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Case report and review of literature of a patient with CML with atypical E14a3 transcript masquerading as BCR-ABL negative CMPD.

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Abstract

CML with atypical transcript is a rare entity. Patient in this case report was diagnosed as BCR-ABL negative chronic myeloproliferative disorder (CMPD) and started on hydroxyurea. The diagnosis was made on the basis of bone marrow aspiration, biopsy and a negative qualitative PCR for typical BCR-ABL transcript. He presented to our oncology unit 2 years after starting hydroxyurea with progressive splenomegaly and leukocytosis. Cytogenetic evaluation showed presence of 9,22 translocation. Qualitative PCR for atypical transcript followed by sanger sequencing from blood showed presence of e14a3 transcript. This is possibly 24th reported case of CML with this atypical transcript in literature. Initially after starting imatinib he had CHR but during follow up, due to absence of cytogenetic response he was switched to Nilotinib. He achieved Complete cytogenetic response (CCyR) to Nilotinib, but the response was lost at one year. Dasatinib was started after Bcr-Abl kinase domain mutation analysis showed E255K mutation. Presently at 1 year after starting Dasatinib, this patient has achieved CCyR and molecular remission which was confirmed by karyotyping, FISH and RT PCR for variant transcript. This case highlights the importance of cytogenetics or FISH evaluation for 9,22 translocations in every patient of BCR-ABL negative CMPD.

Keywords:

Atypical transcript, CML, e14a3/b3a3 transcript, Cytogenetics, Bcr-Abl negative CMPD.

Background

Chronic myeloid leukaemia (CML) is a hematopoietic disorder characterized by the malignant expansion of bone marrow stem cells. Its cytogenetic hallmark is a reciprocal t(9;22)(q34;q11) chromosomal translocation that creates a derivative 9q+ and a small 22q–, known as the Philadelphia (Ph) chromosome. The latter harbours the BCR-ABL fusion gene encoding a chimeric Bcr-Abl protein with a deregulated tyrosine kinase activity1,2.

In this case report we highlight the importance of cytogenetic evaluation in patients with BCR-ABL negative Chronic Myeloproliferative Disorder (CMPD). Commercially available PCR probes for typical BCR-ABL transcripts will miss patients with CML having atypical transcripts. To the best of our knowledge this patient with e14a3 transcript is 24th reported case of this transcript in CML in the world and possibly 6th from india3,4,5.The limited number of cases could also be due to underdiagnosis.

Present case also discusses the challenges associated with monitoring response to TKI in these patients.Cytogenetic assessment from marrow was done to assess response in this case.

Clinical outcomes in this subset of CML with atypical transcript appears similar to other patients with CML ,with caveat being limited number of cases and no standardized criteria to measure depth of response.

Case Report

39 years old male was diagnosed as BCR-ABL negative CMPD on the basis of bone marrow aspiration, biopsy and negative qualitative PCR for typical Bcr-Abl transcripts. He was started on hydroxyurea. He came to our oncology unit due to increasing splenomegaly and persistent leukocytosis, 2 years after starting hydroxyurea. His recent CBC showed Hb-8.7g/dl, White blood count-107550/mm3and platelet-1,82000/mm3 .

Patient's previous investigations were reviewed, report of cytogenetics was not available.

A repeat bone marrow aspiration and bone biopsy was done along with cytogenetics. Bone marrow aspiration and biopsy showed a markedly hypercellular marrow with myeloid predominance and left shift-possibly CMPD. Cytogenetics showed presence of 9,22 translocation(Figure 1). Repeat qualitative PCR for typical Bcr-Abl transcripts was negative.

He was started on imatinib and achieved complete hematological response in 1 month.

Qualitative PCR for atypical transcript followed by sanger sequencing for the type of Bcr-Abl transcript showed a rare transcript E14a3(Figure 2).In view of transient febrile illness and accompanying thrombocytopenia imatinib was stopped for 2 weeks by the treating physician. He was restarted on imatinib after consultation with us. As quantitative PCR for monitoring cannot be performed in presence of rare translocation, cytogenetics was used for response evaluation. At 6 months repeat cytogenetic evaluation in bone marrow showed 100 % metaphase showing 9,22 translocations.

In view of failure to achieve cytogenetic response , patient was advised for nilotinib. He could not get the drug for next 3 months in view of non-availability from approved pharmacy in government set up under which he was being treated, so imatinib was continued.

Nilotinib was started at 400 mg twice daily after 3 months of cytogenetic evaluation. Repeat cytogenetic evaluation at 6 months after starting Nilotinib showed CCyR(Figure 3). Patient was continued on Nilotinib. Repeat cytogenetic evaluation at 1 year of starting Nilotinib showed loss of CCyR.

