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PROGNOSTIC EXPRESSION OF CD7 AND CD56 WITH THE MOLECULAR ABNORMALITIES AND POST INDUCTION STATUS IN AML

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Abstract: Chromosomal abnormalities contribute to the classification of the acute leukemia and important prognostic factor for achievement of complete remission, risk of relapse. leukaemia-associated Immunophenotypes (LAIPs) is useful for a better prognostic evaluation, therapeutic approach and also for MRD studies in AML. Immunophenotypic studies carried out on AML patients demonstrated that several antigens expressed on AML cells as CD7, CD34, CD56 and other represent important independent prognostic factors that affect the clinical outcome of these patients. Prognostic group expression also associated with Complete Remission rates and molecular abnormality. **Aims**: The study correlation between induction status and molecular abnormalities in prognostic groups based on expression of CD7 and CD56. **Material and Method**: 100 patients diagnosed with AML were included out of which 13 cases did not receive any form of treatment. The WBC count, molecular abnormality, FAB diagnosis, MRD data, gender, age, clinical and outcome data were collected for each patient. Response to therapy was assessed according to standardized NCCN criteria. **Result and Conclusion**: It is concluded that immunophenotypically AML can be divided prognostic ally into 4 groups with group CD56+CD7+ showing highest number of complete remission in group 4(CD56- CD7-) while lowest remission in Group 1 (CD56+ CD7+). Molecular and cytogenetic abnormalities also have prognostic effect with the presence of CD56 and CD7. However more data and studies are needed for a significant result.

Keywords: AML, MRD, molecular abnormalities, CD56, CD7.

INTRODUCTION:

Acute myeloid leukemias could be considered as a heterogeneous group of disorders which often present with different morphological, immunophenotypic and cytogenetic patterns.[1– 5] For better prognostic evaluation identification of these characteristics is required which helps in therapeutic approach and MRD studies. The leukemic cells which are left behind after chemotherapy and cannot be identified with morphologic study is defined as the Minimum Residual disease (MRD). Immune-based methodologies for detection of MRD depend on establishing leukemia-associated aberrant immunophenotype (LAIP), at diagnosis or relapse and use this information at specified time points for detection of MRD in a different from normal approach. The leukemia-associated immuno-phenotypes (LAIPs) are very infrequently present on normal blood or bone marrow (BM) cells.[6-10] Different immunophenotypic studies carried out on AML patients demonstrated that several lymphoid antigens expressed on AML cells, represent important independent prognostic factors that affect the clinical outcome of these patients[11–19] .

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CD56 expression is considered by some authors to have prognostic value in AML patients and is apparently associated with short overall survival,21-25 lower CR rates21,24and shorter duration of CR.20,21,24 Baer et al. showed that CD56 expression in AML with t(8;21) (q22;q22), usually associated with a high CR rate and prolonged disease-free survival, was in fact significantly correlated with a short CR period.22 CD7 is expressed in 30% of AML cases and CD7 positivity is linked with poor prognosis in myeloid malignancies.26,27 Prognostic significance within AML has consistently been shown for mutations in the NPM1, CEBPA, AML-ETO and FLT3 genes alone or in combination in patients28-33

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OBJECTIVES :

To categories AML based on expression of CD7 and CD56 at diagnosis into prognostic groups and correlated the prognosis of these groups with the morphological remission status of BM and also with the molecular status (FLT3-ITD, NPM1, CBF and AML-ETO)

MATERIAL AND METHODS:

Patients

BM and peripheral blood samples from patients diagnosed with AML in DRBRAIRCH, AIIMS, New Delhi, India. The study was done from December, 2013 to March, 2016 from peripheral blood and bone marrow obtained from 100 newly diagnosed AML patients. Routine diagnostic flow cytometer was performed on fresh PB samples. The study was done on the 87 patients as the 13 cases did not receive any of treatment. Diagnosis of patients was based on morphology, immunophenotyping, and cytogenetics. The WBC count, FAB diagnosis, gender, age, clinical and outcome data were collected for each patient. cytogenetics and molecular abnormalities analysis was done on the same samples received for flowcytometry.

