Diagnosis and Classification

3

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Introduction

Non-Hodgkin lymphomas (NHL) are typical tumors for the pediatric pathologist to be familiar with as they represent around 7–10% of pediatric malignancies, the fourth most common one in children, being more frequent between 15 and 19 years of age [1–3]. These lymphomas could arise in any tissue, nodal or extranodal, and the pathologist should be prepared to diagnose its histological type according to the updated revised 2016 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues [4]. When dealing with a suspicious diagnosis of NHL, the pathologist should follow four major rules:

1. Eliminate the most frequent entities that might have a peculiar clinical or histological presentation before suggesting a rare subtype
2. Have the knowledge of entities that have not been described or are very rare in this age group, and consider therefore a reactive process mimicking NHL before assessing NHL
3. Consider a potential immune deficiency with an expansion of B or T cells before assessing a diagnosis of NHL, and avoid making a diagnosis without the full clinical information. It is therefore crucial before any diagnosis to rule out concurrent Cancer Predisposition Syndromes [5], Primary or Secondary Immunodeficiencies or Genetic diseases.
4. Ensure the use of ancillary techniques to confirm the diagnosis.

In this chapter, we will present the new updated 2016 revision of the WHO, discuss the importance of sampling (cytology, needle biopsy, open wedge biopsy) for diagnosis, and present the different techniques considered today as being instrumental for an optimal diagnosis of childhood and adolescence NHL.

The Revised 2016 Classification of Lymphoid Neoplasms

This revised 4th edition of the WHO classification includes some changes linked to a better understanding of some entities with a common agreement consensus between members of the European Association for Haematopathology, the Society for Hematopathology (US), as well as an international clinical advisory committee. The importance of an international nomenclature is crucial when comparing NHL arising in children within different countries, and the pathologist classifying the tumor should stick to these entities. There is no space for a pediatric classification of NHL. Although some entities are predominantly described in children, they can arise rarely among patients >18 years of age. However, a few entities are different (clinically and molecularly) from the classical adult type, giving rise in the WHO nomenclature to a "pediatric-type" to underline this specificity. On the other hand, some entities described in adults (mantle cell lymphoma, angioimmunoblastic T-cell lymphoma) have not been clearly described in children. The WHO classification underlines very strongly the importance of multiparametric approach to classify a tumor and this is even more important considering childhood NHL. The clinical presentation, the age, the morphologic pattern, the phenotype by immunohistochemistry and if possible flow cytometry, the genetic profile (translocation, gain or loss, mutations, expression profiling, etc.), and presence of blood tumor cells are all important factors to consider in the final diagnosis. This multiparametric concept should lead to collaborative efforts between pathology, hematopathology, and molecular and cytogenetics laboratories for an optimal

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diagnosis of childhood and adolescence NHL. At least, all these data should be gathered in a unified and integrated consolidated report, with the pathology report. The pathologist facing a suspicion of childhood and adolescence NHL should be familiar with the whole NHL classification. This classification differentiates precursor cell lymphoid neoplasm (Table 3.1) (either B, T, or NK lineage) from mature B-cell (Table 3.2) and T-cell neoplasms (Table 3.3) and immune deficiency-related lymphoproliferative disorders (Table 3.4). The revised WHO classification differentiates also provisional entities that need more data and studies to consider them as well as distinct entities. In addition, we believe, considering children NHL that it is crucial to differentiate entities well described in children from the ones classically not reported in children or rarely described and we decided to mention this differentiation in the tables (Tables 3.1, 3.2, 3.3, and 3.4). To us, this distinction is important to avoid a misdiagnosis when facing a morphologic lesion suspicious of a lymphoma entity non-described in children. In such cases, it is important to eliminate a reactive lymphoid process that may mimic a rare or non-described NHL subtype. For example, primary immune deficiencies such as children with *RAG-1* hypomorphic deficiency might have huge polyclonal expansion of T cells in the spleen or bone marrow mimicking mature peripheral T-cell lymphoma (Fig. 3.1). When dealing with a diagnosis of NHL in children, few histological subtypes cover more than 80–90% of the cases, and these are mainly aggressive lymphoid neoplasms: lymphoblastic B or T, Burkitt and diffuse large B-cell lymphomas, anaplastic large cell lymphomas, and post-transplant or primary immune deficiency-related lymphoproliferations. However, some of these classical pediatric subtypes might present with an unusual histological or clinical pattern, in unusual sites, that can be challenging for diagnosis. This is the case, for example, of a primary central nervous system ALK+ anaplastic large cell lymphoma, presenting with a small cell variant morphology (Fig. 3.2). Nevertheless, other rare subtypes are classically described among children and might be difficult to diagnose requiring full clinical information and ancillary techniques such as double staining combining in situ hybridization and immunophenotyping or molecular techniques.

