Review paper

Comprehensive histopathological diagnostics of aggressive B-cell lymphomas based on the updated criteria of the World Health Organisation's 2017 classification

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Revision of the fourth edition of the World Health Organisation (WHO) Classification of Haematopoietic and Lymphatic Tissues, which was published in 2017, introduced important changes updating the biology, pathology, genetics, and clinical presentation of aggressive B-cell lymphomas. High grade B-cell lymphomas (HGBLs) replaced B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, the new provisional entity Burkitt-like lymphoma with 11q aberration was identified, and some categories were upgraded, e.g. EBV-positive diffuse large B-cell lymphoma, not otherwise specified. Still the histopathological diagnostics is based on morphology and immunoprofile, but to define the HGBLs evaluation of *MYC*, *BCL2*, and *BCL6* gene statuses is required. According to the presented WHO criteria, in the comprehensive histopathological diagnostics of aggressive B-cell lymphomas a highly specialised diagnostic team including a pathologist, a molecular biologist, a geneticist, a haematologist, and immunophenotyping technicians is needed.

Key words: aggressive B-cell lymphomas, high grade B-cell lymphomas, Burkitt-like lymphoma,11q aberration.

Introduction

Aggressive lymphomas from mature B cells are a heterogeneous group of diseases that differ in biological, pathological, and clinical features. The World Health Organisation (WHO) classification updated at the end of 2017 distinguishes 18 entities (Table I) with distinct clinical, pathological, and

Table I. Aggressive, mature B-cell lymphomas according the World Health Organisation's 2017 classification

Aggressive, mature B-cell lymphomas
Diffuse large B-cell lymphoma, not otherwise specified Germinal centre B-cell type Activated B-cell type
T-cell/histiocyte-rich large B-cell lymphoma
Diffuse large B-cell lymphoma, topographic site related Primary mediastinal (thymic) large B-cell lymphoma Primary diffuse large B-cell lymphoma of the central nervous system Primary cutaneous diffuse large B-cell lymphoma, leg type Intravascular large B-cell lymphoma
Diffuse large B-cell lymphoma, EBV related EBV-positive diffuse large B-cell lymphoma, NOS Diffuse large B-cell lymphoma associated with chronic inflammation Lymphomatoid granulomatosis
Large B-cell lymphoma with terminal B-cell differentiation Plasmablastic lymphoma ALK-positive large B-cell lymphoma Primary effusion lymphoma HHV8-positive diffuse large B-cell lymphoma, NOS#
Burkitt lymphoma Burkitt-like lymphoma with 11q aberrations#
High grade B-cell lymphoma High-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements High-grade B-cell lymphoma, NOS
Mantle cell lymphoma Blastoid or pleomorphic variant
B-cell lymphoma, unclassifiable, with features intermediated between diffuse large B-cell lymphoma and classic Hodgkin's lymphoma

 # provisional entities

genetic features [1]. Some types of aggressive B-cell lymphomas are relatively frequent, i.e. diffuse large B-cell lymphoma, not otherwise specified (DLBCL, NOS) which represents 25-35% non-Hodgkin's lymphomas depending on the population; others occur rarely and refer to specific groups of patients. Prognosis and response to therapy are different in each clinical-pathological unit. Although currently used treatment regimens are effective in the majority of patients, about 30% of patients develop progression to incurable disease [1, 2]. The results of genetic and molecular research conducted in recent years allow a better understanding of the mechanisms underlying the clinical and biological diversity of these tumours and provide valuable information about new therapeutic options, and at the same time allow improvement of diagnostic criteria. The aim of this study is to present practical diagnostic recommendations necessary for the correct diagnosis of aggressive B-cell lymphomas. The novelty concerning the three aggressive B-cell lymphomas has been discussed in detail: DLBCL, NOS, high-grade B-cell lymphomas (HGBLs), and Burkitt lymphoma/Burkitt-like lymphoma with 11q aberration (BL/BLL,11q); for the remaining units, the most important features regarding clinical and pathological presentation as well as genetic changes are highlighted [1, 2]. Moreover, general rules for preservation of biological material and tips on supporting diagnostic techniques (immunohistochemistry, flow cytometry, genetics) are given.

