World Journal of Genetics & Molecular Biology

Iris Publishers

Case Report

Unmasking B-ALL: A Case Identified by Molecular Genetics Over Molecular Cytogenetics

Serena Albertazzi[1], Mirjam Lista[1], Federica Estersini[1], Kokou EA Ayissou[1], Alessia Campagna[2] and Rossella Caselli[1]*

[1]*Medical Genetic Laboratory, IRCCS Humanitas Research Hospital, Rozzano – Milan, Italy*

[2]*Heamatology Unit, IRCCS Humanitas Research Hospital, Rozzano – Milan, Italy*

***Corresponding author:** Rossella Caselli, Medical Genetic Laboratory, IRCCS Humanitas Research Hospital, Via Manzoni 56, Rozzano (MI) 2089, Italy.

Received Date: February 06, 2025

Published Date: February 18, 2025

Abstract

B-cell acute lymphoblastic leukaemia (B-ALL) is an haematological malignancy that occurs in both children and adults, characterized by lymphoblasts uncontrolled proliferation. Genetically, it is marked by chromosomal abnormalities and genetic alterations that affect lymphoblasts differentiation and proliferation. Advances in genomic profiling techniques have identified more specific genetic alterations and mutations driving B-ALL, leading to the development of novel therapies. A cytogenetic diagnostic workflow on adult patients includes KMT2A rearrangements, ETV6::RUNX1, TCF3::PBX1, TCF3::HLF, BCR::ABL1 translocations.

In this case report, we present the first adult case in our cohort, where a canonical BCR::ABL1 rearrangement is identified only with genetic molecular analysis and not by FISH hypothesizing a masked cryptic rearrangement.

Keywords: Cytogenetics; Molecular genetics; B-ALL; FISH; BCR:ABL1; Oligo-SNPs Array; Real Time-PCR; Chromothripsis.

Abbreviations: B-ALL: B-cell Acute Lymphoblastic Leukaemia; FISH: Fluorescence In Situ Hybridization; Oligo-SNPs Array: Oligonucleotide Single – Nucleotide Polymorphisms Microarrays RT-PCR: Real Time – Polymerase Chain Reaction; WHO-HAEM: World Health Organization – classification of Haematolymphoid Tumors; NGS: Next-Generation Sequencing; CN-LOH: Copy Neutral Loss of Heterozygosity; BM: Bone Marrow

Introduction

Acute lymphoblastic leukaemia (ALL) is a hematologic neoplasm involving B or T lymphoblasts characterized by an expansion of abnormal, immature lymphocytes and their progenitors in bone marrow, blood, and extramedullary sites [1]. ALL heterogeneity features are recently revealed by several genomic studies, which lead to a re-classification in the 5th edition of WHO Classification of Haematolymphoid Tumors (WHO-HAEM5 2022) as further distinct subgroups with specific molecular entities, helped also by next-generation sequencing (NGS) and cytogenetic characterization which improve comprehensive genomic profiling [1,2]. This is decisive for risk stratification and for monitoring clonal evolution during all phases of the disease; it is also important to understand

better both target therapies development and drug resistance mechanisms [2].

B-ALL genetic profile is characterised by chromosomal alterations (chromosomal rearrangements ad/or ploidy status alteration) that could result in chimeric genes and in some cases in genes upregulation by juxtaposition with a strong enhancer [1]. Among recurrent gene alterations and chromosomal abnormalities, that play a central role in tumour progression, there are KMT2A rearrangements, ETV6::RUNX1, TCF3::PBX1, TCF3::HLF, BCR::ABL1 translocations, associated with patient clinical features and treatment responsiveness. Hyperdiploid/hypodiploid chromosomal pattern evaluation is equally important for prognosis [3, 4].

Genetic diagnostic workflow in adult B-ALL includes chromosome banding analysis, screening for recurrent fusion genes by FISH/RT-PCR and oligo-SNPs array to evaluate the presence of multiple whole chromosome CN-LOH.

