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Micro *BCR::ABL1* 재배열을 동반한 만성골수백혈병 1예: 위음성을 피하기 위한 역전사 PCR 검사 시 주의사항

A Case of Chronic Myeloid Leukemia with Micro *BCR::ABL1* Rearrangement: Precaution in Reverse Transcription PCR to Prevent False Negativity

김서완[1] · 김홍경[1] · 신새암[1] · 정해림[2]
Seo Wan Kim, M.D.[1], Hongkyung Kim, M.D.[1], Saeam Shin, M.D.[1], Haerim Chung, M.D.[2]

연세대학교 의과대학 진단검사의학교실[1], 혈액내과학교실[2]
Department of Laboratory Medicine[1], Yonsei University College of Medicine, Severance Hospital, Seoul; Division of Hematology, Department of Internal Medicine[2], Yonsei University College of Medicine, Severance Hospital, Seoul, Korea

We report a patient negative for *BCR::ABL1* in qualitative reverse transcription (RT)-PCR but subsequently reported to be positive for t(9;22)(q34;q11.2) in conventional karyotyping. The patient was finally diagnosed with micro-type chronic myeloid leukemia after re-examining RT-PCR and performing targeted RNA sequencing. Through this case, we highlight the risk of false negativity when interpreting RT-PCR to detect micro-type fusion. Upon re-examining RT-PCR results, the patient's internal control band was thicker than others. After extending the electrophoresis run time, a 911-bp internal control band and a target band around the level of 1.0 kb were separated. We confirmed a fusion breakpoint (*BCR* exon 19 and *ABL1* exon 2) by targeted RNA sequencing, and it corresponds to 1,012 bp-sized e19a2 (c3a2) type among four micro-type fusion transcripts that RT-PCR HemaVision® kit M6B can detect.

Key Words: Chronic myeloid leukemia, *BCR::ABL1*, p230, Thrombocytosis, False negative

INTRODUCTION

Chronic myeloid leukemia (CML) is caused by increased tyrosine kinase activity resulting from *BCR::ABL1* fusion. A chimeric mRNA transcripted from this fusion gene is translated into constitutively active tyrosine kinase and induces hyperproliferation of myeloid lineage cells, leading to leukemia. According to the breakpoints, fusion transcripts can be classified into several types with different molecular sizes. The breakpoint of *ABL1* rarely shows variation and is located at the upstream region of exon 2 (a2). In contrast, the *BCR* gene has three breakpoint cluster regions (bcr): major, minor, and micro-bcr (M-, m-, and μ-bcr) [1]. Exons 13 and 14 in M-bcr are the breakpoints in the majority of patients with CML, resulting in fusion transcripts of e13a2 (b2a2) and e14a2 (b3a2), respectively [2]. The m-bcr is located upstream of the M-bcr and produces e1a2, e1a3, e6a2, and e6a3 transcripts. These transcripts are translated into lower molecular weight proteins than those of the major types. However, it is observed in 1.0% of patients with CML and is more closely associated with Philadelphia chromosome-positive B-cell acute lymphoblastic leukemia [3, 4]. The last and rare breakpoint, μ-bcr, is located downstream of M-bcr and gives rise to e19a2 (c3a2), e19a3 (c3a3), e20a2 (c4a2), and e20a3 (c4a3) transcripts, which are translated into higher molecular weight

Corresponding author: Saeam Shin, M.D.
https://orcid.org/0000-0003-1501-3923
Department of Laboratory Medicine, Yonsei University College of Medicine, Severance Hospital, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea
Tel: +82-2-2228-2453, Fax: +82-2-2227-8353, E-mail: saeam0304@yuhs.ac

Co-corresponding author: Haerim Chung, M.D.
https://orcid.org/0000-0002-7926-9285
Division of Hematology, Departments of Internal Medicine, Yonsei University College of Medicine, Severance Hospital, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea
Tel: +82-2-2228-1989, Fax: +82-2-393-6884, E-mail: SANHO23@yuhs.ac

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proteins than those of major-types [5].

