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LETTER TO THE EDITOR

Complex Karyotype in a Pediatric Patient with Double-Hit *BCL6* High-Grade B-Cell Lymphoma

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To the Editor:

Double-hit (DH) high-grade B-cell lymphoma (HGBCL) is a non-Hodgkin lymphoma (NHL) characterized by the presence of *MYC* and *BCL2* or *BCL6* rearrangements. The World Health Organization classification [1] only recognizes DH-HGBCL with *MYC* and *BCL2* rearrangements, due to their biologic features and genetic characteristics. In contrast, the International Consensus Classification [2] includes both DH-HGBCL with *MYC* and *BCL2* (HGBCL-DH-BCL2) rearrangements and with *MYC* and *BCL6* (HGBCL-DH-BCL6) translocations, but the last one as a provisional entity. Particularly, HGBCL-DH-BCL6 is characterized by the involvement of extranodal sites, a germinal center immunophenotype that is less likely to be $CD10^+$ but more likely to be $IRF4/MUM1^+$, less complex karyotypes, as well as different gene expression profiles and mutational landscape than HGBCL-DH-BCL2 [3–6]. HGBCL is a relatively frequent NHL subtype in adults, but is a very uncommon entity in pediatric patients, representing approximately 1% cases in several published series [6–10].

In this report, we present a 13-year-old male patient admitted to our hospital in November 2018. He presented a complex clinical picture including generalized bone pain, a history of clavicle and rib fracture in the last month, weight loss of 10 kg in the last 3 months, and asthenia. No adenopathy or visceromegaly was detected. Magnetic resonance imaging showed a bone lesion in the proximal third of the humerus (3 × 1.4 cm). Hematological studies revealed: white blood cell count 6.9 × 10^9/L (36% neutrophils, 30% lymphocytes, 14% monocytes), hemoglobin 9.9 g/dL, hematocrit 26%, and platelet count 230 × 10^9/L. Laboratory tests indicated spontaneous tumor lysis syndrome: urea 1.81 g/L, creatinine 4.47 mg/dL, uric acid 8.63 mg/dL, calcium 14.3 mg, phosphate 8.9 mg/ dL, lactate dehydrogenase 1890 U/L. Bone marrow (BM) aspiration showed 90% blast cell infiltration. Flow cytometry analysis of BM revealed a major abnormal mature large B-cell population expressing CD45, CD19, CD22, CD20, CD38, CD10 (Becton Dickinson, USA), and surface lambda light chain (Dako Corporation, Carpintero, USA). Cytogenetic analysis showed a complex karyotype: 47~49, XY, +X, t(3;16;14)(q21q27;q24;q32), t(8;9)(q24;p13), t(11;15)(q23;q22), +12, +18, −19, +21[cp18]/46, XY[2] (Figure 1A; Supporting Information). Fluorescence in situ hybridization (FISH) confirmed translocations and *MYC* rearrangement (Figure 1B–E); t(11;15)(q23;q22) was not previously described in this type of NHL [11]. It was decided to start with the pediatric NHL protocol of the Argentine Group for the Treatment of Acute Leukemias (GATLA)-BFM (Berlin–Frankfurt–Münster) [12–14]. After the first three blocks, new clinical deterioration was observed, with intense bone pain and renal failure, requiring hemodialysis. Imaging evaluation showed bilateral renal infiltration, as well as involvement of the vertebral bodies, liver, and testicles. Renal biopsy showed a diffuse proliferation of mostly medium-sized cells. By immunohistochemistry the neoplastic cells were positive for CD20, PAX5, BCL6, and BCL2, the last one supported by the