BCR-ABL1 kinase domain mutation analysis was done which suggested E255K mutation. This mutation is known to be sensitive to Dasatinib, so the drug was started at 100 mg once daily. He had thrombocytopenia after starting dasatinib, but the drug was continued with careful monitoring of platelet count, with a goal to keep platelet count more than 50000/mm3. Response evaluation at one year after starting dasatinib showed complete cytogenetic response. Repeat RT PCR for atypical transcript at this timepoint is negative for E14a3 transcript (Figure 4) which is suggestive of good molecular response as well.

Discussion

Patients with CML have Ph chromosomal abnormality and majority of the fusion transcripts are e13a2 and e14a2 (M-bcr) which produce a 210 kDa protein when transcribed2. Less commonly patients have, e1a2 (m-bcr) and e19a2 (μ-bcr) transcripts.

Atypical breakpoints have also been sporadically reported and can be grouped into four categories: BCR breakpoints originating within introns that lie outside M-bcr, m-bcr, or μ-bcr fused to ABL a2; BCR breakpoints occurring within exons fused to ABL a2; typical BCR breakpoints (M-bcr, m-bcr, or μ-bcr)

fused to ABL breakpoints located downstream of a2; and transcripts containing intervening sequences between BCR and ABL a26.

Worldwide approximately 2–4% of patients harbour atypical BCR-ABL1 transcripts lacking ABL1 exon2 (e13a3 or e14a3) or resulting from atypical BCR breakpoints (e.g.: e1a2, e6a2, e8a2, or e19a2) that may yield a false negative PCR using routine primer sets in qualitative or quantitative reverse transcriptase PCR protocols[16].

In this case report we describe a patient diagnosed as CML based on cytogenetic analysis after being initially treated as BCR-ABL negative CMPD, due to absence of typical Bcr-Abl transcript by RT-PCR. Further evaluation after confirmation of 9,22 translocation on conventional cytogenetics with RT PCR for atypical transcript demonstrated the presence of an e14a3 (also known as b3a3) BCR-ABL fusion.

This patient with e14a3 transcript CML is the 24th reported case of this transcript in CML in the world and possibly 6th from india3,4,5.In a paper by A.K.Arun et al. from the department of Haematology, CMC Vellore to assess frequency of rare fusion transcripts in CML, amongst 1260 patients with CML, 4 cases of e14a3 fusion transcript were noted with a incidence of 0.3%5.

The limited number of cases reported for these atypical transcripts, may be due to underdiagnosis. Missing the diagnosis of CML in presence of atypical transcript will result in missed treatment opportunity and also result in diagnosis of CML in accelerated or Blast stage, thus leading to inferior outcome.

The quantitative RT-PCR assay is used to monitor disease response and progression after treatment with TKI in CML. Monitoring response to TKI in patients with atypical transcripts is challenging, due to inability of conventional probes to detect these transcripts. Caution in interpretation of negative result as molecular remission should be kept in mind. Cytogenetic assessment from marrow was done to assess response and change of treatment in this case.

The Outcome specific to CML in patients with BCR-ABL e14a3 fusions are difficult to define because of the limited number of cases reported. Table 1 shows the Characteristics of CML patients with b3a3 reported in literature. Median age of diagnosis in reported cases is 45.5 years which is lower than that reported for classical CML. Majority of the cases of atypical transcript are reported in males. Reported cases appear to respond well to treatment with imatinib but prospective long term follow up is unavailable.

BCR-ABL1 kinase mutation analysis can be performed in the presence of atypical transcript and can guide in selection of alternate TKI in the absence of response to initial and subsequent TKI. BCR-ABL1 kinase

mutations can be detected with sensitivities of about 20% by Sanger sequencing and of about 3% by NGS. The greater sensitivity of NGS enables the early detection of clinically relevant BCR-ABL1 resistance mutations[17,18]. NGS is the recommended technology to detect BCR-ABL1 resistance mutations in patients not responding adequately to TKI.

Treatment free remission (TFR) which is certainly emerging as one of the goals in treatment of CML cannot be offered to these patients with rare transcripts as monitoring of response using bcr-abl transcript is not available. Presence of atypical transcript is exclusion criteria for TFR[16].

Present case highlights the challenges in community setting and low-income countries in procuring 2nd generation TKI when imatinib fails. Patient having failed imatinib could not start Nilotinib for 3 months because of unavailability of the same from empaneled government facility and patient could not afford to buy the drug. Delayed initiation among individuals even with cost-sharing subsidies suggests that out-of-pocket costs may be a barrier to timely initiation of therapy among individuals diagnosed with CML and certainly availability of cheaper generics will help in making the 2nd generation TKI more accessible and improve compliance to treatment[19].

