

Diagnosing lymphoma in a setting with a high burden of infection: a pediatric case of Epstein-Barr virus-associated aggressive B-cell lymphoma with t(8;14) (q23;q32) and extensive necrosis mimicking tuberculosis

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ABSTRACT

The association of lymphoma with necrotic granuloma can pose diagnostic challenges and delay treatment, especially in settings with a high burden of infection. In these settings, the timely use of cytogenetic and molecular methods is most relevant. Here, we report a case of B-cell lymphoma with t(8;14) in a 5-year-old male child. The lymphoma was associated with necrotic granuloma and was initially misdiagnosed as tuberculosis. Polymerase chain reaction was used to detect clonal lymphoproliferation and to rule out *Mycobacterium tuberculosis* infection. Tumor cells harbored Epstein-Barr virus and expressed CD20, CD10, BCL6, and Ki67 (30%), leading to the diagnosis of B-cell lymphoma with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma.

Keywords: Non-Hodgkin lymphoma. Tuberculosis. Necrotic granuloma. Epstein-Barr virus.

INTRODUCTION

Non-Hodgkin lymphomas (NHLs) are a heterogeneous group of neoplasms that are usually diagnosed on the basis of clinical, histological, and immunohistochemical (IHC) characteristics. Molecular biology tools are required to confirm the diagnosis of a small but significant group of NHLs⁽¹⁾. In this group, molecular clonality analyses are relevant for differentiating reactive conditions from lymphoma, especially when the latter must be distinguished from infection-associated reactive processes⁽¹⁾⁽²⁾.

Granulomas are local inflammatory reactions that usually occur in association with infectious diseases and other disorders, but can occasionally occur in association with neoplasms⁽³⁾. Granulomas are characterized by the presence of histiocytes, which resemble epithelial cells; accordingly, the terms *epithelioid* or *sarcoid-like* are applied to granulomas. When

histiocytes aggregate in nodules that exhibit central necrosis, the granulomas are called *necrotic* or *caseating*⁽³⁾. Necrotic granulomas pose diagnostic challenges because they can be difficult to distinguish from tuberculosis, as a consequence of their overlapping pathological findings⁽⁴⁾⁽⁵⁾. Epithelioid granulomas are relatively common in Hodgkin lymphoma and also manifest in cases of NHL, although less frequently⁽³⁾. However, necrotic granuloma is an uncommon finding in lymphoma⁽⁵⁾ and, when present, it is a recognized cause of misdiagnosis and delayed treatment.

Here, we report a pediatric case of B-cell lymphoma that was positive for Epstein-Barr virus (EBV) and associated with necrotic granuloma. Further, we describe the algorithm that was used for the difficult differential diagnosis between tuberculosis and subtypes of lymphoma.

CASE REPORT

A 5-year-old boy was admitted to the Hospital Universitário Oswaldo Cruz, Recife (Northeast Brazil) with an enlarged left cervical region and a 4-month history of evening fever. Blood analyses showed a leukocyte count of 13,600/mm³, a platelet count of 254,000/mm³, and a hemoglobin level of 11.2g/dl without any immature or neoplastic circulating cells. The patient's lactate dehydrogenase level was 589U/l (reference range, 150-380 U/l). Computed tomography scans

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of the chest and abdomen were normal. Prophylactic antibiotic therapy was prescribed and, since the child's condition did not alleviate, a lymph node biopsy was performed. The initial clinicopathological and epidemiological risks factors (exposure, family history, and geographic risk area) pointed to the diagnosis of tuberculosis. The differential diagnoses were fungal infection and lymphoma.

Tuberculostatic agents were prescribed despite an inconclusive tuberculin test. At low magnification, histopathology of the biopsied lymph node showed large areas of necrosis with about 30% of areas preserved. The necrotic areas were surrounded by epithelioid histiocytes and multinucleated cells (Langhans' giant cells). In the preserved areas, most of the cells were typical lymphocytes. However, groups of atypical medium and large cells were also observed, as characterized by a basophilic cytoplasm, a single centrally located vesicular nucleus with fine chromatin, and 2-3 membrane-bound nucleoli (**Figure 1 A-D**). Ziehl-Neelsen staining for acid-fast bacilli and Grocott hexamine-silver staining for fungi were negative.

Immunostaining was performed with a panel of monoclonal antibodies, including CD20 (L26), CD3, CD30 (Ki-1), BCL2 (124), MUM1 (MUM1p), CD138 (MI15), MIB1 (Dako, Carpinteria, CA, USA), CD10 (56C6), BCL6 (P1F1), and TdT (Novocastra, Leica Biosystems, Nussloch, Germany). Detection of primary antibodies was performed with a labeled streptavidin

biotin visualization system (Dako), except for MIB1/Ki67 immunostaining, which was extensively repeated using an Envision kit (Dako). Large lymphocytes were observed to express CD20 (**Figure 1E**), CD10 (**Figure 2A**), and BCL6, but were negative for CD3 (**Figure 2B**) TdT, CD30, CD138, MUM1, and BCL2. These findings were compatible with mature B-cell lymphoma. Ki67 was expressed by about 30% of the cells (**Figure 1F**).