Preservation of biological material

Various materials are evaluated in the diagnosis of lymphoid tumours. Samples most often originate from the main structures of the lymphatic system, which include lymph nodes, spleen and thymus, Waldeyer's ring (lymphatic tissue band surrounding the oral throat with palatal tonsils, and lingual and pharyngeal tonsils), appendix, and Peyer's patches in the ileum. Other areas containing lymphatic tissue concern: bone marrow, mediastinum, liver, skin, pleura and gonads. Patients admitted with suspicion of lymphoma for pathological diagnosis require biological material protection also for molecular, and/or cytogenetic tests. Therefore, it is necessary to provide a standard operating procedure for material processing, including the following major steps:

– tissue obtained for testing should be fresh and processed according to type of material; entirely excised lymph nodes should be cut into at least 5-mm thick slices along the long axis to ensure optimal penetration of a fixative;
– description of fresh material should include the size, colour, texture, and the presence or absence of macroscopically visible haemorrhages, necrosis fields, or nodular architecture;
– touch imprint cytological slides are made from freshly cut material surface and are fixed in alcohol or dried in the air;
– for cytogenetic tests (karyotype) a fresh tissue sample or viable cells from fine-needle aspiration biopsy (FNAB) should be collected into a sterile container with cell culture medium and antibiotics, always considering the requirements of the laboratory performing the test;
– immunophenotyping by flow cytometry (FCM) requires protection of a fresh tissue sample or viable cells from FNAB in an appropriate transport medium (e.g. RPMI-1640), always considering the requirements of the laboratory performing the test;
– always indicate the type of fixative used and if possible determine the estimated time from tissue col-

lection to fixation, because this is important for the recovery of RNA and phosphorylated proteins;
– for histopathological examination it is recommended to use a fixative based on 10% buffered formaldehyde (so-called formalin); most suitable for additional studies such as immunohistochemistry and fluorescence in situ hybridisation (FISH); to avoid dilution and formalin buffering problems the use of commercially available ready-to-use (RTU) solutions is strongly advised;
– to ensure optimal antibody reactivity preservation (for immunohistochemistry) extension of fixation time (over 24 hours for small tissue sections fixated in formalin) should be avoided;
– if the size/volume of biological material and infrastructure of the diagnostic centre allow, some samples should be biobanked through deep freezing (short-term storage in –80°C freezers, long-term storage in liquid nitrogen at –170°C). The freezing technique should be complementary to further material processing methodology; the most popular techniques are tumour snap frozen sections or tumour viable cell freezing.

General remarks on pathological diagnostics of aggressive B-cell lymphoma:

– histopathological examination sustains a gold standard in diagnostics; however, in most cases precise determination of lymphoma type requires at least one additional method including immunophenotyping, and molecular and/or cytogenetic tests [4, 5, 6, 7, 8, 9, 10]; in cases where material is inadequate or insufficient for diagnostic purposes, the clinician should receive feedback and justify the above condition;
– in certain circumstances, when a lymph node is not easily accessible for surgical biopsy and in patients requiring immediate treatment, FNAB biopsy in conjunction with FCM, karyotype, FISH for major translocation, PCR for *IGH*, and *TCR* gene rearrangement may be sufficient for diagnosis;
– haematopatholgy consultation should include review of all slides with at least one paraffin block representative of the tumour. Re-biopsy is recommended if material is non-diagnostic;
– immunophenotyping can be performed by immunohistochemistry or flow cytometry [8]; each method has its own advantages and disadvantages. Flow cytometry is fast (hours) and quantitative method with evaluation of multiple antigens simultaneously. Antigen detection, however, does not allow to correlate with tumour architecture and its cytological features. Immunohistochemistry requires hours, sometimes days, and the quantitative assessment is subjective, but its most important feature is the possibility of correlation of reaction with architecture and tumour cytology. Moreover, not all antibodies are available for immunohistochemical assessment, especially for fixed tissues, but the advantage of this method is the possibility of using it in the archival materials embedded in paraffin. Both techniques can be used in the lymphomas diagnostics and are a source of clinically relevant information (e.g. identification of molecules necessary for the use of targeted therapies such as CD20);
– the importance of molecular research in lymphoid malignancies is constantly growing and allows the determination of clonality and the origin of neoplastic cells. In specific entities, these tests are necessary to make a definitive diagnosis i.e., Burkitt lymphoma, Burkitt-like lymphoma, or high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements;
– for an interpretation of immunohistochemical reactions a descriptive semi-quantitative scale is adopted: (+) positive reaction, (+/–) partially positive reaction, (–/+) positive reaction in a few, (–) negative reaction in the majority of cells; the percentage of positive cells for each staining may be presented in square brackets;
– panB panel includes antibodies: CD19, CD20, CD22, CD79a, PAX5;
– typical DLBCL immunophenotype includes: CD45(+), CD20(+), CD3(–) Ki-67 > 40% positive cell nuclei;
– panel of IHC antibodies to establish DLBCL diagnosis and GCB vs. non-GCB origin: CD20, CD3, CD5, CD10, BCL2, BCL6, Ki-67, IRF4/MUM1, MYC with or without cell surface analysis by flow cytometry: κ/λ, CD45, CD3, CD5, CD19, CD10, CD20, CD71;
– additional IHC studies to establish lymphoma subtype: Cyclin D1, PAX5, CD30, CD15, CD138, CD38, Epstein Barr virus (EBV) *in situ* hybridisation (ISH), EBV/LMP1, ALK, HHV8, SOX11, CD23, BOB1, OCT2, CD56, κ/λ, EMA;
– the following cutoff points are recommended according to WHO classification: BCL2 ≥ 50% strong positive cells, MYC ≥ 40% strong positive cell nuclei, for CD10, BCL6, IRF4/MUM1 ≥ 30% positive cells/nuclei. The importance of choosing an appropriate antibody clone, especially among those that show reaction liability (i.e. BCL2, CD10, MYC), is highlighted. For routine pathological diagnostics only certified antibodies (in vitro diagnostics, IVD) simultaneously with positive and negative controls are advised;
– presence of EBV virus should be confirmed by intra-tissue hybridisation (EBER-ISH); it is a superior technique to immunohistochemistry (LMP1) (Fig. 1);
– karyotype and/or FISH for *MYC, BCL2, BCL6* rearrangements are recommended especially in cases

