CASE REPORT

The importance of minimal residual disease for detection of late relapse in B-precursor acute lymphoblastic leukemia

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Abstract

Background: The minimal residual disease (MRD) level in patients with B-precursor acute lymphoblastic leukemia (B-ALL) is the strongest independent predictor of relapse and survival. Assessment of MRD plays a crucial role in the treatment of B-ALL.

Case report: We performed long-term monitoring of a 30-year-old woman with B-ALL of standard risk for MRD using multiparametric flow cytometry (MFC). After five years of monitoring, molecular relapse of the disease was confirmed.

Conclusion: This case illustrates that more extended monitoring for MRD, even by only MFC when other newer sophisticated diagnostics are not available, is essential in detecting early relapse in patients with B-ALL of standard risk. HIPPOKRATIA 2022, 26 (1):38-40.

Keywords: Minimal residual disease, B-precursor acute lymphoblastic leukemia, late relapse, multiparametric flow cytometry

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Introduction

B-precursor acute lymphoblastic leukemia (B-ALL) is a heterogeneous disease characterized by lymphoid progenitor cells' malignant transformation and proliferation in the bone marrow, blood, and extramedullary sites[1]. Despite the high complete remission rate induced by standard chemotherapy, the cure rate of patients with B-ALL is considerably lower in adults than in children[1]. Accurate diagnosis and risk stratification are crucial for the treatment approach in B-ALL. Risk stratification of B-ALL plays a vital role in the therapy selection, including allogeneic hematopoietic stem cell transplantation (allo-HSCT). Besides the well-established clinical, cytogenetic, and molecular parameters, assessment of minimal (measurable) residual disease (MRD) by either multiparametric flow cytometry (MFC) or polymerase chain reaction (PCR) is crucial for refining risk stratification and assessing the treatment response in ALL[2,3]. Positive MRD is associated with a higher relapse rate[4-6]. MRD monitoring is of paramount importance for early molecular relapse detection, which allows clinicians to apply additional therapy in order to eradicate MRD and prevent clinical disease relapse [bone marrow (BM), blasts ≥5 %][7].

MRD is defined as the post-therapy leukemic cells' persistence at levels below morphologic detection[8]. MRD evaluation by MFC is based on detecting populations with leukemia-associated aberrant immunophenotypes and achieves a sensitivity of 10^{-3} to 10^{-4}. Conversely, real-time quantitative PCR (RQ-PCR) sensitivity is 10^{-3} to 10^{-5} based on detecting clonal rearrangements of immunoglobulin (Ig) genes[8]. Several studies have shown a high concordance between MFC and PCR-based assays[9]. In addition, MFC is a rapid and accurate procedure with sufficient sensitivity and specificity and is widely used in hospitals. New sophisticated methods, such as next-generation genome sequencing assays, have been developed, which are even more sensitive for detecting molecular relapse[10]. However, the clinical significance of next-generation sequencing is still unknown. The optimal timing and sensitivity of these assays and integration with other indicators in treatment decisions of various ALL subgroups need to be refined in further studies.

We report a case of B-ALL in a patient with standard risk, in whom a molecular relapse was detected after five years of MRD follow-up using MFC. This report aims to illustrate the importance of prolonged MRD monitoring, which allows clinicians to commence treatment timeously in patients who experience a relapse.

Case report

A 30-year-old woman was admitted to the hematology clinic in May 2013. Her complete blood count at diagnosis showed: white blood cell count 2.6×10^9/L, hemo-

globin 87 g/L, and platelet count 36 × 10^9/L with 23 % blasts in a peripheral blood smear. A BM aspirate revealed infiltration with 57 % lymphoblasts. Immunophenotyping by MFC (BD FACSCalibur, CellQuest Software, San Jose, CA, USA) of the BM nucleated cell (BM/NC) specimen showed that the leukemic cells expressed the following antigens: nTdT^{+low}, HLADR$^{+hetero}$, CD34^{+high}, CD38^{+low}, CD19$^{+intermed}$, cCD79a^{+low}, CD22$^{+intermed}$, CD24^{+low}, CD10$^{+hetero}$, CD33^{+low}, CD45^{+low}/SSC^{low}. Karyotype analysis revealed a normal female karyotype in 20 mitoses. Real-time PCR (RT-PCR) using the BM specimen excluded both t(9;22)/*BCR-ABL1* fusion transcripts and t(4;11)/*MLL-AF4*. MFC analysis of cerebrospinal fluid was negative for leukemic cells. According to the WHO classification[11], the patient was diagnosed with B-ALL, B-II (common) ALL subtype, with a standard level of risk of relapse.

The patient was treated with eight cycles of the hyper-fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (hyper-CVAD) regimen. The last cycle was administered in December 2013. She achieved complete remission (CR) with MRD negativity (leukemic cells <0.1 % NC/BM) in July 2013 after two cycles of chemotherapy. MRD was assessed using 4-color MFC (BD FACSCalibur, BD CellQuest Pro Software), with a two antigens combination (CD24^{+low}, CD34^{+high}, CD10$^{+hetero}$, CD45^{+low}/SSC^{low}), and (CD34^{+high}, CD33^{+low}, CD10$^{+hetero}$, CD45^{+low}/SSC^{low}), sensitivity level of 0.1 % leukemic cells. The patient completed two years of maintenance therapy in February 2016. Since February 2014, she has undergone regular 6-monthly MRD monitoring. She remained MRD-negative until July 2018, when MRD was detected by MFC (CD24^{+low}, CD34^{+high}, CD10$^{+hetero}$, CD45^{+low}/SSC^{low}, 0.27 % BM/NC). At the time, the only treatment option in our center was allo-HCST. However, the patient refused transplantation as an option. We continued to monitor MRD with MFC. The MRD levels in BM were monitored every three months from October 2018 to April 2019. The MRD values varied from 0.25 to 0.75 % (Figure 1). Throughout this period, the patient remained in cytomorphological CR.

