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Case Report

Increased immature T-cells detected by flow cytometry in post chemotherapeutic patients with acute myeloid leukemia, a case report and small series study

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Abstract: Detection of minimal/measurable residual disease (MRD) in bone marrow specimens by flow cytometry is widely used in patients with T cell acute lymphoblastic leukemia (T-ALL). It plays a central role in guiding treatment and assessing prognosis. However, the occurrence of a normal physiologic reactive immature T-cell population in treated bone marrow is unknown. To investigate this, we examined 14 post chemotherapeutic bone marrow specimens with a T-ALL MRD flow cytometry panel. This included 9 acute myeloid leukemia (AML) and 5 T-ALL cases. Immature T-cells are defined as surface CD3 negative cells that coexpress cytoplasmic CD3 (cyCD3) and terminal deoxynucleotidyl transferase (TdT), or as cells that express CD34 with coexpression of multiple T-cell markers. Immature T-cells were present in 1 of 9 AML cases (11%), between day 20-31 post chemotherapy. Follow-up of this patient who had 4.00% cyCD3+ TdT+ immature T-cells, showed the population gradually decreased to 0.50% at day 31, 0.15% at day 46, and was undetectable (0.00%) at day 116. This population remained undetectable at the most current follow-up on day 147. This pilot study shows that a low level of cyCD3+ TdT+ immature T-cells may be present in post chemotherapeutic regenerating bone marrow and can be detectable by flow cytometry. Thus, extra caution should be taken when interpreting T-ALL MRD results, especially between days 20-31 post chemotherapy.

Keywords: Immature T-cells, minimal residual disease, acute myeloid leukemia

Introduction

Lymphocyte precursors originate from bone marrow and fetal liver. Immature T lymphocytes migrate to the thymus, and mature by moving through the sub-capsular area, cortex, and medulla. Mature T lymphocytes are eventually released into peripheral blood and migrate to lymphoid organs/tissue [1]. Immature T lymphocytes are usually undetectable by flow cytometry in healthy bone marrow. The utilization of flow cytometry on bone marrow specimens in detection of MRD plays an essential role in guiding treatment protocols and stratifying prognostic risks for AML [2], B-cell lymphoblastic lymphoma/leukemia (B-ALL) [3, 4] and T-ALL [5].

The principal methods of MRD detection include multiparametric flow cytometry (MFC), quantitative Polymerase Chain Reaction (qPCR), and Next Generation Sequencing (NGS). Among them, MFC is considered less sensitive than qPCR and NGS (0.01% vs 0.001% vs 0.0001%, respectively) [6, 7] and is less standardized [8]. Yet, it remains a powerful method to monitor treatment response and to stratify risks in ALL patients due to its accessibility in most clinical facilities, short turnaround time and its relatively low cost. One caveat in detecting MRD in ALL patients by flow cytometry or qPCR, compared with NGS, is the limited capability of differentiating residual neoplastic blasts from regenerative change following chemotherapy, which causes concern of false positivity in real practice [9, 10]. The present case we encountered recently demonstrates this caveat in practice, highlighting the necessity of taking caution when interpreting MDR data in acute leukemia post treatment. To explore regenerative changes in the bone marrow following chemotherapy,

