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유세포분석기 세포 분류 후 FISH 기법을 이용한 만성골수백혈병과 필라델피아 양성 B-림프모구백혈병의 감별: 증례보고

Differentiation between Chronic Myeloid Leukemia in B-Lymphoblastic Crisis and B-Cell Acute Lymphoblastic Leukemia with *BCR::ABL1* Fusion Using the FISH Technique after Flow Cytometry Cell Sorting: A Case Report

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Distinguishing between chronic myeloid leukemia (CML) in B-lymphoblastic crisis and B-cell acute lymphoblastic leukemia (B-ALL) with *BCR::ABL1* fusion is important in establishing treatment plans, since hematopoietic stem cell transplantation is usually advised in the former case but is more situational in the latter. However, the morphological and flow cytometric similarities between leukemic blast cells make the clear diagnostic distinction between these two diseases challenging. Nonetheless, the fact that the *BCR::ABL1* gene fusion is present only in lymphoblasts in B-ALL, whereas it is also observed in non-blast cells (e.g., neutrophils) in CML in B-lymphoblastic crisis, can be leveraged to obtain a definitive diagnosis. However, although various methods to exploit this key feature difference have been proposed, a widely accepted standardized protocol has not yet been established. This case report introduces a new protocol that enhances the convenience and accuracy of making this disease distinction. We present the case of a patient whose initial diagnosis of B-ALL was re-evaluated after flow cytometry was used to sort bone marrow cell populations into blasts and neutrophils, and these cells were subsequently separately tested for the *BCR::ABL1* fusion using FISH. This approach led to a final diagnosis of CML in B-lymphoblastic crisis.

Key Words: Philadelphia-positive B-cell acute lymphoblastic leukemia, Chronic myeloid leukemia in B-lymphoblastic crisis, Flow cytometry, B-cell acute lymphoblastic leukemia with *BCR::ABL1* fusion

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INTRODUCTION

When a patient presents with increased lymphoblast counts in either their peripheral blood or bone marrow aspirate and concurrently tests positive for the Philadelphia (Ph) chromosome, B-cell acute lymphoblastic leukemia (B-ALL) with *BCR::ABL1* fusion and chronic myeloid leukemia (CML) in B-lymphoblastic crisis are among the malignant diseases that must be considered in the differential diagnosis. However, the differentiation of these malignancies based solely on morphological evaluation and flow cytometry is difficult, because the leukemic blast cells in both diseases can appear similar and share myeloid/lymphoid surface markers in flow cytometry [1]. In addition to considering the clini-

cal presentation and progression of the disease, several laboratory protocols involving FISH can be used to aid in achieving a definitive diagnosis [1-3]. In this case report, we showcase a new variation of the FISH protocol that can be used to resolve the aforementioned ambiguities. To the best of our knowledge, this diagnostic protocol has yet to be reported in the literature. By performing separate FISH assays on two flow cytometrically sorted cell populations, we re-evaluated a B-ALL diagnosis to CML in B-lymphoblastic crisis in the patient. This study was approved by the institutional review board of Yongin Severance Hospital, Yongin, Korea (IRB No. 9-2024-0010).

CASE REPORT

A 69-year-old male with dyspnea and general weakness was referred to Yongin Severance Hospital in September 2022. The complete blood count revealed leukocytosis, anemia, and thrombocytopenia (white blood cell count: 55.35×10^9/L; hemoglobin: 66 g/L; platelet count: 50×10^9/L). On initial evaluation, hepatosplenomegaly and lymphadenopathy were not observed. The blasts counts were increased in the peripheral blood (24% of all nucleated cells) without basophilia and in the bone marrow aspirate (87.6% of all nucleated cells). Flow cytometric analysis identified an abnormal blast population expressing CD34, cCD79a, CD19, CD38, CD7, HLA-DR, CD33, cCD22, and CD10. Karyotyping showed t(9;22)(q34;q11.2) without additional chromosome anomaly, and quantitative reverse transcription-nested PCR (qRT-PCR) analysis indicated major *BCR::ABL1* rearrangement (b2a2 subtype).

