Relapse/refractory paediatric B-ALL case with CD19-phenotype switching indicating the importance of appropriate diagnostics approach and targeted treatment adjustment – case report

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Case Report

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Abstract

Background:

The case reported presents a rare CD19⁻ phenotype shift of acute lymphoblastic leukaemia clone during the course of relapse/refractory ALL in a paediatric patient. We explore possible reasons promoting CD19 negative cell selection, including discrete mutations and anti-CD19 treatment, which is gaining importance as targeted therapies such as blinatumomab enter standard treatment protocols.

Case presentation:

A 9-year-old male patient was admitted to the Department of Pediatric Hematology, Oncology and Transplantology, Medical University of Lublin, Poland with fatigue, anaemia and hepatosplenomegaly, and was subsequently diagnosed with B lymphocyte acute lymphoblastic leukaemia. Initial standard genetic analysis did not show significant chromosomal aberrations, and the patient underwent chemotherapy in line with the intermediate-risk protocol. After initially achieving remission, the disease relapsed, and the patient required hematopoietic stem cell transplantation (HSCT). In-depth retrospective microarray analysis performed at this point revealed additional risk factors including hyperdiploidy. After staying in remission for several months, a second recurrence was diagnosed which prompted targeted treatment application (Blinatumomab) and subsequent HSCT. The third leukemic relapse diagnosed shortly after second HSCT limited treatment options to last-resort CAR T cell therapy in Germany. Subsequent immunophenotyping revealed lack of CD19 expression by ALL clones and disqualified the patient from treatment. The patient died in October 2019 from disease progression.

Conclusions:

The case we report highlights the importance of in-depth molecular diagnostics and monitoring of relapse/recurrent ALL cases in order to identify and manage as many potential risk factors as possible during treatment. This gain importance as selective targeted treatments use increases, as antigenic phenotype and its changes directly influence the efficacy of such treatments, and therefore patients’ prognosis.

Introduction

Advances in immunophenotyping and genetic screening techniques have contributed to a shift in the understanding of acute lymphoblastic leukaemia (ALL) as a heterogenous group of diseases defined as malignancies of the lymphoid line of white blood cells (WBCs) characterised by a rapid development of large numbers of immature lymphocytes. On a molecular level however, multiple different heritable and acquired mutations of genes involved in lymphoid cell proliferation can lead to either dysregulating the cell cycle or accumulating subsequent harmful mutations (1). Many of those mutations have been
described and linked to disease development, such as \textit{C-MYC} translocation or \textit{ETV6::RUNX1} and \textit{BCR::ABL1} fusion genes, but some are less common and often include numerical mutations, such as hyperdiploidy. Different combinations of those changes are reflected in ALL clone phenotype through the expression of different antigens and receptors (2).

Multiple evidence suggests that \textit{de novo} mutations in tumour progenitor cells may lead to a phenotypic shift during treatment or in case of a relapse (3). As a treatment escape process, changes in phenotype often lead to an emergence of a less differentiated clone, and can involve myeloid lineage switching (4). Determining the unique immunophenotype is important not only for diagnostic, but also treatment purposes. While chemotherapy remains the first line of treatment for ALL, multiple targeted therapies involving enzyme inhibitors and immunomodulation, such as Blinatumomab and CAR T cell, rely solely on identification of specific cell surface markers. Targeted therapies are often used as a supportive treatment to haematopoietic stem cell transplantation (HSCT) for relapsed ALL, showing a significant increase in overall survival rates (5). Even though the predictive value of specific antigenic combinations on treatment outcome remains to be assessed for novel methods such as CAR-T cell, key antigens must be expressed to induce response based on treatment mechanism. The presence of such targets must be reassessed, possibly during treatment cycle and always in case of a relapse.