EBV was detected in tumor cells by EBV-encoded RNA *in situ* hybridization (**Figure 2C**), with a latency I pattern defined by the negativity of LMP1 immunorexpression. Testing for *Mycobacterium tuberculosis* was performed with a sensitive nested-PCR assay⁽⁶⁾ and produced a negative result.

B- and T-cell clonality were investigated by using polymerase chain reaction (PCR) assays to examine immunoglobulin heavy chain (IGH) and T-cell receptor gamma gene rearrangements in DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tissue. An IGH monoclonal gene rearrangement was detected by PCR, confirming the diagnosis of B-cell NHL (**Figure 2D**).

The presence of t (14;18) and t (8;14) were investigated by fluorescence *in situ* hybridization (FISH) using LSI® IGH/BCL2 CEP®18 and MYC/IGH CEP®8 probes, while *BCL6* rearrangements were investigated using the LSI® *BCL6* Dual Color Break Apart Probe (Vysis-Abbott, Des Plaines, IL, USA).

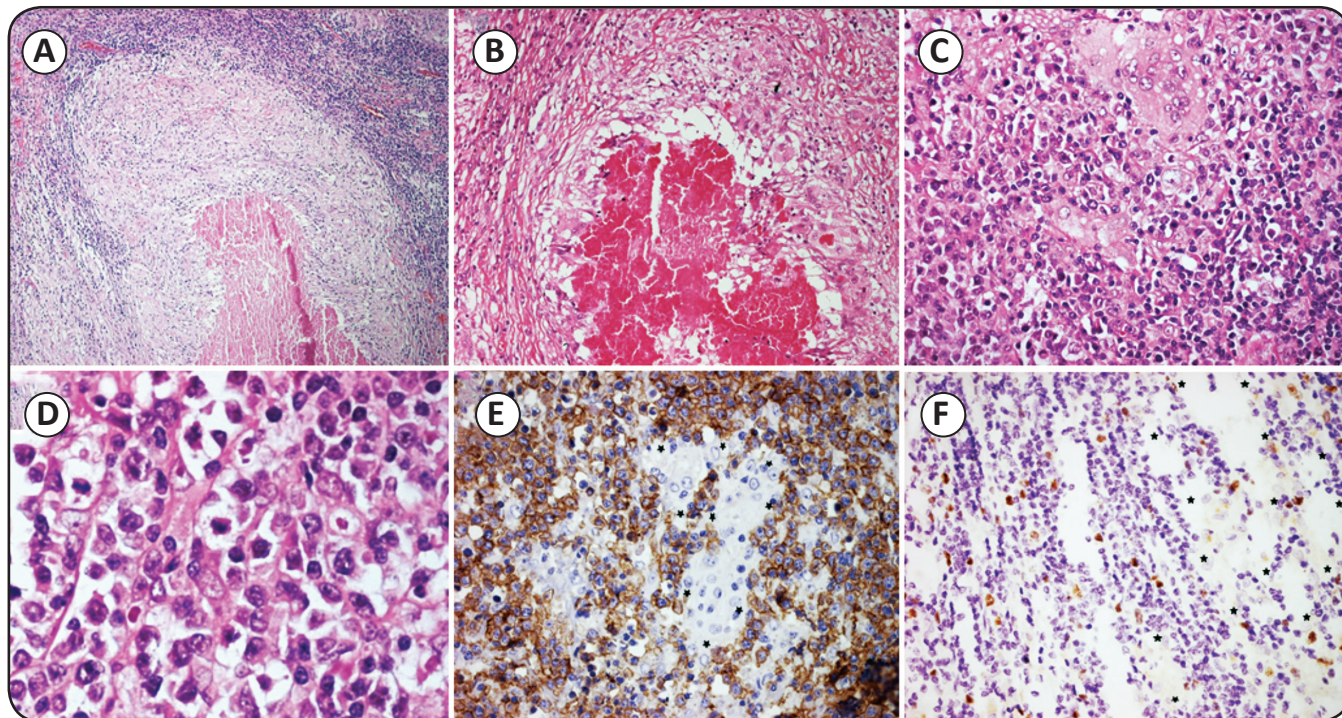


FIGURE 1 - Histopathological and immunohistochemical characteristics. A and B: Large areas of necrosis with granulomatous reaction (hematoxylin and eosin [HE]; original magnification 100× and 200×, respectively). C: Typical lymphocytes, epithelioid granuloma, and neoplastic cells evident in the few preserved areas (HE, original magnification 200×). D: Atypical malignant lymphocytes characterized by appreciable amounts of cytoplasm and a predominantly single, centrally located nucleus (HE; original magnification 400×). E: Atypical lymphocytes showing CD20 membrane immunorexpression (original magnification, 400×). Stars indicate granulomas. F: Nuclear immunorexpression of Ki-67 in a few neoplastic cells (original magnification, 400×). As previously, stars indicate granulomas.

