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Pattern of Expression of PML-RARα Transcripts in Acute Promyelocytic Leukemia Patients and Its Correlation with Overall Survival: A Retrospective Study from Western India

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Abstract

Introduction Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia with specific molecular pathogenesis, clinical features, and treatment. It is cytogenetically characterized by translocation t (15;17) (q24;q21). The location of breakpoints within the PML gene determines the formation of distinct promyelocytic leukemia-retinoic acid receptor α (PML-RARα) transcript subtypes which have prognostic significance. Breakpoints in intron 6 result in the long (L or bcr-1) subtype, those in exon 6 produce the variant (V or bcr-2) subtype, and breakpoints in intron 3 lead to the short (S or bcr-3) subtype.

Objectives The aim of our study was to determine the frequencies of the different PML-RARα transcripts in patients with APL at baseline and to investigate the impact of bcr-3 transcript as a prognostic factor on survival in newly diagnosed patients with APL.

Materials and Methods A retrospective study was conducted that included 54 newly diagnosed patients with APL. Clinicopathological parameters were evaluated in all cases for prognostic significance with overall survival. Real-time quantitative polymerase chain reaction was used for the quantification of PML-RARα transcripts.

Results Out of 54 patients, 53 (98.1%) patients expressed bcr-3 transcript either alone or in combination with either bcr-1 or bcr-2 transcripts. Twenty-one patients (38.9%) in our study showed expression of bcr-3 transcript only. Twenty eight (51.9%) patients in our study expressed all the three transcripts. Out of the 54 patients, 20 (37%) patients died of disease, out of which 19 patients died before completion of induction therapy. Complete remission was obtained in 34 patients (63%) after induction therapy. The survival rate for patients expressing the bcr-3 transcript alone was 66.66% as compared with 60.71% for patients expressing bcr-3 transcript in combination with other two transcripts. The survival rate for patients receiving all-*trans* retinoic acid (ATRA) in combination with arsenic trioxide was far better than patients receiving ATRA alone.

Keywords
- acute promyelocytic leukemia
- PML-RARα transcripts
- bcr-3 transcript

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Conclusion The total leucocyte count is an independent prognostic factor in patients with APL as it was statistically significant with overall survival in our study. The bcr transcript either alone or in combination with bcr-1 and/or bcr-2 transcripts was the most frequent pattern observed.

Introduction

Acute promyelocytic leukemia (APL) is a distinctive subtype of acute myeloid leukemia characterized by specific molecular pathology, clinical features, and treatment approaches. Cytogenetically, it is characterized by translocation t (15;17) (q24; q21), which is a balanced translocation. This translocation results in the fusion of retinoic acid receptor α (RARα) gene on chromosome 17 with promyelocytic leukemia (PML) gene on chromosome 15.[1] RARα breakpoints are seen in intron 2. Breakpoints within the PML gene which are located at intron 6, exon 6, and intron 3 result in the formation of three different PML-RARα transcripts which are referred to as long (L or bcr-1), variant (V or bcr-2), and short (S or bcr-3), respectively.[2]

The most common breakpoint subtype of PML is bcr-1 (breakpoint cluster region) or long form, which occurs at intron 6, and is identified in 45 to 55% of cases of APL. The second most common subtype is the short form, or bcr-3, at intron 3 of PML, and is seen in 35 to 45% of cases. The least common subtype is the variable form, or bcr-2, occurring at exon 6 of PML, and accounts for 5 to 10% of cases.[3]

Reverse transcriptase polymerase chain reaction (RT-PCR) remains the gold standard method for genetic confirmation of APL, as it allows the identification of the specific PML/RARα transcript subtype.[4] Conventional karyotype and fluorescence in situ hybridization are the other techniques that can be used for confirmation of diagnosis. Isoform-specific quantitative real-time PCR allows for the evaluation of response during therapy, minimal residual disease monitoring, and detection of molecular relapse during the follow-up period.[5,6] Posttreatment leukemic relapse is indicated by re-appearance of bcr transcripts.

