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Very late disease relapse in patient with B-common acute lymphoblastic leukemia treated with allogeneic stem cell transplantation: does clonal evolution play a role?

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Introduction

BCR-ABL1-like acute lymphoblastic leukemia (*BCR-ABL1*-like ALL) is a high risk disease subtype characterized by a gene expression profile similar to Philadelphia positive (Ph+) B-cell ALL, but lacking the distinguishing molecular fusion gene — *BCR-ABL1* [1, 2]. *BCR-ABL1*-like ALL has been incorporated as a separate entity into the 5th edition of the World Health Organization's classification of Hematolymphoid Tumors due to its therapeutic and prognostic significance [3]. The nature of the underlying genetic features of *BCR-ABL1*-like ALL is heterogenous, and therefore diagnosis remains challenging [4].

BCR-ABL1-like ALL patients often relapse early after initial treatment, but late and very late recurrences also occur [5, 6]. They are predominantly reported in the pediatric population, while in adults they are seldom observed [5, 7]. The relapse rate within the first three years from achieving complete remission (CR) among adults with B-cell ALL (B-ALL) varies from 35% in standard risk patients to 50% among high risk subjects [5]. The relapse rate beyond three years and five years in patients who achieve CR is estimated at c.3.8% and 2.8%, respectively [5, 8].

Herein, we present a case of a very late disease relapse with features of *BCR-ABL1*-like ALL which were absent at the first manifestation of the disease.

Material and methods

The presented case was identified during a study of immune, molecular and cytogenetic characteristics of *BCR-ABL1*-negative, *KMT2A*-rearrangement negative patients with B-cell ALL admitted to the Department of Hematology and Bone Marrow Transplantation of Poznań University of Medical Sciences in Poznań, Poland (study funding: Poznań University of Medical Sciences Doctoral School Large Research Grant No. DGB 3/2021).

The expression of thymic stromal lymphopoietin receptor (TSLPR), predictive of the rearrangement of the *CRLF2*, with an anti-TSLP antibody (Invitrogen™) was performed using the 10-color multiparameter flow cytometry method (FCM; BD FacsCanto II Ilyric™). The karyotype analysis was performed using G banding (GTG). Fluorescent *in situ* hybridization (FISH) studies were performed on the interface nuclei using break-apart probes for CRFL2, JAK2, EPOR, ABL1, ABL2 (CytoCell®, Cambridge, UK) and for BCR-ABL1, KMT2A, and PDGFRb (Vysis, IL, USA) and, additionally, for IGH and P2RY8 in the *CRLF2* rearranged (*CRLF2*-r) cases (CytoCell®, Cambridge, UK). At least 100 interphase nuclei were scored for each probe by two independent examiners. Analysis of the *JAK2* exon 16 sequence was conducted using DNA extracted from whole-blood leukocytes at the time of the diagnosis with the use of a QIAmp DNA Mini Kit (Qiagen) and high-resolution melt analysis (HRMA). For the variant type identification screened by HRMA, Sanger sequencing was applied using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Thermo Fisher Scientific) and the following primers: forward: 5'-TGCTCCAGTACTTGTGGACTGA-3' and reverse: 5'-CCACTGCCCAAGTAAAGCTTAG-3'.

To identify *BCR-ABL1*-like cases, we implemented an algorithm combining multiparameter flow cytometry (FCM), karyotype analysis using G banding and FISH. In a prospective analysis, the expression of TSLPR was evaluated by FCM. Patients expressing TSLPR on leukemic blasts were enrolled for the FISH analysis with a CRLF2 break-apart probe. Patients lacking the TSLPR expression were recognized as non-*CRLF2*-rearranged (non-*CRLF2*-r) and, subsequently, proceeded to analysis with the remaining FISH probes for other *BCR-ABL1*-like ALL features (JAK2, EPOR, ABL1, ABL2, PDGFRb).

In retrospective analysis, the patients were primarily examined for the presence of *CRLF2* rearrangement with a FISH probe. In *CRLF2*-r cases, the next step included an

analysis with IGH and P2RY8 FISH probes to identify the fusion gene. Non-*CRLF2*-r cases proceeded to the analysis with the remaining FISH probes. This study was approved by the Poznań University of Medical Sciences Bioethical Committee (Resolution No 705/20).