Confirming the micro-*BCR::ABL1* transcript, which originated from breakage in μ-bcr, is crucial for differential diagnosis because micro-type CML can express variable hematological presentations, including neutrophilic CML (CML-N), acute leukemia, and essential thrombocythemia [6, 7]. There are few longitudinal studies evaluating the incidence of patients with micro-type CML in Korea, and only several sporadic reports are available [8-10]. However, in the comprehensive analysis of *BCR::ABL1* transcripts in Korean patients with CML, the micro-type incidence was 0.73% [11]. We report a Korean patient with CML expressing an e19a2 (c3a2) *BCR::ABL1* fusion transcript. This work was approved by the Institutional Review Board of Severance Hospital, Seoul, Korea (IRB no. 4-2021-1566).

CASE REPORT

A 57-year-old man was referred to our center with leukocytosis (white blood cell: 35.6×10^9/L), thrombocytosis (platelet: $1,637\times10^9$/L), hepatomegaly, and weight loss. The initial differential complete blood count (CBC) performed at our center was as follows: neutrophil 72.2%, lymphocyte 12.0%, monocyte 2.5%, eosinophil 5.2%, and basophil 8.1%. A left shift in granulocytes (promyelocyte 2.0%, myelocyte 2.0%, metamyelocyte 3.0%, band 3.0%, segmented neutrophil 56.0%), a small number of blasts (2.0%), and increased eosinophils (7.0%) and basophils (7.0%) were noted in a peripheral blood smear (Fig. 1A). Bone marrow (BM) showed hypercellularity (>80.0%), and markedly increased small-sized megakaryocytes (Fig. 1B). The blasts in the BM were counted as 2.0% of all nucleated cells. There was no evidence of myelofibrosis or dysplastic features. The patient was suspected to have CML in the chronic phase and was started on hydroxyurea at 500 mg/day. The result of qualitative reverse transcription (RT)-PCR (HemaVision® kit; DNA Technology, Aarhus, Denmark) analysis of *BCR::ABL1* fusion showed negativity. This qualitative RT-PCR is a nested PCR that amplifies cDNA synthesized from RNA using two primer sets (first set: M6B PCR-I primers and M8F PCR-I primers,; second set: M6B PCR-II primers and M8F PCR-II primers). M6B primers cover six major- and four micro-type fusions, while M8F primers cover four minor-type fusions. *JAK2, MPL,* and *CARL* mutations were not detected. The patient was tentatively diagnosed with atypical CML, and the daily hydroxyurea dosage was increased to 1,000 mg. Approximately three weeks later, the patient's platelet count surged to $2,123\times10^9$/L. At this moment, the karyotype was reported as 46, XX,t(9;22)(q34;q11.2) [20]. The drug was promptly changed to a 2nd generation tyrosine kinase inhibitor (TKI), dasatinib at 100 mg/day. We reviewed the initial qualitative RT-PCR result and performed quantitative RT-PCR (qPCR using the Ipsogen BCR-ABL1 Mbcr IS-MMR kit; Qiagen, Hilden, Germany). When we carefully examined the results of gel electrophoresis, the internal control (IC) band looked thicker than those of other patients (Fig. 2A). After extending the electrophoresis run time up to 50 minutes, two distinct bands were revealed, a 911-bp IC band and a target band (Fig. 2B). During gel electrophoresis, we applied a molecular size ladder (Genepia, 100 bp plus #D1001-4, Korea) as a reference to estimate the size of the target band. The separated target band was located near the fourth band from the top (corresponding to a 1.0 kb size). As aforementioned, four micro-type fu-

