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CORRELATION OF MRD STATUS AND RELAPSE IN ACUTE MYELOID LEUKEMIA

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Abstract: The presence of minimal residual disease (MRD) after achievement of complete remission (CR) on completion of induction therapy predict relapse in acute myeloid leukemia (AML). These results suggest a correlation between relapse and MRD at the time of morphologic remission. Here we wanted to establish the earlier supported theory whether MRD and relapse provide independent prognostic information. **Patients and Methods**: Analyzed data from 87 adults with AML who achieved CR after induction therapy were used. Bone marrow samples were collected after achieving complete remission and MRD was determined by 5-color multiparameter flow cytometry. **Results**: A total of 87 patients achieved morphological CR and 28 of which relapsed or refractory AML. 40 patients (48.2%) had flow Cytometric evidence of MRD out which 16(18.3%) relapsed, while 24(27.5%) didn't relapsed incident rate are more for MRD+ case with higher hazardous ratio. **Conclusion:** MRD level are linked with the prognostic factor for relapse in AML patients.

Keywords: MRD, flowcytometry, Relapse, RFS

INTRODUCTION:

Acute myeloid leukemia (AML) is a heterogeneous disease with a variable response to therapy and achieved, complete remission (CR) rates range from 20% to 90%. Relapse is the most common event ultimately leading to death, occurs in 10% to 95%.[1-3] Risk stratification is determined by several patients and disease-related factors assessed at diagnosis. Many factors such as age, de novo versus secondary AML, cytogenetics, and aberrations in the molecular abnormalities are of limited predictive value. Not mutually exclusive, an alternative means is to incorporate information gained as treatment has started, such minimal residual disease (MRD) as measured by multiparameter flow cytometry (MFC). There is more evidence that MRD level after induction therapy is independently associated with relapse and survival.[6-14] The prognostic significance of response to induction therapy has also been recognized, with a recent study showing that achievement of CR, is independently associated with longer relapse-free survival (RFS).[15]The work described here is a study that correlate the relationship between MRD and clinical response and relapse by flowcytometry.

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MATERIAL AND METHODS:

Patients

BM and peripheral blood samples were used in all of our studies in this study from patients diagnosed with AML in DRBRAIRCH, AIIMS, New Delhi, India. The study was done from December 2013 to March 2016 with 100 newly diagnosed AML patients. Routine diagnostic flow cytometry was performed on fresh PB and/or BM samples. The study was done on the 87 patients as the 13 cases did not receive any form of treatment and/or were lost to follow up after initial diagnosis. Diagnosis of patients was based on morphology, immunophenotyping, and cytogenetics. The WBC count, FAB diagnosis, gender, age, clinical and outcome data were collected from 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 samples from all patients were 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 were obtained from patients with solid tumors 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 cytomorphological analysis was performed using a bone marrow smear or a peripheral blood smear from all patients and stained with Jenner-Giemsa stain.

Immunophenotyping

For flow cytometric analysis, at least 200,000 events were acquired at follow up (day 30) in all cases from each sample tube and stored as list mode data files. For all the specimens, five-color FCM was performed on Coulter FC500 instrument [Beckman Coulter (BC), Hialeah, FL, USA]. Each seven tubes containing, five different antibodies, with CD34 and CD45 as backbone markers antibody panel was used as shown in Table 1. All antibodies were purchased from Beckman Coulter (BC), Hialeah, FL, USA.

Table 1.panel of antibody for the AML

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

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.

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 was done daily 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[9,10,28]. 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 were determined. LAIP may disappear after the treatment at time of MRD analysis and maturation pathways was also used as LAIP.[29]Gating strategy included 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 were gated with different parameters and LAIP identified. 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 buffer was acquired for a period of expected time of sample acquisition and number of event noted down and then control normal sample stained with AML panel was acquired for events that were detected in the region of interest. 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 analyse 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 for patients 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.

RESULT:

A total of 87 patients achieved morphological CR (by 2008 WHO criteria) and 28 of which relapsed or refractory AML, of these 87 patients. **Table1** lists the detailed characteristics of the 83 patients. 40 patients (48.2%) had flow Cytometric evidence of MRD out which 16(18.3%) relapsed, while 24(27.5%) didn't relapsed. Median follow-up was 24 months; 55 of these patients remained in remission while 67 patients are still alive. Their relationship suggested that relapse and MRD might provide similar, overlapping information regarding risks of relapse, RFS.