The data was collected from the records section of DRBRAIRCH, AIIMS and the follow up and clinical data retrieved from patient file. MRD data for all patients was received as a part of routine diagnostic protocol. No additional tests were performed for this study. Response to therapy was assessed according to standardized criteria.Ten bone marrow samples obtained from patients with solid tumors and lymphoma, uninvolved by disease and post-induction regenerating marrows from patients with acute lymphoblastic leukemia were used as controls to obtain the normal expression pattern of the markers used in the study.

Morphological analysis

The cyto-morphological analysis was performed using a bone marrow smear or a peripheral blood smear from EDTA sample and stained with May Grunwald-Giemsa.

Immunophenotyping

For flow cytometric analysis, at least 200,000 events were acquired at follow up in all cases from each tube and data were stored in list mode. For all the specimens, five-color FCM was performed on Coulter FC500 instrument [Beckman Coulter (BC), Hialeah, FL, USA]. Each seven tube containing, five different antibodies, with CD34 and CD45 as backbone markers. The antibody panel used was shown in Table 1. All antibodies were purchased from Beckman Coulter (BC), Hialeah, FL, USA. The bone marrow samples were collected in EDTA and processed within 4 hours of receipt of sample by the standard stain-lyse-wash method. For staining procedure the combinations of antibodies were added to 1x106 cells and incubated for 20 min at room temperature in dark. The volume of antibodies added was calculated after titration. The red cells were then lysed by ammonium chloride based solution (in house preparation) and washed twice by phosphate buffered saline

Table 1.panel of antibody for the AML

Five color combination	
1. MPO/c79a/ 34/c3/45	Screening panel
2. 64/117/34/2/45	Extended panel
3. 9/HLA-DR/34/13/45	
4. 15/117/34/11b/45	
5. 34/33/19/56/45	
6. 7/34/14/64/45	
7. 38/117/34/4/45	
8. 36/HLA-DR/34/33/45	

The cells were finally suspended in 0.05%PFA solution. The instrument setup was done by Flow Check fluorospheres (BC, Hialeah, FL, USA) for alignment, Flow Set beads (BC, Hialeah, FL, USA) for voltage standardization and compensation was also done. Alignment done on daily basis to ensure precise flow of cells through the laser beam intersection, and the fluid stream so that each detector gave maximal and reproducible signals from the standard particles or cells. The same instrument settings were used for samples. For data analysis boolean logic gating was used to identify the leukemia-associated immunophenotype (LAIPs) at time of

diagnosis and at time of MRD quantification in the post-induction marrow[9,10,34]. In LAIP, four

type of aberrant phenotype analyses according to the publish literature i.e. asynchronous expression, cross-lineage expression (CD56, CD7), under expression and over-expression.

LAIP may disappear after the treatment at time of MRD analysis and maturation pathways was also used as LAIP.[35]Gating strategy include the debris exclusion by time gate (for taking continuous sample stream). On CD45/SS plot at intermediate/low side scatter region a gate was formed, followed by back gating on the CD34+ population and removal of CD19+ hematogones from analysis. these cells with different parameter were used to identified LAIP.

Instrument cleaning was a crucial step to reduce background level of noise below the threshold that would interfere the rare events. For identification of rare event accuracy, staining bufferacquired for a period of expected time of sample acquisition and number of event noted down and then normal sample stained with AML panel is acquired for events that are detectable in the region of interest. This exercise should be performed periodically or any time when there is an unexpected result. It is important to acquire a saline or sheath fluid before MRD collection to ensure no carryover is there.

