

Letter to the Editor

Chronic myeloid leukemia with a rare fusion transcript, b2a3 (e13a3) BCR-ABL1: A report of four cases from India

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Dear Editor,

Chronic myeloid leukemia (CML) is characterized by reciprocal t(9:22) (q34; q11) translocation, leading to the generation of the BCR-ABL1 protein which plays a crucial role in CML pathogenesis. The BCR-ABL1 fusion gene is the molecular hallmark and causative event of CML. More than 95% of CML patients express the transcript e14a2 (b2a2) or e13a2 (b3a2), namely major BCR-ABL1 coding for p210 protein. Approximately 1%–2% of CMLs show e1a2 (minor BCR-ABL1) coding for p190 protein and <1% of cases show e19a2 (micro BCR-ABL1), encoding a 230-kDa protein.

Another rare fusion transcript e13a3 (b2a3) coding for p203 protein is also reported^[1,2] This uncommon fusion transcript, i.e., b2a3, is formed due to the fusion of BCR breakpoint at exon 13 with ABL point of exon 3 instead of more commonly involved exon 2, resulting in the generation of p203 protein, which is rarely seen CML.^[3] To date, only nine CML cases with this rare fusion transcript have been reported worldwide.^[4–10] These infrequent fusion transcripts may escape

detection or may be misdiagnosed when the commonly used techniques which detect typical fusion transcripts are used.^[11]

Here, we describe the clinicohematological profile and treatment response of four cases of Philadelphia (Ph)-positive CML with b2a3 fusion transcript. All the four patients presented to Hematology Outpatient department, AIIMS New Delhi, India, between January 2013 and December 2017, with chief complaints of generalized body weakness, early fatigability, left-sided abdominal mass, and night sweats for 2–4 months. The general characteristics and hematological parameters are summarized in Table 1. Based on clinical features and hematological parameters including peripheral smear (PS) findings, provisional diagnosis of CML was made [Table 1]. Three of the cases had CML-CP, and one patient (case 2) had *de novo* myeloid blast crisis. The patient (case no 2) had *de novo* myeloid blast crisis and was initially considered acute leukemia with probable diagnosis of CML-BC. This case had organomegaly (splenomegaly and hepatomegaly) at presentation. Our diagnosis was confirmed by multiplex nested reverse transcriptase polymerase chain reaction (MPX-RT PCR) and was later confirmed by bone marrow biopsy study which showed abnormal megakaryocytes (dwarf) in addition to blasts.

Fluorescence *in situ* hybridization (FISH) for cytogenetics and MPX-RT PCR for BCR-ABL fusion transcript was advised. Cytogenetic analysis by FISH revealed t(9:22) (q34; q11.2), and no other chromosomal abnormality was detected. Using MPX-RT PCR fusion transcript b3a2 (472 bp), b2a2 (397 bp),

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e19a2 (808 bp), and e1a2 (310 bp) was negative, but it showed unexpected band at 230 bp [Figure 1]. Real-time quantitative PCR was also negative for b3a2, b2a2, e19a2, and e1a2 fusion transcript. On the basis of these findings, CML with atypical fusion transcript was suspected, and DNA sample of the patients was further processed for DNA sequencing. Sequencing of the amplified product was performed as per the instructions of The BigDye® Terminator v3.1 Cycle Sequencing Kit (ABI, USA) on an ABI Genetic Analyzer 3730 (ABI, Japan) reagents (Applied Biosystems). The samples were placed in autosampler tray, and the purified reaction product was electrophoresed at 50°C in ABI 310 genetic analyzer. At the end of the run, the sequence was analyzed with sequence analysis software which confirmed b2a3 fusion transcript [Figure 2]. The patients were started on imatinib 400 mg/day as first-line treatment. Written informed consent was obtained from all the patients.

Results and Discussion

In all the four cases, CML was suspected on the basis of complete history, general physical examination, and characteristic PS findings. Using multiplex RT-PCR, an unexpected band at 230 bp was observed and hence impression of atypical fusion transcript was made [Figure 1]. Our findings were later confirmed by DNA sequencing which revealed b2a3 fusion transcript [Figure 2]. The final diagnosis of CML with atypical fusion transcript b2a3 was reported.