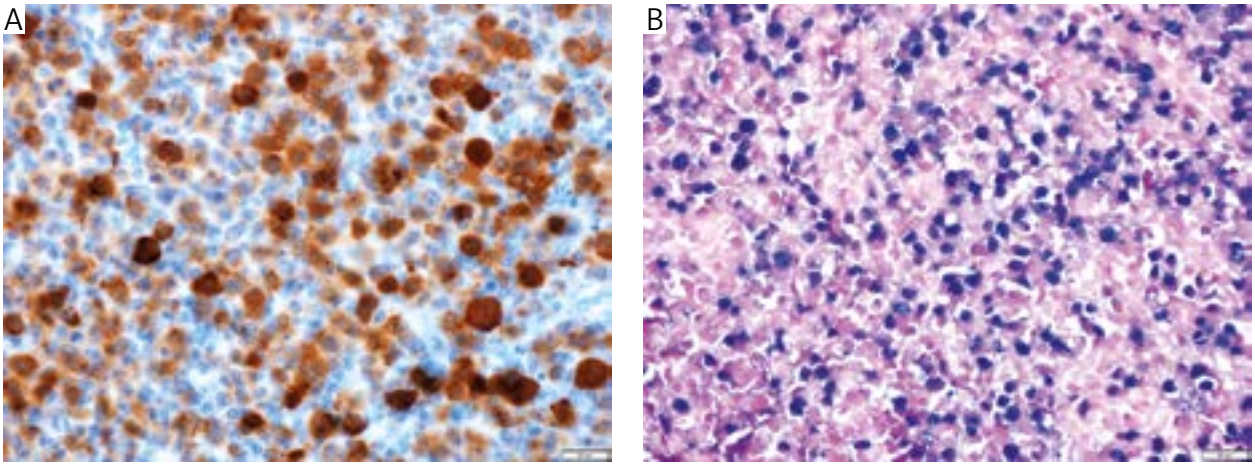


Fig. 1. EBV detection: A) immunohistochemical staining with LMP1 and B) EBER-ISH method (magnification 400×)

with double expression of MYC and BCL2 and/or having a GCB phenotype and high-grade morphology;
– despite advanced diagnostic methods, still there is a group of cases whose image does not meet all criteria and "escapes" from diagnostic guidelines; the pathologist, taking into account the clinical presentation and course, should maximally narrow the differential diagnosis. It is worth remembering that the most powerful predictive factor determining the therapeutic approach remains the pathologically confirmed type of lymphoma;
– diagnosis of lymphomas requires close cooperation between haematopathologists and specialists in flow cytometry and genetics as well as clinicians (oncologists, haematologists).