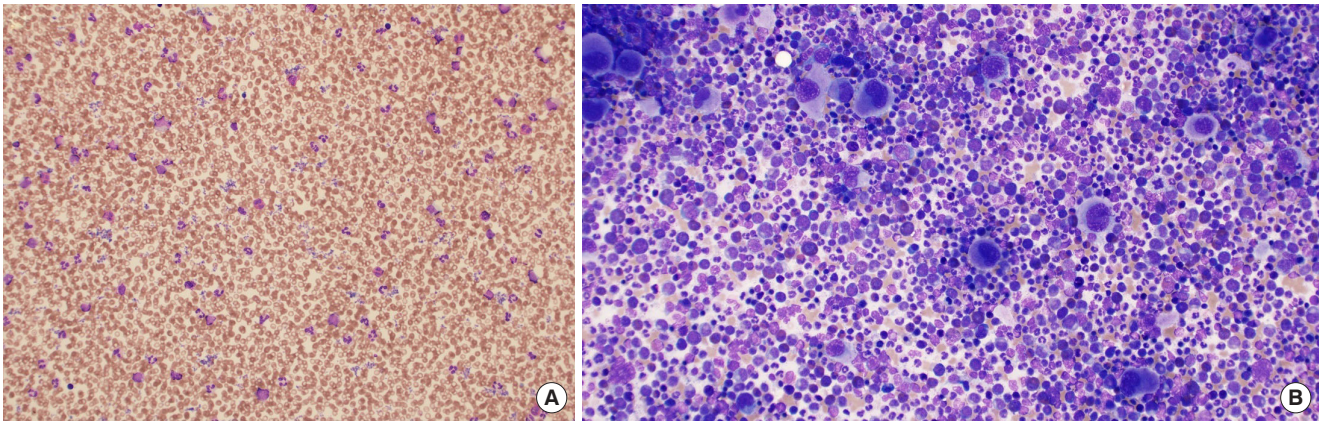

Fig. 1. Presentation of (A) peripheral blood smear (×200 magnification) and (B) a bone marrow aspiration at initial diagnosis (×200 magnification).

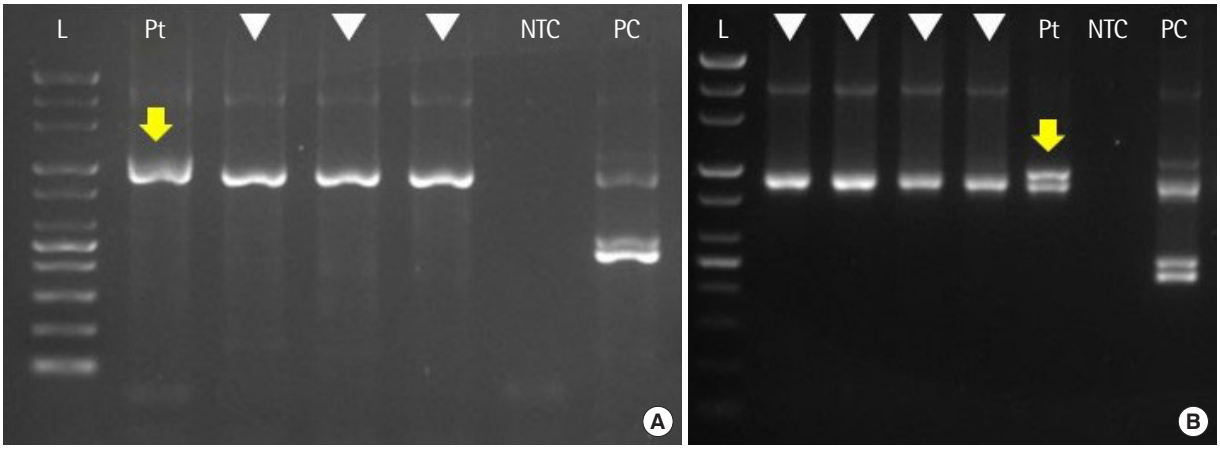

Fig. 2. Qualitative *BCR::ABL1* RT-PCR results using a HemaVision® kit (DNA Technology, Aarhus, Denmark). (A) Gel electrophoresis of the M6B lane at initial diagnosis. The index patient's IC band (yellow arrow) appeared thicker than that of other patients (triangles) without the *BCR::ABL1* transcript. (B) Repeated gel electrophoresis with a prolonged run time of up to 50 min revealed a separate band with a larger molecular size (1,012 bp, yellow arrow) than the IC band (911 bp).
Abbreviations: L, molecular size ladder (3.0 kb, 2.0 kb, 1.5 kb, 1.0 kb, 800 bp, 600 bp, 500 bp, 400 bp, 300 bp, 200 bp, and 100 bp size from the top); Pt, index patient; NTC, no template control; PC, e13a2 type positive control with 472 bp size.