APL patients are divided into low-, intermediate-, and high-risk categories based on the total leucocyte count (TLC) at presentation, which is also an important prognostic factor. At diagnosis, a TLC count greater than 10,000/μL places patients into a high-risk subset, whereas those with TLC count less than or equal to 10,000/μL are further classified into low- and intermediate-risk categories based on platelet count cut-offs of $>$40,000 and $\leq$40,000/μL.[7]

Combination of chemotherapy with arsenic trioxide (ATO) and all-*trans* retinoic acid (ATRA) has shown excellent outcomes with approximately more than 90% complete remission rates. In spite of these results, the incidence of death rate continues to remain high with this leukemia, which is particularly more frequent in patients who belong to the high-risk category. High-risk features in APL are defined by TLC count $>10 \times 10^9$/L at presentation, CD34 expression by flow cytometry, bcr-3 transcript expression on PCR, and FLT3-internal tandem duplication (ITD) mutations.[8]

Thus, we investigated the frequencies of the PML-RARα transcripts in 54 APL patients at baseline and the impact of bcr-3 transcript as a prognostic factor on survival in newly diagnosed patients with APL.

Need of the study: our present study evaluates the impact of bcr-3 transcripts on newly diagnosed APL patients as well as certain pathological and clinical risk factors such as TLC and platelet count. In APL, intensification of therapy in addition to conventional ATRA is based on these factors.

Materials and Methods

Sample Size

Newly diagnosed patients with APL during the period from January 2021 to October 2023.

Inclusion Criteria

All newly diagnosed patients with APL in which quantification of PML-RARα transcripts was done were included. Patients were divided into low- and high-risk groups on the basis of TLC at the time of diagnosis.

The treatment protocol was decided on the basis of risk stratification. Low-risk patients received combination of ATRA and ATO, while high-risk patients were treated with chemotherapy and ATRA. Treatment decision was not based on the pattern of transcripts.

Exclusion Criteria

Patients without PML-RARα transcripts at baseline were excluded.

Fifty-four newly diagnosed patients with APL were studied using bone marrow examination, flow cytometry, and cytogenetic analysis of t(15;17). Real-time quantitative PCR was used for the quantification of all the three different PML-RARα transcripts.

Peripheral blood was used for RNA extraction using an RNA extraction kit (Qiagen, Hilden, Germany). A complementary DNA (cDNA) synthesis kit (Thermo Scientific) was used for converting RNA to cDNA. Quantification was done on the Aria Mx instrument using Qiagen Ipsogen PML-RARα kits.

Statistical Analysis

Statistical analysis was done using SPSS software version 22 (IBM Company, Armonk, New York, United States). The difference between variables was analyzed by the χ^2-test. Overall survival (OS) was determined using the Kaplan–Meier analysis and was measured from the start of treatment to the either last follow-up date or to the date of death.

Ethical Approval

This study is approved by the Institutional Review Committee (IRC/2024/P-52, July 9, 2024).

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Results

Out of 54 patients, 31 (57.4%) were females and 23 (42.6%) were males. The median age was 34 years and the age range was 05 to 72 years. In total, 25 (46.3%) patients had TLC count $\leq$10,000 cells/mm^3 and were categorized as low risk and the remaining 29 (53.7%) had TLC count $>$10,000 cells/mm^3 and were categorized as high risk. Platelet count was $\leq$40,000 cells/mm^3 in 45 (83.3%) patients, whereas 9 (16.7%) patients had platelet count $>$40,000 cells/mm^3. Bone marrow morphological examination showed 52 (96.3%) patients having hypergranular variant of promyelocytes and only 2 (3.7%) had microgranular variant of promyelocytes. Cytogenetic analysis showed 49 (90.7%) patients were positive for t (15;17), whereas 5 (9.3%) patients showed variant positivity for t(15;17). Flow cytometry was also done in all the cases and 44 (81.5%) patients showed the classic immunophenotype of APL with 2 (3.7%) patients showing CD34 positivity, 5 (9.3%) with HLA-DR positivity, and 3 (5.5%) patients with HLA-DR and CD34 positivity.

Out of the 54 patients, 53 (98.1%) patients expressed bcr-3 transcript either alone or in combination with other transcripts. In our study, the bcr-3 transcript alone was detected in 21 (38.9%) patients. A total of 28 (51.9%) patients in our study expressed all the three transcripts (►**Table 1**).

Nine patients (16.6%) who were treated with only ATRA succumbed to disease and hence could not receive further treatment. Twenty five (46.3%) patients were treated with combination of ATRA and ATO. A total of 20 patients received chemotherapy, out of which, in 12 (22.2%) patients chemotherapy was given in combination with ATRA and in 8 (14.8%) patients it was given in combination with ATRA and ATO. Chemotherapy was given in 6 (21.4%) patients expressing combination of transcripts in contrast to 12 (57.1%) patients showing isolated bcr-3 transcript.

