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【 CASE REPORT 】

Aleukemic Extramedullary Blast Crisis as an Initial Presentation of Chronic Myeloid Leukemia with E1A3 BCR-ABL1 Fusion Transcript

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Abstract:

Right neck swelling and pain occurred in a 49-year-old man. A Blood count showed a slight increase in platelet count without leukemoid reaction. After a biopsy of the cervical mass and bone marrow aspiration, a diagnosis of extramedullary blast crisis (EBC) of chronic myeloid leukemia (CML) was made. Fluorescence *in situ* hybridization (FISH) analysis showed a BCR-ABL1 fusion signal, but results of real-time polymerase chain reaction (RT-PCR) for major and minor BCR-ABL1 transcripts were negative. We identified a rare e1a 3 BCR-ABL1 fusion transcript. Administration of dasatinib resulted in disappearance of the extramedullary tumor. This is the first reported case of CML-EBC with e1a3 transcript. An aleukemic extramedullary tumor can be the initial presentation of CML.

Key words: e1a3, extramedullary blast crisis, Philadelphia (Ph) chromosome, BCR-ABL1

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Introduction

The Philadelphia (Ph) chromosome, which results from reciprocal translocation of chromosomes 9 and 22, is the hallmark of chronic myeloid leukemia (CML) (1). As a result of the translocation, the BCR-ABL1 fusion gene is formed, and the BCR-ABL1 fusion protein causes constitutional tyrosine kinase activation and drives cell proliferation. There are variations in the break points of BCR and ABL transcripts. Major BCR-ABL1 (a fusion of BCR exon 13 or 14 and ABL1 exon 2) is positive in most cases of CML, while minor BCR-ABL1 (a fusion of BCR exon 1 and ABL exon 2) is positive in some cases of CML and in some cases of acute B-cell lymphoblastic leukemia (B-ALL) (2). We experienced a case with cervical lymphadenopathy, which turned out to be an extramedullary blast crisis (EBC) of CML. Fusion of BCR exon 1 and ABL1 exon 3 (e1a3) was detected in this case. So far, 26 cases of e1a3 BCR-ABL1-positive leukemia have been reported in the literature with ALL in 17 cases, CML in 8 cases, and acute myeloid leukemia (AML) in 1 case. A leukemic extramedullary mass has not been reported so far, and our case seems to be a very rare case. Here we report a case of CML presenting with an e1a3 fusion variant with EBC as an initial presentation.

Case Report

A 49-year-old man was referred to our hospital due to right neck swelling and pain. He had been aware of these symptoms for 2 months and had visited a nearby otolaryngology clinic. He was administered antibacterial agents and steroids for suspected infection or necrotizing lymphadenitis (Kikuchi disease). However, his symptoms persisted and he was referred to the Otolaryngology Department of Hokkaido University Hospital. He was then referred to our department

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Table 1. Laboratory Data at the First Consultation.

WBC	8,500 /μL	PT	11.4 s	TP	7.5 g/dL	Cr	0.64 mg/dL
Blast	0.0 %	APTT	28 s	ALB	4.3 g/dL	Na	140 mEq/L
Neutro	65.8 %	Fbg	664 mg/dL	T-Bil	0.7 mg/dL	K	4.8 mEq/L
Lympho	28.5 %	D-dimer	1.98 mg/dL	D-Bil	0.1 mg/dL	Cl	104 mEq/L
Mono	4.7 %			AST	23 U/L	Ca	9.4 mg/dL
Eosino	0.5 %			ALT	37 U/L	CRP	3.51 mg/dL
Baso	0.5 %			LD	221 U/L	sIL-2R	278 U/mL
RBC	524×10^4 /μL			ALP	419 U/L		
Hb	16.1 g/dL			γ-GTP	90 U/L		
Plt	35.5×10^4 /μL			BUN	15 mg/dL		

WBC: white blood cell, Neutro: neutrophil, Lympho: lymphocyte, Mono: monocyte, Eosino: eosinophil, Baso: basophil, RBC: red blood cell, Hb: hemoglobin, Plt: platelet, PT: prothrombin time, APTT: activated partial thromboplastin time, Fbg: fibrinogen, TP: total protein, ALB: albumin, T-Bil: total bilirubin, D-Bil: direct bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LD: lactate dehydrogenase, ALP: alkaline phosphatase, γ-GTP: γ-glutamyl transpeptidase, BUN: blood urea nitrogen, Cr: creatinine, Na: sodium, K: potassium, Cl: chlorine, Ca: calcium, CRP: c-reactive protein, sIL2-R: soluble interleukin-2 receptor