In May 2019, RQ-PCR analysis of Ig gene rearrangement of a BM sample showed MRD at the level of 2 %, which confirmed a molecular relapse. The patient received one cycle of blinatumomab in December 2019, and molecular remission, assessed by both RQ-PCR and MFC, was achieved. She subsequently underwent matched unrelated donor allo-HSCT in March 2020. Since then, she has remained in complete molecular remission.

Discussion

Monitoring of MRD in patients with ALL can significantly improve their survival, primarily due to early detection of relapse. Approximately 30-50 % of patients with B-ALL have persistent MRD after the initial treatment phase and have a higher risk (70-100 %) of hematologic relapse[2-5,8]. Conversely, MRD-negative patients have a much less incidence of hematologic relapse (2-8 %)[4,5,6]. Patients with MRD reappearance have a worse outcome than those with MRD persistence[2]. Although our patient remained MRD-negative for five years, a late relapse was detected. Therefore, it is essential to closely monitor these patients to initiate therapy in time. This is especially important in the era of blinatumomab and inotuzumab ozogamycin. Using these monoclonal antibodies leads to the re-achievement of MRD negativity and better survival of these patients[12,13]. Namely, MRD negativity before HSCT is the most potent known predictor of better posttransplantation outcome[14,15].

Blinatumomab is a bispecific T-cell-engaging monoclonal antibody contrived to form a synapse between CD3 T cells and CD19 B cells that redirects host T cells to cancer cells expressing cell surface antigen[13,14]. Bi-

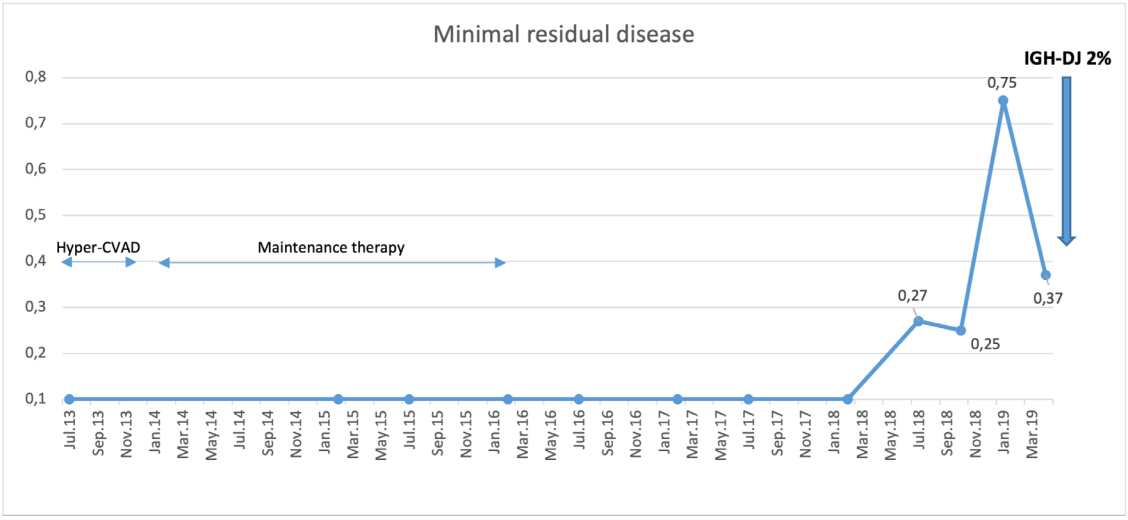

Figure 1: Graph demonstrating the minimal residual disease monitoring of the reported case during time from therapy to molecular confirmed relapse.

Hyper-CVAD: hyper-fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone regimen, IgH: Immunoglobulin heavy-chain.

specific T-cell engagers (BiTEs) has been approved for treating relapse and refractory B-ALL and MRB-positive B-ALL by the US Food and Drug Administration and the European Medicines Agency. However, it is unavailable in some countries due to its high cost.

According to current standard recommendations for treating adults with B-ALL, in patients with standard risk who do not undergo HSCT during their first remission, MRD should be assessed from a BM aspirate at least at the following points of treatment: i) at the end of induction, ii) in the early consolidation therapy phase (after approximately three months of therapy), and iii) every three months after that, for at least three years[15]. We speculated that a more extended MRD follow-up period might help detect early relapse.

This case study has some limitations. First, MRD monitoring using MFC is more subjective and less sensitive than PCR or next-generation genome sequencing. Conversely, in many countries, including ours, PCR and next-generation genome sequencing diagnostics for MRD monitoring are unavailable. Therefore, our success at detecting an early relapse using 4-color MFC, standardized for a detection level of 0.1 % leukemic cells, shows that this method can be used in the absence of other methods. Second, although this case may encourage clinicians to monitor their patients for a more extended period and respond promptly, larger, well-designed studies are required to provide more precise recommendations if a molecular relapse is detected.

In summary, considering the late molecular relapse in our patient with standard risk B-ALL, even longer MFC-based monitoring of MRD could be informative in individuals who are not candidates for allo-HSCT in the first CR. This case report is an excellent initial hypothesis for further research, precisely showing the significance of long-lasting MRD monitoring. Moreover, clinicians must continue refining the application of MRD response approaches to complex ALL therapy and carefully consider its limitations on predicting long-term effects[10].

Conflict of interest

The authors declare that they have no conflict of interest.

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