
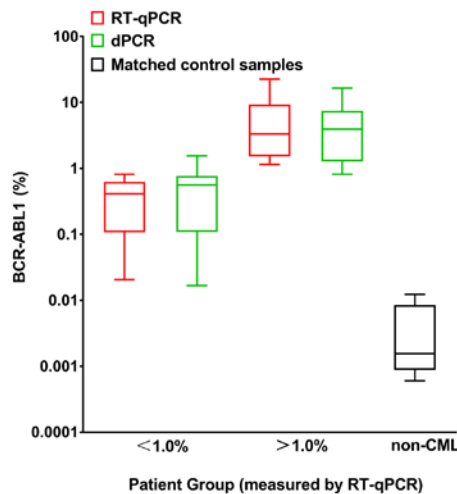

Figure 3. Disease levels measured by RT-qPCR (*BCR-ABL1*[IS] <1.0%, and *BCR-ABL1*[IS] >1.0%) are shown with the matched non-CML controls. The NTC reactions of matched control samples were generated with water in place of cDNA. The box plots present the median and interquartile range with the whiskers presenting the minimum and maximum data points from 2 replicates for each sample. RT-qPCR, reverse transcription quantitative-PCR; dPCR, digital PCR; CML, chronic myeloid leukemia.

generally a better agreement between dPCR and RT-qPCR when measuring *BCR-ABL1* absolute values than when

measuring for *ABL1* (7). These results suggest that *ABL1* may be a good choice for a reference gene for RT-qPCR, rather than the ideal internal reference gene for dPCR (14).

In conclusion, the detection results of the *BCR-ABL1* gene in patients with CML using dPCR apply well with the results obtained by RT-qPCR, particularly in the detection of low abundance *BCR-ABL1* gene (*BCR-ABL1*[IS] <1.0%). dPCR has advantages for patients with CML, who have a deep molecular response, as the results of dPCR can be applied as a supplement to RT-qPCR before planning TKIs discontinuation (16).

Acknowledgements

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

ZY and QS conceived and designed the study. HZ, YH and QS collected the samples and carried out the research. ZY, HZ, QS, SZ, KZ, QW, JC, MN, JQ and HC analyzed the data and wrote the manuscript. JC and MN revised the manuscript. ZY, QS, JQ and HC participated in the translation of the manuscript. The study was conducted under the guidance of KX, LZ and ZL. All the authors accepted the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Clinical Trials of the Affiliated Hospital of Xuzhou Medical College (approval number, XYFY2015-KL005-01). Certain patients received hematopoietic stem cell transplantation. No organs/tissues were obtained from prisoners. Patients donated hematopoietic stem cells in the Affiliated Hospital of Xuzhou Medical College. The informed consent was signed by all participants.

Patient consent for publication

Written informed consent was provided by all patients.

Competing interests

The authors declare that they have no competing interests.

References

1. Apperley JF: Chronic myeloid leukaemia. Lancet 385: 1447-1459, 2015.
2. Cross NC, White HE, Colomer D, Ehrencrona H, Foroni L, Gottardi E, Lange T, Lion T, Machova Polakova K, Dulucq S, *et al*: Laboratory recommendations for scoring deep molecular responses following treatment for chronic myeloid leukemia. Leukemia 29: 999-1003, 2015.
3. Pallera A, Altman JK, Berman E, Abboud CN, Bhatnagar B, Curtin P, DeAngelo DJ, Gotlib J, Hagelstrom RT, Hobbs G, *et al*: NCCN guidelines insights: Chronic myeloid leukemia, version 1.2017. J Natl Compr Canc Netw 14: 1505-1512, 2016.
4. Branford S, Cross NC, Hochhaus A, Radich J, Saglio G, Kaeda J, Goldman J and Hughes T: Rationale for the recommendations for harmonizing current methodology for detecting BCR-ABL transcripts in patients with chronic myeloid leukaemia. Leukemia 20: 1925-1930, 2006.
5. Ramsden SC, Daly S, Geilenkeuser WJ, Duncan G, Hermitte F, Marubini E, Neumaier M, Orlando C, Palicka V, Paradiso A, *et al*: EQUAL-quant: An international external quality assessment scheme for real-time PCR. Clin Chem 52: 1584-1591, 2006.
6. Zhang T, Grenier S, Nwachukwu B, Wei C, Lipton JH, Kamel-Reid S and Association for Molecular Pathology Hematopathology Subdivision: Inter-laboratory comparison of chronic myeloid leukemia minimal residual disease monitoring: Summary and recommendations. J Mol Diagn 9: 421-430, 2007.
7. Alikian M, Whale AS, Akiki S, Piechocki K, Torrado C, Myint T, Cowen S, Griffiths M, Reid AG, Apperley J, *et al*: RT-qPCR and RT-digital PCR: A comparison of different platforms for the evaluation of residual disease in chronic myeloid leukemia. Clin Chem 63: 525-531, 2017.
8. Branford S, Fletcher L, Cross NC, Müller MC, Hochhaus A, Kim DW, Radich JP, Saglio G, Pane F, Kamel-Reid S, *et al*: Desirable performance characteristics for BCR-ABL measurement on an international reporting scale to allow consistent interpretation of individual patient response and comparison of response rates between clinical trials. Blood 112: 3330-3338, 2008.
9. Foskett P, Gerrard G and Foroni L: Real-time quantification assay to monitor BCR-ABL1 transcripts in chronic myeloid leukemia. Methods Mol Biol 1160: 115-124, 2014.
10. Huggett JF, Cowen S and Foy CA: Considerations for digital PCR as an accurate molecular diagnostic tool. Clin Chem 61: 79-88, 2015.
11. Whale AS, Cowen S, Foy CA and Huggett JF: Methods for applying accurate digital PCR analysis on low copy DNA samples. PLoS One 8: e58177, 2013.
12. Wang WJ, Zheng CF, Liu Z, Tan YH, Chen XH, Zhao BL, Li GX, Xu ZF, Ren FG, Zhang YF, *et al*: Droplet digital PCR for BCR/ABL(P210) detection of chronic myeloid leukemia: A high sensitive method of the minimal residual disease and disease progression. Eur J Haematol 101: 291-296, 2018.
13. Zagaria A, Anelli L, Coccaro N, Tota G, Casieri P, Cellamare A, Impera L, Brunetti C, Minervini A, Minervini CF, *et al*: BCR-ABL1 e6a2 transcript in chronic myeloid leukemia: Biological features and molecular monitoring by droplet digital PCR. Virchows Arch 467: 357-363, 2015.
14. Mori S, Vagge E, le Coutre P, Abruzzese E, Martino B, Pungolino E, Elena C, Pierri I, Assouline S, D'Emilio A, *et al*: Age and dPCR can predict relapse in CML patients who discontinued imatinib: The ISAV study. Am J Hematol 90: 910-914, 2015.
15. Goh HG, Lin M, Fukushima T, Saglio G, Kim D, Choi SY, Kim SH, Lee J, Lee YS, Oh SM and Kim DW: Sensitive quantitation of minimal residual disease in chronic myeloid leukemia using nanofluidic digital polymerase chain reaction assay. Leuk Lymphoma 52: 896-904, 2011.
16. Taylor SC, Laperriere G and Germain H: Droplet digital PCR versus RT-qPCR for gene expression analysis with low abundant targets: From variable nonsense to publication quality data. Sci Rep 7: 2409, 2017.