The patient was initially diagnosed with B-ALL with t(9;22)(q34;q11.2); *BCR::ABL1* and was therefore prescribed induction chemotherapy (vincristine, daunorubicin, and prednisolone) and the tyrosine kinase inhibitor (TKI) imatinib mesylate. Follow-up bone marrow examination at 1-month post induction revealed a hypercellular marrow (70%) with myeloid hyperplasia and 4.6% blasts. The qRT-PCR-assayed *BCR::ABL1* transcript level was 0.1% on the International Scale. However, the disease relapsed 11 months after the initial diagnosis, prompting its re-evaluation. A

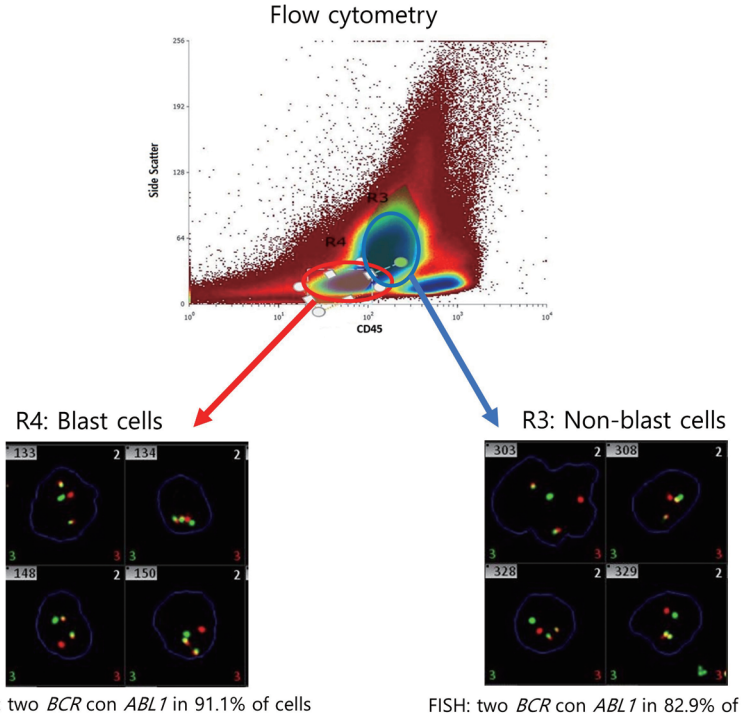

Fig. 1. Flow cytometry diagram depicting the combined flow cytometry/fluorescence *in situ* hybridization (FISH) protocol used in our case. The patient bone marrow sample was first analyzed and sorted using the S3e™ Cell Sorter (Bio-Rad, Hercules, CA, USA). The x-axis represents the CD45 signal; the y-axis represents the side scatter. The population with a weak CD45 signal and low side scatter was designated as R4 and represents blast cells. The other significant population was designated as R3 and represents non-blast cells. Each cell population was subjected to FISH analysis, whereupon *BCR::ABL1* translocation was observed in both types of cells.

bone marrow examination was performed, followed by flow cytometric sorting of the blast cell and neutrophil (non-blast) populations guided by CD45 and side scatter patterns. Subsequent separate FISH analyses of the blast and non-blast cells confirmed the presence of the *BCR::ABL1* fusion in both populations (Fig. 1).

Remarkably, 91.1% of 234 blast cells and 82.9% of 175 neutrophils exhibited the *BCR::ABL1* fusion, solidifying the diagnosis of CML in B-lymphoblastic crisis. This case highlighted the enhanced diagnostic precision of this integrated approach of combining flow cytometric cell sorting and FISH analysis, which will be particularly valuable in scenarios with increased blast cell counts.

After the final diagnosis, the TKI was changed from imatinib to dasatinib, and hematopoietic stem cell transplantation (HSCT) was discussed in the event the patient proved refractory to the new drug regimen. Fortunately, treatment was effective and the patient showed negative minimal residual disease (as per qRT-PCR-based *BCR::ABL1* measurements) approximately 4 months after dasatinib treatment. The patient continues to visit the clinic as an outpatient and has not required HSCT. A timeline of the disease diagnoses, treatments, and monitoring for this patient is provided in Fig. 2.