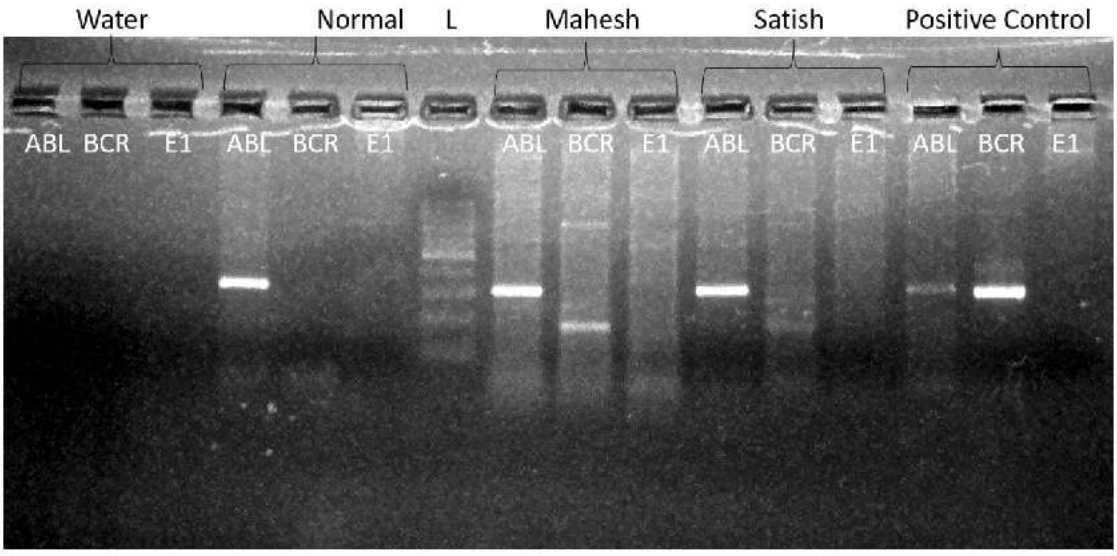

Fig. 1 RT-PCR for the BCR-ABL1 fusion, e14a3 transcript with band size of 186 bp. Lane (Water): negative control sterile H2O. Lane (Normal): Normal control. Lane (L): 100-bp DNA ladder. Lane (Mahesh): CML patient current case with e14a3 transcript an atypical transcript. Lane (Satish): BCR-ABL-negative case. Lane (Positive Control): BCR-ABL-positive Control with e13a2 major transcript.

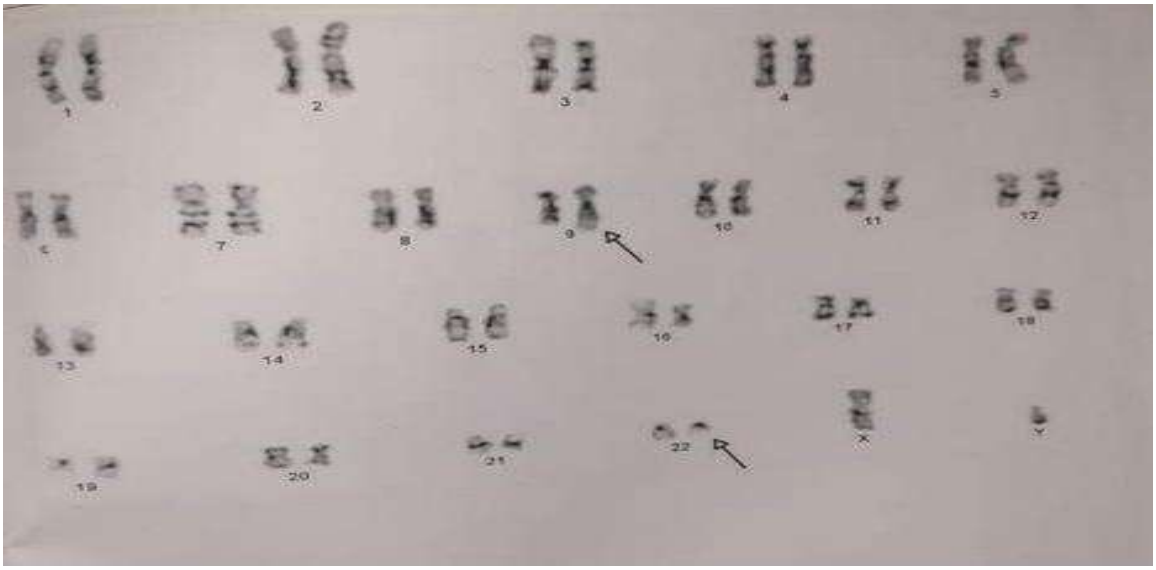

Figure 2: Conventional karyotype showing the classic t(9;22) at the time of initial diagnosis. Arrows represent the abnormal derivative chromosomes resulting from this apparently balanced translocation.

