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Clinicopathological Significance of Common Genetic Alterations in Patients With Acute Promyelocytic Leukemia

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BRIEF COMMUNICATION

Clinicopathological Significance of Common Genetic Alterations in Patients With Acute Promyelocytic Leukemia

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Abstract

Objective/Background: **Acute myeloid leukemia (AML) is one of the common forms of hematological malignancy and acute promyelocytic leukemia (APL) is a unique subtype of AML conferring favorable prognosis. We aimed to determine the prevalence and prognostic impact of Fms-like tyrosine kinase 3 (*FLT3*), nucleophosmin 1 (*NPM1*) mutation, epidermal growth factor receptor (*EGFR*), and flow marker's expression in patients with APL.**

Methods: **In the present study, 165 de novo APL patients were molecularly characterized for promyelocytic leukemia (PML) breakpoint and additional genetic alterations. Reverse transcriptase polymerase chain reaction (PCR) and real-time PCR assays were used to detect genetic alterations.**

Results: ***PML/RARα* was detected in 29/165 (17.5%) samples with breakpoint cluster region 1 (bcr1) in 17/29 (58.5%) and bcr3 in 12/29 (41.5%) samples. The prevalence of *FLT3-ITD*, *NPM1*, and *EGFR* were detected in 5/29 (17.5%), 11/29 (38%), and 5/29 (17.5%) patients, respectively. Patients expressing bcr-3 hybrid transcript had lower overall survival compared with bcr1 ($p = .254$). White blood cell (WBC) count was significantly higher in bcr3 in comparison with bcr1 patients ($p = .002$). Patients with positive *EGFR* expression ($p = .042$) and higher WBC ($p = .002$) were significantly associated with poor survival ($p < .05$).**

Conclusions: **We documented the higher prevalence of bcr1 and confirmed that the association of *FLT3-ITD* significantly reduced the chances of survival in APL. The mortality rate of bcr3 was comparatively higher than that of bcr1. Higher WBC count and *EGFR* expression were significantly associated with poor survival.**

Keywords: APL, Co-prevalence, Genetic alterations, Prognosis

1. Introduction

Almost 92% of acute promyelocytic leukemia (APL) cases show classical t(15;17) translocation whereas 5% shows nonclassical genetic abnormalities. Classical translocation results in three different transcripts: breakpoint cluster region 1 (bcr1), breakpoint cluster region 2 (bcr2), and breakpoint cluster region 3 (bcr3). Most frequent transcripts are bcr1 and bcr3, whereas bcr2 is rare [1]. A higher prevalence of bcr1 over bcr3 was reported by different studies in different ethnic populations [2]. Fms-like tyrosine kinase 3 (*FLT3*) and nucleophosmin 1 (*NPM1*) genes are the two most common genetic alterations in acute myeloid leukemia (AML) with worse and favorable prognosis, respectively [3]. Epidermal growth factor receptor (*EGFR*) plays a significant role in several epithelial

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cancers with high expression. The presence of *EGFR* has been associated with worse outcome in AML [4]. Immunophenotyping has a diagnostic and prognostic impact on AML subtypes [5]. ATRA/ATO- (all *trans*-retinoic acid/arsenic trioxide) or ATRA-based induction therapy is the current gold standard treatment for APL patients.

The paucity of studies from India relating to the clinical significance of other genetic alterations and flow markers in APL patients prompted us to carry out this study. We are reporting the possible influence of *FLT3*, *NPM1* mutation, *EGFR*, and flow marker's expression on APL patients from a cohort in North East India.

2. Materials and methods

2.1. Samples

Bone marrow and/or peripheral blood was collected from 165 AML patients at the Department of Clinical Haematology, Gauhati Medical College & Hospital (GMCH; Assam, India) with written informed consent (90 males and 75 females; age range, 1–84 years).

2.2. Cytomorphology, cytogenetics, and flow cytometry

Bone marrow and peripheral blood smears were stained as per standard techniques, including May–Grunwald–Giemsa stains and myeloperoxidase following French–American–British (FAB) and World Health Organization (WHO) criteria [6]. Karyotyping was done at diagnosis according to the International System for Human Cytogenetic Nomenclature [7], and flow cytometry was performed from peripheral blood or bone marrow samples according to the presence and availability of blast cells as described [8].