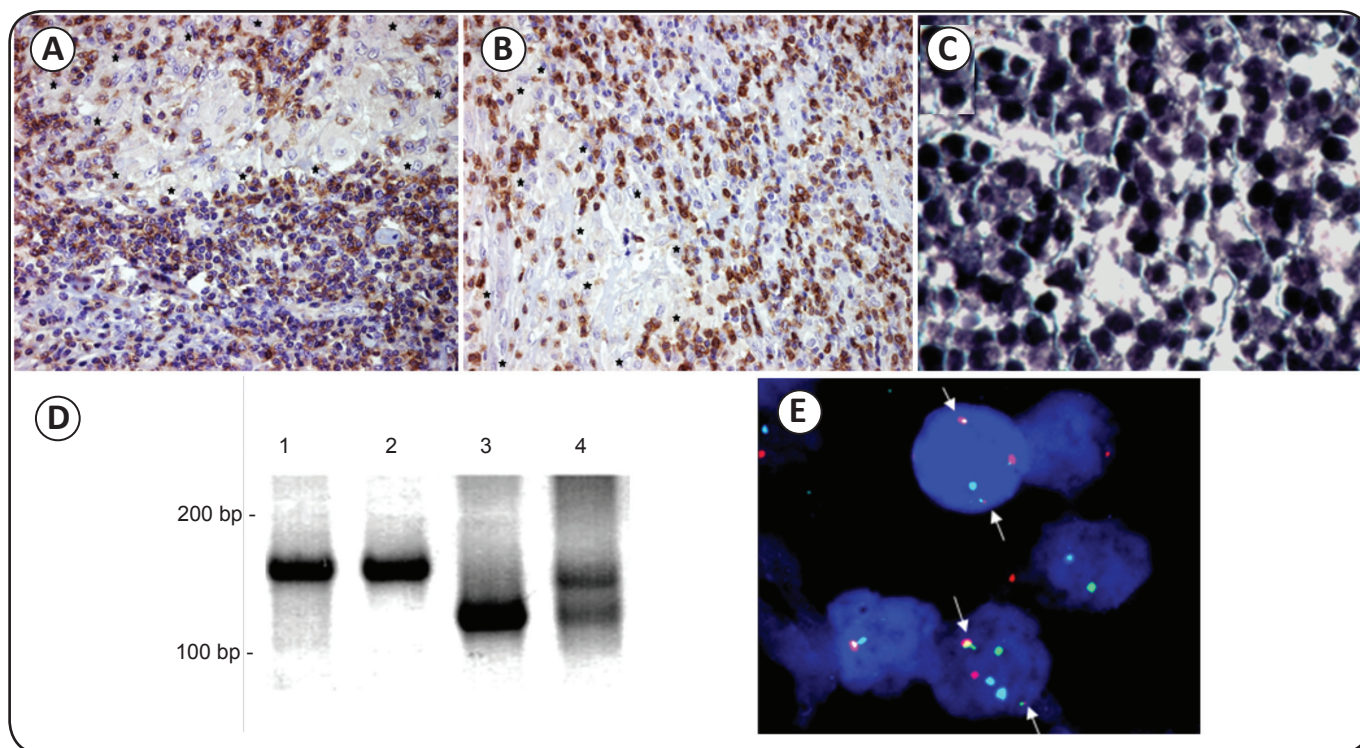


FIGURE 2 - Immunomolecular features. A: Neoplastic lymphocytes showing CD10 immunoexpression (original magnification, 400×). B: CD3+ reactive lymphocytes represented by membranous brown staining (original magnification, 400×). C: Epstein-Barr virus-encoded RNA *in situ* hybridization showing intense blackish nuclear staining in malignant lymphocytes (original magnification, 400×). D: Fluorescence *in situ* hybridization (FISH) images of paraffin-embedded interphase nuclei counterstained with DAPI. Tumor cells with t (8;14) were detected with a combination of IGH (green), MYC (red), and chromosome 8 centromere (aqua) probes. Derived translocation products with adjacent MYCIGH signals are indicated by arrows. The analysis was performed with an Olympus X-60 microscope with a monochromatic CCD camera (Photometrics, Tucson, AZ, USA) and QUIPS software (Vysis-Abbott, Des Plaines, IL, USA); E: Analysis of IGH gene rearrangements using FR3/JH PCR amplification. Lanes 1 and 2: the patient's clonal PCR product; DNA extracted from paraffin-embedded tumor samples (crude lysate and 1:10 dilution, respectively). Lane 3: positive control. Lane 4: polyclonal control (reactive hyperplasia); 8% polyacrylamide gel electrophoresis and silver staining.