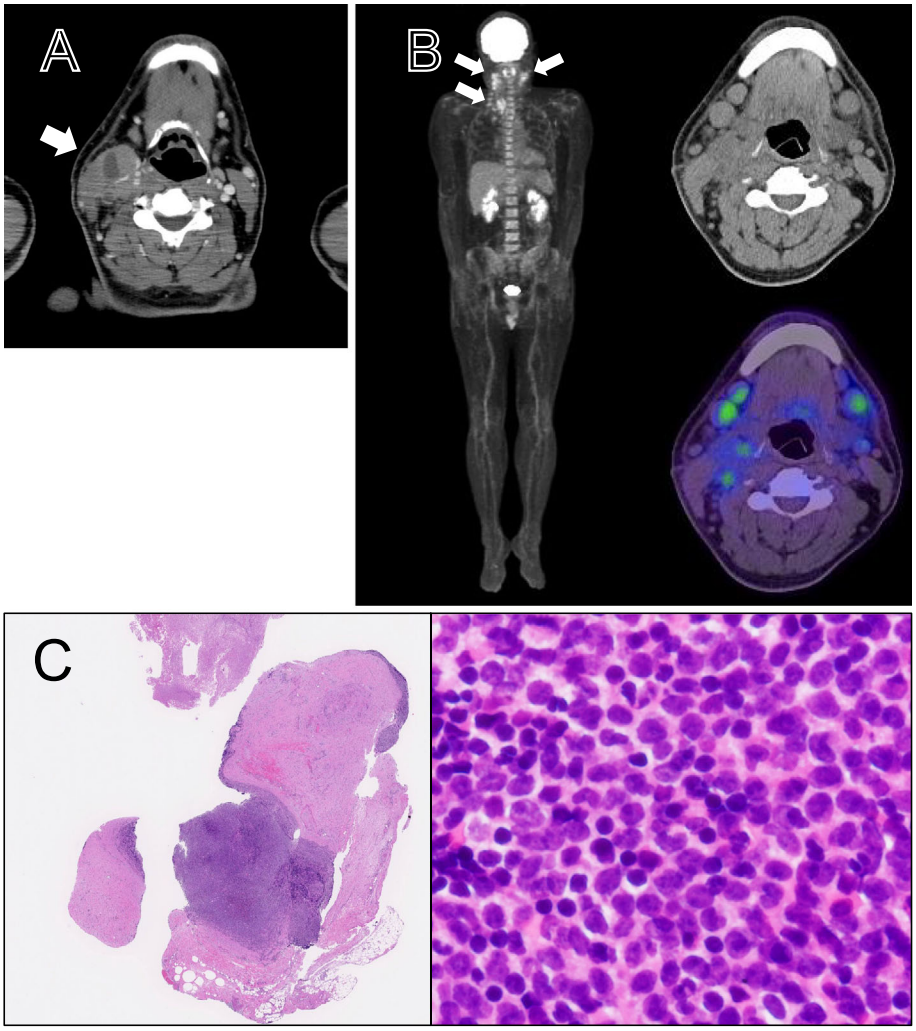

Figure 1. A: A CT scan showed a supraclavicular mass with a low-density area (arrow). B: PET-CT showed FDG uptake at cervical and supraclavicular masses (arrows). C: Histopathology (Hematoxylin and Eosin staining). Left: Most of the tissue specimen was necrotic (loupe view). Right: Dense aggregates of atypical mononuclear cells were found in some areas (original magnification ×40).

because he was suspected of having malignant lymphoma. He had chronic hepatitis C but no other medical history. Sustained virological response had been achieved by previous direct acting antivirals. A complete blood count (CBC)

