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Original Article

Flow cytometric detection of minimal residual disease in B-lineage acute lymphoblastic leukemia by using “MRD lite” panel

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ABSTRACT

Background: Normal B lymphoid precursors that express CD19, CD10, and/or CD34 are highly sensitive to corticosteroids and after two weeks of remission-induction therapy, they form less than 0.01% of the bone marrow population. More than 0.01% of such cells indicate minimal residual disease (MRD). MRD “lite” panel uses only three antibodies, namely CD19, CD10, and CD34 for MRD detection in cases of B-lineage acute lymphoblastic leukemia (B-ALL) expressing CD19, CD10, and/or CD34 by flow cytometry.

Methods: Fifteen cases of B-ALL were studied for MRD at Day 19 of remission-induction therapy by employing a simplified MRD detection protocol using a 3-color fluorochrome conjugated antibody panel (CD19, CD10, and CD34) on bone marrow aspirate samples.

Results: All cases at diagnosis expressed CD19, CD10, and CD34. Of fifteen patients, five (33.33%) were MRD negative with less than 0.01% of mononuclear cells and remaining ten cases (66.66%) were MRD positive, with the level of 0.01% to less than 0.1% cells.

Conclusion: The MRD assay used in this study is a simplified method for detecting MRD at Day 19 of remission-induction therapy for B-lineage ALL. This MRD assay is an effective and useful methodology in cases of B-ALL expressing CD19, CD10, and/or CD34 by flow cytometry.

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Introduction

Minimal residual disease (MRD) refers to the presence of recalcitrant leukemic blasts in the peripheral blood or bone marrow, which is beyond the lower limit of morphologic detection by light microscopy and can only be detected by highly sensitive assays, be it polymerase chain reaction based or flow cytometry based.¹ PCR-based and flow cytometric MRD

technologies have been developed over the past two decades with their inherent advantages and disadvantages.^{2–9} Detection of MRD would aid in the patient management by either intensifying therapy in MRD positive cases or de-intensifying and thereby reducing treatment-related mortality and morbidity in MRD negative cases. Prospective studies in large series of patients have demonstrated a strong correlation between MRD levels during clinical remission and treatment outcome.^{10,11} For MRD studies, leukemia-associated immunophenotypes (LAIP)

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can be identified in most cases with a sensitivity of 0.01%, for which a large number of antibodies are required. Conventional flow cytometry uses 6–8 colors for MRD detection in B-lineage acute lymphoblastic leukemia (B-ALL).² This study was aimed to assess MRD during treatment (remission-induction) in B-ALL expressing CD10 and/or CD34 at diagnosis and risk stratify the patients as per MRD positivity/negativity respectively using a limited panel of antibodies namely MRD “lite” panel.

The concept of MRD “lite” is based on the fact that normal B lymphoid precursors that express CD19, CD10, and/or CD34 are highly sensitive to prednisolone/dexamethasone which form the backbone of all remission-induction protocol for acute lymphoblastic leukemia (ALL) and after two weeks of remission-induction therapy, these normal B lymphoid precursors form less than 0.01% of the bone marrow population.¹² Presence of CD19+ cells with CD10 and/or CD34 expression at Day 19 remission-induction therapy would in all probability indicate MRD. This panel uses only three antibodies, namely CD19, CD10, and CD34 and it is easy to interpret the results.

Material and methods

Fifteen cases of B-ALL were enrolled as a part of this study from 10 June to 9 July 2010 at St Jude Children's Research Hospital. All the cases were studied for MRD at Day 19 of remission-induction therapy by employing a simplified MRD detection protocol developed at St Jude Children's Research Hospital using a 3-color fluorochrome conjugated antibody panel. Bone marrow aspirates were collected in preservative free heparin and stored at room temperature before separation. Mononuclear cells (MNCs) were separated by density gradient using conventional Ficoll technique. Mononuclear cells were labeled with anti-CD19 conjugated to peridinin chlorophyll protein (PerCP)-Cy5.5, anti-CD10 conjugated to phycoerythrin (PE), and anti-CD34 conjugated to fluorescein isothiocyanate (FITC) (all antibodies are from BD Biosciences, San Jose, CA). Following 10-min incubation, the cells were washed twice with phosphate buffered saline containing 0.5% bovine serum albumin and 0.5% sodium azide and then fixed with 0.5% formaldehyde. The events were acquired and analyzed with a FACS Scan or a FACS calibur flow cytometer using Cell Quest software (Beckton Dickinson, San Jose, USA). 50 cell cluster with LAIP is defined as a clone and on acquiring 500,000 events, the sensitivity achieved was 0.01%. To prove a sensitivity of 0.01%, leukemic samples were serially diluted in normal bone marrow samples to pick out 0.01% cells of interest. At the time of the MRD assay (i.e.) on D + 19, cells were labeled with the panel of antibody combination and 500,000 events were acquired and analyzed in each sample. After gating on CD19+ cells, the percentages of CD19+ cells expressing CD10 and/or CD34 were determined. Data was recorded in blinded manner with the observer being unaware of the patient's clinical status except for the diagnostic immunophenotype of the disease.

Results

All of our 15 cases at diagnosis were B-lineage ALL and the diagnostic immunophenotyping were CD19+ cells that also

Table 1 – CD19+ve cells expressing CD10 and/or CD34 along with their percentage on Day 19 bone marrow aspirate in cases of B-ALL.