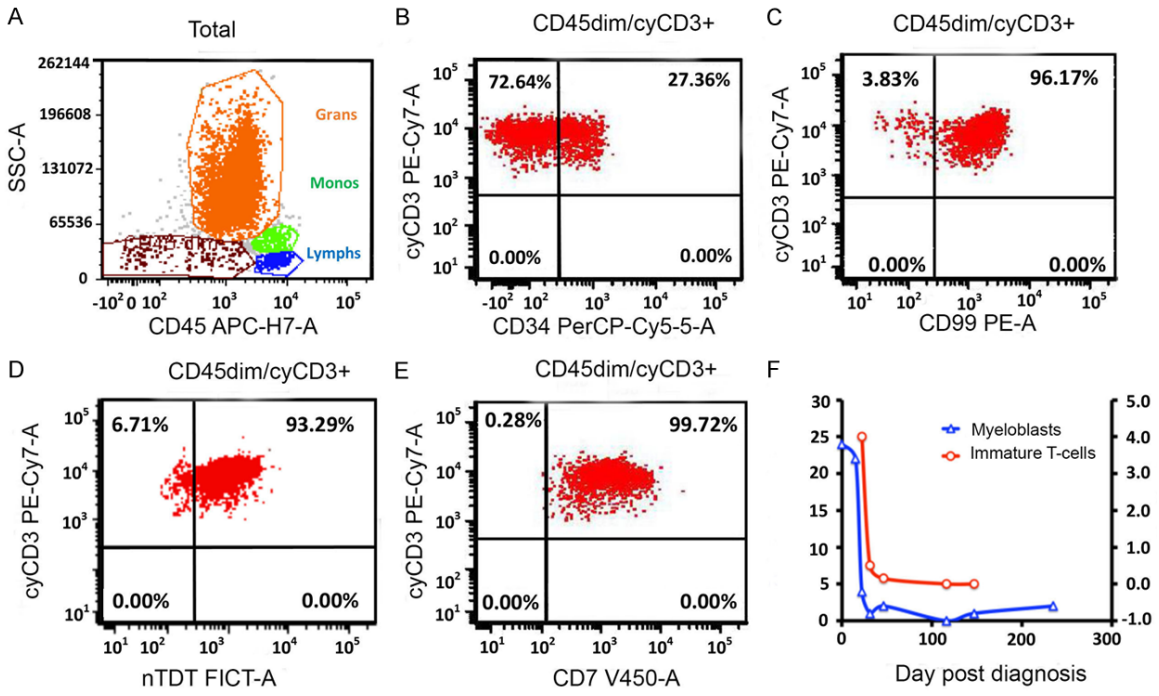

Figure 1. Immature T cells and myoblasts in AML marrow at day 22 post induction chemotherapy. A. Bone marrow specimen. B-E. Partial CD34+, cyCD3+, CD99+, TdT+, and CD7+ immature T-cells. F. In this AML case, % immature T-cell population (red) and residual myeloblasts (blue) decreased over time and remained detectable until day 46 post chemotherapy (0.15% of total events).

we phenotyped 9 AML and 5 T-ALL post chemotherapeutic marrow specimens with a flow cytometry T-ALL MRD panel. This panel included markers for cyCD3, TdT, CD34, CD1a, CD2, CD4, CD7, CD8 and CD99.

Case

This study was performed with approval from the Institutional Review Board of The University of Kansas Medical Center and patient informed consent were obtained for this study. Our patient was a 57-year-old male with a history of ulcerative colitis, previously treated with Mesalamine and Azathioprine, was diagnosed at The University of Kansas Medical Center with FLT3-ITD positive AML. He was treated with 7+3 (Cytarabine + Idarubicin) induction therapy and Midostaurin/Gemtuzumab on day 4. Bone marrow biopsies were performed at day 22, 31, 46, 116, and 147. T-ALL MRD flow cytometry panel revealed a population (4.00%) of immature T-cells positive for cyCD3, TdT, CD2, CD7, CD99, and partial dim CD34 at day 22. The population gradually decreased to 0.50% at day 31, 0.15% at day 46, and was undetectable (0.00%) at day 116. This population remained undetectable at the most resent follow-up on day 147 (**Figure 1**). We then further performed the same T-ALL MRD panel on 8 additional patients with AML and 5 patients with T-ALL. Results showed immature cyCD3+ TdT+ T-cell populations were absent at day 20 to day 31 in additional AML cases (**Tables 1**, **2**).

Discussion

The recently published consensus for MRD in AML patients, from *The European LeukemiaNet MRD Working Party,* establishes the use of MRD for risk analysis at an early time point prior to consolidation therapy and recommends using 0.1% as the cutoff value for differentiating MRD positive and MRD negative patients [11]. Our case shows that regenerating bone marrow in patients post chemotherapy can have increased immature T lymphocytes as high as 4.00% on day 22. This increase is most likely reflective of a reactive process to chemotherapy, since they gradually trended down to an undetectable level. There was no evidence of T-ALL during future follow-ups, with the most recent at day 147. T-ALL MRD studies in the remaining 8 AML cases, following

Table 1. Percentage of immature T-cells[a] in marrow of T-ALL cases

T-ALL case#	Day 1 Immature T-cells (%)	Day 20-30 Immature T-cells (%)	Original Immunophenotype
1	23.00	0.60	CD2/7/34
2	94.30	1.00	CD2/4/8 TdT
3	85.00	0.19	CD4/5/7/10 TdT
4	96.00	2.00	CD2/5/7/34 Tdt
5	88.34	0.14	CD5/7/13/33/34

[a]Immature T cells are positive for cyCD3, TdT, CD34, CD2, CD7 and CD99, and negative for CD4, CD5, CD8 and CD1a (n = 5).

Table 2. Percentage of immature T-cells[a] and myeloblasts[b] marrow in AML cases*

AML case#	Day 1 Myeloblasts (%)	Day 20-31 Immature T-cells (%)	Day 20-31 Myeloblasts (%)
1 (present case)	24.00	4.00	1.00
2	21.00	0.00	1.34
3	49.00	0.00	2.20
4	50.00	0.00	1.80
5	11.00	0.00	4.70
6	2.50	0.00	8.00
7	79.00	0.00	0.38
8	13.00	0.00	5.80
9	63.00	0.00	0.94

[a]Immature T-cells positive for cyCD3, TdT, CD34, CD2, CD7 and CD99, and negative for CD4, CD5, CD8 and CD1a. [b]Myeloblasts positive for CD34, CD13, and CD33. *All cases are cyCD3 positive, MPO, cyCD22, and cyCD79a negative.

induction chemotherapy were all negative; This is consistent with the traditional concept that bone marrow represents a "memory reservoir" for T-cells and T lymphoblasts are usually not detectable in this compartment except in rare conditions such as T-ALL [12]. The significance of our study is to show that the number of immature T-cells, under rare circumstances, can be significant enough to be detected in regenerative marrow by flow cytometry. Unfortunately this might be misinterpreted as a positive MRD in T-ALL. These immature T cells can create a potential diagnostic pitfall in T-ALL MRD detection. Therefore, extra caution should be taken to avoid misinterpreting T-ALL MRD results, especially during day 20-31 post chemotherapy and when the immunophenotype differs from the original T-ALL. For low-level MRD results, confirmatory tests by other methods such as qPCR and NGS would be advantageous.

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Disclosure of conflict of interest

None.

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