DISCUSSION

Distinguishing between *de novo* B-ALL with *BCR::ABL1* fusion and CML in B-lymphoblastic crisis is crucial for informing treatment decisions, as patients with the latter disease are typically advised to receive a brief course of TKI therapy together with chemotherapy followed by allogeneic HSCT, whereas patients with *de novo* B-ALL with *BCR::ABL1* fusion who respond satisfactorily to chemotherapy and TKI are typically not considered for HSCT [4-7].

Most patients with CML are initially diagnosed in the chronic phase. Approximately 5% of patients are diagnosed in the accelerated phase/blast phase, and of those individuals, 20–30% are in the lymphoblastic crisis phase [8, 9]. In these patients, the presence of basophilia, myeloid hyperplasia, and micromegakaryocytes may be suggestive of underlying CML. However, CML in B-lymphoblastic crisis can potentially be misdiagnosed as *de novo* B-ALL with *BCR::ABL1* fusion without a documented history of CML [1]. Both diseases exhibit similar flow cytometric profiles, making a definitive diagnosis challenging. However, they can be distinguished according to several differences at the time of diagnosis [1, 2, 7, 9-14] (Table 1). The isoforms of *BCR::ABL1* may aid

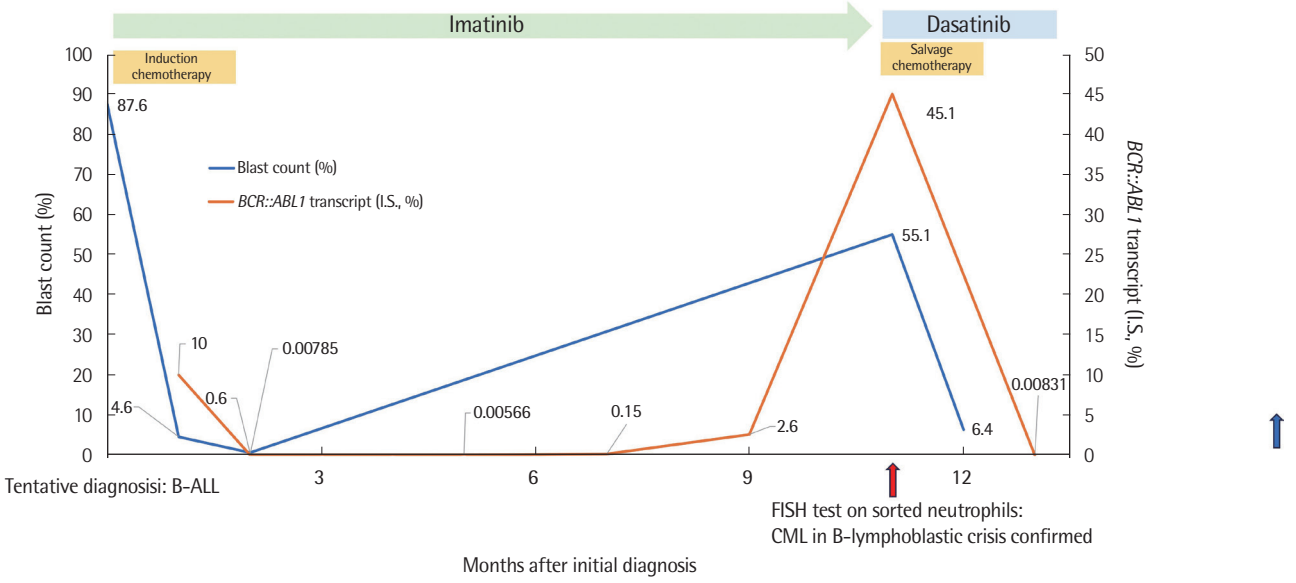

Fig. 2. Chronological timeline of the diagnoses, follow-up tests, and treatments. After the initial diagnosis of B-cell acute lymphoblastic leukemia (B-ALL), the patient underwent induction chemotherapy. The disease status was monitored with periodic *BCR::ABL1* quantification tests, and an abnormal elevation was detected at 11 months after induction. FISH assays were performed separately on the flow cytometrically sorted blast and neutrophil populations, leading to the final diagnosis of chronic myeloid leukemia (CML) in B-lymphoblastic crisis. A second-line tyrosine kinase inhibitor regimen was added, and the patient showed complete response.
Abbreviation: I.S., International Scale.

