Case report

Minor p190 Fusion Transcript in CML- A case report

Short running title**:** In CML minor (p190) fusion transcript

Abstract:

Objective: Detection of minor (p190) BCR-ABL fusion protein in a CML patients.

Case Report: A 26-year-old female presented with complaints of pain abdomen and episodes of vomiting. On examination her vitals were stable and there was moderate splenomegaly. The peripheral blood and bone marrow cytological features were consistent with myeloproliferative neoplasm. Fluorescent in situ hybridization (FISH) for BCR ABL was positive, with 87% of cells showing fusion transcript. However, Real Time Polymerase chain reaction (RT-PCR) for p210 BCR-ABL was found to be negative. In view of high morphologic index of suspicion for CML, further molecular analysis was carried out for BCR/ABL fusion transcript variants along with JAK2 V617F mutations.

Conclusion: The patient was found to be positive for BCR/ABL p190 e1a2 fusion transcript, while BCR/ABL p210 fusion transcript was negative.

Introduction

Chronic myeloid leukemia (CML) is characterized by the Philadelphia (Ph) chromosome which exists in three principal forms (P190, P210, and P230) that arise from distinct breakpoints in the BCR gene on chromosome 22, resulting in translocation of BCR exon 1, exons 1–12/13 , or exons 1–19 , respectively, to the c-ABL gene on chromosome 9 (1). The majority of patients (>90%) with CML express a 210-kDa BCR-ABL (t (9; 22)(q34;q11) protein, while patients with Ph^+ ALL commonly express a 190-kDa BCR-ABL protein (2). Only a subgroup of patients with CML express 190 kDa BCR-ABL fusion protein and its presence indicates poor response to

treatment (3). We report a patient with CML expressing minor BCR ABL transcript (p190) due to its rarity and prognostic implications.

Case report

A 26-year-old female presented with complaints of pain abdomen and episodes of vomitings. On examination her vitals were stable and there was moderate splenomegaly. Laboratory investigations showed leukocytosis of 234 × 10^9/L, hemoglobin (Hb) level of 10.7g/dL and platelet count of 2.25× 10^9/L. Bone marrow evaluation showed promyelocytes (03%), myelocytes (13%), metamyelocytes (09%), eosinophils (08%), basophils (12%), blasts (06%), neutrophils (43%) and lymphocytes (03%) (Figure1). The peripheral blood and bone marrow cytological features were consistent with myeloproliferative neoplasm. Cytogenetic analysis failed due to non availability of analyzable metaphases. Fluorescent in situ hybridization (FISH) for BCR ABL was positive, with 87% of cells showing fusion transcript. However, Real Time Polymerase chain reaction (RT-PCR) for p210 BCR-ABL was found to be negative. In view of high morphologic index of suspicion for CML, further molecular analysis was carried out for BCR/ABL fusion transcript variants along with JAK2 V617F mutations (GeneLab). The patient was found to be positive for BCR/ABL p190 e1a2 fusion transcript, while BCR/ABL p210

fusion transcript was negative. The Jak2V617F mutation analysis was also tested negative (Table I).

Transcript expression load for BCR-ABL p190 e1a2 (m-bcr) was found to be 63.4% by quantitative RT-PCR. She was started on Veenat 400 mg/day. On follow-up at 3 months, her copy number for the e1a2 transcript by qRT-PCR (Genelab) was found to be 15.44%. The dosage of Veenat was escalated to 600mg/day. At 6 months follow-up her e1a2 transcript load decreased to 6.5% estimated by qRT-PCR (Genelab), continued on treatment with same dosage. There was reduction in transcript load at 9 months follow-up with 3.72% of p190 e1a2 transcript levels. She was evaluated for imatinib resistance (Oncquest) and the results were positive for E255V in P-loop of ABL1 kinase domain of BCR/ABL1 transcript. The study was approved by the institutional ethics committee (EC Reference No: IEC/2019/162).

Discussion

Majority of CML patients express 210-kDa BCR-ABL (t (9; 22)(q34;q11) protein and minor transcript positivity is rare. In our institute, which is a tertiary care cancer center, minor transcript constituted 1.78% (1/56) of newly diagnosed CML patients tested by real time pcr. This finding was in agreement with the reported frequency in literature [3-5].