Out of the 54 patients, 20 (37%) patients died, of which 19 patients died before completion of induction therapy. Complete remission was obtained in 34 patients (63%) after induction therapy.

Table 1 Frequency distribution of different PML-RARα transcripts

PML-RARα transcripts	Frequency ($n=54$)
bcr-1 + bcr-2 + bcr-3	28 (51.9%)
bcr-2 + bcr-3	02 (3.7%)
bcr-1 + bcr-3	02 (3.7%)
bcr-3	21 (38.9)
bcr-2	01 (1.8%)

Table 2 Clinicopathologic characteristics of bcr-3-positive cases alone and in combination with bcr-1 and bcr-2 transcripts

Clinicopathologic parameters	bcr-1 + bcr-2 + bcr-3 ($n=28$)	bcr-3 ($n=21$)
Total leukocyte count		
$\leq$10,000 cells/mm^3	16 (57.1%)	08 (38.1%)
$>$10,000 cells/mm^3	12 (42.9%)	13 (61.9%)
Platelet count		
$\leq$40,000 cells/mm^3	22 (78.6%)	20 (95.2%)
$>$40,000 cells/mm^3	06 (21.4%)	01 (4.8%)
Morphology		
Hypergranular	28 (100.0%)	19 (90.5%)
Microgranular	00 (0.0%)	02 (9.5%)
Cytogenetics		
Classic t(15;17)	26 (92.8%)	19 (90.5%)
Variant t(15;17)	02 (7.2%)	02 (9.5%)
Immunophenotyping		
Classic IPT	24 (85.7%)	15 (71.4%)
CD34 positive	01 (3.6%)	01 (4.8%)
HLA-DR positive	00 (0.0%)	05 (23.8%)
CD34 and HLA-DR positive	03 (10.7%)	00 (0.0%)
Treatment		
ATRA	05 (17.9%)	04 (19.1%)
ATRA + chemotherapy	03 (10.7%)	07 (33.3%)
ATRA + arsenic trioxide	17 (60.7%)	05 (23.8%)
ATRA + arsenic trioxide + chemotherapy	03 (10.7%)	05 (23.8%)

Abbreviation: ATRA, all-*trans* retinoic acid.

In our study, we also compared the clinicopathologic characteristics of bcr-3-positive cases alone and in combination (►**Table 2**).

Survival Analysis

In our study, we studied the effects of different variables on OS. OS was determined using the Kaplan-Meier analysis. Patients having TLC count $>$10,000 cells/mm^3 belonged to the high-risk category and had significant differences in OS compared with patients having TLC count $\leq$10,000 cells/mm^3 ($p=0.007$; ►**Fig. 1**). Patients expressing bcr-3 transcript alone had a survival rate of 66.66% as compared with 60.71% for patients expressing bcr-3 transcript in combination with other two transcripts (►**Fig. 2**). However, the median OS in patients with combination of transcripts was 5 months as compared with 9 months for patients expressing bcr-3 transcripts alone. The survival rate for patients receiving ATRA in combination with ATO was far better than patients receiving ATRA alone (►**Fig. 3**). The

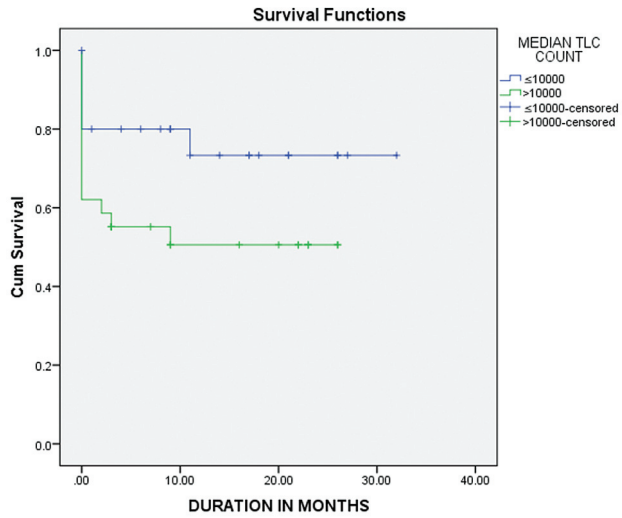

Fig. 1 Kaplan–Meier overall survival of APL patients according to TLC counts. APL, acute promyelocytic leukemia; TLC, total leukocyte count.

