Ig/T-cell receptor clonality testing as a method for Minimal Residual Disease monitoring in T cell- Acute Lymphoblastic Leukemia patients

Irina Panovska-Stavridis[1], Nevenka Ridova[1], Martin Ivanovski[1], Sanja Trajkova[1], Aleksandra Pivkova-Veljanovska[1], Dushko Dukovski[1], Lazar Chadievski, Zlate Stojanoski[1], Marija Popova-Labachevska[1], Bozidar Kocoski[1], Simona Stojanovska-Jakimovska[1], Milche Cvetanoski[1], Milena Grivchevska[1], Viktorija Kostojchinoska[1], Mario Jakjimovski[1], Ilina Hamamdzieva[1], Elizabeta Krestevska – Bozinovik[2], Aleksandar Dimovski[2]

[1] Clinical Center "Mother Teresa" - University Clinic of Hematology - Skopje, University Ss. Cyril and Methodius - Medical Faculty – Skopje, Skopje – Republic of North Macedonia

[2] Center for Biomolecular Pharmaceutical analyses, University Ss. Cyril and Methodius -Faculty of Pharmacy, Skopje, Skopje, Republic of North Macedonia

Background

Assessment of early response to therapy by minimal residual disease (MRD) monitoring in Acute Lymphoblastic Leukemia (ALL) has been proven to be one of the strongest prognostic factors for the disease outcome in ALL patients. Therefore, MRD status at specific time points during the induction period is long-ago incorporated in risk-stratification and risk-directed treatment approaches in pediatric ALL regimens, defining the intensity of postinduction therapy. In addition, data emerging from most recent studies emphasize the clinical significance of MRD status and suggest three-point MRD assessment for better determination of the risk groups, remission status and indications for hematopoietic stem cell transplantation (HSCT)[1].

Numerous techniques, starting with cytomorphology, may be used for MRD assessment in ALL. Nowadays, with current therapeutic approaches, majority of patients manage to achieve morphological remission. However, cytomorphology is insufficient in defining the residual tumor burden, which requires quantitative methods able to detect one blast in a background of up to one million cells. Currently, two standardized methods are generally used for MRD detection in ALL setting: Polymerase Chain Reaction (PCR) based immunoglobulin (Ig) or T cell receptor (TCR) clonality testing and multiparametric flow cytometry. Both techniques provide high sensitivity and specificity and both are reproducible and widely applicable. Table 1 enlists the main features, advantages and disadvantages of these methods[2].

Objective

Taking into consideration the previous studies highlighting the clinical importance of PI MRD in pediatric patients, in this single-center retrospective study, we describe disease status and outcome in patients with T-ALL older than 14 in correlation with post-induction residual disease response. In our study, MRD status was assessed by using molecular approach – detection of TCR genes rearrangements. We present the therapeutical implications of MRD status.

Materials & Methods

TCR gene analyses were performed in 11 adults with confirmed T-ALL. These patients were treated at the University Clinic for Hematology – Skopje between January 2018 and January 2022. CR rearrangements in bone marrow samples were analyzed by multiplex fluorescent PCR amplifications of the regions: TCRB-Vß-Jß, Dß-Jß, TCRG-Vγ-Jγ, TCRD-Vδ-Dδ-Jδ, Dδ-Dδ, Vδ-Dδ and Dδ-Jδ and subsequent capillary electrophoresis of PCR-products by automatic DNA analyzer, following EuroClonality/BIOMED-2 guidelines. Adolescents and young adults, which is a total of 6 patients, received BFM-based protocol. The remaining 5 adults were treated with Hyper-CVAD/high dose metotrexat (HDM) and cytarabine (ARA-C) regimen. MRD status was assessed at the end of the induction cycle. More precisely, in patients receiving BFM regimen, molecular MRD was evaluated on day 33 and day 78. In patients treated with Hyper-CVAD, TCR clonalty was tested at the completion of the first cycle of therapy. (Figure 1)

Table 1	Multiparametric Flow Cytometry	Ig/TCR mutplex PCR
Evaluation method	Targeting leukemia associated immunophenotype	Tracking Ig/TCR rearrangements
Substrate used	Suspension of live cells – unstable	DNA - stable
Processing	Immediate	Stable samples - delayed
Time required for analysis	Hours	Days
Sensitivity	4 colors – 1 x 10^{-4} 8 colors – 1 x 10^{-6}	1 x 10^{-4} to 1 x 10^{-6}
Applicability	~95%	>95%
Cost	More affordable	More expensive
Main advantage over the other method	Analysis bone marrow status and heterogeneous populations	High sensitivity & specificity – blast specific aberrations
Main disadvantage over the other method	Requires high number of viable cells to reach sensitivity level	Requires patient specific samples and probes False negative results in oligoclonal rearrangements
Standardization	Euro Flow Consortium	Euro-MRD Consortium (ESG – MRD- ALL)

Results

TCR rearrangements were detected in all 11 patients. All patients were negative for additional molecular prognostic markers. TCRB and TCRG genes rearrangements were slightly dominant over TCRD (64,3% vs. 50%). Due to different recommendations for MRD assessment depending on the therapy regimen, we will present both groups separately.

Adolescents and young adults: The mean age in patients treated with BFM protocol was 24 years, range 14-35. Risk groups were defined as medium and high risk, as no T-ALL patiens were defined as standard risk group. Patients showing good response to prednisolone and morphological presense of <10% blasts on day 15 and less than <1% blasts on flow cytometry on day 33 were stratifed as standard risk group. Although current recommendations have narrowed the indications for hematopoeitic stem cell transplantation (HSCI), especially in patients stratified into medium risk group, all our patients who achieved remission procedeed to HSCT.