Table 3.1 2016 WHO classification of non-Hodgkin lymphoma, precursor lymphoid neoplasms

B-lymphoblastic lymphoma/leukemia, NOS
B-lymphoblastic leukemia/lymphoma, NOS with recurrent genetic abnormalities
T-lymphoblastic leukemia/lymphoma
NK-lymphoblastic leukemia/lymphoma[a]

Bold: well described NHL in the pediatric population
[a]Provisional entity according to WHO

Table 3.2 2016 WHO classification of non-Hodgkin lymphoma, mature B-cell lymphomas

Chronic lymphocytic leukemia/small lymphocytic lymphoma, Monoclonal B-cell lymphocytosis, B-cell prolymphocytic leukemia, Splenic marginal zone lymphoma, Hairy cell leukemia, Splenic B-cell lymphoma/leukemia, unclassifiable[a]*, Splenic diffuse red pulp small B-cell lymphoma*[a]*, Hairy cell leukemia-variant*[a]*, Lymphoplasmacytic lymphoma (Waldenstrom macroglobulinemia), Mu heavy chain disease, Gamma heavy chain disease*
Alpha heavy chain disease
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
Nodal marginal zone lymphoma**, Pediatric nodal marginal zone lymphoma**[a]
Follicular lymphoma, In situ follicular neoplasia, *Duodenal-type follicular lymphoma*
Pediatric-type follicular lymphoma
Large B-cell lymphoma with IRF4 rearrangement[a]
Primary cutaneous follicle center lymphoma, Mantle cell lymphoma, In situ *mantle cell neoplasia*
Diffuse large B-cell lymphoma (DLBCL), NOS Germinal center B-cell type
Diffuse large B-cell lymphoma (DLBCL), NOS Activated B-cell type, *T-cell/histiocyte-rich large B-cell lymphoma*, Primary DLBCL of the CNS, *Primary cutaneous DLBCL, leg type*, EBV-positive DLBCL, NOS, EBV+ Mucocutaneous ulcer[a]*, DLBCL associated with chronic inflammation, Intravascular large B-cell lymphoma, Primary effusion lymphoma*, HHV8-positive DLBCL, NOS
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma
ALK-positive large B-cell lymphoma, Plasmablastic lymphoma
Burkitt lymphoma
Burkitt-like lymphoma with 11q aberration[a]
High-grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements, High-grade B-cell lymphoma, NOS
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

Bold: well described NHL in the pediatric population; Italic: classically not described below 18 years; Non-Bold Non-Italic: rarely described in children
[a]Provisional entity according to WHO

This is the case, for example, of an optimal diagnosis of chronic active Epstein–Barr virus (EBV) infection of T and NK cell type, systemic form, recently individualized in the revised WHO classification in which it is crucial to demonstrate that EBV-positive cells are of the T lineage (Fig. 3.3). It might be important at a national and/or international level such as the EICNHL (European Intergroup for Children NHL, with Japan and Hong Kong) and the North American Children's Oncology Group (COG) to have validated database of these rare NHL subtypes requiring both the analysis of those cases by a panel of pathologists (with detailed phenotypical and molecular analyses of the case) and very precise clinical information such as clinical Case Report Form (CRF) created by a panel of clinicians interested in rare subtypes. For example, the differentiation of chronic active

Table 3.3 2016 WHO classification of non-Hodgkin lymphoma, mature T and NK cell lymphomas