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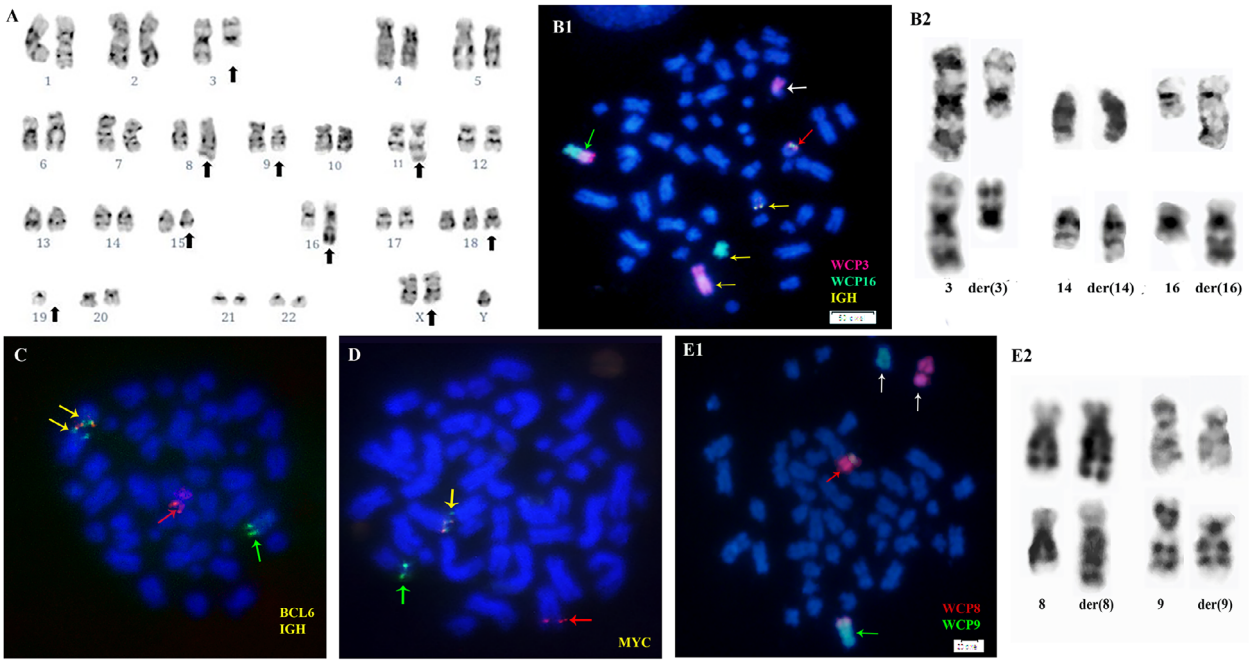

FIGURE 1 | (A) Complex karyotype of our patient showing translocations: t(3;16;14)(q21q27;q24;q32), t(8;9)(q24;p13), and t(11;15)(q23;q22), trisomy 18, monosomy 19, and gain of a chromosome X (arrows). (B1) Metaphase hybridized with WCP3 (whole chromosome paintings 3) (red), WCP16 (green), and IGH break-apart probes (LiVE-LEXEL, Buenos Aires, Argentina) showing the complex t(3;16;14) translocation. Normal chromosomes 3, 14, and 16 yellow arrows, der(16) green arrow, der(14) red arrow, and der(3) white arrow. (B2) Partial karyotypes showing translocation t(3;16;14)(q21q27;q24;q32). (C) Metaphase hybridized with BCL6 and IGH break-apart probes (Zytovision, Germany) showing normal chromosomes 3 and 14 (yellow arrows) and double fusions on der(14) (green arrow) and der(16) (red arrow). (D) Metaphase hybridized with MYC break-apart probe (Zytovision, Germany) showing MYC gene rearranged. Normal chromosome 8 (yellow arrow), der(8) (red arrow), and der(9) (green arrow). (E1) Metaphase hybridized with WCP8 (red) and WCP9 (green) painting probes (LiVE-LEXEL, Buenos Aires, Argentina) showing t(8;9)(q24;p13). Normal chromosomes 8 and 9 (white arrows), der(8) (red arrow), der(9) (green arrow). (E2) Partial karyotypes showing translocation t(8;9)(q24;p13).

presence of trisomy 18, and negative for CD3, CD10, MUM1, and TdT (Figure 2). MYC expression was observed in 5% of lymphoid cells. A germinal center B-cell-like (GCB) phenotype, suggestive of GCB molecular subtype, was defined. The Ki67 proliferation index was 95%. A diagnosis of relapsed/refractory HGBCL was made. The patient had no histoidentical donor. Second-line treatment with rituximab plus etoposide, cytarabine, cisplatinum, and methylprednisolone was started, followed by a third line with gemcitabine, vinorelbine, and pegylated liposomal doxorubicin, both with no response. He presented disease progression and clinical deterioration, and died 6 months after diagnosis was started.

In our country, lymphomas represent the third most common cause of childhood cancer (11.6% of all cases), with 28.25% of them NHL [15]. BCL6 is a transcriptional suppressor that has a leading role during germinal center formation and lymphomagenesis, participating in B-cell differentiation and proliferation [16, 17]. Particularly, pediatric cases with a HGBCL-DH-BCL6 with very complex karyotypes, like our patient, are very uncommon in the literature [11]. Our case showed a complex *BCL6* rearrangement, not previously reported in the literature, which only includes two cases with a complex translocation involving *BCL6* gene [t(3;14;18)(q27;q32;q21)], both in adult patients with diffuse large B-cell lymphoma [18, 19].

MYC gene products are transcription factors that control the expression of many genes. They are essential for multiple cellular functions including apoptosis, cell cycle regulation, proliferation, DNA repair, differentiation, stress response, metabolism, and protein synthesis [4, 20, 21]. Approximately 40% of HGBCL present non-*IG::MYC* translocations, commonly associated with complex karyotypes and probably related to disease progression [6, 22–24]. There are different genes involved in non-*IG::MYC* translocations, particularly *PAX5*, *ZCCHC7*, and *ZBTB5* at 9p13 [6, 18, 22]. Our case showed t(8;9)(q24;p13), commonly associated to HGBCL-DH-BCL2 [11, 22], in which the breakpoint on 9p13 was centromeric to *PAX5* gene (Figure S1). The literature shows less robust *MYC* expression and transcription levels in non-*IG::MYC* translocations than those observed in *IG::MYC* rearrangements [18, 24, 25], similar to the low *MYC* expression observed in our patient.