This case report aims to demonstrate, through an example of a relapse/refractory paediatric B-ALL case, a need for in depth genetic analysis using microarrays and immunophenotyping at multiple points during treatment, to ensure the most effective approach and realistic prognosis. We describe a rare case of CD19 negative B-ALL phenotype acquired over the course of treatment and multiple relapses in a 9-year-old patient, which disqualified him from CAR T cell therapy, eventually leading to his death. Based on available literature we try to assess whether additional risk factors could be inferred from cytogenetic analysis, and how to effectively monitor a recurrent disease for phenotype changes. This is particularly important in 2023, as presented anti-CD19 treatment methods are now much more available through standard treatment protocols, which might increase the incidence of resistant cases, requiring close monitoring.

\textbf{Case Presentation}

In October 2015 a 9-year-old male was admitted to the Department of Paediatric Haematology, Oncology and Transplantology of the University Children Hospital in Lublin. The patient was referred to the hospital by a GP after the CBC results suggested a malignant disease. The boy presented with tiredness anaemia, neutropenia, and hepatosplenomegaly. Peripheral blood smear and myelogram revealed 43% and 93% blasts respectively. The patient was diagnosed with B-ALL. Immunophenotyping showed expression of CD10, CD19, CD22 and CD79a. Cytogenetic analysis revealed a normal karyotype with no structural or numerical aberrations of chromosomes. There was no evidence of \textit{BCR::ABL1}, \textit{ETV6::RUNX1} fusion genes or \textit{KMT2A} and \textit{TCF3} rearrangements. However, additional signals were observed from molecular probes complementary with \textit{ETV6}, \textit{RUNX1} and \textit{TCF3}, which suggested a hyperdiploidy, despite seemingly normal karyotyping result (Figure 1). Retrospectively, and outside the standard treatment protocol,
microarrays were performed using the Affymetrix GeneChip 2.7 HD which provided further evidence for hyperdiploid karyotype (Figure 2). The patient was classified into an intermediate risk group based on his age (<9 years old) and initial response to treatment. Peripheral blast count on the 8th day of chemotherapy was <1000/µl, and percentage of blasts in myelogram at 15 days of treatment was <2%. Intensive chemotherapy according to ALL-IC-BMF 2009 protocol concluded in July 2016.

In January 2017 very early isolated BM relapse was diagnosed, and a subsequent genetic analysis of somatic karyotype and microarrays revealed previously non-existent karyotype changes: 46,XY,t(9;17) (p10;p10). There was evidence of complex gain/loss events as well as duplication of several autosomes including chromosomes 1, 2, 4, 5, 8, 9, 21 and deletion of the short arm of chromosome 17 with a loss of heterozygosity (LOH). Additional genetic tests confirm a pathogenic loss of function mutation TP53 V173L. Based on IntReALL definition of standard and higher risk strategy group classification the patient was classified into the high-risk (HR) group due to very early relapse. According to IntReALL, all HR patients are eligible for allogeneic hematopoietic stem-cell transplantation after achieving a complete second remission. Chemotherapy for HR patients was introduced and after achieving a complete remission, HSCT from the patient’s sister was performed. The post-transplant period was not complicated, and the patient presented with full donor chimerism.

February 2018 brought a second recurrence. Karyotyping showed 46,XY//46,XX (68% XY/32% XX), which was expected after allo-HSCT from sister donor. Fluorescent in-situ hybridization (FISH) revealed no structural rearrangements, however there was a signal missing from ETV6 and an additional signal from RUNX1. Microarrays were not performed at this stage due to mixed chimerism (32% of donor cells). At this point, IntReALL HR 2010, version 2.0, HIA block was introduced. Additionally, three courses of blinatumomab were administered between 13th April and 4th August 2018. The first cycle was complicated by fever and neurological symptoms, such as aphasia and hemiparesis. This required ceasing blinatumomab and implementing dexamethasone and mannitol treatment. After achieving improvement of the patient’s clinical state, therapy with reduced doses of blinatumomab was continued and was further well-tolerated. The patient received next two cycles of blinatumomab, and additional tests showed unsuccessful treatment response. In September 2018 the patient underwent the second HSCT from the same family donor without post-transplant complications.