T-cell prolymphocytic leukemia, T-cell large granular lymphocytic leukemia, *Chronic lymphoproliferative disorder of NK cells*[a], Aggressive NK cell leukemia
Systemic EBV+ T-cell Lymphoma of childhood
Chronic active EBV infection of T- and NK-cell type, systemic form
Hydroa vacciniforme-like lymphoproliferative disorder
Extranodal NK/T-cell lymphoma, nasal type
Adult T-cell leukemia/lymphoma, Enteropathy-associated T-cell lymphoma, Monomorphic epitheliotropic intestinal T-cell lymphoma, Indolent T-cell lymphoproliferative disorder of the GI tract[a]
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis fungoides, *Sezary syndrome*
Primary cutaneous CD30-positive T-cell lymphoproliferative disorders
Lymphomatoid papulosis
Primary cutaneous anaplastic large cell lymphoma, Primary cutaneous gamma-delta T-cell lymphoma, Primary cutaneous CD8-positive aggressive epidermotropic cytotoxic T-cell lymphoma[a], *Primary cutaneous acral CD8-positive T-cell lymphoma*[a], Primary cutaneous CD4-positive small/medium T-cell lymphoproliferative disorder[a]
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma, Follicular T-cell lymphoma, Nodal peripheral T-cell lymphoma with TFH phenotype
Anaplastic large cell lymphoma, ALK-positive
Anaplastic large cell lymphoma, ALK-negative
Breast implant-associated anaplastic large cell lymphoma[a]

Bold: well-described NHL in the pediatric population; Italic: classically not described below 18 years; Non-Bold Non-Italic: rarely described in children

[a]Provisional entity according to WHO

Table 3.4 2016 WHO classification of non-Hodgkin lymphoma, post-transplant lymphoproliferative disorders (PTLD)

Plasmacytic hyperplasia PTLD
Infectious mononucleosis PTLD
Florid follicular hyperplasia PTLD
Polymorphic PTLD
Monomorphic PTLD (B- and T/NK-cell types)
Classical Hodgkin lymphoma PTLD

Bold: well described NHL in the pediatric population

EBV infection of T-cell type, systemic form, from the systemic EBV-positive T-cell lymphoma of childhood is very complex and could be better validated in the future by an international pathology and clinical study. Rather than using ICD-9 or ICD-10 codes for the creation of the database [6], it seems important to follow the ICD-O codes related to the WHO 2016 classification and to review old cases taking into account this new classification.

Cytology, Needle Biopsy, or Open Wedge Biopsy for Diagnosis?

A dogma for NHL diagnosis is that we need histopathology and therefore a tissue biopsy for the diagnosis. However, in children, in very few selected cases, with a typical clinical presentation, cytology with phenotype by flow cytometry and genetic profiling allows an adapted clinical management. This is the case for Burkitt lymphoma when the clinical presentation, the flow phenotype, and the FISH for MYC translocation are all typical and for lymphoblastic B-cell or T-cell lymphoma when the leukemic phase or marrow involvement (too low to be considered as acute lymphoblastic leukemia) is present at diagnosis and considered sufficient for the diagnostic laboratory for cytology, immunophenotyping, and molecular evaluation. What is really important in this cytological approach is that any unusual feature (clinical, cytological, phenotypical, or molecular) should lead to a histological evaluation.

The use of core needle biopsy for histological diagnosis of pediatric tumors is increasingly performed as it is the case in adult tumors, although at a lower frequency. A recent prospective study for diagnosis on non-nephroblastoma solid intraabdominal tumors has clearly shown the significant advantage of open wedge biopsy over needle biopsy for diagnosis [7]. However, a single-center retrospective study of 396 image-guided percutaneaous needle biopsies performed in children for pediatric tumors showed a diagnostic accuracy of 91%, underlining however the importance of 4 passes, and of the ability to freeze at least one core for molecular diagnostic tools [8]. Concerning lymphoma diagnosis suspicion, the ideal management of tissue sample requires touch imprints (Cytology, FISH), flow cytometry, freezing tissue for molecular techniques, and of course large amount of tissue for histopathological and immunohistochemical studies. Since lymphoma can arise as a primary tumor in any tissue (i.e., bone, skin), it might be useful to stain one imprint and to send a fresh sample for flow cytometry when lymphoma cannot be ruled out (i.e., small cell round tumor). These requirements highlight the importance of large amount of tissue samples for diagnosis and explain why an open wedge biopsy is preferred over a needle biopsy by most pathologists. Nevertheless, a diagnosis of NHL can be made on most needle biopsies if all the morphological and immunohistochemical criteria of a well-defined entity are present, but the pathologist should not make a definite diagnosis in all other cases. In addition, it is very difficult to exclude a diagnosis of lymphoma on a needle biopsy if the biopsied tissue is normal (i.e., normal architecture of lymph node and normal distribution of B and T cells) and if clinical suspicion is strong. Therefore, core needle biopsies performed by an interventional radiologist could be a

