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A new dual translocation of chromosome 14 in a pediatric Burkitt lymphoma/leukemia patient: t(8;14) and t(14;15)

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ABSTRACT

Burkitt lymphoma/leukemia (BL/L) is an aggressive mature B-cell malignancy cytogenetically characterized by the translocation t(8;14)(q24;q32) or its variants, which determines the juxtaposition of the *MYC* oncogene to one of the three immunoglobulin loci. In addition to *MYC* translocations, different secondary genetic abnormalities have been described, some of them with prognostic significance. However, dual translocations of chromosome 14, except those involving chromosome 18, are very rare events in this pathology. Herein, we present the coexistence of translocations t(8;14) and t(14;15) in a pediatric BL/L patient. To our knowledge, this is the first report of a translocation t(14;15)(q32;q22) as a secondary alteration in a BL/L patient. The patient had multiple complications at diagnosis but he evolved favorably reaching complete remission. The description of new secondary alterations in this pathology as well as their impact on clinical evolution, add information to the biological characterization of BL, contributing to a higher accuracy in the diagnosis and/or prognosis of the disease.

Introduction

Burkitt lymphoma (BL), including its leukemic counterpart Burkitt leukemia (≥25% bone marrow involvement), is an aggressive mature B-cell malignancy, characterized by specific histopathological, immunophenotypic and genetic features [1]. It is uncommon in adults but frequent in children and adolescents. BL together with its leukemic form is named Burkitt lymphoma/leukemia (BL/L). Cases presenting a tumor mass at extranodal site with bone marrow involvement are considered stage IV disease, which is associated with a poor prognosis. Chromosome translocations involving *MYC* gene located at chromosome 8q24, are highly characteristic but not specific of this pathology. Translocation t(8;14)(q24;q32) that juxtapose *MYC* gene with the immunoglobulin heavy chain locus on chromosome 14q32 is the most frequent rearrangement (80% of cases), followed by its variants involving kappa (2p12) (15%) and lambda (22q11) (5%) light-chain genes. In addition to *MYC* rearrangements, different secondary genetic abnormalities have been described, some of them associated to unfavorable prognosis, suggesting their importance in refining risk stratification for BL/L patients [2–7]. Among them, concomitant dual chromosome 14 translocations, others than t(14;18)(q32;q21), are very rare events in this pathology [8]. In this report, we present to our knowledge, the first case with t(14;15)(q32;q22) as a secondary alteration in a pediatric patient with BL/L.

Case report

A 6-year-old patient was admitted to our Hospital in December 2019. He presented vomiting and abdominal distension for a week. Physical examination revealed lower limb edema and abdominal mass at the right iliac fossa. Hematological data showed: white blood cells count 7 × 10^9/L (4% myelocytes, 52% neutrophils, 36% lymphocytes, 6% monocytes and 2% eosinophils), hemoglobin 9 gr/dL, hematocrit 27.4%, platelets count 179 × 10^9/L. Lactate dehidrogenase (LDH) 13,110 IU/L, urea 0.79 g/L, creatinine 1.24 mg/dL, uric acid 15.6 mg/dL, calcium 8.1 mg/dL, phosphatemia 4.48 mg/dL. Total proteins and albumin in normal range. Abdominal ultrasound showed: liver and spleen with normal echogenicity and preserved size, kidneys with diffuse increased echogenicity. An abnormal abdominal effusion and the intestinal loops without peristalsis as

