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SHORT COMMUNICATION

T-ALL Minimal Residual Disease Using a Simplified Gating Strategy and Its Clinico-hematologic Correlation: A Single Center Experience from North India

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Abstract The presence of minimal residual disease (MRD) is one of the strong predictors of disease outcome in various hematological malignancies including B-ALL and T-ALL, independent of pre-therapeutic risk factors. There is scant Indian data on MRD by flowcytometry in T-ALL including gating strategies, clinical correlation etc. The primary aim of this retrospective observational study was to define the clinico-hematologic characteristics and prognostic significance of patients with ETP/near-ETP versus non-ETP immunophenotype, especially in terms of minimal residual disease at different time points as well as event-free survival (1 year). Baseline hematologic characteristics along with post-induction (Day-35) and post-consolidation (Day-78) MRD in bone marrow samples from newly diagnosed T-ALL patients were studied. 14.3% patients had ETP-ALL immunophenotype, 11.4% were near-ETP ALL patients and the remaining 74.5% were of non-ETP subtype. The ETP/near ETP patients was significantly associated with higher risk of MRD positivity ($> 0.01\%$) at the end of induction in comparison to the non-ETP patients ($p = 0.033$). Also, these patients showed a trend towards proclivity to anemia ($p = 0.06$) and higher rates of induction failure ($p = 0.07$) However, no difference was observed between the two subgroups in terms of age, high TLC, thrombocytopenia, adverse cytogenetics, steroid responsiveness on Day + 8 of induction, MRD-positivity $> 0.01\%$ at the end of consolidation and EFS-1 year. Through this preliminary study, it can be stated clearly that ETP status is associated with MRD $> 0.01\%$ post-induction but has no significant impact on long-term survival of these patients.

Keywords Minimal residual disease · Acute lymphoblastic leukemia · Immunophenotyping

The presence of minimal residual disease (MRD) is one of the strong predictors of disease outcome in various hematological malignancies including B-ALL and T-ALL, independent of pre-therapeutic risk factors and is used for guiding clinical decisions in current treatment protocols [1]. There is scant Indian data on MRD by flowcytometry in T-ALL including gating strategies, clinical correlation etc. Moreover, few western studies have differed in their observations regarding the impact of ETP-immunophenotype on prognostic outcome in T-ALL patients. With this in background, the primary aim of this retrospective observational study was to define the clinico-hematologic characteristics and prognostic significance of patients with ETP/near-ETP versus Non-ETP immunophenotype, especially in terms of minimal residual disease at different time points as well as event-free survival (1 year). Baseline hematologic characteristics along with post-induction (Day-35) and post-consolidation (Day-78) MRD in bone marrow samples from newly diagnosed T-ALL patients from January 2017–June 2018 treated under COG-AALL0232 protocol were studied. Events included relapse and disease-related deaths. Statistical analysis was done

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using SPSS software version 23 and p value < 0.05 was considered as statistically significant.

MRD was performed using bulk lysis method on FACS-Canto II 8 color, 3 laser flowcytometer (BD Biosciences) and analyzed using FACS Diva software (BD) (Panel shown in Table 1). At least 0.5–1 million events were acquired to achieve a sensitivity of 0.01%. MRD detection in T-ALL is theoretically easier since most stages of normal T-thymocyte maturation occur in the thymus and hence, presence of immature T-cell subset in blood or bone marrow should be considered aberrant. However, difficulty arises mainly from loss of immaturity markers on the abnormal blast population following induction chemotherapy. MRD detection thus relies heavily on capturing aberrant immunophenotype of T-cell antigens such as CD2, CD3 (cytoplasmic and surface), CD5, CD4, CD8, CD7 etc which are relatively stable antigens even in the absence or reduced preservation of immaturity associated antigens like CD99, TdT, CD34 and CD10 [2].

Sequential gating to identify the population of interest was applied as follows (Fig. 1):

Doublet exclusion resulting in singlet gating was followed by exclusion of debris, red cells and platelet clumps on FSC/SSC plot (i.e. viable cell gating). Mononuclear cells were then gated on SSC/CD7 scatter plot to exclude granulocytes, B-lymphocytes and monocytes. However, the challenge still remains to distinguish the T-lymphoblasts from mature T cells with loss of surface CD3, NK cells and regenerating myeloblasts with dim CD7 expression. Thus, the gated CD7 positive cells were further analyzed by separately selecting the two populations i.e. CD7+/surface CD3- and CD7+/surface CD3 + on subsequent plots. NK cells (CD16+/CD56+/CD5−/CD38+/surface CD3−/CD7+/CD4−/CD8 variable) were excluded by invert gating on next two scatter plots i.e. cytoCD3/CD16 + CD56 and CD5/CD38 respectively. The remaining population which would comprise of lymphoblasts, residual NK cells and mature T cells with loss of surface CD3 was plotted on CD4/CD8 and CD99/CD48 scatter plots respectively to delineate the abnormal T-cell clone. MRD percentage was calculated as a ratio of the abnormal T-cell population to the total number of non-debris events. T-MRD levels $>$ 0.01% was taken as clinically significant. Two important points to be highlighted related to MRD standardization and reporting are: (a) Ten normal bone marrow samples such as ITP, Lymphoma staging etc and ten stressed marrows such as B-ALL/AML on chemotherapy were run in initial stages for standardization of MRD testing. (b) The major confusion exists in separating NK cells with CD16−/CD56−/CD8 variable/CD4−/ CD5−/CD38 variable immunophenotype from T-lymphoblasts in the absence of more specific NK cell antigens such as CD94. A close follow-up and repeat MRD is advised in such dubious cases.