Follow-up bone marrow examination executed in April 2019, revealed 94% of blasts. After diagnosing a third leukemic recurrence, the patient was redirected for further testing and qualification for CAR T cell therapy in Germany. He was also qualified to the INFORM (Individualized Therapy For Relapsed Malignancies in Childhood) registry based on inclusion criteria: relapsed ALL with >40% blasts in BM. Subsequent analysis revealed lack of CD19 expression, CD22 overexpression, CDKN2A/B deletion, PIK3R1, MYC mutation, and SYK overexpression which disqualified the patient from CAR T cell therapy. Based on CD22 expression inotuzumab ozogamicin treatment was introduced in July 2019, but he did not respond to the first cycle (3 doses). The second cycle brought partial response with rising WBC counts. Due to treatment resistance and lack of subsequent effective solutions, therapy was ceased, and palliative care was introduced. The patient died in October 2019 because of disease progression.
Discussion And Conclusions

Despite good survival rates upon diagnosis of acute lymphoblastic leukaemia in children, relapsed/recurrent ALL is associated with a dismal prognosis and remains a leading cause of death from childhood cancer. Targeted therapies, including engineered T cell therapies are a new strategy that allows an increasing number of patients to establish durable complete remission (6). Both blinatumomab and CAR T cell therapy are based on interaction of the drug with CD19 target on malignant cells. Blinatumomab is a bispecific T-cell engager (BiTE), which enables patient's own T cells to recognize and neutralise CD19+ cells, through combining two binding sites: CD3 site for the T cell and CD19 site for the target cells (7). Chimeric T cell receptors are engineered receptor proteins that enable T cells to target a specific antigen, in this case CD19. Expression of CD19 on ALL clone cells is a necessary provision to ensure treatment efficacy. The patient was initially qualified both for Blinatumomab and CAR T cell therapy based on immunophenotyping at diagnosis and after relapses. The first treatment proved inefficient, while he was disqualified from the latter based on CD19 negative phenotype discovered in Germany.

As CD19 is a B cell marker expressed on all stages of lineage development, CD19 negative B ALL is extremely rare, with only eight cases described in literature (8). This makes CD19 one of the key markers used in diagnostics and characterisation of B cell malignancies, with the WHO including CD19 as a lineage-defining marker for B-ALL. The early expression also contributes to CD19 being used in minimal residual disease (MRD) monitoring, and it has been brought up that lack of CD19 expression can lead to a delay in relapse diagnosis. Hence, Ghodke et al. suggest using at least 4 other B cell markers (CD10, CD20, CD22, CD79a) in follow-up monitoring (8), which is routinely done in our clinic and has contributed to early diagnoses of relapses in our patient.

The exact mechanism of CD19− relapse in our patient remains unknown. Initial presentation at diagnosis showed a normal karyotype, which did not suggest a higher relapse risk. We speculated that the karyotype could appear normal due to a highly heterogenic ALL clone population, with possible blood cell contamination. During diagnosis and testing, one sample collected from the patient must be divided into multiple specimens, creating a bottleneck effect, and preventing full spectrum analysis. Upon diagnosing a relapse, retrospective microarray analysis was performed from genetic material isolated at diagnosis, which together with FISH analysis were sufficient to state that numerical mutations were present, resulting in a hyperdiploid karyotype. Those aberration usually associated with a favourable prognosis, however about 20% of patients relapse (9).

A more disturbing and potentially pathogenic mutation was undetectable using a standard karyotype and FISH analysis according to diagnostic protocol. Microarray analysis and Normal Diploid overlay revealed LOH at short arm of chromosome 17 which rose concern of changes in TP53 gene. Subsequent INFORM analysis confirmed a loss of function TP53 V173L mutation. It is a missense substitution mutation in exon 5 of TP53, which results in amino acid change p.Val173Leu. The change is located in a highly conserved TP53 residue that is known to be functional, and in silico was shown to affect TP53 activity.
This mutation was previously described in patients with a TP53-related disorder; it occurs in a region with several other missense mutations described as pathogenic for Li Fraumeni syndrome (10).