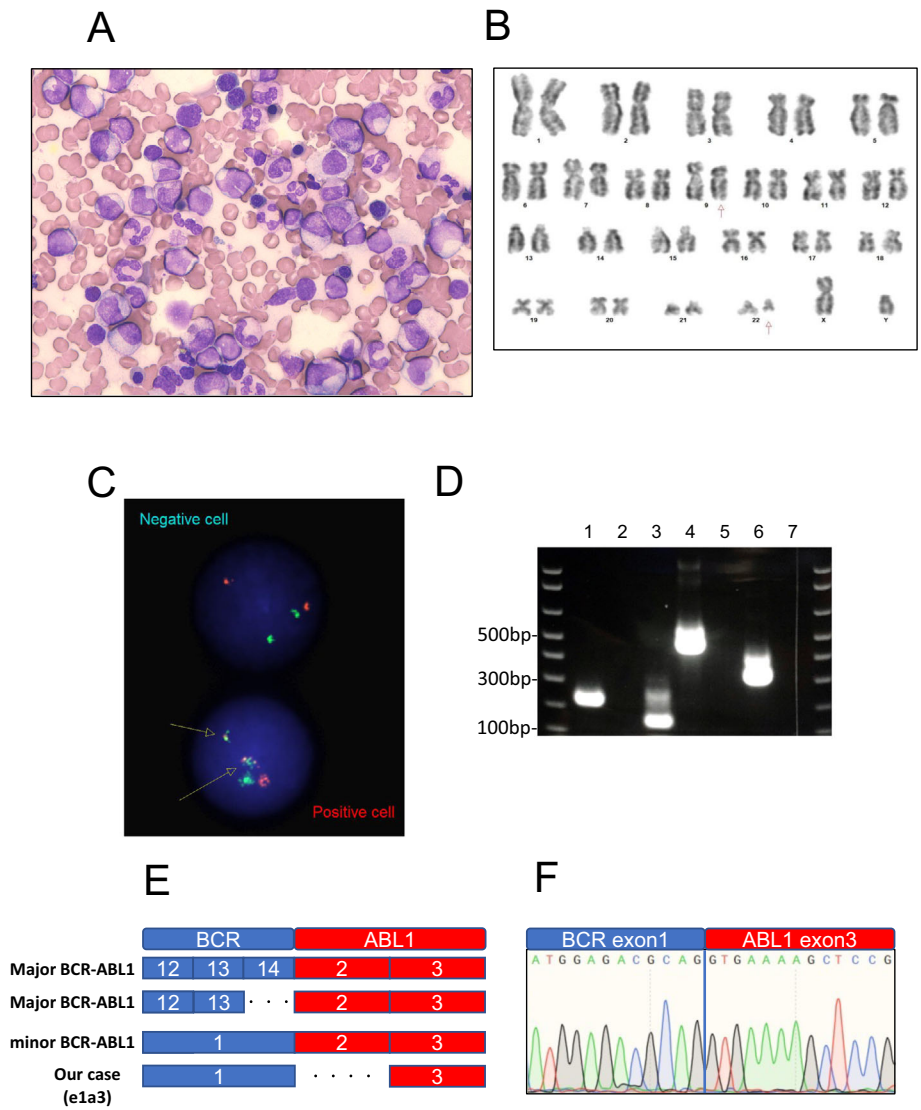

Figure 2. **A: Bone marrow smear obtained at presentation, compatible with CML (May-Giemsa, ×100). B: G-band of BM showed Philadelphia chromosome (the most dominant karyotype). C: FISH showed typical BCR-ABL1 fusion signal. D: RT-PCR, lane 1: GAPDH (internal control of the patient sample), lane 2: major BCR-ABL1 of the patient sample, lane 3: minor BCR-ABL1 of the patient sample, lane 4: major BCR-ABL1 (positive control), lane 5: major BCR-ABL1 (negative control), lane 6: minor BCR-ABL1 (positive control), lane 7: minor BCR-ABL1 (negative control). The minor BCR-ABL1 is detected as a 320-bp size band, but a smaller band of about 120-bp was detected in this case. E: Schema of the transcript of our case. F: Sanger sequence of the a1e3 transcript.**

at the first visit showed a slightly elevated platelet count but no other obvious abnormal findings (Table 1). A computed tomography (CT) scan revealed several swollen lymph nodes in his neck and supraclavicular fossa. Enhanced scanning showed that the lymph nodes had a low-density area on an image and appeared to be necrotic (Fig. 1A). ^{18}F-fluorodeoxyglucose positron emission tomography-computed tomography (^{18}F-FDG PET-CT) showed FDG uptake (standardized uptake value: 4-6) at the sites of lymphadenopathy (Fig. 1B). A biopsy of the cervical mass was performed. Histological examination revealed dense aggregates of atypical mononuclear cells surrounded by massive coagulation necrosis, suggestive of hematolymphoid malignancy (Fig. 1C). An immunohistochemical examination showed that the mononuclear cells had the following phenotypes: CD43+, CD56+, CD68+, CD123+, MPO+, and Lysozyme+. A histopathological diagnosis of myeloid neoplasm was made. Bone marrow (BM) aspiration was then performed, and examination of the BM revealed hypercellular marrow with serial increase of maturating granulocytes without evidence of lymphoma (Fig. 2A). The results of a BM smear tests were as follows: 3.8% blasts, 4.2% progranulocytes, 16.6% myelocytes, 6.8% metamyelocytes, 14.6% stabs, 16.0% segs, 0.6% eosinophils, 1.2% basophils, 12% lymphocytes, 1% plasma cells, 6.2% monocytes and 17% erythroblasts. BM flow cytometry showed no significant increase in blasts or abnormal cells. Karyotype analysis of BM showed 46,XY,t(9;22)(q34;q11.2) [11]/46,idem,add(12)(p