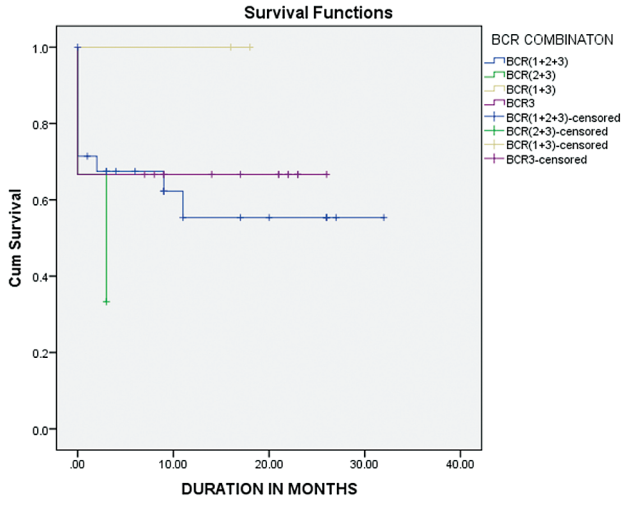

Fig. 2 Kaplan–Meier overall survival of APL patients according to PML-RARα fusion gene transcripts. APL, acute promyelocytic leukemia.

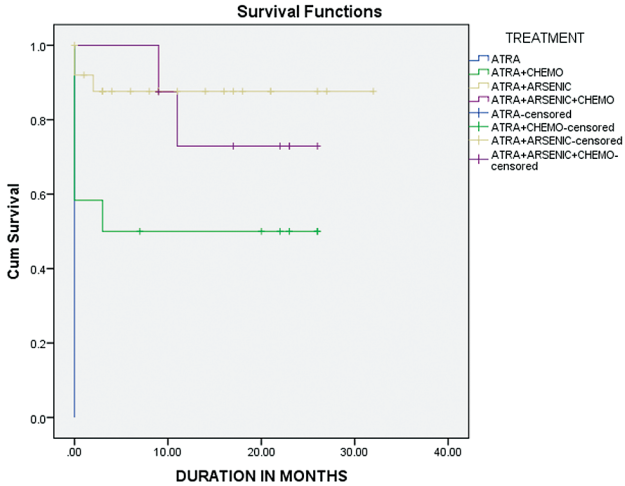

Fig. 3 Kaplan–Meier overall survival of APL patients according to treatment. APL, acute promyelocytic leukemia.

survival rate was also better for patients receiving ATRA and ATO in combination with chemotherapy (►**Table 3**).

Discussion

Depending on the location of breakpoints within the PML gene, APL shows three different transcripts (bcr-1, bcr-2, and bcr-3), which can be seen either singly or in combination. Monitoring of these transcripts is considered as an important part of treatment management in APL.

We aimed to study the pattern and distribution of these transcripts in APL and their effect on survival in our study.

The most frequent pattern of expression of transcripts in our study was a combination of all three transcripts (bcr-1 + bcr-2 + bcr-3) seen in 28 patients (51.9%), followed by bcr-3 alone in 21 patients (38.9%).

Another study conducted by Baba et al studied 45 cases of APL, of which bcr-1 expression was seen in 26 patients, followed by bcr-3 in 16 patients and bcr-2 in 3 patients. Combination patterns were not described in this study.[9]

Rasekh et al studied expression of bcr-3 and bcr-1 in 118 patients with APL, of which bcr-3 was seen in 31.4% and bcr-1 in 52.5%.[10] Similarly, Nath et al also analyzed bcr transcript expression in 29 patients with APL, of which 17 patients had bcr-1 and 12 had bcr-3 expression.[11] Expression of bcr-2 transcripts and combination patterns was not described as in our study.

Of hematological parameters, survival was better in low-risk patients with TLC count $\leq$10,000 cells/mm^3 (p-value = 0.007). Other parameters such as platelet count, age, gender, and immunophenotyping were not statistically significant with OS in our study.

Median survival of patients expressing bcr-1 + bcr-2 + bcr-3 transcripts was 5 months as compared with 9 months in patients only expressing bcr-3 transcripts. However, OS was not statistically significant with pattern of transcripts ($p = 0.445$).