In translocation of t(9;22), Abl part in the chimeric protein is constant while the Bcr portion varies greatly, resulting in different sizes of the Bcr sequence , hence it is not only a reflection of the site of breakage but may also be a result of alternative splicing [6]. That BCR-ABL transcript type and levels in association with pattern of secondary genetic changes can largely predict blast phenotype, with e1a2/p190 BCR-ABL expression nearly always exclusively driving lymphoid transformation, as supported by experimental studies [7]. In vivo experiments have shown that both p210 and p190 can give rise to CML or B-ALL depending on whether stem cells or committed progenitor cells are transduced. It is possible, therefore, that the rarity of p190 in human CML is not a direct consequence of the activity of this fusion, but rather that BCR intron 1 breaks may be much more frequent in ALL because they are formed by a lymphoid-specific mechanism. Genomic breakpoints in p190 and p210 BCR–ABL indicate distinct mechanisms of action [8].

E255V in P-loop of ABL1 kinase domain of BCR/ABL1 transcript being positive in our patient, is a clinically relevant mutation and has been previously reported in patients who develop resistance to imatinib mesylate [9]. Poor prognosis of CML patients expressing minor BCR ABL transcript, with survival ranging from 3 months to 9 years was reported [10]. Treatment with imatinib, as frontline therapy, achieved complete hematological response showing no molecular

remission as in our patient and other studies [3, 11]. Only one patient with Philadelphia negative, p190 BCR–ABL positive CML showed excellent response to imatinib treatment [12].

A higher number of patients need to be evaluated along with the monitoring of response to tyrosine kinase inhibitors with high end techniques and understand the resistance to therapy, to determine the prognostic significance.

Conclusion: In CML patients BCR-ABL t(9;22) translocations variants need to be tested, as these variants would help in quantitative analysis at follow up. The rare variants would aid in determining the prognostic significance, as the presence of pl90 BCR ABL indicates resistance to imatinib therapy.

References:

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Table I: Chromosomal alterations for t(9;22) in Chronic myeloid leukemia and Jak2 Mutation

No.	**Chromosomal alteration**	**Genes involved**	**Fusion gene**	**Result**
1	t(9;22)(q34;q11)		b2a2	Not detected
		MAJOR (p210)	b2a3	Not detected
		BCR(22q11) and ABL (9q34)	b3a2	Not detected
			b3a3	Not detected
2	t(9;22)(q34;q11)	**MINOR (p190)**	**e1a2**	**Detected**

		BCR(22q11) and ABL (9q34)	e1a3	Not detected
3	t(9;22)(q34;q11)	MICRO (p230) BCR(22q11) and ABL (9q34)	e19a2	Not Detected
	Jak2		V617F	Not Detected

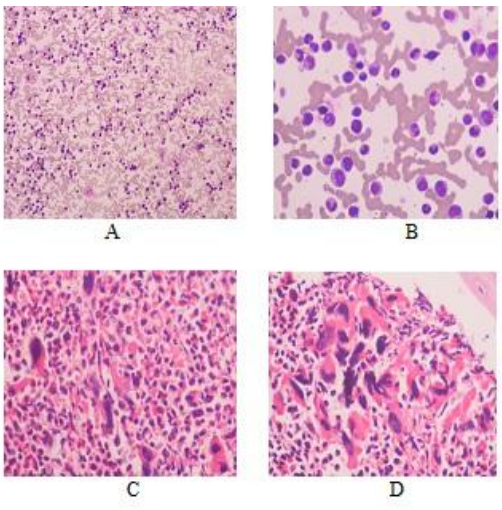

Fig 1 : A - Peripheral smear showing overwhelming leukocytosis (Leishman 4X)

B - Peripheral smear showing shift to left and basophilia (Leishman 40X)

C - Bone marrow biopsy: Myeloid hperplasia(H&E stain 40X)

D - Bone marrow biopsy: Focal clustering of megakaryocytes(H&E 40X)