Case Presentation

A 70 years old man was admitted to our hospital with acute leukaemia diagnosis: bone marrow analysis and immunophenotype were consistent with B-ALL subtype with presence of 85% lymphoblasts. According to internal clinical protocol we performed from bone marrow (BM) sample conventional cytogenetic and FISH analysis from uncultured cells and nuclei extraction respectively. Karyotype analysis was performed using standard QFQ banding procedure and no numerical or structural alterations were found, although below the recommended minimum number of metaphases (Figure 1). Interphase FISH analysis, performed with specific probes for BCR::ABL1 fusion gene (Metasystem XL BCR/ABL1 plus DF), KMT2A locus (Metasystem XL MLL BA) and TCF3::PBX1/TCF3::HLF fusion genes (Cytocell E2A/PBX1 plus DF), didn't show recurrent rearrangements for investigated loci evidencing only copy number alterations for loci BCR, KMT2A, PBX1 and HLF (Figure 2).

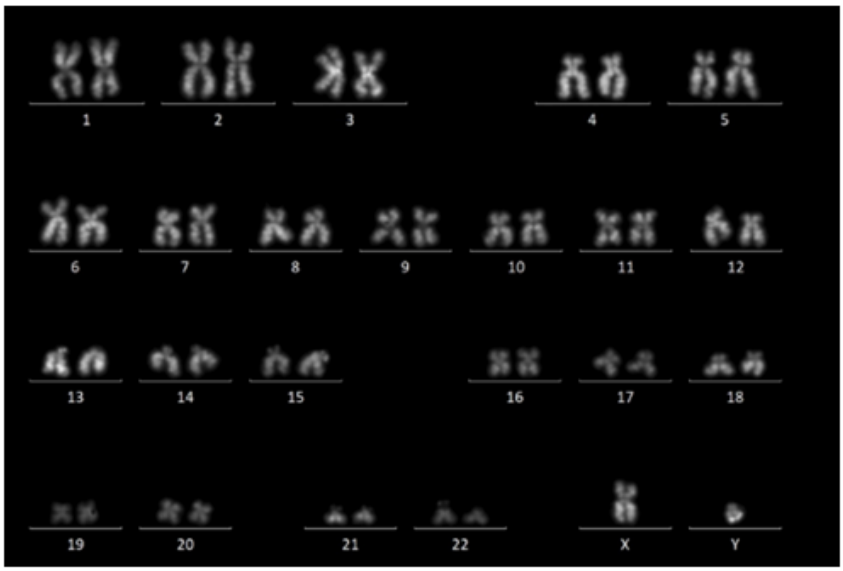

Figure 1: Conventional cytogenetic analysis – Q banding normal karyotype.

Based on the observed signal pattern detected in the FISH assay a second level analysis was performed with a-CGH+SNPs on the DNA extracted on a BM second sample taken four days later steroid therapy. It was hybridised on Surprint G3 Cancer CGH+SNPs 4×180K platform (Agilent Technologies) (Figure 2D), and the data, analysed with Cytogenetics' v.5.0.1.6 software (Agilent Technologies), didn't show any ploidy alteration despite expectations. RT-PCR molecular analysis, performed with Test easy PGX ready BCR-ABL fusion kit (Diatech Pharmacogenetics), detected p190 canonical transcript of BCR::ABL1 fusion gene.