Baba et al's study showed prognostically significant differences in OS with bcr transcripts, which was 100% with bcr-2 transcripts, 88.5% with bcr-1 transcripts, and 56.3% with bcr-3 transcripts.

Nath et al also highlighted higher induction death rates in patients expressing bcr-3 transcripts (34%) in comparison to bcr-1 transcripts (12%).

In our study of 54 patients with APL, bcr-3 was detected in 53 cases, either alone or in combination. Effect of bcr-3 alone was not found to be statistically significant with survival as compared with other studies, which could be explained as combination of all three transcripts was the most common pattern in our study. Hence true impact of bcr-3 alone could not be assessed with survival.

We also observed that in our study, 28 patients showed combination of all three transcripts in contrast to 21 patients showing isolated bcr-3 transcripts, which leads to a possible hypothesis that the prognostic impact of bcr-1 and bcr-2 transcripts cannot be ignored. However, larger cohorts of patients and longer follow-up periods are needed to test this hypothesis.

Table 3 Effect of different variables on overall survival in APL

Variables		Total	Death	Alive		*p*-Value
				N	%	
Overall		54	20	34	62.96	–
Median age	≤34	28	10	18	64.28	0.843
	>34	26	10	16	61.53	
Gender	Female	31	13	18	58.06	0.367
	Male	23	07	16	69.56	
TLC count	≤10,000 cells/mm^3	25	06	19	76.00	0.007
	>10,000 cells/mm^3	29	14	15	51.72	
Platelet count	≤40,000 cells/mm^3	45	15	30	66.70	0.206
	>40,000 cells/mm^3	09	05	04	44.40	
Morphology	Hypergranular	52	19	33	63.46	0.657
	Microgranular	02	01	01	50.00	
Cytogenetics	Classic t(15;17)	49	18	31	63.26	0.765
	Variant t(15;17)	05	02	03	60.00	
Immunophenotyping	Classic IPT	44	17	27	61.36	0.798
	CD34 positive	02	00	02	100.00	
	HLA-DR positive	05	02	03	60.00	
	CD34 and HLA-DR positive	03	01	02	66.66	
PML-RARα transcripts	bcr-1 + bcr-2 + bcr-3	28	11	17	60.71	0.445
	bcr-2 + bcr-3	02	01	01	50.00	
	bcr-1 + bcr-3	02	0	2	100.00	
	bcr-3	21	07	14	66.66	
	bcr-2	01	01	00	00.00	
Treatment	ATRA	09	09	00	00.00	<0.000
	ATRA + chemotherapy	12	06	06	50.00	
	ATRA + arsenic trioxide	25	03	22	88.00	
	ATRA + arsenic trioxide + chemotherapy	08	02	06	75.00	

Abbreviations: APL, acute promyelocytic leukemia; ATRA, All-trans retinoic acid; TLC, total leukocyte count.

This is, to our knowledge, as of today, the only study which has assessed the correlation of combination patterns of bcr transcripts with survival. Patients with combination of all three transcripts were doing worser than patients with bcr-3 transcripts alone, as highlighted by the Kaplan–Meier curve (►**Fig. 2**), even though *p*-value was not found to be statistically significant. Hence, we conclude that bcr-3 has major prognostic impact on OS because the median OS of patients with bcr-3 transcripts alone was also only 5 months.

Future Directions

Cytogenetic and molecular abnormalities such as FLT3-ITD along with PML-RARα transcripts can be studied in patients with APL to evaluate association with prognosis and hence, modification of therapy can be based on presence of poor prognostic molecular markers.

Conclusion

High TLC is a bad prognostic factor in APL as it was statistically significant with OS in our study. Bcr-3 transcript either alone or in combination with bcr-1 and/or bcr-2 transcripts was the most frequent pattern observed. No statistically significant association was observed between OS and pattern of transcripts in our study. Combination of all three transcripts together was noted in our study only till date. Although the results in our study were found to be statistically insignificant, further exploration of combination of patterns is needed to study their prognostic significance.

Study Limitation

Larger cohorts of patients need to be studied to establish prognostic significance of various transcripts on OS and management. Previous studies have not reported

combination of transcripts. Hence comparison with other studies was not possible. Comparisons between bcr transcripts and clinical features were not done in our study.

Conflict of Interest
None declared.

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