Statistics

A log binomial model was used to analyses the data. The outcomes of interest were response after induction and relapse after achieving CR. Whether the patient was LAIP positive or negative was included in each model as a predictor. A number of potential confounders were identified (age at diagnosis, gender, MRD, cytogenetic risk group and molecular abnormalities) and both unadjusted and adjusted risk ratios were calculated to compare prognostic group with MRD and risk. OS was measured from the date of diagnosis until date of death or last date available and RFS for patients who achieved CR was measured from the date of diagnosis to relapse while LFS forpatients who achieved CR was measured from the date of CR to relapse. OS, RFS and LFS were plotted by Kaplan-Meier method; differences between curves were analysed by the log-rank test. The log-rank test was used to validate equality of the survival distributions. Cox-regression was also used to obtain the hazard ratio. This analysis was performed in STATA version 11.1. A p-value $\leq$ 0.05 was required for statistical significance.

DISCUSSION:

Our study showed that the CR status was low for the Group 1(CD56+ CD7+), which was supported by Tiftik N etal, 2004[44] study that CD7 and CD56 positivity at diagnosis associated with

low remission rate and biological aggressiveness in a significant proportion of patients. In our study the molecular abnormalities like NPM1, FLT3, AML-ETO and CBF was correlated in the prognostic group considering group 4 (CD56-CD7-) as a control group. NPM1 and FLT3 was more profoundly present in group1 (CD56+ CD7+) (28.5 p=0.38 and 37.5 p=0.11respectively) while AML-ETO was more express in group2(CD56+ CD7-) (30.7% p=0.48) similar result was seen by Paietta E et al. 2012[37] and also by Pradeep singh chauhan et al. 2013[36] that demonstrated that immunophenotypically NPM1 mutation was associated with the lack of CD34 (p< 0.001) and HLD-DR expression (p<0.001), while FLT3/ITD mutation was positively associated with the expression of CD7 (p = 0.04) and FLT3/ITD mutation was found to be inversely associated with AML/ETO fusion gene (p = 0.04).Harry Dang et al. 2010 [38] also reported same result that CD56 and CD7 both are equally express FLT3 and NPM1 mutations. CBF was less frequently seen in the all groups (12.4% p=0.88) as it expressed favorable prognosis as reported by Sinha C et al. 2015[39] that CBF-AMLs are considered to have relatively good prognosis compared to other leukemia subtypes, they are a heterogeneous group of disorders and modern therapy frequently leads to relapse and the associated morbidity and mortality. All results were statistically not significant due to small sample size. In our study cytogenetic analysis was done in prognostic group and cytogenetic abnormalities associated with unfavorable prognosis in group 2 (CD56+ CD7-,p=0.267, 14.2%) and group 3 (CD56- CD7+, p=0.267, 11.1%) and this result was similar to those reported by Raspadori D et al. 2001 [39],Kita K et al.1993[40] and Del Poeta G et al.1994[41] where a cytogenetic analysis was associated with a significant correlation between CD56 or CD7 expression and cytogenetic abnormalities associated with unfavorable prognosis was documented both in univariate and multivariate analysis. Similarly Ogata et al. 2001[43] found that CD7 positivity did not adversely affect the OS or DFS in the favourable or intermediate cytogenetic category. In support our study by Ana Paula Alegretti et al. 2011[42]asignificant correlation between CD56 expression and cytogenetic abnormalities associated with unfavorable prognosis was documented both in univariate and multivariate analysis.

SUMMARY AND CONCLUSION:

Among the all prognostic groups, group 1(CD56+ CD7+) has the least cases of complete remission in first induction while the group 4(CD56- CD7-) has achieved more complete remissions. The molecular abnormalities like NPM1 and FLT3 was most common in Group 1(CD56+ CD7+) (28.5% and 37.5% cases respectively) whereas AML ETO was positive in Group 2(CD56+ CD7-) (30.7% cases), CBF mutation was equally present in all four groups. Cytogenetic study showed that Group 2(CD56+CD7-) with 3 (14.2%) cases had the most unfavorable cytogenetic profile and least unfavorable profile was that in group1 with 1 case (10%). Though the result was not significant and more work is needed with significant number of cases, the study however suggested that the presence of CD56+ and CD7+ antigens together had poor CR than their individual presence and NPMI and FLT3 was more associated with the group had both CD56+ and CD7+ positivity.

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