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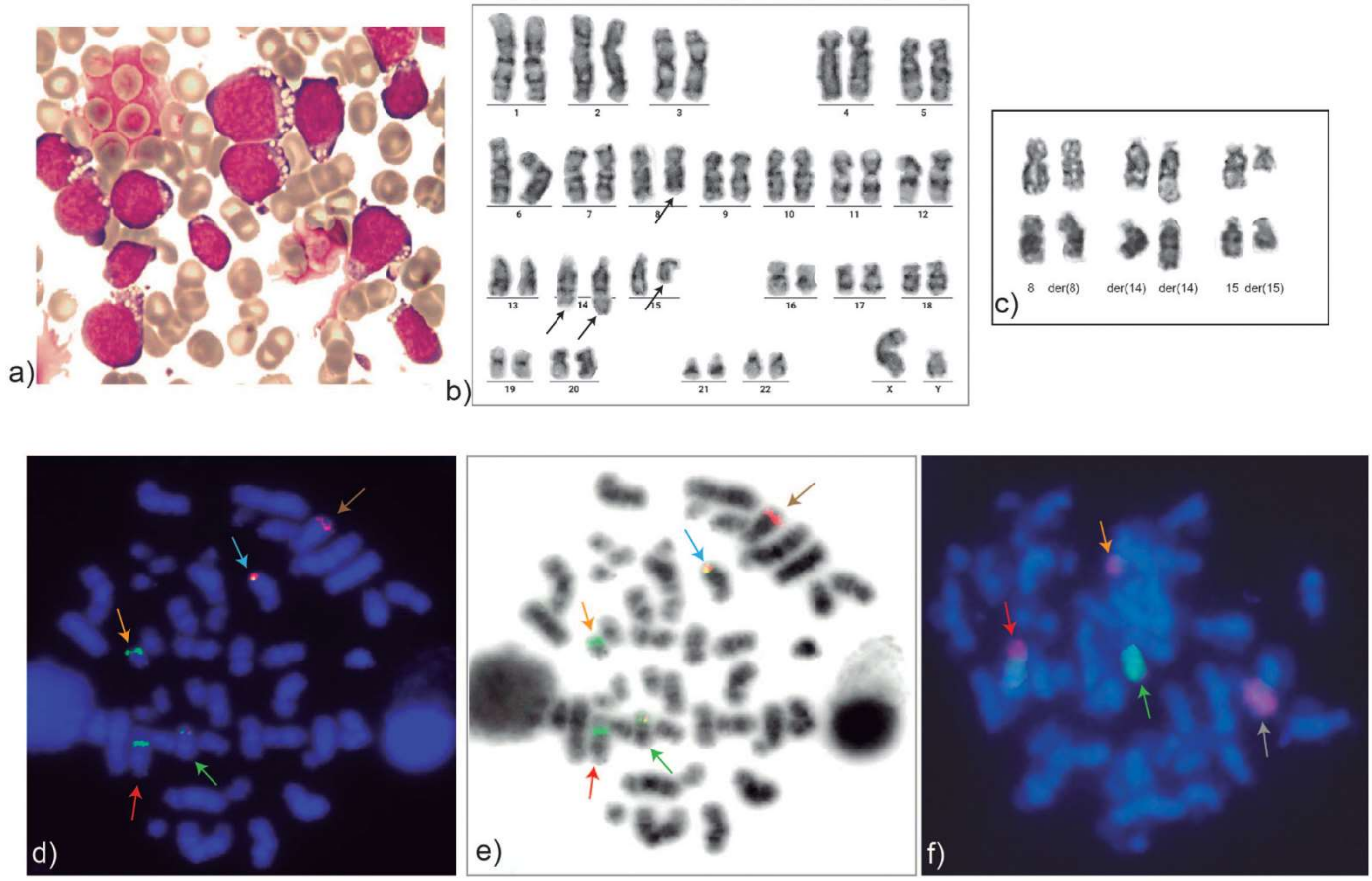

Fig. 1. (a) Bone marrow smear showing infiltration of lymphoid blasts cells with abundant cytoplasmic vacuoles; (b) G-banding karyotype showing translocations t(8;14)(q24;q32) and t(14,15)(q32;q22) (arrows); c) Partial karyotypes showing t(8;14)(q24;q32) and t(14,15)(q32;q22); d-e) Metaphase and reverse DAPI-G-banded metaphase hybridized with the IGH(14q32)/MYC(8q24) translocation dual fusion DNA probe (Cytocell, Oxford, UK) showing normal chromosome 8 (light brown arrow), der(8)t(8;14) (light blue arrow), der(14)t(8;14) (green arrow), der(14)t(14;15) (red arrow) and der(15)t(14;15) (orange arrow); f) Metaphase hybridized with the whole chromosome painting PCT14 and PCT15 DNA probes (Live-Lexel, Buenos Aires, Argentina) showing normal chromosome 15 (gray arrow), der(14)t(8;14) (green arrow), der(14)t(14;15) (red arrow) and der(15)t(14;15) (orange arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