Table 2. Primer Sequences for Nested PCR.

Major BCR-ABL	Forward primer (5'-3')	Reverse primer (5'-3')
First round	GAGTCACTGCTGCTGCTTATGTC	TTTTGGTTTGGGCTTCACAC
Second round	CACGTTCCTGATCTCCTCTGAC	ACACCATTCCCCATTGTGATTAT
Minor BCR-ABL	Forward primer (5'-3')	Reverse primer (5'-3')
First round	CGCTCTCCCTCGCAGAACT	TTTTGGTTTGGGCTTCACAC
Second round	ACTGCCCGGTTGTCGTGTC	ACACCATTCCCCATTGTGATTAT

Table 3. Cases of e1a3 BCR-ABL1 Transcripts Reported in Literature.

Case	Age	Sex	Diagnosis	Karyotype (the most dominant karyotype was shown)	Extramedullary lesions	Therapy	Transplant	References
1	43	M	ALL	46,XY,del(9)(p22)t(9;22)(q34;q11),del(20)(q13)	NA	IMA+chemo	allogeneic	(12)
2	65	M	ALL	46,XY,t(9;22)(q34;q11)	NA	IMA+chemo	-	(12)
3	76	M	ALL	NA	NA	Dasa	-	(13)
4	NA	M	ALL	46,XY,t(1;21)(p36.1;q22),t(2;7)(p12;p13),t(9;22)(q34;q11.2)	NA	IMA+chemo	allogeneic	(14)
5	NA	M	ALL	46,XY,ider(9)(q10)t(9;22)(q34;q11.2),der(22)t(9;22)	NA	IMA+chemo	-	(14)
6	62	F	ALL	NA	NA	IMA	NA	(15)
7	25	F	ALL	46,XX,t(9;22)(q34;q11)	NA	NA	allogeneic	(16)
8	62	M	ALL	46,XY,t(9;22)(q34;q11.2)	NA	chemo	autologous	(17)
9	64	F	ALL	46,XX	NA	chemo	-	(17)
10	31	M	ALL	44,XY,dic(3;9)(q27:p11),t(9;22)(q34:q11), -7	NA	IMA+chemo	allogeneic	(17)
11	61	F	ALL	NA	NA	IMA+chemo	-	(17)
12	48	M	ALL	46,XY	NA	chemo	-	(17)
13	45	F	ALL	NA	NA	IMA+chemo	allogeneic	(17)
14	NA	NA	ALL	NA	NA	NA	NA	(18)
15	1	NA	ALL	NA	NA	NA	NA	(19)
16	39	NA	ALL	NA	NA	NA	NA	(20)
17	NA	NA	ALL	NA	NA	NA	NA	(21)
18	68	M	AML	46,XY,t(9;22)(q34;q11)	NA	Dasa→Pona	-	(22)
19	NA	NA	CML-CP	NA	NA	NA	NA	(23)
20	NA	NA	CML-CP	NA	NA	NA	NA	(24)
21	NA	NA	CML-CP	NA	NA	NA	NA	(25)
22	41	F	CML-CP	46,XX,t(9;22;17)(q34;q11;q24)	NA	IFN-α→IMA	-	(26)
23	64	F	CML-CP	NA	NA	IMA	-	(26)
24	75	F	CML-CP	46,XX,t(9;22)(q34;q11.2)	NA	-	-	(27)
25	68	F	CML-CP	46,XX,t(9;22)(q34;q11)	NA	IFN-α+HU	-	(28)
26	80	M	CML-BC	46,XY,t(9;22)(q34;q11.2)	NA	IMA	-	(29)
Our case	49	M	CML-BC	46,XY,t(9;22)(q34;q11.2)	YES	Dasa		