Diffuse large B-cell lymphoma, not otherwise specified

Diffuse large B-cell lymphoma, not otherwise specified (DLBCL, NOS) is morphologically, clinically and biologically heterogeneous. In developing countries and in selected Eastern European countries, such as Poland it represents 25-35% of non-Hodgkin's lymphomas and is a constant leader among all aggressive mature B-cell lymphomas [3, 4]. The identification of two molecular subtypes of this lymphoma based on gene expression profiling (GEP) was one of the most important achievements in understanding its diversity. Subtyping DLBCL, NOS is based on different cell origins and includes lymphomas derived from germinal centre cells (GCB) or from activated B-cells (ABC) [5]. Additionally, these DLBCL subgroups vary in the activated molecular pathways, chromosomal changes, and the occurrence of somatic mutations. In GCB type, the activation of the PI3K/AKT signalling pathway and over-expression of BCL6 protein are observed, whereas in ABC type there is a constitutive activation of the NF-κB pathway in the course of various mechanisms. Unique genetic changes, especially mutations such as *GNA13* and *EZH2* in GCB and *MYD88*, *CARD11*, and *CD79B* in ABC are found, respectively [6, 7]. Chromosomal translocations in DLBCL involving regions with *BCL6* (3q27), *BCL2* [t(14;18)(q32;q21.3)], and *MYC* (single hit) comprise, respectively, approximately 30% (with predominance in ABC subtype), 20-30% (more commonly in GCB subtype), and 8-14% (similar distribution among ABC and GCB subtype) [8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18]. It is estimated that approximately 50% of DLBCL with *MYC* translocation demonstrate *BCL2* and/or *BCL6* rearrangement and that cases should be transferred to the high-grade B-cell lymphoma category [1, 19, 20]. DLBCL with typical morphology and isolated *MYC* translocation usually present higher mitotic index, but still such cases meet the criteria of diagnosis of DLBCL, NOS [8, 21]. The pathologists are also discouraged from preselecting aggressive B-cell lymphomas to FISH testing upon Ki-67 result, while its range can be variable [21]. Biological differences translate into a distinct clinical course; so far in the majority of studies, a worse prognosis was observed among patients with DLBCL, ABC in comparison with GCB subtype. Moreover, the latest results of clinical trials concerning combining R-CHOP regimen with bortezomib, lenalidomide, and ibrutinib in the ABC subtype significantly stress the potential benefits of such treatment [22, 23]. The optimal method for the molecular classification of DLBCL is the study of the gene expression profile, but it requires fresh tissue and is not widely used in routine diagnostics. Due to its clinical impact, pathologists are still obligated to precisely identify the molecular subtype. An up-to-date Hans algorithm based on a panel of antibodies (CD10, BCL6, and IRF4/MUM1) determined by immunohistochemistry is available in most pathology departments and should be continuous-

ly applied [24, 25, 26, 27]. The compliance of IHC with GEP reaches about 80-90%, but a small group of about 10% of DLBCL cases "slip out" of molecular classification and the ABC/GCB match is not possible [28]. One of the limitations of IHC is also standardisation between haematopatholgy centres, including quality of staining and evaluation reproducibility among pathologists [28, 29]. Recently developed GEP tests based on RNA extracted from formalin-fixed and paraffin-embedded tissues (e.g. the Lymph2Cx assay from NanoString Technologies, Seattle, WA, USA) can identify DLBCL molecular subtypes [30]. Moreover, those methods are characterised by high compliance level with GEP microarrays and satisfactory reproducibility between laboratories [30, 31, 32]. Possibly, tests based on GEP will be a more precise diagnostic method than currently approved by the WHO IHC methods in future [1, 20]. The summary of DLBCL, NOS characteristic is presented in Table II.