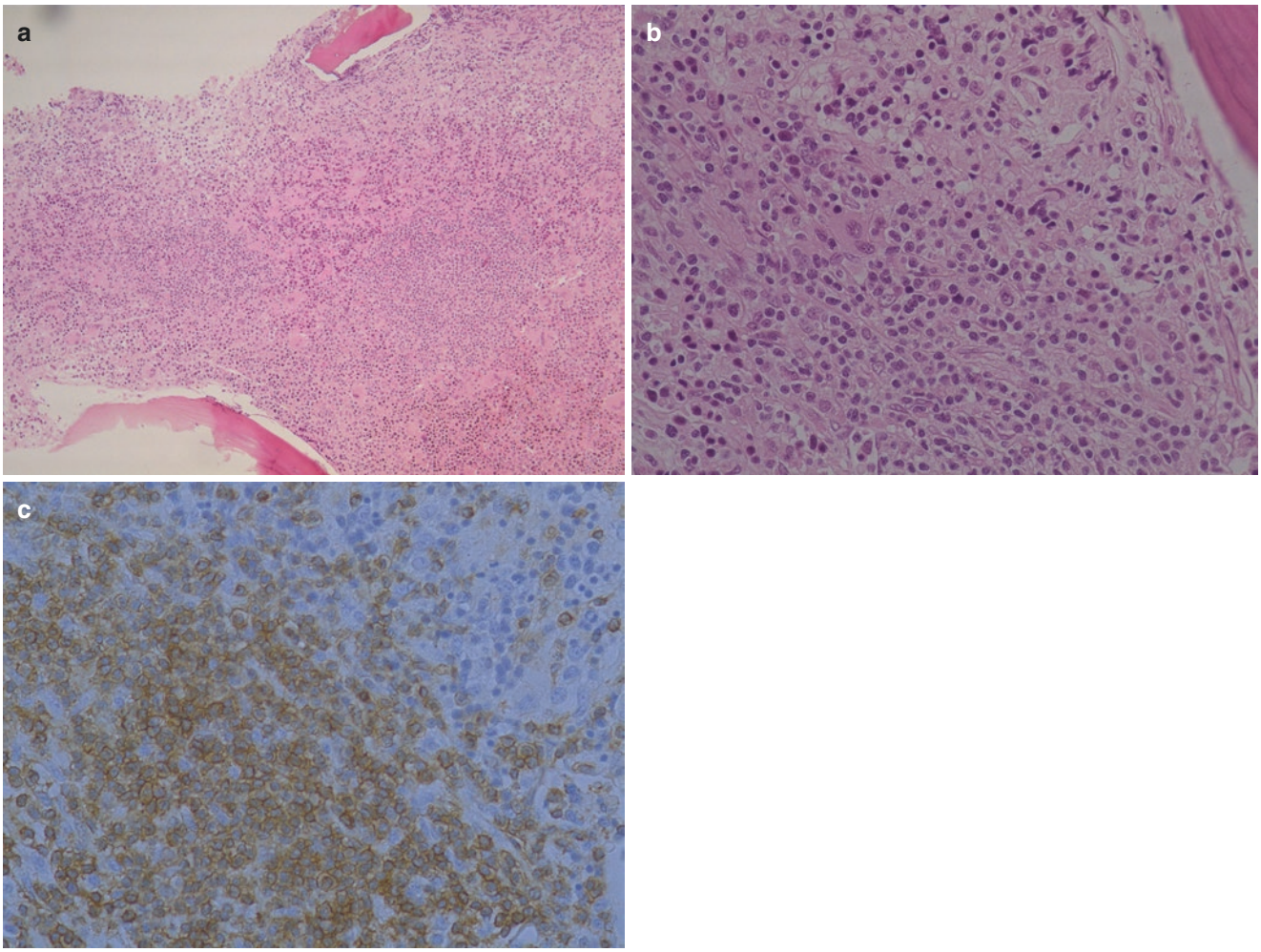

Fig. 3.1 Polyclonal Gamma delta T-cell expansion in the bone marrow of a 13-year-old *RAG1* hypomorphic patient with pancytopenia mimicking lymphoma. (**a**) at low magnification, infiltrate of small lymphoid cells destroying the normal architecture of the bone marrow (H &E stain). (**b**) at higher magnification this dense infiltrate is made of small lymphoid cells. (**c**) Most of these lymphoid cells are CD3-positive T cells

reasonable first diagnostic procedure in close collaboration with the pathologists and clinicians; however parents need to be informed that in a minority of cases, an open wedge biopsy might be needed for an optimal diagnosis. Open wedge biopsy is often the rule when dealing with chronic superficial adenopathies suspicious of a rare indolent pediatric lymphoma subtype (such as follicular lymphoma pediatric type) or in the context of possible primary immunodeficiency disease associated with a lymphoproliferation.

Ancillary Techniques

When dealing with the possible diagnosis of lymphoma, in addition to the classical paraffin-embedded analysis of histopathology, immunohistochemistry, and immunophenotyping, other techniques are currently required to classify the NHL according to 2016 WHO Classification. The immunohistochemical profile performed should stick to the phenotype described for each entity. When dealing with an undifferentiated small−/medium-sized blue cell tumors, in addition to the classical panel for non-lymphoid tumors, a first screen comprising CD79a, CD3, TdT, and CD30 will help to diagnose the most frequent lymphoid neoplasms. EBER in situ hybridization to detect the presence of EBV in tumor cells is widely used in the case of suspicious immune deficiency-related lymphoproliferations or EBV-associated lymphomas. The setup by the pathology laboratory of colorimetric double stain CD3/EBER, CD79a/EBER, CD20/EBER, and CD8/EBER to demonstrate the lymphoid lineage affected by EBV is crucial, as there is often B- and T-cell expansion in EBV-associated lymphoproliferations. Clonality tests by PCR to look for *IgH*, *Kappa*, *Lambda*, *TCR gamma*, and *TCR beta* rearrangements are very useful in difficult cases of B- or T-cell lymphoma or when discussing lymphoid expansions mimicking lymphoma; in most