ALL: acute lymphoblastic leukemia, CP: chronic phase, BC: blast crisis, IMA: imatinib, Dasa: dasatinib, Pona: ponatinib, IFN-α: interferon-α, HU: hydroxyurea, NA: not available

11.1)[6]/46,XY[3] (Fig. 2B), and fluorescence *in situ* hybridization (FISH) test results were positive for BCR-ABL1 fusion signal (82.4%, 412/500) (Fig. 2C). Real-time polymerase chain reaction (RT-PCR) of the BM was negative for major and minor BCR-ABL1, but primer set for minor BCR-ABL1 amplified a smaller band than the expected 320-bp band (Table 2 and Fig. 2D), and the Sanger sequence of the PCR product revealed an e1a3 BCR-ABL1 fusion transcript (Fig. 2E, F). The G-band of the cervical mass was not obtained due to poor proliferation, but the results of the FISH test showed 70% BCR-ABL1-positive cells. Based on these findings, a definitive diagnosis of CML was made. The percentage of blast cells in peripheral blood increased to 10% about a month after the first visit and the blast count criteria met the accelerated phase criteria. On the other hand, the extramedullary leukemic mass formation was categorized as blast crisis in the European LeukemiaNet criteria (3). The patient was administered dasatinib at a dose of 140 mg/day. After the start of treatment, shrinkage of the cervical mass and disappearance of blast cells in peripheral blood were observed, and the percentage of BCR-ABL1 transcripts was cautiously followed. Six months after the

start of treatment, BCR-ABL1 determined by FISH decreased to 0.8%. According to the European LeukemiaNet criteria, it was classified as Complete Cytogenetic Response (CCyR) and Optimal at the moment.

Discussion

In most CML patients, the two major BCR-ABL1 transcripts are e13a2 and e14a2, which encode the P210 oncoprotein. CML can have other BCR-ABL1 transcripts including minor BCR-ABL1 (e1a2) (2), micro BCR-ABL1 (e 19a2) (4), and other rare types. In addition to e1a3, atypical BCR-ABL1 translocations including e6a2 (5), e8a2 (6), e9a1 (7), e12a2 (8), e13a3 (9), and e14a3 (10) have been reported in CML, but they are extremely rare. Forty patients (1.7%) with rare BCR-ABL1 transcripts were identified from a cohort of 2,331 CML patients: 4 types of rare transcripts including e1a2 (0.9%), e19a2 (0.4%), e13a3 (0.1%), and e14a3 (0.3%) were identified (11). The e1a3 BCR-ABL1 transcript observed in this case has been reported in 17 cases of ALL, 8 cases of CML, and 1 case of AML (Table 3) (12-29). Among the 8 cases of CML, there was no case in which an extramedullary mass was formed without an increase in blood cells in peripheral blood as in our case. Our case is the first reported case in which e1a3 CML presented as EBC. Expression of CD56 might contribute to the extramedurally mass formation, because CD56 is an adhesion molecule.

The e1a3 BCR-ABL1 transcript lacks ABL1 exon 2 and lacks the SRC homology 3 (SH3) domain encoded by ABL1 exon 2 (30). Since the SH3 domain negatively regulates the SH1 domain, which is a kinase region, it is thought that a deficiency of the SH3 domain promotes tumorigenesis (31). However, only a few cases of CML lacking exon 2 have been reported so far, and the characteristics and prognosis of the clinical course have not been clarified.

Also, a concern for cases with these rare BCR-ABL1 transcripts is that they can be overlooked in routine tests. By using the primer corresponding to the ABL exon 2 sequence, it may not be possible to detect a BCR-ABL1 transcript having a cut point in ABL exon 3 as in this case. If major BCR-ABL1 or minor BCR-ABL1 is negative despite the existence of the Ph chromosome, it is necessary to consider the presence of a rare BCR-ABL1 transcript variant. In this case, it was possible to identify the e1a3 BCR-ABL1 transcript by using Sanger sequencing. Clarifying a fusion transcript is important to find a minimal residual disease marker, although a method for quantitative evaluation has not yet been established.

In conclusion, Ph-positive leukemia with an e1a3 fusion transcript is a very rare disease, but there may be more potential cases and an accumulation of cases will deepen the understanding of the characteristics of the disease. EBC of CML should be considered as a differential diagnosis even in a case that shows almost normal CBC.

The authors state that they have no Conflict of Interest (COI).

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