High-grade B-cell lymphomas

High-grade B-cell lymphoma (HGBL), with gene expression profile intermediate between molecular signatures of Burkitt lymphoma (BL) and non-BL (mostly DLBCL), is a heterogeneous group of aggressive, mature B-cell lymphomas, which should not be classified separately due to biological and clinical reasons [1, 2]. High-grade B-cell lymphoma is a newly introduced category in the updated 2017 WHO classification, which primarily replaces B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma (BCLU, DLBCL/BL) and at the moment comprises two entities: HGBL with *MYC* and *BCL2* and/or *BCL6* chromosomal rearrangements (HGBL, R) and high grade B-cell lymphomas, not otherwise specified (HGBL, NOS). HGBL, R is also called double/triple-hit lymphoma (DH/THL). Most patients develop *de novo* HGBL, R, while a minority have a history of FL that progress to DH/THL secondarily, presumably by acquisition of a *MYC* translocation [1]. Double/triple-hit lymphomas are characterised by various morphologies including DLBCL, BL and intermediate features between DLBCL and BL (DLBCL/BL). HGBL, which lack co-occurring *MYC* and *BCL2* and/or *BCL6* rearrangements, falling in the category HGBL, NOS; such cases appear as blastoid or DLBCL/BL morphology (eventually resembling more closely BL than DLBCL), and in up to half of DLBCL/BL cases *MYC* rearrangements as one separate hit are found [1, 2, 33, 34] (Fig. 2). Former criteria for BCLU, DLBCL/BL were vague, and the diagnosis was not used uniformly, limiting its utility as a diagnostic category [1, 2]. The morphological appearance should always be specified in a comment in the pathological report referring to HGBL, R and HGBL, NOS because the DLBCL morphology may predict a better outcome compared to DLBCL/BL [35]. Neither morphology nor proliferation index assessed by Ki-67 have sufficient sensitivity and specificity to identify DH/THLs. MYC and BCL2 protein expression, although independently valuable prognostic factors, are similarly not ideal predictors of DH/THLs, and hence FISH (whenever the karyotype assessment is not available) is the recommended modality to identify DH/THLs (Fig. 3) [1, 2]. Despite the fact that a clear consensus has not yet been reached to provide molecular testing guidelines, the 2017 WHO update strongly advises that all DLBCL cases should undergo genetic studies for the detection of *MYC*, *BCL2*, and *BCL6* rearrangements. Nevertheless, some pathologists and clinicians suggest that only cases with a GCB phenotype and/or high-grade morphology or with > 40% MYC immunohistochemically positive cells are worthy of deeper molecular testing [1] (Fig. 2). Nearly 100% of DHLs with *MYC* and *BCL2* rearrangements harbour the t(14;18) translocation, which results in BCL2 overexpression by IHC and flow cytometry. BCL2 protein is expressed (in 30% of DHLs with *MYC* and *BCL6* rearrangements) or overexpressed (occurrence of *BCL2* extra copy/amplification) in a higher proportion of DLBCL and HGBL, NOS. That phenomenon is often associated with a concomitant expression of MYC, but still IHC evaluation cannot be used as a surrogate marker for DHL. Most DLBCL and HGBL do not carry both rearrangements (*MYC* and *BCL2*) and are referred to as "double-expressor lymphomas" (DELs) [1, 2, 33, 36]. DH/THL and DEL patients usually progress more rapidly, are resistant to R-CHOP chemo-immunotherapy [33, 35], and have very poor prognosis. Moreover, such cases may harbour *TP53* mutations or deletion, frequently observed in *MYC*/*BCL2* DHLs and blastoid morphology [37, 38, 39]. Worse prognosis in HGBL, R and HGBL, NOS compared with the DLBCL groups were published lately [38, 40]. DH/THLs show a common GCB immunophenotype (CD10+/CD81+^{higher}/ BCL6+/CD44– or CD44+/–^{dim}) by FCM, often presenting decreased expression of CD20 or CD19, over expression of CD38 and BCL2 (Fig. 4) [41]. An aggressive biologic behaviour and poor clinical outcome of DH/THL may shortly influence to therapy selection including still controversial more intensive regimens initiation and CNS-directed prophylaxis consideration. Under consideration is cytogenetic testing by metaphase or cytogenetic analysis by karyotyping if biological material (fresh cells from FNAB or tissue surgical section) is available. As far as possible, a comprehensive histopathological diagnostics of HGBL should include IHC, FISH, FCM and cytogenetic studies (Table III) [42, 43, 44].