well as an image of a fixed intestinal loop in the right iliac fossa with 1 cm thickened wall was noted. Pleural ultrasound found pleural effusion of 2.5 cm. Bone marrow aspiration showed marked hypercellularity with 100% infiltration of lymphoid blast cells with abundant vacuoles (Fig. 1a). Serological test for antibodies specific for Epstein-Barr virus (EBV) antigens was positive for viral capsid antigen (VCA) IgG but negative for VCA IgM. Bone marrow flow cytometry immunophenotyping revealed a major abnormal B-cell population expressing CD19, cytoplasmic CD79a, CD45 dim, CD20, CD10, bright CD38 (Becton-Dickinson Biosciences, San Jose, CA) and surface kappa light chain (Dako, Carpinteria, CA), and negative for TdT, CD34 (Becton-Dickinson Biosciences, San Jose, CA) and lambda light chain (Dako, Carpinteria, CA). Flow cytometry analysis was also performed in a sample of pleural fluid with similar results. A Becton Dickinson FACSCanto II flow cytometer was used for the acquisition; the files were analyzed using Infinicyt software (v1.7, Cytognos, Salamanca, Spain). Thus, a diagnosis of stage IV BL/L was performed. The patient presented a rapid deterioration of the clinical condition requiring admission to the pediatric intensive care unit (ICU). He started with the prephase of the Pediatric Non-Hodgkin Lymphoma 2017 protocol of the Argentine Acute Leukemia Treatment Group (GATLA) – BFM (Berlin-Frankfurt-Münster) [9–11]. During his stay at the ICU, he presented an abdominal compartment syndrome requiring surgical abdominal decompression. He received Rasburicase and appropriate measures for tumor lysis syndrome. The patient presented renal failure with hemodialysis requirement for three days. Not central nervous system involvement was found. Despite the multiple complications in admission and elevated LDH, the patient evolved favorably with good response to treatment, achieving complete remission.

Methods

Cytogenetic and FISH (fluorescence in situ hibridization) analysis

Cytogenetic study was performed on bone marrow cells cultured in RPMI 1640 medium supplemented with 20% fetal calf serum during 24 h at 37 °C. G-banding technique was used. Chromosome abnormalities were described according to the International System for Human Cytogenomic Nomenclature [12]. The karyogram was performed using the Celleric SAS software.

FISH analysis was performed on the same material used for cytogenetic studies according to manufacturer's protocols. The IGH(14q32)/MYC(8q24) translocation dual fusion probe (Cytocell, Oxford, UK) and, the locus specific OLE13q14D13S319 and the whole chromosome painting PCT14 and PCT15 DNA probes (LiVE-Lexel, Buenos Aires, Argentina), were used. Image acquisition was performed using the Cytovision 3.9 Software (Applied Imaging Corporation, California, USA).

Results

Cytogenetic analysis on bone marrow cells showed a 46,XY,t(8;14)(q24;q32),t(14;15)(q32;q22)[20] karyotype (Fig. 1b and c). Normal metaphases were not found. FISH analysis using IGH/MYC probes confirmed two dual fusion signals corresponding to t(8;14)(q24;q32) as well as two green signals, one on the homologous der(14) and the other on der(15) corresponding to the t(14;15)(q32;q22). One red signal orthotopically located on chromosome 8 was also observed (Fig. 1d and e). The presence of t(14;15) was confirmed using the PCT14 and PCT15 DNA probes (**Fig. 1**f). The analysis with the D13S319 probe showed a normal pattern of signals.