FISH analysis revealed *MYC*/IGH fusion signals in tumor cells (Figure 2E), and ruled out the presence of t(14;18) and *BCL6* rearrangements.

In summary, classical and molecular diagnoses ruled out infectious agents, while analyses of clonality, morphology, and immunohistochemistry together led to the final diagnosis of B-cell lymphoma bearing t (8;14). The total period that had elapsed from the onset of symptoms to the final diagnosis was 5 months and 12 days. The patient was treated according to a Brazilian pediatric NHL protocol, achieving complete remission for 20 months. However, relapse ensued and he underwent allogeneic bone marrow transplantation. After 70 months of follow-up, the patient is in complete remission.

Ethical approval for the publication of this case report was granted by the Hospital Universitário Oswaldo Cruz. The patient's guardians provided written informed consent for the publication of the details of his diagnosis and treatment.

DISCUSSION

In this case, the diagnosis of lymphoma was problematic because clinical and epidemiological characteristics of tuberculosis were both present. Further, the histopathological analysis was compromised by the scarcity of malignant cells, which were masked by large extensions of necrosis and granuloma. In agreement with previous reports^{(4) (5)}, the coexistence of granuloma and lymphoma was an important factor in the delayed diagnosis of lymphoma and treatment of the present case.

Clonality studies were relevant to achieving a definitive diagnosis in our case, indicating that methods of detecting clonality should be included early in differential diagnosis schemes for similar cases. PCR assays for B- and T-cell clonality detection are highly standardized methods that can be implemented in the routine diagnosis of lymphoma and

applied to DNA that has been extracted from various sources, including FFPE specimens^{(1) (2)}. Of note, PCR results that are relevant to the diagnosis of lymphoma should be interpreted in the context of both clinical and pathologic findings. For example, these findings can help to determine the nature of areas of lymphoid proliferation outside the context of a germinal center in a lymph node after IHC analysis.

In our case, diagnostic management also required the use of conventional and molecular methods to rule out infectious agents. The use of molecular methods is important, since associations with tuberculosis have been previously reported in cases of leukemia and lymphoma that exhibit necrotic granuloma⁽⁷⁾ and, in some of these cases, the association was only disclosed after *Mycobacterium*-specific PCR assays.

In the present case, once NHL had been conclusively diagnosed, it was still challenging to determine whether the NHL belonged to the Burkitt lymphoma (BL) or diffuse large B-cell lymphoma (DLBCL) subclassifications. The diagnosis of BL was favored by CD10, CD20, and BCL6 expression, as well as by the presence of t(8;14) in tumor cells. In contrast, the diagnosis of DLBCL with *MYC* rearrangement was favored by the low proliferative index (~30%), which was mainly relevant because BL should be diagnosed with a Ki67 fraction close to 100%⁽⁸⁾, the disease's indolent clinical behavior, and the morphologic feature of atypical cells.

A small subset of mature B-cell lymphomas has characteristics that are intermediate between BL and DLBCL, although these lymphomas occur more frequently in adults than in children⁽⁹⁾. Moreover, *MYC* rearrangement is observed in up to 25% of DLBCL, while a fraction of otherwise classical BL may not harbor a *MYC* translocation^{(8) (9)}. The present case extends the description of B-cell lymphomas with intermediate characteristics to include pediatric lymphoma with some pathological features that may reflect the participation of infectious factors, such as EBV.

In fact, the pathophysiology of granuloma is poorly understood. Inflammatory reactions to tumor-associated antigens and the production of cytokines by tumor cells have both been proposed to have etiologic roles for granulomas^{(3) (5) (7)}. Granulomas have also been described in association with EBV in a subgroup of nonendemic BL, as a consequence of T-cell-mediated response to EBV-associated antigens⁽¹⁰⁾. The authors of that report proposed that their patients' granulomas would have been consequences of inflammatory CD4+ T-cell-mediated responses against EBV nuclear antigen 1 epitopes, highlighting the complex nature of this pathological condition.

In conclusion, our case exemplifies some of the obstacles that can be encountered during the diagnosis of high-grade childhood NHL, especially in settings with high burdens of infection. Furthermore, this case shows how molecular techniques can be included in the diagnostic workflow in an appropriate manner. Diagnostic algorithms can be improved by timely use of molecular analyses and interpreting their results in the context of histopathological findings.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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