Table II. Characteristics of diffuse large B-cell lymphoma, not otherwise specified

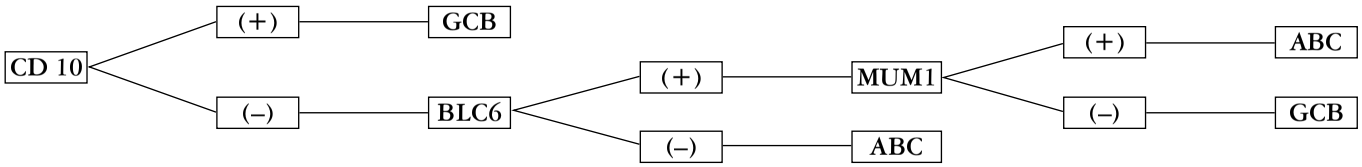
	Diffuse large B-cell lymphoma, not otherwise specified
Presentation	
Clinical	25-35% adult non-Hodgkin lymphomas; Elderly patients; Median age the 7th decade [rare cases in children and young adults]; M > F; Most cases primary DLBCL, NOS [de novo]; Secondary DLBCL, NOS - transformation from less aggressive lymphoma [CLL/SLL, FL, MZL, NLPHL]; Risk factors: immunodeficiency, EBV-infection [3% in western to 10% in Asian / Latin American populations; cases with EBV positivity in most lymphoma cells should be diagnosed as EBV-positive DLBCL, NOS/another specific type of EBV-positive lymphoma]; Rapidly enlarging tumour mass at single/multiple nodal/extranodal sites; ~50% of patients are in stage I or II disease – inclusion PET/CT raised up the initial stage
Pathological	**Morphology** Three most common variants: Centroblastic [medium-sized to large, oval to round vesicular nuclei with fine chromatin, 2-4 nucleoli close to nuclear membrane, cytoplasm scant and amphophilic/basophilic]; Immunoblastic [a single centrally located nucleolus, more abundant basophilic cytoplasm; immunoblasts with plasmacytoid differentiation may be observed]; Anaplastic [large to very large cells with bizarre nuclei, some cells may resemble: HRS cells/anaplastic large cell lymphoma/undifferentiated carcinoma; may show a sinusoidal and/or cohesive growth pattern]; Rare morphological variants: myxoid stroma, fibrillary matrix, pseudorosette formation, spindle-shaped/signet ring lymphoma cells Architecture Diffuse or partial [interfollicular and/or less commonly sinusoidal] nodal involvement; Infiltration of perinodal tissue [often]; Sclerosis [broad/fine bands]; Background may contain high number of T cells and/or histiocytes
IHC	PanB(+), surface and cytoplasmic immunoglobulins [50-75%, IgM(+)>IgG(+)>IgA(+], CD5(–/+) [5-10%, positive cases usually DLBCL de novo not CLL/SLL transformation], CD30(–/+) [10-20% anaplastic variant], cyclin D1/SOX11(–) [rare cases with cyclin D1/SOX11 weakly positive without CCND1 translocation], CD10(+/–) [30-50%], BCL6(+/–) [60-90%], IRF4/MUM1(+/-) [35-65%], BCL6 and IRF4/MUM1 co-expression in 50% of cases, FOXP1(–/+) [20%; more often in ABC subtype with IRF4/MUM1(+) and BCL2(+) without t(14;18)(q32;q21.3)], GCET1(+/–) [40-50%, in GCB subtype], LMO2(+/–) [45%, more often in GCB subtype with CD10(+), BCL6(+), HGAL(+) but IRF4/MUM1(–) or BCL2(–)], BCL2(+/–) [47-84%], p53(+/–) [20-60%, expression more frequent than mutation], Ki-67 high [≥ 40% up to > 90% in some cases]
Hans algorithm	

Table II. Cont.

Genetic									
	Rearrangements								
	BCL2		***BCL6***		***MYC, single bit***				
ABC subtype	< 5%		25-30%		5-8%				
GCB subtype	40%		15%		5-8%				
	Mutations								
	MYD88	**CD79B**	**CARD11**	***KMT2D***	***CREBBP***	***GNA13***	***EZH2***		
ABC subtype	35%	20-25%	10-15%	35%	10%	→	→		
GCB subtype	→	→	10-15%	40%	30%	25%	20-25%		
	Pathways								
ABC subtype	NFκB activation								
GCB subtype	PI3K/AKT								

*→ *Rare/Uncommon*

Burkitt Lymphoma and Burkitt-like lymphoma with 11q aberration

Burkitt lymphoma (BL) is defined by the WHO classification as a highly aggressive lymphoid neoplasm, often presenting with extra nodal site involvement or as an acute leukaemia composed of monomorphic, medium-sized B-cells with basophilic cytoplasm and a high mitotic index. Translocation involving the *MYC* oncogene (8q24) and immunoglobulin *IG* genes is the constant features in 90% of cases [1]. Case analysis without *MYC* rearrangement have shown at least several mechanisms for the alternative activation of *MYC* i.e. microRNA, amplification, transcriptional increase of *MYC* activity. Results of next-generation sequencing revealed the BL profile of somatic mutations; in about 70% of classic BL mutations of transcription factor *TCF3* (*E2A*) and its negative regulator *ID3* were found and its role in PI3K pathway signalling was observed. Furthermore *CCND3, RHOA, TP53, ARID1A* and *SMARCA4* mutations were identified in 30% of patients with BL.