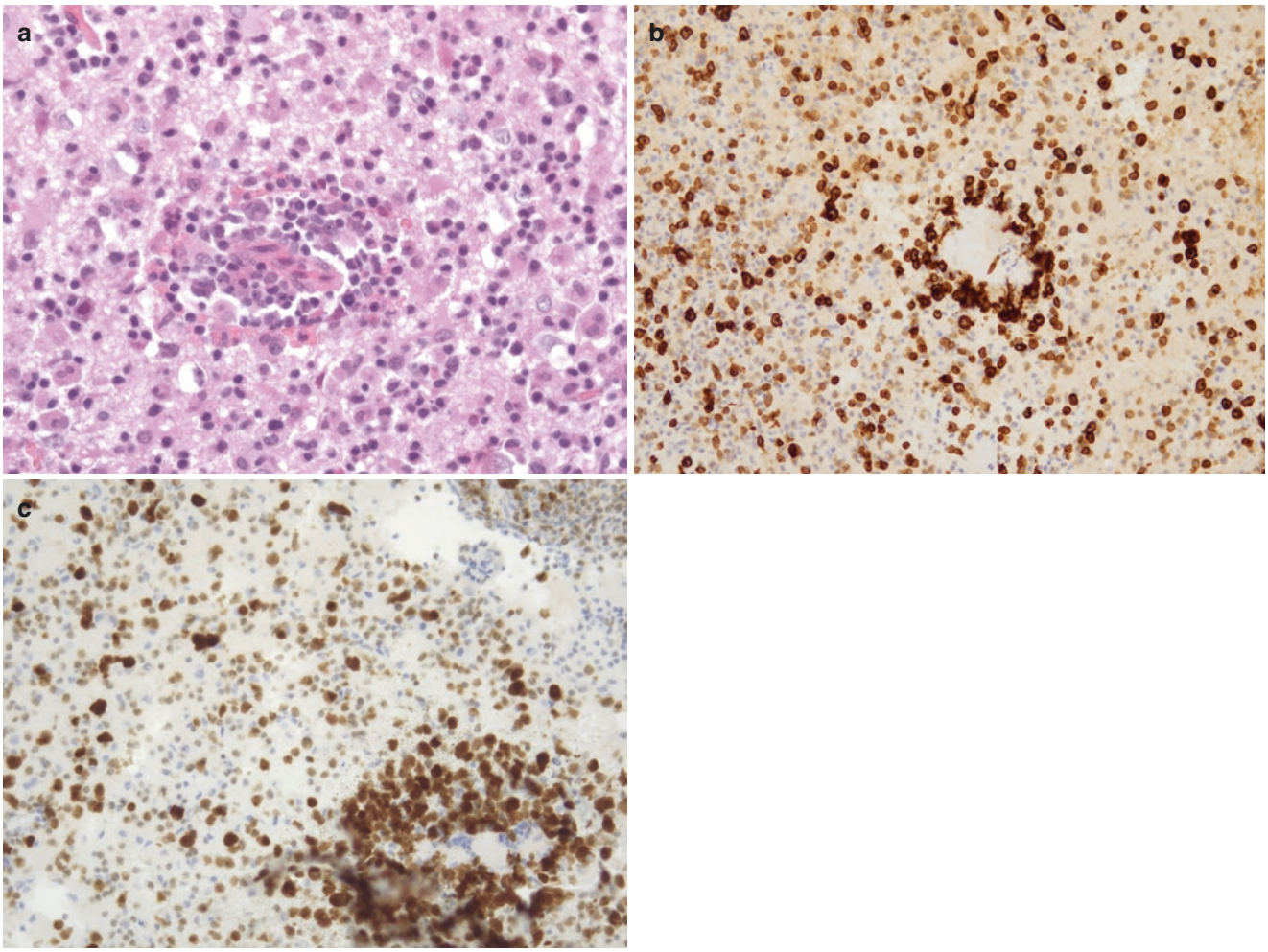

Fig. 3.2 Anaplastic large cell lymphoma, small cell variant occurring at presentation in the central nervous system (CNS) in a 5-year-old child. (**a**) Small lymphoid cell infiltration in a perivascular predominant topography of the central nervous associated with an interstitial infiltrate. (**b**) Strong expression of CD30 by small lymphoid cells. (**c**) Nuclear expression of ALK by tumor cells