Results and case medical history

A 32-year-old male was diagnosed with a relapse of B-ALL 13 years after treatment with chemotherapy and an allogeneic hematopoietic stem cell transplantation (allo-HSCT). He was diagnosed at the age of 19 with B-common ALL in October 2008. Intensive polychemotherapy according to the Polish Adult Leukemia Group 2007 protocol was given immediately after disease diagnosis. The treatment resulted in the first complete remission (CR1) with negative measurable residual disease (MRD). Allo-HSCT from a matched unrelated donor (MUD) was performed in August, 2009. The pre-transplant conditioning regimen consisted of total body irradiation (TBI) and cyclophosphamide (120 mg/m^2). Thereafter, the patient remained in long-term remission for 13 years.

In July 2022, leukocytosis, anemia and thrombocytopenia were noted. FCM of bone marrow nuclear cells revealed 84% of lymphoblasts expressing B-common phenotype with high expression of TSLPR (99%). A subsequent GTG cytogenetic analysis revealed complex karyotype. Analysis with FISH probes showed the presence of rearrangement of the *CRLF2* (*CRLF2*-r) gene. An additional study with FISH probes identified *CRLF2-IGH* fusion (Figure 1). Due to the frequent co-occurrence of *JAK2* mutation encountered in *CRLF2*-r positive *BCR-ABL1*-like cases, the *JAK2* exon 16 sequence was analyzed using Sanger sequencing. The study confirmed the presence of single nucleotide variant LRG_612:c.2049A>C(p.Arg683Ser) with a variant allele frequency (VAF) of c.26%. Interestingly, a study of the samples collected at the initial disease manifestation for the presence of *CRLF2-r* was negative. Moreover, other cytogenetic aberrations characteristic of *BCR-ABL1*-like ALL were absent (Figure 1).

Due to the disease relapse, intensive chemotherapy according to the Polish Adult Leukemia Group ALL7 protocol for newly diagnosed patients was given. The patient achieved CR2 with negative MRD after induction therapy. The MRD was still undetectable after consolidation chemotherapy. In fact, the patient is proceeding to allo-HSCT in CR2.