2.3. Molecular studies

Real-time polymerase chain reaction (PCR) was performed for *PML/RARα* (promyelocytic leukemia/retinoic acid receptor alpha; Hs03043651_ft for bcr1 and Hs03024794_ft for bcr3) and *EGFR* (Hs01076078_m1) using *TaqMan* inventoried assays. *FLT3* and *NPM1* mutations were detected as previously described [9].

2.4. Statistical analysis

All statistical analyses were performed using statistical packages SPSS 22.0 (SPSS Inc., Chicago, IL, USA) and Epi Info 2000 (Centers for Disease Control and Prevention, Atlanta, GA, USA). A two-sided p value < 0.05 was considered statistically significant.

3. Results

PML/RARα t(15;17) was detected in 29/165 (17.5%; 95% confidence interval [CI], 12.5–24.1%) patients. Among them, 14 patients were male (48%) and 15 patients were female (52%). Real-time PCR results exhibited bcr1 in 17 patients 17/29 (58.5%) and bcr3 in 12 cases 12/29 (41.5%). Of 29 APL patients, 5 patients were *FLT3-ITD* positive (17.5%). *NPM1* mutants were detected in 11 patients (38%), which was a striking observation among APL patients, and *EGFR* expression was detected in 5 patients (17.5%). *FLT3-D835* mutations were not detected in APL patients.

Induction outcome and association of demographic, laboratory, immunophenotype, and other clinical characteristics with survival.

The overall induction death rate in this series was 6/29 (20.7%) (Table 1). The median survival for the patients with *FLT3-ITD* negative was observed to be significantly higher (median survival, >27 months) compared with patients with *FLT3-ITD* positive (median survival, 0.5 months; 95% CI, 0.13–0.86; log-rank test $p < .001$). The induction death rate was higher in patients with bcr3 than in patients with bcr1 (34% vs. 12%, $p = .344$). The overall complete remission (CR) rate was 17/29 (58.7%), and there was higher CR in patients with bcr1 compared with patients with bcr3 (65% vs. 50%, $p = .681$). The exact median survival of *PML/RARα* patients could not be reached owing to the shorter follow-up duration; however, in bcr1 positive patients, the overall median survival was recorded as 27 months (median survival, >27 months; mean survival, 20 months; 95% CI, 14.9–26) compared with the median survival > 26 months (mean survival, 15 months; 95% CI, 8.2–22.7) in bcr3 positive, and the difference was not statistically significant (log-rank $p = .254$). Patients with positive *EGFR* expression (log-rank test $p = .042$) and higher white blood cell (WBC) count ($p = .002$) were significantly associated with poor survival ($p < .05$). However, mortality or death % was insignificant between positive and negative *EGFR* patients. Similarly, positive M3v, HLA-DR (human leukocyte antigen, DR isotype), CD34, CD56, and CD2 were associated with poor survival, but were not statistically significant (data not shown because of the very small number present in each respective group). Moreover, sex (log-rank test $p = .654$) and age (log-rank test $p = .357$) did not significantly ($p > .05$) influence overall survival (OS); however, OS

Table 1. Association between demographic, laboratory, immunophenotype, and other clinical characteristics with bcr1 *and* bcr3.

Demographic, laboratory, and clinical characteristics	APL-specific *PML/RARα* ($n = 29$)		
	bcr1 ($n = 17$) *n* (%)	*bcr3* ($n = 12$) *n* (%)	*p*
Sex			
Male	9 (53)	5 (42)	0.550
Female	8 (47)	7 (58)	
	bcr1 Mean ± SD [median (range)]	*bcr3* Mean ± SD [median (range)]	*p*
Age (y)	24.94 ± 16.40 [20.0 (6–65)]	30.71 ± 16.39 [28.0 (8.5–65)]	0.359
WBC count, $\times 10^9$/L	26.74 ± 40.69 [13.3 (9–170)]	44.07 ± 44.25 [22.4 (15.7–150)]	0.002
Platelet count, $\times 10^9$/L	43.31 ± 34.58 [32.7 (7–134)]	43.32 ± 41.12 [29.9 (8–150)]	0.626
Hemoglobin level, ×g/dL	8.36 ± 1.91 [8.3 (3.3–10.7)]	7.54 ± 2.45 [7.5 (4–11.4)]	0.321
Blast count, %	39.35 ± 9.54 [39 (28–60)]	42.75 ± 16.0 [41.5 (25–75)]	0.842
	bcr1 Positive, *n* (%)	*bcr3* Positive, *n* (%)	*p*
CD34	0 (0)	2 (16.7)	0.163
CD56	1 (5.9)	1 (8.3)	0.998
CD2	1 (5.9)	0 (0)	0.995
HLA-DR	0 (0)	1 (8.3)	0.414
FLT3-ITD	2 (11.8)	3 (25)	0.622
NPM1	4 (23.5)	7 (58.3)	0.057
Hypogranular or M3v	1 (5.9)	2 (16.7)	0.553
EGFR gene expression	3 (17.6)	2 (16.7)	0.992
	bcr1 *n* (%)	*bcr3* *n* (%)	*p*
Early death	2 (12)	4 (34)	0.344
Complete remission	11 (65)	6 (50)	0.681