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Table 1. Comparison between CML in B-lymphoblastic crisis and *de novo* B-ALL with *BCR::ABL1* fusion

Characteristics	CML in B-lymphoblastic crisis	*De novo* B-ALL with BCR::ABL1 fusion	References
Incidence	2.2% present with *de novo* lymphoblastic crisis; lymphoid blast crisis accounts for 30% of CML B-lymphoblastic crisis	2–4% of B-ALL (< 15 years old) 10% of B-ALL (15–39 years old) 25% of B-ALL (40–49 years old) 20–40% of B-ALL (> 50 years old)	[1, 10]
Onset age	Median age of diagnosis is 67 years but may present at any age Natural history of CML is a chronic phase for 3–5 years followed by rapid progression to the fatal blast phase	Incidence of B-ALL with *BCR::ABL1* fusion rises with age; occurs in approximately 50% of patients older than 50 years	[7, 11]
Cell of origin	Granulocyte-macrophage progenitor	Pre-B-cell	[1]
Clinical features	Fever, weight loss Anemia, thrombocytopenia, leukocytosis Splenic enlargement	Symptoms of bone marrow failure: thrombocytopenia, anemia, neutropenia Frequent lymphadenopathy/hepatosplenomegaly Bone pain, arthralgia may be prominent	[1, 9, 10]
BCR::ABL1 transcript/Protein isoform	e14a2 (b3a2)/p210: ~62.1% e19a2/p230: 39.8% e13a2 (b2a2)/p210: ~37.9% e1a2/p190: 16.9%	e1a2/p190: ~70% e13a2 (b2a2)/p210: 17.5% e14a2 (b3a2)/p210: 12.5% p190 isoform in most childhood cases and approximately half of adult cases	[2, 10, 12]
Additional chromosomal abnormality	Deletion in chromosome 9p Deletion of p16/CDKN2A Numerical gains and breakpoints involving chromosomes 1q and 7p	Greater number of chromosomal abnormalities Gains in chromosome 9	[13]
Frequent mutations	*IKZF1* (55%) *CDKN2A/B* (50%) *RUNX1* (25–35%) *BCOR* (15–25%)	*IKZF1* (70%) *CDKN2A/B* (45%) *PAX5* (30–40%) *BTG1* (18%) *RB1* (14%) *EBF1* (13%) *ETV6* (5%)	[14]

Abbreviations: CML, chronic myeloid leukemia; B-ALL, B-cell acute lymphoblastic leukemia.

diagnosis, since the p210 isoform is common in CML whereas the p190 isoform predominates in *de novo* B-ALL. The most reliable diagnostic method is to investigate the cell lineages that harbor the *BCR::ABL1* translocation, given that CML in B-lymphoblastic crisis involves both myeloid and lymphoid cells since it is fundamentally a cancer originating from a myeloid progenitor [2]. Discrepancy between the percentage of blast cells in bone marrow aspirates and that of *BCR::ABL1*-positive cells in the interphase FISH test would indicate the presence of non-blast *BCR::ABL1*-positive clones, providing indirect evidence in support of CML [2]. Discordance between the percentage of blast cells and the size of *BCR::ABL1* clones can also be supportive of CML [7].

Our patient was diagnosed with a CML in B-lymphoblastic crisis for several reasons. First, the p210 *BCR::ABL1* fusion was detected, and it was not restricted to lymphoblasts but also presented in neutrophils at relapse. Second, after 1 month of induction, the bone marrow showed relatively high cellularity with myeloid hyperplasia and residual blasts, implying disease initiation from a myeloid lineage rather than a lymphoid one. In this case, we sorted bone marrow cells into blast and neutrophil populations using flow cytometry and then used the FISH assay to confirm CML. The CD45 biomarker and side scatter profile were used to distinguish the two populations, because the blast cell population displays a weak CD45 signal and low side scatter, allowing effective sorting. Several diagnostic methods for investigating *BCR::ABL1* fusion in separate cell populations have been introduced in the past, such as identifying the nuclei of each cell type using 4′,6-diamidino-2-phenylindole (DAPI) staining or culturing each cell type to observe the occurrence of Ph translocation in neutrophils [2, 15]. However, differentiating cells by their nuclei requires visual inspection of many cells and can be challenging owing to artifacts, and cell culturing by lineage takes considerable time and effort. We expect our proposed method to be more convenient and definitive in cases where patients have greatly increased blast cell counts, where the relative scarcity of band/segmented neutrophils could make their visual localization using the

DAPI-based FISH protocol challenging.