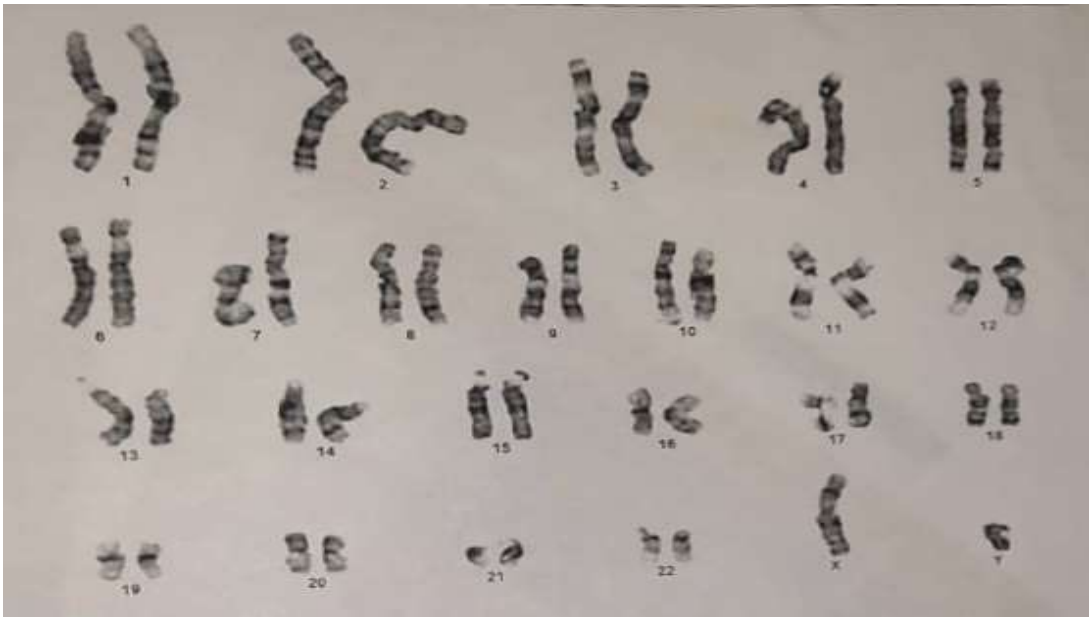

Figure 3: Conventional karyotype showing CCyR after treatment with Nilotinib.

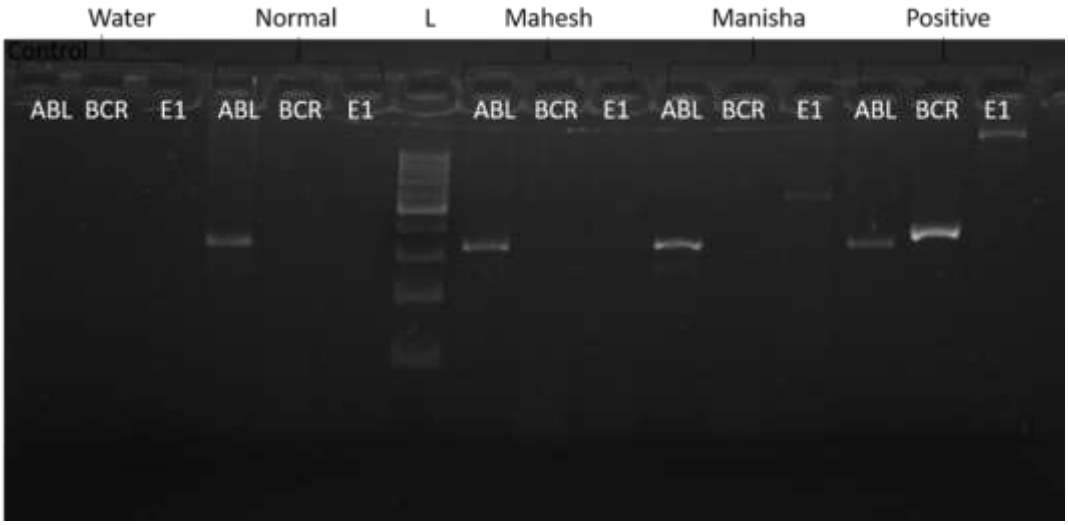

Figure 4: RT-PCR for the BCR-ABL1 fusion, e14a3 transcript with band size of 186 bp. Lane (Water): negative control sterile H2O. Lane (Normal): Normal control. Lane (L): 100-bp DNA ladder. Lane (Mahesh): CML patient current case with atypical e14a3 transcript is not seen after 1 year Dasatinib. Lane (Manisha): BCR-ABL-negative case. Lane (Positive Control): BCR-ABL-positive Control with e13a2 major transcript.