*p-values computed using Pearson chi-square test and Fisher's exact test, unpaired and Mann–Whitney U tests.
APL = acute promyelocytic leukemia; *EGFR* = epidermal growth factor receptor; *FLT3* = Fms-like tyrosine kinase 3; HLA-DR = human leukocyte antigen, DR isotype; *NMP1* = nucleophosmin 1; *PML/RARα* = promyelocytic leukemia/retinoic acid receptor alpha; SD = standard deviation; WBC = white blood cell; y, year.

was longer in younger patients (aged $\leq$ 20 years) and females, respectively. Furthermore, in 2/12 patients with bcr3, additional deletion of 7q was found.

3.1. Treatment protocol and monitoring of minimal residual disease

All APL patients were treated at the Department of Clinical Haematology, GMCH, as per the ASH International Committee Acute Promyelocytic Leukaemia Protocol (IC-APL2006) [10]. In our study, three patients (1 bcr1 patient and 2 bcr3 patients) died because of severe bleeding before the induction therapy could be started. Minimal residual disease was analyzed in 20 patients using real-time PCR to confirm the CR, and results revealed the absence of *PML/RARα* fusion transcripts in 17 patients. Three patients were detected to be positive after induction therapy, but they died before the consolidation therapy could be started. Four patients discontinued the treatment and follow-up with two patients was lost. CR was achieved after induction and consolidation therapy for 17/29 (58.7%) of patients.

4. Discussion

We assessed the prevalence and prognostic impact of APL patients. The associations of other genetic alterations have also been demonstrated to understand any possible role in disease outcome. The incidence of *PML/RARα* transcripts detected in this population was 17.5%, which is in line with other studies in Asian and Western countries. In our study, the prevalence of bcr1 was higher compared with bcr3, a pattern that reflects earlier reports [2]. No significant characteristic differences were observed between bcr1 and bcr3 for age, sex, hemoglobin concentration, platelet, and blast count except for WBC count. Patients expressing bcr3 were found to be associated with lower survival period. One possible cause could be additional cytogenetic changes in bcr3 patients compared with bcr1 patients [11].

An earlier study reported the presence of *FLT3-ITD* in APL with a significantly lower OS [12]. In our study, three patients died during the treatment period (1 bcr1 patient and 2 bcr3 patients), which can be correlated to their association with *FLT3-ITD*. Swaminathan et al [13] showed that the incidence rate of NPM1 mutations was higher (45%) in Indian APL patients, and a similar trend (38%) was also observed in our study, which indicates that *NPM1* mutation might be frequent in Indian APL patients. The expression of *EGFR* in AML is poorly defined and the role of *EGFR* in AML remains contradictory [14]. Out of 29 patients, 3 bcr1 patients and 2 bcr3 (5/29; 17.5%) patients showed *EGFR* expression, which confirmed the presence of *EGFR* in AML. Sun et al. [4] showed *EGFR* confers worse clinical outcomes in AML, and we are in agreement with their studies. In the current study, CD13, CD33, and CD117 were detected positive in all cases, indicating their potential use as a therapeutic target in treatment of patients with APL in the future. The expression of CD56 in APL patients can be considered a poor prognostic marker [15]. In our study, two patients with CD56 were detected and both died, which can be correlated to their classification under the high-risk category (WBC, $>10 \times 10^9$/L; platelets, $\leq 40 \times 10^9$/L).

In conclusion, we found higher prevalence of bcr1 over bcr3 and confirm that the association of *FLT3-ITD* significantly reduces the chances of survival in APL. APL patients with bcr3 variants have a short survival period compared with those with the bcr1 variant. *EGFR*, *CD56* expression, and higher WBC count are significantly associated with poor survival in patients with APL.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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