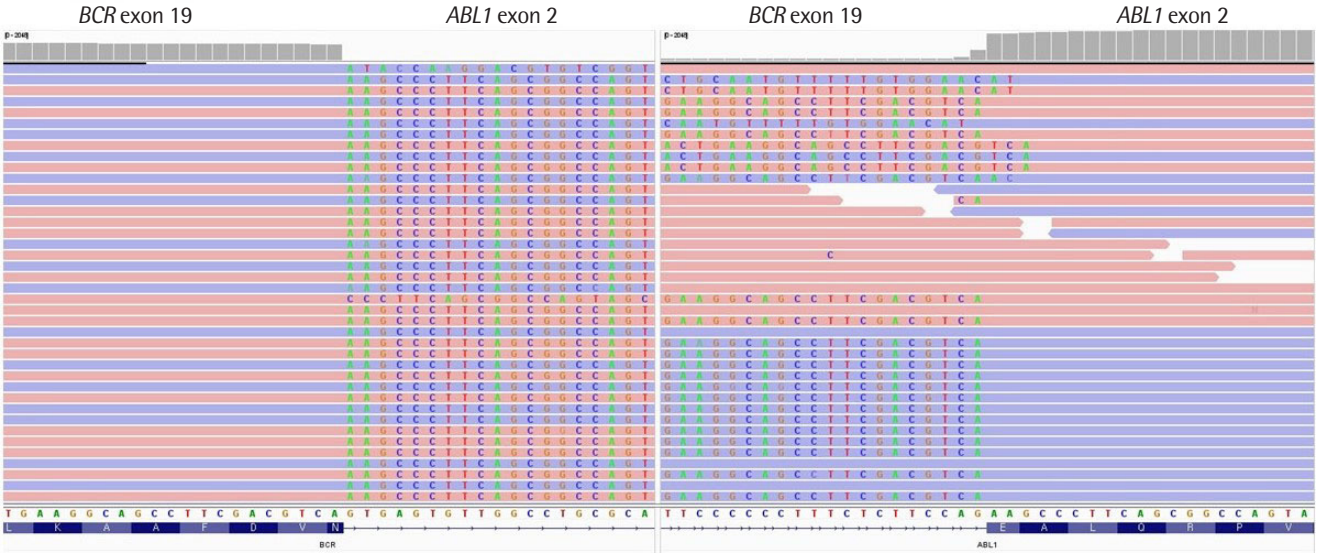

Fig. 3. Targeted RNA sequencing analysis (FusionPlex Pan-Heme Panel; ArcherDX, Boulder, CO, USA) confirmed breakpoints in *BCR* exon 19 and *ABL1* exon 2. The fusion reads of *BCR::ABL1* numbered 634 (64.63 % of total reads). Breakpoint visualization was performed with the Integrative Genomics Viewer (Broad Institute, Cambridge, MA, USA).

sions could be detected by performing nested RT-PCR using M6B primer sets: 1,147-bp-sized c4a2 type (fusion between *BCR* exon 20 and *ABL1* exon 2), 1,012-bp-sized c3a2 type (fusion between *BCR* exon 19 and *ABL1* exon 2), 973-bp-sized c4a3 type (fusion between *BCR* exon 20 and *ABL1* exon 3), and 838-bp-sized c3a3 type (fusion between *BCR* exon 19 and *ABL1* exon 3). We confirmed a fusion breakpoint of *BCR* exon 19 and *ABL1* exon 2 using targeted RNA sequencing (Fig. 3), which refers to a 1,012-bp-sized target band. The initial quantitative RT-PCR analysis showed an international scale (IS) of 5.18% for the *BCR::ABL1* transcript. After three months of TKI therapy, there was a 2.5-log reduction from baseline (IS 0.0149%). After six and nine months of treatment, IS values of 0.000225% and 0.000137% were measured, respectively; at 12, 15, and 18 months of treatment, negative results were observed for the *BCR::ABL1* transcripts. In addition, the CBC complete blood count at 18 months of treatment was within

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the normal range, showing white blood cells of 8.03 $\times 10^9$/L, hemoglobin of 124 g/L, and a platelet count of 302×10^9/L. Currently, the patient is steadily taking 100 mg/day of dasatinib.