Loss of function mutations have been reported in connection with cases of CD19\(^{-}\) relapses after/during anti-CD19 treatments with CAR T cell therapy, due to a dysfunctional or absent transmembrane domain of CD19 surface antigen. These have been linked to the initial occurrence of relapse itself, being present in nearly all malignant cells (11). A similar mechanism might have arisen as a result of selection during blinatumomab treatment for the third relapse, to which the patient showed poor response. A relatively small undetected CD19\(^{-}\) fraction could have dominated the tumour cell population after targeting CD19\(^{+}\) cells. This remains a hypothesis, as our clinic does not have access to genetic analyses performed after blinatumomab treatment.

Genetic analysis revealing potentially pathogenic mutations in key regulating suppressor genes such as TP53 as well as identifying cells with accumulated mutations should raise additional concerns of worse prognosis due to relapses and loss of surface antigens. The structure of tumour cell population may change rapidly as a treatment evasion mechanism, and therefore we recommend repeating the analysis after implementing another drug with the same target as a previous treatment. Disturbing evidence presented by Orlando et al. suggest that mutations resulting in CD19\(^{-}\) relapses could have been completely undetectable in samples collected as close as 1 month prior to clinical relapse (11), which requires further confirmation and measures to improve early diagnosis. This is especially important for patients belonging to high-risk groups, such as ones treated previously with a CD19 targeting protocol. It is important to note that in 2016 blinatumomab treatment was not refundable, and had to be paid for by the patient’s family. This has resulted in treatment delay, but carries other possible implications. As of 2023 blinatumomab is included in standard treatment protocols for relapsed ALL and is therefore much more widespread. Based on our experience with this patient, anti CD19 treatment may induce pressure for CD19 negative clonal selection. Hence, we could see an increase in recurrent CD19 negative ALL, resistant to last-resort treatments such as CAR T cell therapy, increasing the need for close monitoring.

The case presented highlights the importance of in-depth genetic analysis using microarrays and close monitoring of ALL immunophenotype as a prognostic factor for ALL treatment outcome and relapse risk. While standard diagnostic protocols provide evidence for the most common pathogenic mutation types, some of the changes can remain undetected. Microarrays are a useful diagnostic tool that we recommend using as early as at initial diagnosis, as they can help reveal discrete mutations, which can have a fundamental role in risk stratification, and therefore approach to treatment.

**List Of Abbreviations**

ALL – acute lymphoblastic leukaemia

B-ALL – acute lymphoblastic leukaemia derived from B lymphocytes

BiTE – bispecific T-cell engager
Declarations

Ethics approval and consent to participate

Research has been performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee at Medical University of Lublin (komisja.bioetyczna@umlub.pl). Further information and documentation are available to the Editor on request by contacting the corresponding author.

Consent for publication

Consent for publication was obtained from the legal guardians of the underaged patient according to institutional consent form.

Availability of data and materials

Medical history of the patient including test results and information on performed procedures are not available publicly, but access can be requested through contacting the corresponding author of the manuscript.

Competing interests

The authors declare that there were no competing interests.

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Authors’ contributions

A.P. analysed and interpreted the medical history and was primarily responsible for literature search, writing the sections: Abstract, Introduction, Discussion and Conclusions and Reference. AP prepared all the necessary parts of the manuscript for presentation.; P.J. analysed the medical history and was primarily responsible for writing the section: Case presentation.; B.S. was responsible for carrying out genetic analysis to produce and analyse genetic results. BS explained and annotated genetic test results used in the presentation.; M.L. Conceptualisation; Reviewing and Editing; Overseeing genetic analysis.; A.Z.P. Conceptualisation; Reviewing and Editing; All authors reviewed the manuscript.

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None.

References


**Figures**

**Figure 1**

FISH analysis showing additional gene signals: (A) 4 copies of KMT2A and (B) 4 copies of BCR and 2 copies of ABL.
Figure 2

(A) Normal karyotype seen at diagnosis; (B) Microarray suggesting first evidence of hiperdiploidy; (C) Further karyotype generated using Normal Diploid overlay – duplication of most chromosomes is evident, with a noticeable LOH at short arm of chromosome 17; (D) Karyotype analysis at first relapse; Own work using Chromosome Analysis Suite, 2017 and Affymetrix GeneChip 2.7 HD.