Recently, a subset of *MYC* translocation-negative aggressive B-cell lymphomas resembling BL, characterized by proximal gains and distal losses of the long arm of chromosome 11 was described [45, 46]. In the 2017 WHO classification, these *MYC*-negative lymphomas were recognized as a new provisional entity, "Burkitt-like lymphoma with 11q aberration" (BLL,11q) [1] (Fig. 5). *MYC*-negative BLL,11q shows a number of clinico-pathological similarities to *MYC*-positive BL, but also harbour some significant differences in immunoprofile [1, 45, 46, 47, 48, 49, 50]. BLL,11q usually express CD43/LMO2/CD56 in IHC and CD16/CD56/CD38/CD45/CD8/CD43 in FCM. That characteristics may contribute to the differential diagnosis of BLL, 11q and BL [49]. The 11q aberrations in BLL, 11q (11q-gain/loss) were described as an inverted duplication of a part of the long arm of chromosome 11 with mono- or biallelic telomeric deletion of 11q (Figure 6) [45, 46, 47, 49]. Coincidence of duplication and deletion of 11q (11q23 and 11q24-qter, respectively) suggests a possibility of simultaneous up-regulation of oncogenes and down-regulation of tumour suppressor genes. The candidate oncogene is commonly up-regulated *PAFAH1B2. FLI1* and *ETS1,* located in the region of deletion, are often down-regulated and/or mutated, and are postulated to be candidate tumour suppressor genes affected by this aberration [46, 47]. The diagnostic algorithm for diagnosing BL and BLL, 11q, besides standard histopathological and immunohistochemical examination, incorporates flow cytometry with a broad panel of monoclonal antibodies as well as genetic analysis (conventional cytogenetic analysis with fluorescence in situ hybridisation and molecular techniques) [1, 48]. Some cases with 11q aberration