Table 1. Correlation of MRD with Relapse

n=87	RELPASE n,%	NOT RELPASE n,%
Total	28 (32.2)	59 (67.8)
MRD+	16(18.3)	24(27.5)
MRD-	12(13.9)	35(40.3)

Table1. show that the after the achieving complete remission, the MRD was analyzed for cases at day thirty and found that there was MRD+ and MRD- case was present in spite of the fact that complete remission achieved. Our study showed that 40(48.2%) cases out of 87 was MRD+ but only 16(18.3%) cases relapsed while 24 (27.5%) of total didn't relapsed. If we saw MRD- cases them we came across that 12(13.9%) case relapsed while 35 (40.3%) did n't.

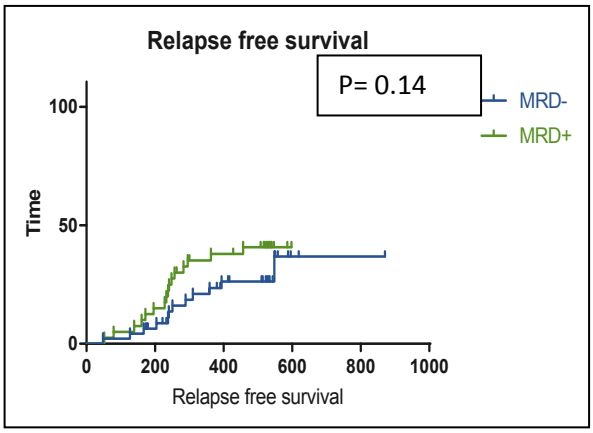

Figure 1. Relapse free survival for MRD

Figure 1 showed that the survival analysis for the MRD as the trend of relapse in the cases. In above survival analysis the green line shows the MRD+ case and blue line shows the MRD- cases. relapse was more frequently seen in the MRD+ case though the MRD- case also have the relapse status but the percentage was more in MRD+ cases . Hazard ratio of MRD- case was 0.60 while for MRD+ was 1.67.

Table 2. survival analysis for relapse free survival

n=87	MRD+ f (%)	MRD- f (%)	P.v
Relapse	16(18.3)	12(13.9)	0.29
Sur. prob. (365 days)	0.32(0.58-0.63)	0.70(0.45-0.85)	
Sur.prob.(455 days)	0.32(0.58-0.63)	0.70(0.45-0.85)	
Incidencerate (per 1000)	2.4	0.6	

MRD+ had the highest incidence rate (2.4) of relapse with 18.3% of patients and also the least survival probability of 0.32. Least incidence rate (0.6) of relapse was seen in MRD- cases (13.9%). Though the result are statistically insignificant.

DISCUSSION:

In treatment of disease, the usefulness of therapy often depends on prognosis. Likewise in AML cases, if standard therapy worsens the disease, the need of the new therapy started the clinical trial. The ability to diagnose absolute prognosis, at least on the basis of commonly measured pretreatment is limited.[22] But with the inclusion of more pretreatment genetic, proteomic data may improve ability of prognosis, done by Bayes law. The rate of peripheral blood blast elimination after induction therapy predicts CR and RFS[14,15] but generally there was no residual blast present after therapy. From last many years MRD detection by flow cytometry or molecular methods has proven to be predictive of relapse and survival,[4-13] and risk-directed therapy based on MRD assessment may improve outcome in high-risk patients,[16-20] although this remains untested in a randomized trial. Another post-treatment parameter response as defined by blood count recovery has also been recognized as prognostically significant,[13] suggesting a effective relationship between MRD and response.Our study demonstrated correlation between MRD and relapse. (Table 1) The patients who achieved CR within complete blood count recovery more frequently had higher levels of MRD than patients achieving CR. This finding suggests that failure of blood count recovery may result from incomplete treatment of AML. The persistent leukemic blasts may disturb the microenvironment and as it act like cytotoxic agents.[21] In any event, delaying therapy in patients with only partial recovery of blood count while marrow with 5% blasts may be unlikely to improve blood counts. Perhaps our most important discoveries are that their shows strong inter-relationship, MRD and response as a independent prognostic information and accounting for these post-treatment factors in comparison of traditional pretreatment prognostic factors. The monosomal karyotype, favorable cytogenetics molecular abnormality and relapsed or refractory AML are associated with greater risk of relapse, whereas favorable cytogenetics are assumed to be linked with a reduced risk of relapse,[8-12]

CONCLUSION:

Our study concluded and suggested that peripheral count recovery and MRD level are linked as independent prognostic factor for relapse and RFS in AML. Information about these post-treatment factors is likely more important than information about several traditional pretreatment prognostic factors and should play a important role in planning post induction therapy. Though the result was not significant but with a large case and more time it will be greatly helped for standardization of methods for MRD determination and time assessment and relapse.

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CONFLICT OF INTEREST:

Authors declared no conflict of interest