	Year	Age, years Sex	WBC	Platelets	Treatment	Splenomegaly	Comments	Reference
1	1994	39/F	9,000	Not reported	No treatment documented	Absent	CML-CP	7
2	1994	43/M	64,800	160,000	High dose chemo, INF, Hu	Not at presentation but developed later on	CML + ALL	4
3	1997	19/M	42,000	381,000	INF+Hu	Not reported	CML-CP	8
4	1999	23/M	95,000	485,000	INF+Hu	No	CML-CP	9
5	1989	51/M	19,900	566,000	INF ×11 mo, followed by ABMT, followed by INH × 16 mo	Not reported	CML-CP	10
6	1996	69/M	18,000	527,000	Hu ×28 mo replaced with busulfan and 6-mercaptopurine	Absent	CML-CP	10
7	2001	69/M	29,900	286,000	INF-alpha	No	CML-CP	11

8	2009	81 /M	28,000	Not reported	Imatinib, followed by dasatinib, followed by Hu	Not reported	CML-CP	12
9	2013	30 /M	45,000	Not reported	Not reported	Not reported	CML	13
10	2004 – 2012	52/M	229,000	590,000	IFN+ Hu ×13 mo, followed by Imatinib, then dasatinib	Not reported	CML-CP	3
11	2004 – 2012	41/M	26,000	414,000	Hu ×1 mo, followed by imatinib	Present	CML-AP	3
12	2004 – 2012	41/M	115,000	798,000	INF + Hu ×19 mo, followed by imatinib	Present	CML -CP	3
13	2004 – 2012	48/F	300,000	435,000	NF + Hu ×15 mo, followed by imatinib, then vincristine, prednisone	Present	CML, progressed to lymphoid blast crisis/ deceased	3
14	2004 – 2012	48/M	98,200	1,072,000	Hu ×1.5 mo followed by ABMT	Present	CML-CP	3
15	2017	40/M	46,420	275,000	Imatinib	Absent	CML-CP	3
16	2011	54/M	13,000	1,320,000	Nilotinib + Hu	Absent	CML-CP	4
17	2016	N/A	N/A	N/A	Imatinib intolerant	N/A	CML-CP	5
18	2016	N/A	N/A	N/A	Imatinib resistant	N/A	CML-Lymphoid Blast crisis	5
19	2016	N/A	N/A	N/A	Imatinib	N/A	CML-CP	5
20	2016	N/A	N/A	N/A	Imatinib	N/A	CML-CP	5
21	2016	52/M	N/A	207000	Nilotinib	Not Reported	CML-CP	15
22	2019	51/M	90000	400000	Imatinib,Nilotinib	Not reported	CML-myeloid Blast crisis	20
23	2019	23/F	64000	-	Imatinib,hydroxyurea	Present	CML-CP	20
24	2020	34/M	107550	182000	Hu followed by imatinib followed by Nilotinib then Dasatinib	Present	CML-CP (Present study)	

INF-interferon therapy; Hu-hydroxyurea; ABMT-autologous bone marrow transplantation, N/A-not available, mo, months. Table adapted from Table 1 from Hu et al. [3]. And Table 1 from Chishti and Sanders et al. [4]

Table 1- Characteristics of CML patients with b3a3 reported in literature:

Conclusion

1) Cytogenetics or FISH study for 9,22 translocations should be a part of work up for all suspected CMPD.

2) Atypical transcripts in CML are rare, but can be misdiagnosed in the absence of adequate workup.

3) In a patient with CML with atypical transcript, a negative quantitative PCR for BCR-ABL using conventional primers should not be misinterpreted as deep molecular response.

4) It is difficult to monitor response in these patients and cytogenetics can be used as a tool to monitor response.

5) BCR-ABL kinase domain mutation can be used in these patients to decide subsequent TKI in the absence of adequate response to initial TKI.

6) CML is a chronic disease and requires possibly lifelong use of TKI in majority of patients. The availability of cheaper generics will improve accessibility and compliance.

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