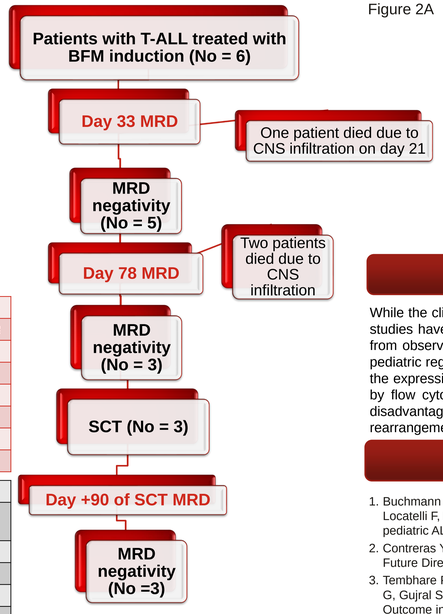
Adults: The mean age in patients treated with Hyper-CVAD protocol was 49 years. One patient died due to intracerebral hemorrhage during the induction protocol. One patient failed to achieve response and he was swithced to FLAG-Ida regimen. Three patients achieved remission and two of them procedeed to SCT and one patient completed POMP maintenance therapy.

Patients treated with BFM regimen – Clinical and disease features

No.	Age	Type of T-ALL	Risk group	TCR – MRD (-) d33	TCR- MRD (-) d78
1.	24	T – ALL (not early precursor T-ALL)	High risk	no	Not assessed
2.	25		Medium risk	Yes	Not assessed
3.	31		Medium risk	Yes	Yes
4.	25		Medium risk	Yes	Yes
5	16		Medium risk	Yes	Yes
6	14	ETP – T - ALL	Medium risk	Yes	Yes

Patients treated with Hyper-CVAD regimen

No.	Age	MRD (-) after first cycle	Further Therapy
1.	51	Yes	3 x Hyper-CVAD – died from intracerebral hemorrhage
2.	61	No	FLAG-ida – died from sepsis
3.	42	Yes	4 x Hyper-CVAD - HSCT
4.	52	Yes	4 x Hyper-CVAD – POMP maintenance therapy
5.	37	Yes	3 x Hyper-CVAD - HSCT



Special considerations in MRD detection in T-ALL

In this study we show that post-induction MRD status directed further therapeutical strategies in our small cohort of patients with T-ALL. T-ALL cases represent less than 20% of newly diagnosed cases of T-ALL and they are associated with worse outcome and higher risk of extramedular involvement compared to B-ALL patients. Therefore, in absence of wider diagnostic studies for risk stratification, our general approach is to transplant the patients with T-ALL who achieve response to chemotherapy in first remission. ALLIC 2020 clearly defines the T-ALL risk groups criteria and defines HSCT indications generally based on MRD determinants. In contrary, time points for MRD detection and general outlines incorporating this status in patients treated with non pediatric patients are imprecise. Large international studies should be conducted in order to precise and standardize time points for MRD monitoring, cut-off levels and optimal methods for MRD evaluation for each different adult treatment protocol.

Figure 1A

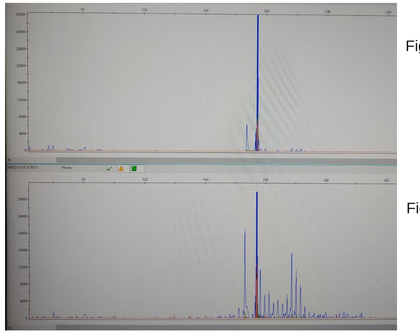


Figure 1B

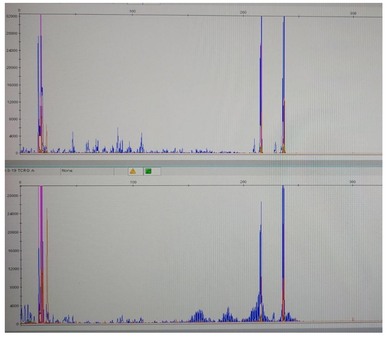


Figure 1A: Diagnostic day 1 TCRD (delta) analysis- monoclonal population;
Figure 1B: Day 33 analysis, polyclonal pattern with enhanced monoclonal spikes.

Figure 2A

Figure 2B

Figure 2A: Diagnostic day 1 TCRG (gamma) analysis- monoclonal population;
Figure 2B: Day 33 analysis, polyclonal pattern with enhanced monoclonal spikes.

Conclusions

While the clinical impact of MRD in B-ALL is well described, the data on clinical significance of MRD status in T-ALL cases are scarce. However, several studies have associated post-induction (PI) MRD positivity with inferior survival and higher risk of disease relapse[3]. In general, these limited data raise from observations seen in pediatric population and predicting disease outcome based on MRD status in adult patients and patients treated with non-pediatric regimens is even more challenging. Another challenge is defining the clinical significant cut-off values. T-ALL immunophenotypes are defined by the expression of early thymic T-cell markers and their presence in peripheral blood or bone marrow is used to determine the residual T-leukemic blasts by flow cytometry. On the other hand, TCR rearrangement assay is well standardized method applicable in majority of T-ALL patients. The main disadvantage is that it requires identification of patient's individual initial aberration and targeting this probes for MRD assessment. Also, this rearrangement is unstable and patients may relapse with clones harboring different rearrangement than those seen at diagnosis[4].

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