Discussion

As reported by the Onco-Pediatric Argentine Registry (ROHA) [13], malignant lymphoma is the third cause of childhood cancer in our country, representing 11.7% of all cases between 2000 and 2016. Among them, 66.3% were non-Hodgkin lymphomas, corresponding 31.4% to BL patients. As known, *MYC* translocations are the genetic hallmark of BL, but additional genomic alterations are needed for BL oncogenesis **[14].** Secondary chromosome abnormalities are observed in 60–90% of cases, some of them with prognostic significance [2–5,15]. On the contrary, the coexistence of dual translocations of chromosome 14, as observed in our patient, is a very uncommon event in this pathology. To our knowledge, there are 20 BL/L reported cases (10 males and 10 females) with both chromosomes 14 rearranged [**8**] (Supplementary Table S1). Interestingly, only 3 cases (15%) were children or adolescents [**16**–**18**], supporting the infrequent observation of dual chromosome 14 translocations in pediatric BL/L patients. At present, it is not clear the impact of the alteration of both IGH locus on selective advantages to the neoplastic clone. A recent study in pediatric BL [**19**] using whole genome sequencing found 8% cases with *IGH* translocations to non-*MYC* partners, supporting that other oncogenes than *MYC* can be activated by juxtaposition with the IGH locus in this pathology. In addition, the authors found that in one case the *IGH* non-*MYC* translocation occurred earlier than the *IGH-MYC* translocation, suggesting that they could cooperate with *MYC* in the pathogenesis of BL.

The literature describes only one pediatric BL patient with a t(14;15) as a secondary chromosomal abnormality, but with breakpoint at 15q15 band [16]. A dup(1)(q12q32), associated with disease progression and poor outcome [2,7], was also observed in his karyotype. This patient had a rapidly progressive clinical course with central nervous system involvement, without response to therapy, and died with severe neutropenia and associated complications. On the contrary, the present case involving chromosome15q22 band, had a favorable clinical course with good response to treatment, achieving complete remission. Interestingly, in a series of BL cases with deletion 13q described by Nelson et al. [17], the authors reported one pediatric case with a t(14;15)(q32;q21) but no information about this patient is available.

The analysis of secondary chromosome alterations in BL patients using comparative genomic hybridization [2] found more changes in cases in the leukemic phase, suggesting that these patients were more predisposed to carry chromosomal alterations than those without bone marrow involvement. Furthermore, Murga Penas et al. [6] detected more structural than numerical changes in both BL cell lines and primary BL samples.

To date, it is not clear whether 15q alterations have a role in BL development. Particularly, the 15q22 region contains several candidate genes [20]. Among them, results of interest *RPL4* (*Ribosomal Protein L4*) and *VPS13C* (*Vacuolar Protein Sorting 13c*) genes. *RPL4* encodes a ribosomal protein that is a component of the 60S subunit, involved in the cellular process of protein translation. It has been found overexpressed in different Burkitt cell lines like Daudi and Raji (biogps.org/#goto=genereport&id=6124) and a recent report [**21**] using BL2 Burkitt cell line showed its role, together other ribosomal proteins, in the transcriptional regulation of *CD40* expression. In reference to *VPS13C* gene, in normal human tissues it has demonstrated to have high expression in lymph nodes and spleen (ncbi.nlm.nih.gov/gene/54,832). This gene plays a role in the protein traffic between organelles, being necessary for mitochondrial function and the maintenance of mitochondrial transmembrane potential. *VPS13C* also participates in the regulation of mitophagia mediated by *PINK1*/*PRKN* in response to mitochondrial depolarization. Several *VPS* including *VPS13C* were found to have mononucleotide repeats, being regions of instability of microsatellites and mutations of *VPS* related to gastric and colorectal cancers [22]. More studies are required to support the possibility that some genes located in 15q22 could be related to lymphoid malignancies and may be involved in leukemogenesis in pediatric lymphomas.

Concluding, to our knowledge, this is the first pediatric BL/L patient with the presence of both t(8;14)(q24;q32) and t(14;15)(q32;q22) translocations. The description of new secondary alterations in this pathology as well as their clinical significance, add information to the biological characterization of BL, contributing to a higher accuracy in the diagnosis and/or prognosis of the disease as well as to refine the risk stratification of BL patients. More information about the concurrence of both translocations, the sequences involved and their impact on gene expression in pediatric BL may be of importance to identify new molecular mechanisms associated to neoplastic development.

CRediT authorship contribution statement

Mariana Quatrin: Methodology, Writing – original draft, Writing – review & editing, Visualization. **Claudia Pasti:** Methodology, Resources. **Silvina Romano:** Resources, Writing – original draft. **Belén Iarossi:** Methodology. **Vanesa Giménez:** Resources. **Virginia Schuttenberg:** Resources. **Alejandra Costa:** Resources, Writing – original draft. **Irma Slavutsky:** Conceptualization, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2021.10.006.

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