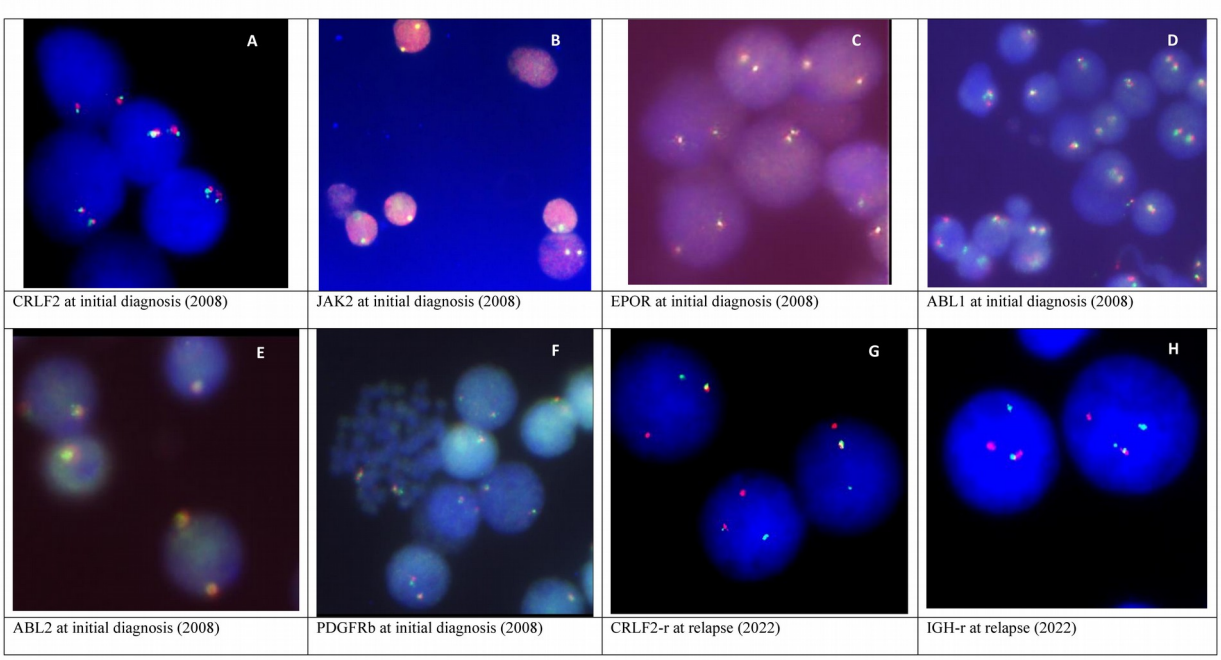

Figure 1. Fluorescent *in situ* hybridization (FISH) analysis of *BCR-ABL*-like rearrangements in presented patient. In normal cell, 2 red/green signals (2R/2G) or yellow (2Y) are observed: **A–F.** FISH analysis with cytokine receptor-like factor 2 gene (CRLF2), Janus kinase 2 gene (JAK2), erythropoietin receptor gene (EPOR), tyrosine-protein kinase ABL1 gene (ABL1), tyrosine-protein kinase ABL2 gene (ABL2), and platelet-derived growth factor beta gene (PDGFRb) break-apart probes on leukemic blasts at initial diagnosis; **G.** FISH analysis with CRLF2 break-apart probe at relapse. A translocation resulting in 1R, 1G, 1Y; **H.** Second step analysis with immunoglobulin heavy *locus* gene (IGH) probe. Cells with 1R, 1G, 1Y are indicative of IGH rearrangement

Discussion

The prognosis for relapsed/refractory B-cell ALL remains dismal, with historical median overall survival (OS) of approximately six months. However, the advent of novel therapies, including monoclonal antibodies (inotuzumab, blinatumomab) and chimeric antigen receptor T-cell therapy (CAR-T) has significantly improved OS in these patients [9].

Although some studies have indicated that late relapses might result in better outcomes than early relapses, the study by Ganzel et al. on 1,909 patients revealed that the outcomes of late recurrences of ALL, i.e. beyond three years of CR, were associated with a poor prognosis, with a five-year OS of 20% [5, 10]. To the best of our knowledge, only one case report of a

relapse of *BCR-ABL1*-like ALL after prolonged remission has previously been reported, although the presence of *BCR-ABL1*-like signature at the initial diagnosis was not examined [6].

The background to late B-cell ALL relapses remains a matter of debate since the mutational mechanisms leading to relapse are yet to be thoroughly investigated. It is currently believed that an ancestral leukemic clone or subclones might undergo evolution, leading to disease recurrence [5, 11]. The analysis by Waanders et al. [11] of 92 cases of relapsed pediatric ALL documented a pattern of clonal evolution, indicating that relapse-driving clones most commonly existed at initial diagnosis as minor clones, and less frequently as major clones. Most of the relapses derived from previously existing clones harboring, or acquiring, drug-resistant mutations [11]. Similar observations were made by Sayyab et al. [12], who reported different patterns of clonal evolution, which supports the hypothesis that relapses originate from evolving clones existing at the initial diagnosis.

Data reporting outcomes of allo-HSCT in *BCR-ABL1*-like ALL is scarce. A retrospective analysis of 56 patients by Aldoss et al. showed that patients with *BCR-ABL1*-like ALL had comparable post-allo-HSCT results to those of other B-cell ALL subjects, with 3-year OS reaching 51% and 50%, respectively [13]. In most *BCR-ABL1*-like ALL cases, genetic alterations which activate kinases and cytokine receptors signaling pathways are present. An excellent example of this are *JAK2* alternations which are present in 1–7% of *BCR-ABL1*-like ALL cases, and are most commonly detected in *CRLF2*-r cases [4, 14, 15].

In our presented case, the single nucleotide variant LRG_612:c.2049A>C(p.Arg683Ser) of *JAK2* gene was detected. The mutation significantly decreases the stability of JAK2 structure, leading to constitutive activation of JAK2, and is implicated in the pathogenesis of B-cell ALL via cooperation with CRLF2 [16, 17]. According to recently published data, this might serve as a potential target for precision therapy, something which has been confirmed by initial reports of the successful application of targeted therapy in combination with chemotherapy and immunotherapy in *BCR-ABL1*-like ALL patients [18].

Conclusions

Our observation confirmed the clonal evolution of B-cell ALL, even in a patient successfully treated with allo-HSCT many years ago. The background of late relapses remains a matter of

debate, although several observations have indicated a role played by the clonal evolution of previously existing minor clones.

We suggest that patients with B-cell ALL should be monitored for a long time after allo-HSCT to provide early detection of disease relapse. We further suggest, in relapsed patients, a detailed immunophenotypic, cytogenetic and molecular analysis should be performed, including screening for *BCR-ABL1*-like features to identify the potentially targetable molecular aberrations acquired during disease clonal evolution. The incorporation of widely available techniques including FCM and FISH enables swifter identification of *BCR-ABL1*-like patients, who may benefit from targeted therapy.

Authors' contributions

AP, KL, AP-Ch, ZK — methodology; AP, KL, ZK, AP-Ch, MK, JK-P, AM — investigation; AP, KL, ZK, MK, AP-Ch, MJ-S, LG — analysis and interpretation of clinical and laboratory data; AP, KL — writing, original draft. All authors read and agreed to published version of manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments and uniform requirements for manuscripts submitted to biomedical journals.

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