A Case with Coexistence of Major and Minor BCR/ABL Fusion Transcript at Lymphoblastic Crisis of Chronic Myelogenous Leukemia in Patients with Major BCR/ABL Positivity during Chronic Phase

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Dear Editor

CML is characterized by the clonal expansion of bone marrow hematopoietic cells and is associated with the Philadelphia chromosome \([1]\). The accelerated phase of CML, which is also known as the blast crisis phase, occurs in patients as the disease progresses. It has been reported that the appearance of an additional chromosomal abnormality is the major pathogenic factor in 63% of the CML cases \([2, 3]\). However, the additional appearance of a minor \(BCR/ABL\) fusion transcript with disease progression has rarely been reported in CML patients. Moreover, the dual expression of the major and minor \(BCR/ABL\) fusion transcripts during blast crisis has been reported in only 4 cases to date \([4, 5]\). Here, we describe a CML patient who exhibited the dual expression of the major and minor \(BCR/ABL\) fusion transcripts during the lymphoblastic crisis (LBC) phase and possessed a mutation in the \(BCR/ABL\) kinase domain.

A 13-yr-old boy was admitted to Asan Medical Center in June 2010 for severe leukocytosis and anemia (white blood cell \(WBC\) count: \(464.1 \times 10^9/L\); hemoglobin: \(68 g/L\)), and splenomegaly. Bone marrow aspiration and biopsy findings were indicative of CML, with 5.4% of myeloblasts demonstrating granulocytic hyperplasia. His karyotype was 46,XY,t(9;22)(q34;q11.2)[20]. FISH analysis for the detection of the \(BCR/ABL\) fusion transcript showed nuc ish (ABL1, BCR)\(\times 3\) (ABL1 con BCR\(\times 2\))[190/200]. We performed reverse-transcriptase PCR for the detection of the major and minor \(BCR/ABL\) fusion transcripts but were able to detect only the major \(BCR/ABL\) fusion transcript (b2a2 type). Quantification analysis using the LightCycler t(9;22) Quantification Kit (Roche Diagnostics, Mannheim, Germany) showed a ratio of 8.00 (normalized copy number, NCN) for the major \(BCR/ABL\) transcript. The patient was thus diagnosed with chronic phase CML, and imatinib treatment was initiated. The patient demonstrated a partial hematologic response for 2 yr after the treatment was started.

In June 2012, the patient was readmitted because he had poor oral intake and diarrhea. A complete blood count (CBC) revealed mild leukocytosis (\(WBC\) count: \(16.1 \times 10^9/L\)) with 47% blasts in peripheral blood and 48% lymphoblasts in bone marrow aspirates. Immunophenotyping results indicated B-cell acute lymphoblastic leukemia. At this time his karyotype had changed to 45,XY,-7,t(9;22)(q34;q11.2)[20], indicating the appearance of monosomy 7. FISH analysis revealed nuc ish (ABL1\(\times 2\), BCR\(\times 3\))(ABL1 con BCR\(\times 1\))[168/200], indicating that 84.0% of the cells showed \(BCR-ABL\) rearrangement with 5`ABL1 dele-
tion. Based on these findings, the patient was diagnosed as being in the LBC phase of CML. Real-time PCR and the BCR-ABL Mbcr IS-MMR and BCR-ABL e1a2 mbcr FusionQuant kits (Ipsogen, Marseille, France) were used to quantify the BCR/ABL fusion transcripts. Ratios of 166.42 (converted to international scale-normalized copy number, IS-NCN) and 1.17 (NCN) were obtained for the major and minor transcripts, respectively (Fig. 1).

The HemaVision kit (Bio-Rad Laboratories, Hercules, CA, USA), which uses multiplex reverse transcriptase PCR, was used to analyze RNA samples from the chronic phase and LBC phase of CML. Only the major BCR/ABL fusion transcript was detected in the chronic phase sample (Fig. 2A and B). Notably however, both the major and minor BCR/ABL fusion transcripts were detected in the LBC phase sample (Fig. 2C-E).

To confirm imatinib resistance, BCR/ABL kinase domain mutation analysis was performed by carrying out reverse-transcriptase PCR and direct sequencing. The results indicated a homozygous missense mutation at the 757th nucleotide, with a substitution of cytosine for thymidine. This mutation introduced a substitution of histidine for tyrosine at the 253rd codon resulting in imatinib resistance. Imatinib was immediately changed to dasatinib, and the patient’s follow-up BCR/ABL evaluation revealed a dramatic decrease in the ratio of the major fusion transcript (0.24 IS-NCN) and negative conversion for the minor transcript, on day 28 after the treatment change.

The CML blast crisis phase is followed by the acquisition of additional chromosomal abnormalities, most notably the double Philadelphia chromosome and the occurrence of trisomy 8 and 17 [4]. A previously published study has reported that the acquisition of the minor BCR/ABL fusion transcript may be related to disease progression and LBC [5]. However, the additional appearance of the minor BCR/ABL fusion transcript during the blast crisis of CML is relatively rare as compared to the other chromosomal abnormalities mentioned above, and has been previously reported in only 4 patients so far [4, 5]. Given that a CML blast crisis more often manifests as myeloid rather than lymphoid, the dual expression of the major and minor BCR/ABL fusion transcripts during the LBC phase in our current case, is a rare phenomenon that is worth reporting.

The HemaVision kit (Bio-Rad Laboratories) has some disadvantages, such as cross-amplification and the generation of non-specific bands on gel electrophoresis. Hence, it is difficult to confirm the dual expression of the fusion transcripts because it uses multiplex reverse transcriptase PCR. Confirmation studies are thus necessary to validate the dual expression of the transcripts. In our case, the quantification of the BCR/ABL fusion transcript revealed that the levels of the minor BCR/ABL fusion transcript were approximately 1% of that of the major BCR/ABL fusion transcript during the LBC phase. This resulted in the formation of a faint minor BCR/ABL band, which could not be easily distinguished from the non-specific bands. In the present case, we detected a BCR/ABL band that was much fainter than the major BCR/ABL band using the HemaVision kit (Bio-Rad Laboratories). Confirmation studies like cloning and

Fig. 1. Detection of the minor BCR-ABL fusion transcript at the lymphoblastic crisis phase by real-time PCR. The amplification curves for the minor BCR-ABL fusion transcript (A) and ABL1 gene (B) are shown. The ratio of the minor BCR-ABL fusion transcript was calculated as 1.17 normalized copy number (NCN).
sequencing of the post-PCR products were not performed in our present case. Instead, we decided to perform a subsequent real-time PCR in order to demonstrate the presence of both, the major and minor \textit{BCR/ABL} fusion transcripts as it is believed to be a more sensitive technique than direct sequencing for the detection of specific genetic abnormalities. In our case, we were able to confirm the presence of the minor \textit{BCR/ABL} fusion transcript primarily by using real-time PCR. This approach is valid because confirmation studies such as cloning or sequencing were not carried out in the 4 cases mentioned in previous reports [4, 5]. Moreover, we successfully confirmed the presence of the additional minor \textit{BCR/ABL} fusion transcript by using reverse-transcriptase and real-time PCR.

In conclusion, we report the dual expression of major and minor \textit{BCR/ABL} fusion transcripts in a patient in the LBC phase of CML, who possesses a mutation in the \textit{BCR/ABL} kinase domain.

\textbf{Authors’ Disclosures of Potential Conflicts of Interest}

No potential conflicts of interest relevant to this article were reported.

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