Case	CD10	CD34	MRD percentage
1	Positive	Negative	0.07
2	Positive	Positive	0.01
3	Positive	Negative	0.06
4	Positive	Negative	0.04
5	Positive	Positive/negative	0.00
6	Positive	Positive	0.05
7	Positive	Positive	0.01
8	Positive	Positive	0.01
9	Positive	Positive/negative	0.00
10	Positive	Positive	0.03
11	Positive	Positive	0.01
12	Positive	Positive/negative	0.00
13	Positive	Positive/negative	0.00
14	Positive	Positive/negative	0.00
15	Positive	Positive	0.01

expressed CD10 as well as CD34. In the bone marrow samples on Day 19 of remission-induction chemotherapy, the percentage of CD19+ cells with CD10 and/or CD34 co-expression among bone marrow mononuclear cells was measured (Table 1). Bone marrow aspirates from T-ALL cases after two weeks of remission-induction therapy were used as controls to look for the percentage of CD19+ cells with CD10 and/or CD34 co-expression and all cases had less than 0.01% of B lymphoid precursors. Of the fifteen patients enrolled in the study, five patients (33.33%) were MRD negative with less than 0.01% of mononuclear cells that showed CD19+ with co-expression of CD10 and/or CD34. The remaining ten cases (66.66%) were MRD positive, with the level of such cells ranging from 0.01% to less than 0.1% (Fig. 1). None of the fifteen B-ALL cases had morphologically recognizable blasts on the bone marrow aspirate smears.

Discussion

Submicroscopic leukemia levels, namely the MRD detection, is no longer a research tool, is becoming the standard of care, and it finds the most important clinical application, namely the prognostication.¹³ Further, MRD detection overcomes the inherent disadvantage of morphology, namely the difficulty in distinguishing ALL cells from the lymphocytes and undifferentiated hematopoietic cells. Those patients achieving molecular remission with leukemic blasts less than 0.01% of nucleated cells in bone marrow at the end of remission-induction therapy fare better than those who did not. And there is a group of patients who show rapid response to therapy with less than 0.01% of leukemic population at the end of two weeks of therapy and are known to be associated with better outcome.¹² As there is a strong concordance between the PCR-based and flow cytometry based methods of MRD assessment, flow cytometry is widely followed as it is a rapid and simple methodology in nearly 95% of the cases. The conventional flow cytometry uses 6–8 colors for MRD detection in B-ALL;² however, we experimented using a limited panel of antibodies, namely MRD “lite” panel to assess MRD during treatment (remission-induction) in B-ALL expressing CD10

Fig. 1 – Flow cytometric assay to measure MRD on Day 19 bone marrow sample in cases of B-ALL. The upper panel shows dot plot analysis at diagnosis while the lower panel shows MRD analysis on D + 19. The gating was based on scatter characteristics and CD19 positive events. At diagnosis, the CD19 positive cells show CD10 expression and dim CD34 expression. On D + 19 MRD analysis, the CD19 positive cells with CD10 expression constitute 0.07% suggesting MRD positivity in this case. In addition, there is loss of CD34 expression after remission-induction.

and/or CD34 at diagnosis and risk stratify the patients as per MRD positivity/negativity respectively.

The presence of CD19, CD10, and/or CD34 positive cells on Day19 of therapy would indicate the slow responsiveness of leukemic blasts and in turn would affect the long-term outcome in such patients. This 3 color MRD “lite” panel would apply to most of the B-ALL cases that express CD10 and/or CD34. In our study, it was brought out that very low levels of MRD (less than 0.01%) noted in five patients while the remaining ten patients had MRD levels between 0.01% and 0.1%. Those patients with very low or negative MRD levels tend to have a better treatment outcome as compared to those with MRD levels more than 0.01%.¹² It is important to identify this subset of patients as measures can be taken to de-intensify the treatment protocols with the aim to reduce the treatment related morbidity and mortality, especially in a developing country like India where intensive chemotherapy is associated with high risk of life threatening infections.

The present study was able to identify ten out of fifteen cases with MRD positivity, even though all these cases were

on remission based on the morphologic criteria. These patients on long-term follow-up may not perform well as compared to the MRD negative group and there is a need to intensify the treatment protocol to achieve a molecular remission or it can be treated with a definitive hematopoietic stem cell transplant. Amidst most sophisticated MRD assays available in many research centers, this study had brought out that a simple 3 color based MRD “lite” assay could risk stratify those B-ALL cases expressing CD10 and/or CD34 and this information can be used to adjust the treatment strategy. This strategy has been tried successfully in Brazil with success and reduced intensity treatment regime being instituted in MRD negative cases.¹² Thus, the proposed 3 color MRD “lite” panel would be an effective MRD assay in cases of B-ALL expressing CD10 and/or CD34. However, it is also emphasized that the MRD “lite” panel is not applicable to B-ALL cases that lack expression of CD10 and CD34 at diagnosis such as a subset of B-ALL with 11q23 (mixed-lineage leukemia gene) abnormalities frequently seen in infants with B-ALL.

Conclusion

The 3-color MRD assay used in this study is a simplified method for detecting MRD at Day19 of remission-induction therapy for B-lineage ALL. However, this method cannot be applied after the remission-induction due to presence of regenerative precursors (hematogones), wherein a comprehensive 6–8 color flow cytometer panel to evaluate leukemia-associated antigens during later part of therapy is then applied. In such instances, an extended panel to detect deviation from normal maturation patterns of expression of CD45, CD10, CD34, CD38, CD20, and CD58 and light chain expression on CD19 positive B cell precursors are useful to detect abnormal populations even in the context of loss of one or more immaturity associated antigens. Modern, evidence-based treatment and management of leukemia is expensive, resource heavy, and needs expertise. Wherever the resource permits, a comprehensive analysis of MRD as per the current practices needs to be followed.

Conflicts of interest

The authors have none to declare.

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