In a total of 1350 BCR-ABL-positive CML patients, 4 (0.01%) cases showed this rare b2a3 fusion transcript encoding a 203-kDa protein. The other rare fusion transcript e19a2 encoding a 230-kDa protein has also been reported in 0.30% of cases of CML patients who presented to the Department of Haematology, AIIMS, New Delhi, India, between January 2013 and December 2017.^[12]

Snyder *et al.* in the year 2004 observed that the estimated frequency of b2a3 variant transcript was 0.9% among all of the BCR-ABL-positive patients.^[2] Biologically, BCR/ABL a3 type transcripts lack part of the ABL SH3 domain, which is believed to contribute leukemogenesis by negatively regulating the kinase domain (SH1) and activating STAT5 signaling. CML with BCR/ABL a3 type transcripts may have a better prognosis because of lacking part of ABL SH3.^[5,13]

Multiplex RT-PCR may fail to detect certain rare BCR-ABL1 fusion transcript type, as many commercially available and laboratory-developed primer sets do not cover such rare fusions.^[14] One of the modifications of RT-PCR is nested

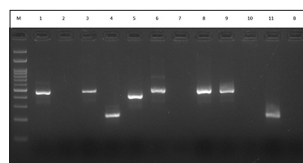


Figure 1: 3% Agarose gel electrophoresis of multiplex reverse transcriptase polymerase chain reaction product, Lane M: 100 bp marker; Lane1: positive control for b3a2 (472 bp), Lane 3,6,8,9: positive for b3a2 (472 bp), Lane 5: positive for b2a2 (397 bp), Lane 4,11: positive for b2a3 (230 bp), Lane 2,7,10: negative for chronic myeloid leukemia and Lane B: negative control

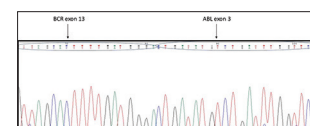


Figure 2: DNA sequencing showing the presence of BCR-ABL b2a3 fusion transcript

Table 1: General characteristics and hematological parameters of patients with b2a3 fusion transcript

Parameters	Case 1	Case 2	Case 3	Case 4
Age (years)	50	12	28	39
Gender	Male	Male	Male	Female
Hb (g/dL)	11.9	7.4	7.7	7
TLC (x10 ⁹ /L)	25	119	122	141
DLC (%)				
Blasts	4	95	1	8
Promyelocyte	6	-	3	2
Myelocyte	10	-	13	13
Meta Myelocyte	12	-	20	15
Band forms	18	-	8	5
Neutrophils	36	1	44	48
Lymphocytes	8	4	5	2
Monocytes	2		1	-
Basophils	4	-	5	7
Platelets (x10 ⁹ /L)	427	85	233	280
Splenomegaly	Absent	Present	Present	Present
Hepatomegaly	Absent	Present	Absent	Absent
CHR (Complete haematological response)	Yes	Yes	Yes	Yes
Imatinib	400mg/day	400mg/day	400mg/day	400mg/day
Last follow up	5 th April 18	5 th April 18	5 th April 18	5 th April 18

Hb=Hemoglobin, TLC=Total leukocyte count, DLC=Differential leukocyte count, CHR=Complete hematological response

multiplex RT-PCR which is used in our laboratory for the detection of various BCR-ABL transcripts and to differentiate between these BCR-ABL breakpoints in CML patients. This method allowed us a reliable detection of typical BCR-ABL

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transcripts, such as b2a2 and b3a2, and also the atypical types, such as transcripts lacking ABL exon a2 (b2a3 and e19a2). Hence, for diagnostic samples, the use of multiplex PCR may be recommended so that several kinds of BCR-ABL and BCR transcripts can be detected simultaneously.

All the patients with fusion transcript b2a3 coding for p203 protein achieved complete hematological remission within 3 months of imatinib therapy (400 mg/day) which was similar to the patients having p210 transcript. No chemotherapy was given as the patients achieved complete hematological response within 3 months of imatinib therapy. All the patients showed improvement in clinical features, reduction in organomegaly, and normalization of white cell count.

At present, all the patients are on regular follow-up and in hematological remission till date [Table 1]. Our findings are in line with Pienkowska-Grela *et al.* who reported a case of 39-year-old male with e13a3 variant CML who reached a complete molecular remission after 8 months of imatinib treatment.^[6] Other studies have also reported a favorable response of patients with b2a3 fusion transcript on imatinib treatment.^[4-10] Furthermore, Jinawath *et al.* reported that CML with BCR/ABL a3 fusion transcripts might have a different response to tyrosine kinase inhibitors compared to CML with BCR/ABL a2 fusion transcripts because of the alterations of tertiary structure.^[14]

Conclusion

Reporting of the patients with this rare b2a3 fusion transcript will provide better insight into understanding the clinicopathological profile and treatment response of these patients. Furthermore, the importance of multiplex nested PCR in combination with sequencing can be helpful for detecting rare BCR/ABL fusion transcripts such as b2a3 fusion transcript. Hence, reporting of more such cases is required to understand the relation between this transcript and treatment outcome.

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Conflicts of interest

There are no conflicts of interest.

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