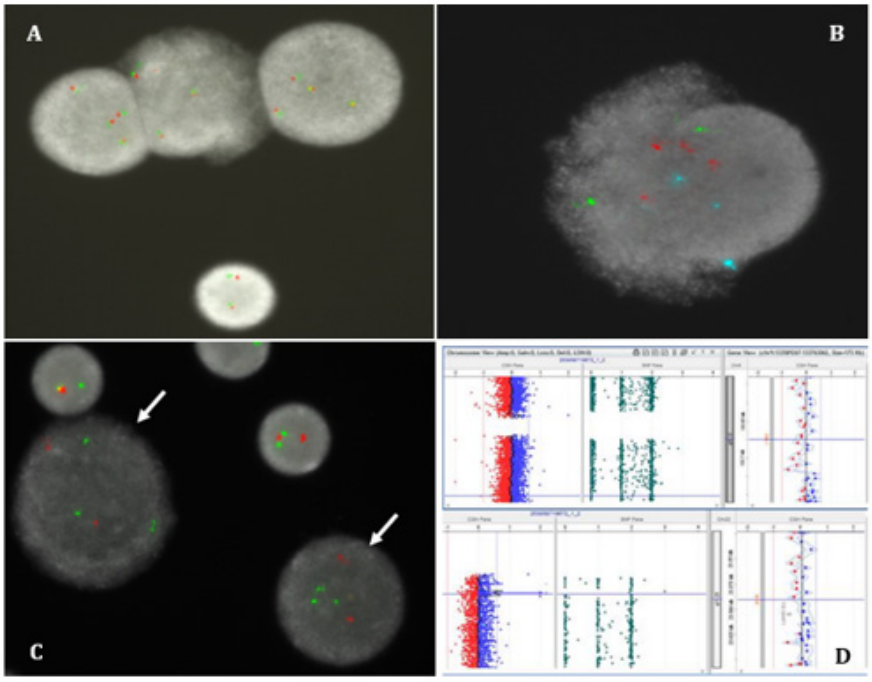

Figure 2: Molecular cytogenetics analysis – FISH results for KMT2A locus, TCF3::PBX1/TCF3::HLF, BCR::ABL1 fusion gene and a-CGH+SNPs result for BCR,ABL1 loci.

Fig2A shows FISH non rearranged pattern signals for KMT2A (11q23.3 – red and green) detecting four copies of KMT2A;

Fig2B shows non rearranged pattern signals for PBX1 (1q23.3 - red), HLF (17q22 - blue), TCF3 (19p13.3 - green) loci detecting four copies of PBX1 and three copies of HLF.

Fig2C shows FISH non rearranged pattern signals for BCR (22q11.23 - green), ABL1(9q34.12 - red) detecting three copies of BCR on blasts (arrow);

Fig2D shows aCGH+SNPs normal results for BCR and ABL1 genes.

Discussion

In contrast to all cases to date analysed, wherein FISH analysis was in alignment with the expectations detecting the rearrangements and/or genes fusion also in presence of non standard pattern, in this case our results led us to suppose the presence of a cryptic chromosomal rearrangement, probably mediated by an elaborate mechanism underlying complex genomic rearrangement such as chromothripsis. This hypothesis is strengthened by the investigations about chromothripsis phenomenon which leads to genome instability detected in 30-50% of cancers; studies conducted in these years tried to understand the contribution of chromothripsis in cancer development and the selective advantages that clonal cells might gain from molecular consequences of chromosomal alterations [5]. Chromothripsis incidence rates reported for hematologic malignancies are moderately lower than those reported in solid tumors for which its association seems to have a significant value for patients' clinical outcomes; it steers the studies towards chromothripsis implications also in hematologic malignancies [5,6]. Chromothripsis is investigated by CGH-array on ALL heterogenic patients cohort (variable age) and it is observed exclusively in adults with a major incidence in chromosome 6 and 15q in B-ALL; all patients had additional cytogenetic abnormalities [6]. Based on this literature data is not possible to exclude chromothripsis' involvement on the other chromosomes, hence we performed a-CGH+SNPs investigation; however, our unexpected inconclusive results are presumably a consequence of steroid therapy which may have masked the tumoral clone. This marks the importance of carrying out timely analyses before any therapeutic treatment.

Despite the proposed hypothesis, our investigative efforts didn't reveal the precise molecular mechanisms that lacked gene fusion detection by FISH that remains pivotal for our future objectives. In conclusion, this case pinpoints the critical necessity of combine cytogenetic and molecular genetic methodologies in a comprehensive evaluation of all B-ALL cases to ensure the detection of all genetic abnormalities. The final purpose is to refine and to enhance diagnostic and prognostic workflow specifically to provide selected therapeutic target in clinical practice.

References

1. Malard F and Mohty M (2020) Acute lymphoblastic leukaemia. Lancet: 395(10230): 1146-1162.
2. Luca Pagliaro, Sai Juan Chen, Daniel Herranz, Cristina Mecucci, Christine J Harrison, et al. (2024) Acute lymphoblastic leukaemia, Nat Rev Dis Primers 10(1): 41.
3. Yasuda T, Sanada M, Tsuzuki S, Hayakawa F (2022) Oncogenic lesions and molecular subtypes in adults with B-cell acute lymphoblastic leukemia. Cancer Sci: 114(1): 8-15.
4. Rack KA, E van den Berg, C Haferlach, HB Beverloo, D Costa, et al. (2019) European recommendations and quality assurance for cytogenomic analysis of haematological neoplasms. Leukemia 33(8): 1851-1867.
5. Simovic-Lorenz M, Ernst A (2025) Chromothripsis in cancer. Nat Rev Cancer 25(2): 79-92.
6. Lagunas-Rangel FA, 2024, Chromothripsis in hematologic malignancies. Exp Hematol: 132: 104172.