Out of a total of thirty-three T-ALL patients, 5/33(14.3%) had ETP-ALL immunophenotype, 4/33(11.4%) were near-ETP ALL patients (same as ETP immunophenotype except for bright CD5 expression) and the remaining 74.5% (26/33) were of non-ETP subtype. Median age at presentation was 2 years (range 3–66years) with a male to female ratio of 8:1. Mean hemoglobin in g/dL was 9.59 ± 2.04. Median TLC and platelet count in $\times$ 10^9/L was 40.3 (range 2.3–200) and 164 (range 19–277) respectively. Normal karyotype was found in 19/26 (73.1%) and 6/9 (66.7%) non-ETP and ETP/Near-ETP patients respectively. The various cytogenetic abnormalitites in T-ALL patients included deletions, trisomies and multiple non-specific translocations including t(1;19), t(11;16), t(6;14), t(13;14), t(1;19) etc. High risk MRD i.e. MRD $>$ 1% was found in 3/9(33.3%) (range 0.03–60) and 2/26(7.7%) (range 0.12–28) ETP/near-ETP and non-ETP patients respectively.

Table 2 shows the baseline demographic and clinico-hematologic characteristics as well as prognostic outcome of ETP versus Non-ETP ALL patients. On univariate analysis, the ETP/Near ETP patients were significantly associated with higher risk of MRD positivity ($>$ 0.01%) at the end of induction in comparison to the Non-ETP patients (p = 0.033). Also, these patients showed a trend towards proclivity to anemia (p = 0.06) and higher rates of induction failure as confirmed by flowcytometry (p = 0.07). However, no difference was observed between the two subgroups in terms of age, high TLC, thrombocytopenia, adverse cytogenetics, steroid responsiveness in terms of blast percentage $<$ 1000/cumm on Day + 8 of induction, MRD-positivity $>$ 0.01% at the end of consolidation and EFS-1 year.

Table 1 T-MRD 8-color panel used

Antibody	CD8	CD5	CD7	CD38	CD16/CD56	CYTOCD3	SCD3	CD4
Fluorochrome	FITC	PE-Cy7	PE	APC-H7	APC	BV421	Per-CP	BV510
Clone (BD)	SK1	L17F12	M-T701	HB7	B73.1/NCAM16.2	UCHT1	SK7	SK3

#A second tube comprising of CD99 and CD48 instead of CD4/CD8 was also used in few cases but provided no additional information in detecting MRD clone