In a closely related context, the distinction between B-ALL with *BCR::ABL1* fusion and CML-like features is increasingly being emphasized in the literature, and the level of discrepancy between post-treatment qRT-PCR results and molecular minimal residual disease measurements (e.g., immunoglobulin/T-cell receptor (Ig/TCR) rearrangement) has been suggested as a distinguishing feature [16, 17]. However, we were unable to incorporate this analysis for this patient since the approach requires Ig/TCR rearrangement testing at the time of diagnosis, which was not performed.

In our case, we sorted cells with flow cytometry using only the CD45 marker. This method is straightforward and we deemed it sufficient for distinguishing non-blast cells, especially neutrophils. However, this protocol has limitations in accurately identifying cell populations. Incorporating additional cell markers specific to blasts, such as CD19 and/or CD34, would likely enable more precise population grouping.

In conclusion, we report an alternative protocol whereby cell sorting and the FISH assay are combined for differentiating between B-ALL with *BCR::ABL1* fusion and CML in B-lymphoblastic crisis. Further evaluation of this new integrated approach and analyses of its comparative performance against various diagnostic protocols will be needed to validate its effectiveness and efficiency.

요 약

일반적으로 환자에서 백혈구 및 모세포 증가 소견이 보이는 경우 급성백혈병이 가장 가능성이 높은 진단이다. 그러나 추가적인 검사에서 *BCR::ABL1* fusion 유전자가 확인되는 경우에는 만성골수백혈병(CML)의 림프구성 모세포기, 그리고 *BCR::ABL1* fusion 양성 급성림프모구백혈병(B-ALL)을 감별진단 해야 한다. 이 두 가지 질환의 감별은 특히 향후 치료의 결정에 중요하다. CML은 주로 Tyrosine kinase inhibitor 치료와 항암요법 시행 후 조혈모세포이식이 필요한 반면 B-ALL with *BCR::ABL1* fusion은 항암 치료반응에 따라 조혈모세포이식을 생략할 수도 있기 때문이다. CML의 림프구성 모세포기와 *BCR::ABL1* fusion 양성 B-ALL은 골수검사를 통한 형태학적인 차이로는 감별이 불가능하다고 알려져 있다. 또한 일반적으로 다양한 혈액암을 분별하는 데 사용되는 유세포검사 또한 이 경우엔 확정적이지 못하다. 두 가지 질환 모두 림프모구가 흡사한 면역표현형을 보이기 때문이다. CML의 림프구성 모세포기와 *BCR::ABL1* fusion 양성 B-ALL의 가장 근본적인 차이점은 *BCR::ABL1*을 보유한 세포군이 서로 다르다는 점이다. CML의 림프구성 모세포기의 경우, *BCR::ABL1*이 림프모구뿐만 아니라 백혈병 전구세포에서 비롯된 호중구에서도 확인된다. 반면 *BCR::ABL1* fusion 양성 B-ALL에서는 오직 림프모구에서만 해당 변이가 관찰된다. 이러한 이유로, 가장 확실한 감별진단을 내릴 수 있는 검사방법은 *BCR::ABL1* fusion을 보이는 세포 종류를 확인하는 것이다. 현재 *BCR::ABL1* fusion 보유 세포군을 파악하는 방법은 표준화되어 있지 않아 몇 가지 사례 보고로 발표되어 있는 상황이다. 본 연구에서는 유세포분석기의 CD45와 측면 산란 측정치를 기반으로 모세포와 모세포가 아닌 세포군을 물리적으로 나누고, 각 세포군에 FISH 검사를 수행하여 *BCR::ABL1* fusion의 유무를 파악하는 방법으로 확진을 내린 환자 사례를 발표하여 추후 이러한 감별진단이 필요한 상황에서 활용할 수 있는 진단 방법을 공유하고자 한다.

Conflicts of Interest

None declared.

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