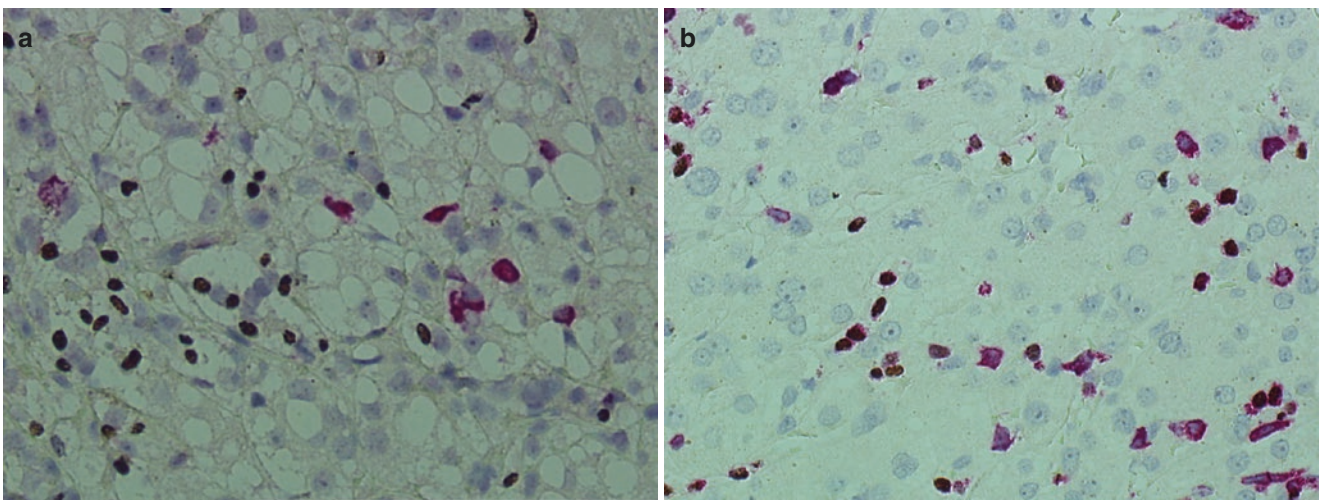

Fig. 3.3 Chronic active EBV infection of the T-cell type, Systemic, liver biopsy in a 15-year-old child with secondary HLH (hemophagocytosis lymphohistiocytosis). (**a**) Presence of a few CD79a-positive B-cell and plasma cells (red) associated with numerous EBER-positive CD79a-negative cells (black nuclear stain), double staining CD79a/EBER. (**b**) the EBER-positive cells (black nuclei) have all CD3-positive cytoplasmic staining (red) whereas some CD3-positive cells are not EBER-positive, double staining EBER/CD3

cases, formalin fixation allows a good interpretation of B- or T-cell receptor repertoire, although DNA extracted from frozen samples might be important to retrieve in some cases. However, the use of these tests should be very cautious as B-cell or T-cell clones can be present in reactive states and false negatives can occur in true lymphomas. A complex question arising these days concerns the use of karyotype analysis for lymphoma diagnosis. This is a costly and labor-intensive technique with the setup of overnight cultures. The difficulty in predicting clinically a potential lymphoma diagnosis in the approach of a pediatric tumor and the knowledge of the major cytogenetic abnormalities arising in different lymphoma subtypes (i.e., translocations) that can be easily diagnosed by interphase FISH have convinced numerous hematological teams to stop the prescription of karyotype as a systematic first approach (with a few exceptions, for example, when clinical presentation is typical of Burkitt lymphoma) and rather to develop alternative molecular techniques. In this respect, the replacement of karyotype by comparative genomic hybridization (CGH) array to evaluate amplifications, deletions, and the complexity of karyotype combined with interphase FISH to diagnose a recurrent translocation is becoming a very efficient approach. The importance of these techniques in childhood and adolescence NHL is underlined by the fact that two new entities, although provisional, are defined by the presence of a translocation detected by FISH such as large B-cell lymphoma (follicular and/or diffuse) with *IRF4* rearrangement, and by proximal gains and telomeric losses detected by CGH array such as Burkitt-like lymphoma with 11q aberration. The use of flow cytometry from fresh cell suspension from a biopsy is very useful for the diagnosis each time there is a suspicion of lymphoma (lymphoblastic, Burkitt, etc.) as the panel of antigens studied is much larger than immunohistochemistry. Nevertheless, it is highly recommended that the diagnosis should never be performed by flow cytometry alone and be integrated with histopathological diagnosis. For example, a monotypic CD19+ CD10+ BCL2 negative Ki67+ 90%, lymphoid population may correspond to the phenotype of a Burkitt lymphoma but also to the phenotype of a follicular lymphoma pediatric-type. In addition, the study of a panel of mutations by next-generation sequencing (NGS), a targeted expression profiling technique from formalin-fixed paraffin-embedded tissue, could help to better define an entity in difficult cases such as in the differential diagnosis between Burkitt lymphoma and DLBCL [9] and/or between PMBL and DLBCL NOS [10]. Cell-free DNA analysis at diagnosis, through blood liquid biopsy, is a promising technique that has been shown in adult DLBCL to reflect with good sensitivity the main genomic abnormalities of NHL in the absence of a leukemic phase and to allow disease response monitoring through clonal evolution analysis in association with FDG-PET scan data [11].

Overall, all these tools should now be implemented to allow an optimal diagnosis of childhood and adolescence NHL. It underlines the importance of a multidisciplinary laboratory approach for the diagnosis of lymphoma gathering pathologists, biological hematologists, and geneticists ideally working together as a diagnostic unit for the diagnosis of lymphoma.

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