Different reports show controversial results about the clinical outcome of adult patients with HGBCL-DH-BCL6 [4, 24]. Some authors found an unfavorable clinical course, while others did not detect differences between patients with HGBCL-DH-BCL6 and HGBCL-DH-BCL2 [22] or observed a better overall survival in HGBCL-DH-BCL6 compared to cases with HGBCL-DH-BCL2 [3, 4, 26]. In addition, some reports did not find differences between cases with *IG::MYC* and non-*IG::MYC* translocations [22, 24], while others detected that non-*IG::MYC* partners had better survival [10, 23, 25, 27], and Li et al. [4] reported an aggressive clinical course for these cases. Our pediatric patient showed a very aggressive clinical course without treatment response and very short survival.

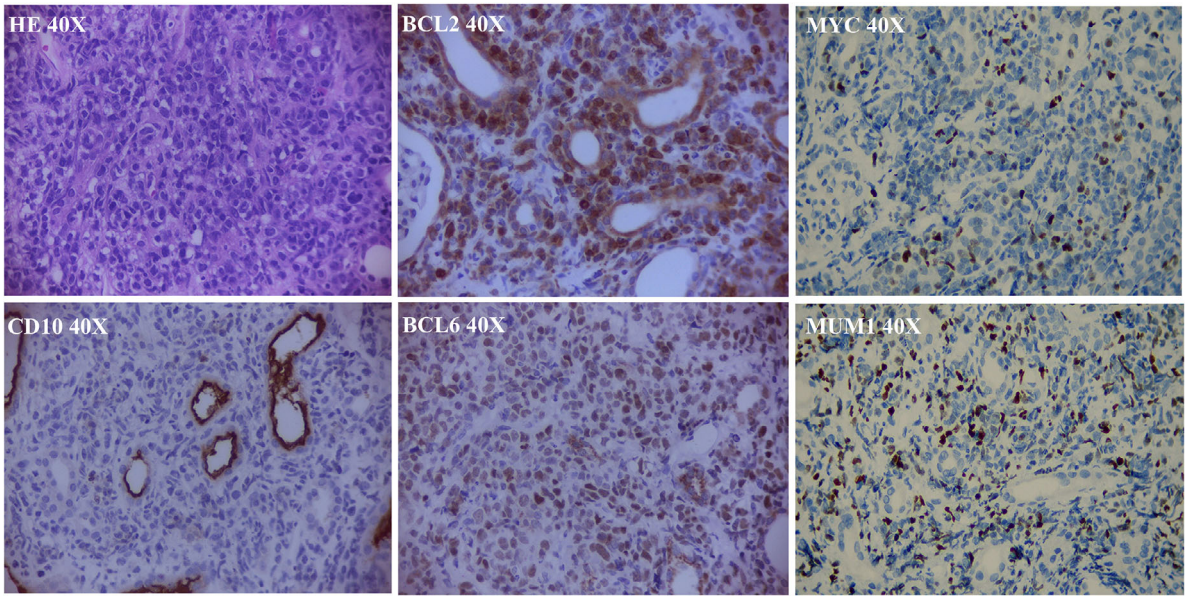

FIGURE 2 | Renal biopsy showing a high-grade B-cell lymphoma with *MYC* and *BCL6* rearrangements. Hematoxylin and eosin (H&E): renal parenchyma diffusely infiltrated by B medium-sized neoplastic lymphoid cells. Immunohistochemistry: neoplastic cells were BCL6 and BCL2 positives, CD10 and MUM1 negatives (0% and 10% [cutoff >30%], respectively) and showed low MYC expression (5%).

Concluding, we present a pediatric case with HGBCL-DH-BCL6 characterized by a new complex *BCL6* rearrangement, a non-*IG::MYC* translocation, and a not previously described t(11;15)(q23;q22), as a part of a complex karyotype that showed a poor outcome.

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Author Contributions

Mariana Quatrin and Irma Slavutsky: conceived and designed the study, wrote the draft of the manuscript, reviewed and edited the final version. Silvina Romano and Virginia Schuttenberg: cared for the patient and were responsible for clinical data. Laura Cabaleiro, Claudia Pasti, and Alejandro Laudicina: participated in cytogenetic and FISH analysis and interpretation of results. Belén Iarossi: performed flow cytometry analysis and interpretation of data. Paula Pérez, María Eugenia Altamirano, and Mauro García Montenegro: contributed with the anatomo-pathologic study. All authors read and approved the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.