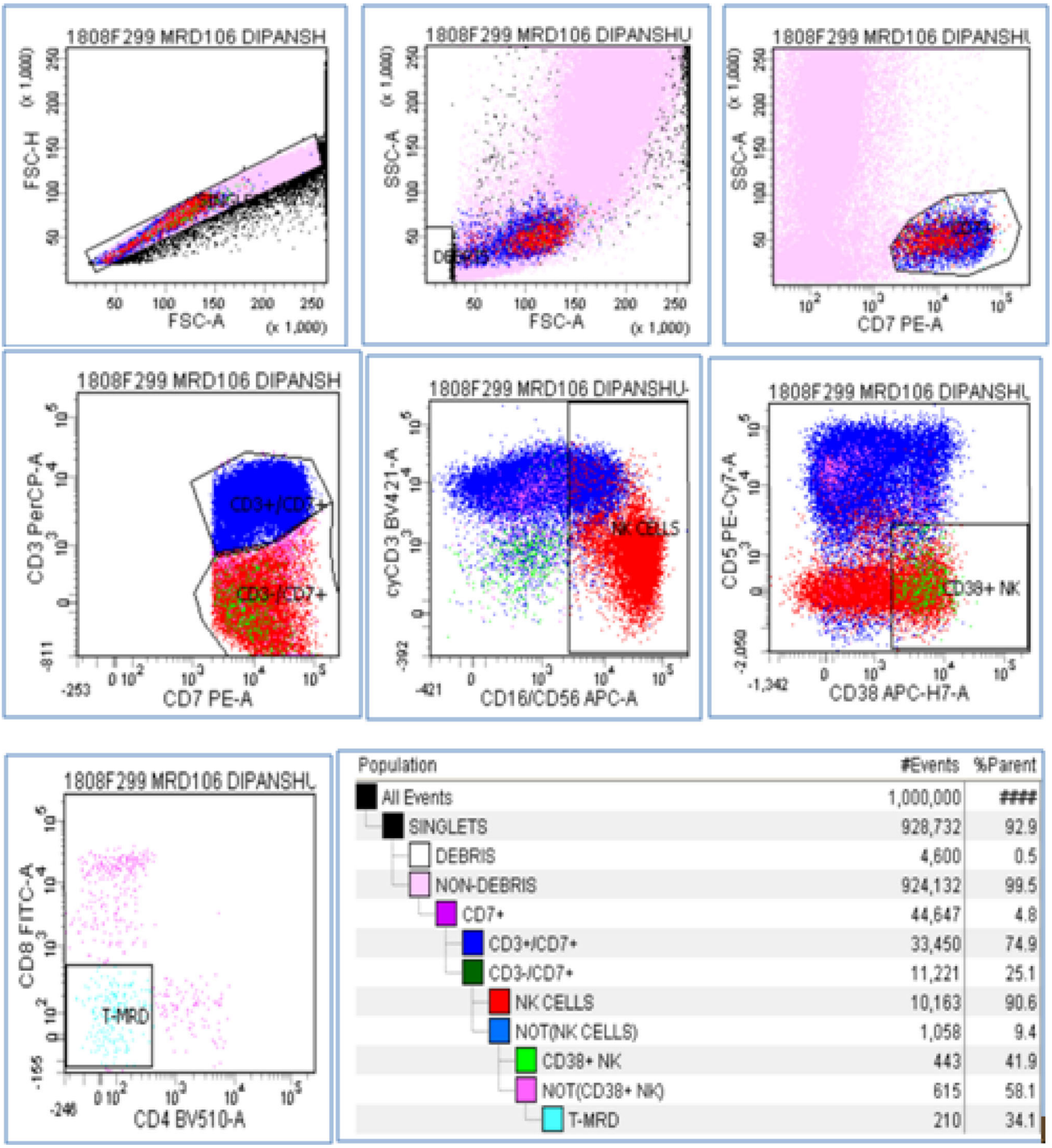

Fig. 1 Sequential gating to identify the population of interest in T-ALL minimal residual disease

Early-T-precursor ALL is a recently described subgroup of T-ALL derived from immature hematopoietic progenitor cells with maturation arrest at a very early stage and retains the ability to differentiate into T-cells and myeloid lineages [3]. It accounts for about 15% of all T-ALL case according to reported literature, similar to the incidence in our study (14.3%). Few previous studies showed significant correlation of ETP immunophenotype with remission failure, hematological relapse and 10-year Overall Survival [4]. However, Patrick et al. showed that although, ETP patients had higher relapse rates, lower TLC, poorer early morphological response and higher incidences of MRD positivity $> 0.01\%$ post-induction, there was no statistically significant difference between the ETP and Non-ETP

Table 2 Clinico-hematologic characteristics and prognostic outcome of T-ALL patients

Variables	ETP/near-ETP ALL	Non ETP-ALL	*P* value
Age (1–10 years)	3/9 (33.3)	4/26 (15.4)	0.25
TLC > 1.0 Lac/cumm	4/9 (44.4)	7/26 (26.9)	0.33
Platelet count < 100 × 10^9/L	3/9 (33.3)	15/26 (57.7)	0.21
Hb < 10g/dL	6/9 (66.6)	8/26 (30.7)	0.06
Adverse cytogenetics	3/9 (33.3)	4/26 (15.4)	0.25
Steroid responsiveness	5/9 (55.5)	21/26 (80.77)	0.14
Induction failure	4/9 (44.4)	4/26 (15.4)	0.07
MRD post-induction > 0.01%	6/9 (66.6)	7/26 (26.9)	0.033
MRD post-consolidation > 0.01%	1/9 (11.1)	Nil	0.12
Median follow up in months	12	14	0.06

#No difference between ETP and Near ETP subtypes so combined for final analysis

subtypes in terms of 5-year EFS and OS [3]. THE COG AALL0434 treatment study by Wood et al. that enrolled 1144 patients of T-ALL from 2007–2014 showed higher induction failure rates in ETP patients (7.8% vs. 1.1%) but the long-term survival in terms of 5-years EFS and OS rates were comparable in both groups (87% and 93% for ETP and 86.9% and 92% for Non-ETP patients respectively). However, they observed that Day + 29 MRD > 0.01% was associated with inferior EFS and OS for all T-ALL patients irrespective of subtype [5]. Similar to the recently published studies, the present study showed strong correlation of ETP-immunophenotype with MRD post-induction > 0.01% but no statistically significant impact of post consolidation MRD and EFS-1 year. The only other Indian study by Tembhare et al. [6] showed poorer EFS for any level of MRD (< 0.01% or > 0.01%) but no effect of ETP status on EFS/OS similar to the findings of the current study.

The major limitations of this study are heterogeneity in terms of age and treatment protocols, smaller sample size and shorter follow-up duration. Through this preliminary study, it can however, be stated clearly that ETP status is associated with MRD > 0.01% but has no significant impact on long-term survival of these patients.

Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical Approval 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.

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