DISCUSSION

RT-PCR is usually performed to identify the *BCR::ABL1* fusion in CML due to its high sensitivity and relatively short turn-around time [12]. However, there is a caveat: the visualization of the IC band from the biotinidase housekeeping gene should be met. This serves as a verification of the RNA's quality and the test's efficiency. As μ-bcr is located downstream of M-bcr, a micro-type fusion transcript has a larger molecular size (838 bp to 1,147 bp) than other types [5]. Since the sizes of the IC band and the micro-type transcript band are similar, misinterpretation as a negative result can occur due to overlap. Therefore, it is essential to compare the thickness or pattern of the IC band with that of the negative control or that of other patients. If a micro-type fusion with a larger molecular size is suspected, the time of gel electrophoresis must be extended sufficiently, and correlation with conventional karyotyping and fluorescence in situ hybridization is highly recommended. In addition, targeted RNA sequencing that detects unknown partner genes and novel breakpoints using universal primers can be a powerful tool for obtaining accurate breakpoint information [13].

The detection of the micro-type variant is essential since it determines the initiation of TKI therapy. In our patient, provisionally diagnosed with atypical CML, improper treatment might have led to a rise in platelet count. The complete blood count dramatically normalized after the medication was changed to dasatinib, and our patient remains in remission. Nonetheless, there is a caveat in interpreting quantification results. A normalized copy number is obtained by dividing the *BCR::ABL1* copy number by the *ABL1* copy number, which is then converted to an IS value. Since an IS value has been established in patients with major-type transcripts, there are no standardized qPCR assays for monitoring molecular response to TKI treatment in patients with the micro-type transcript. For e19a2 (c3a2) type CML, qualitative RT-PCR or nested RT-PCR is generally recommended for monitoring minimal residual disease [14]. However, our patient was followed up by qPCR solely. Although our patient showed a low IS value at initial diagnosis, up to a 4.5-log reduction compared to baseline was noted using qPCR after TKI therapy.

요 약

본 연구진은 정성 역전사 중합효소 연쇄반응 검사에서 *BCR::ABL1* 음성 결과를 보였지만 추후 핵형 검사상 t(9;22)(q34;q11.2)를 발견, 이후 역전사 중합효소 연쇄반응 검사의 재검토와 더불어 타겟 기반 RNA 시퀀싱을 시행하여 micro-type 만성골수성백혈병으로 최종 진단된 환자의 증례를 보고한다. 본 증례를 통해 micro-type 융합 유전자 검출을 위한 역전사 중합효소 연쇄반응 검사 해석 시 위음성의 위험성을 강조하고자 한다. 역전사 중합효소 연쇄반응 검사 재검토 시 환자의 내부 컨트롤 밴드가 다른 환자들에 비해 더 두꺼움을 발견하였다. 전기영동 시간을 연장한 재검사를 시행하였고, 911-bp의 내부 컨트롤 밴드와 1.0 kb 부근에 위치한 타겟 밴드로 분리됨을 확인하였다. 또한 타겟 기반 RNA 시퀀싱을 시행하여 *BCR* 엑손 19와 *ABL1* 엑손 2사이의 절단점을 확인하였으며 이는 역전사 중합효소 연쇄반응 검사의 HemaVision® kit M6B로 검출할 수 있는 4가지 micro-type 융합 유전자들 중 1,012 bp의 e19a2 (c3a2) type임을 확인할 수 있었다.

Conflicts of Interest

None declared.

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