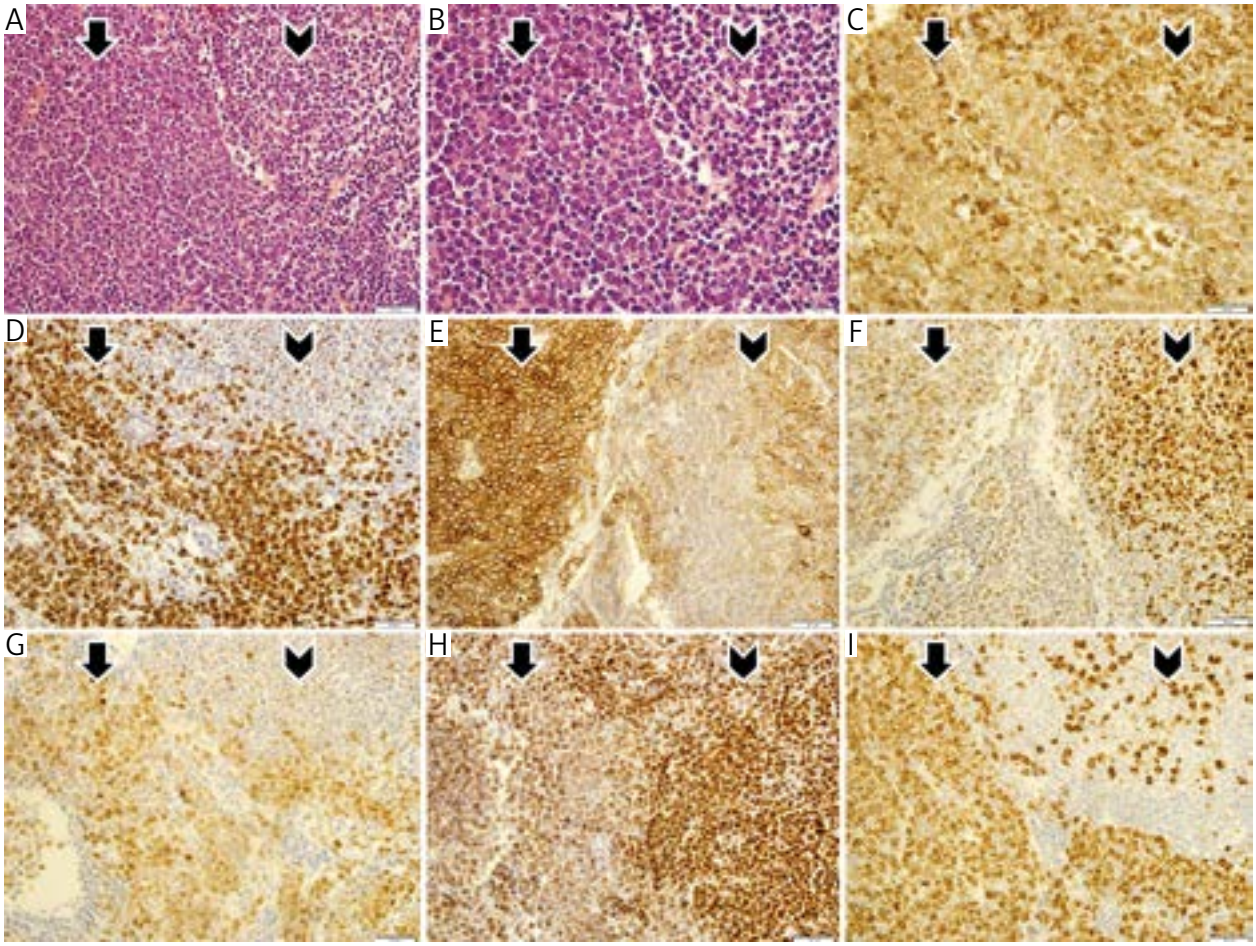


Fig. 2. Histopathology and immunohistochemistry of HGBL, R (A 400×; B, 600×; arrow); a case of a secondary triple-hit lymphoma (transformation from follicular lymphoma – arrowhead; HGBL, R – arrow) with DLBCL/BL morphology. Immunohistochemical staining on HGBL, R cells: C – BCL2(+) weaker, D – MYC(+), E – CD38(+) higher, F – BCL6(+) weaker, G – MUM1(+), H – FOXP1(+) weaker, I – Ki-67 index (>90%) (for all IHC 400×)

also have *MYC* rearrangement and are diagnosed as BL or high-grade B-cell lymphoma, not otherwise specified (HGBL, NOS). From the other point of view, the 11q-gain/loss are not exclusively specific for BLL, 11q [47]. Therefore, the conventional cytogenetic analysis by metaphase karyotyping is needed to provide correct BLL, 11q diagnosis (Table IV) [49].

Other aggressive B-cell lymphomas

Changes in the WHO 2017 classification and diagnostic criteria of other aggressive lymphomas from mature B cells are less significant.

In the group of large B-cell lymphomas associated with infection with EBV the most common lymphoma is EBV-positive diffuse large B-cell lymphoma, not otherwise specified [DLBCL EBV(+), NOS]. In the previous WHO 2008 classification, this unit was called EBV-positive DLBCL of the elderly, due to significantly more frequent occurrence in patients over the age of 50 years with a peak of incidence in the eighth decade of life [51]. Recent studies have shown that these lymphomas may also occur in younger patients (mainly in the third decade of life and almost three times more often in men) [1, 20]. DLBCL EBV(+), NOS is characterised by a different clinical picture and pathological features comparing with DLBCL, NOS. Mainly it occurs in patients with immunological disorders that impair the control of viral infections. Histopathology reveals large atypical B cells that may resemble Hodgkin's and Reed-Sternberg cells, and variable inflammatory infiltrates involving cytotoxic T lymphocytes [CD8(+)], plasmocytes, and histiocytes. In addition, tumour necrosis is present in most cases. Histopathological features may suggest a diagnosis, but it should be confirmed by testing for the presence of EBV virus by *in situ* hybridisation, which is a superior method to IHC staining. EBV is found in most lymphoma cells and is usually in the second and third phase of latency [52]. Pictures of EBER-ISH and EBV-LMP1(IHC) are shown in Fig. 1.

In the group of large B-cell lymphomas classified on the basis of topography, the changes concern mainly primary mediastinal (thymic) large B-cell lymphoma (PMBL) and primary DLBCL of central nervous sys-

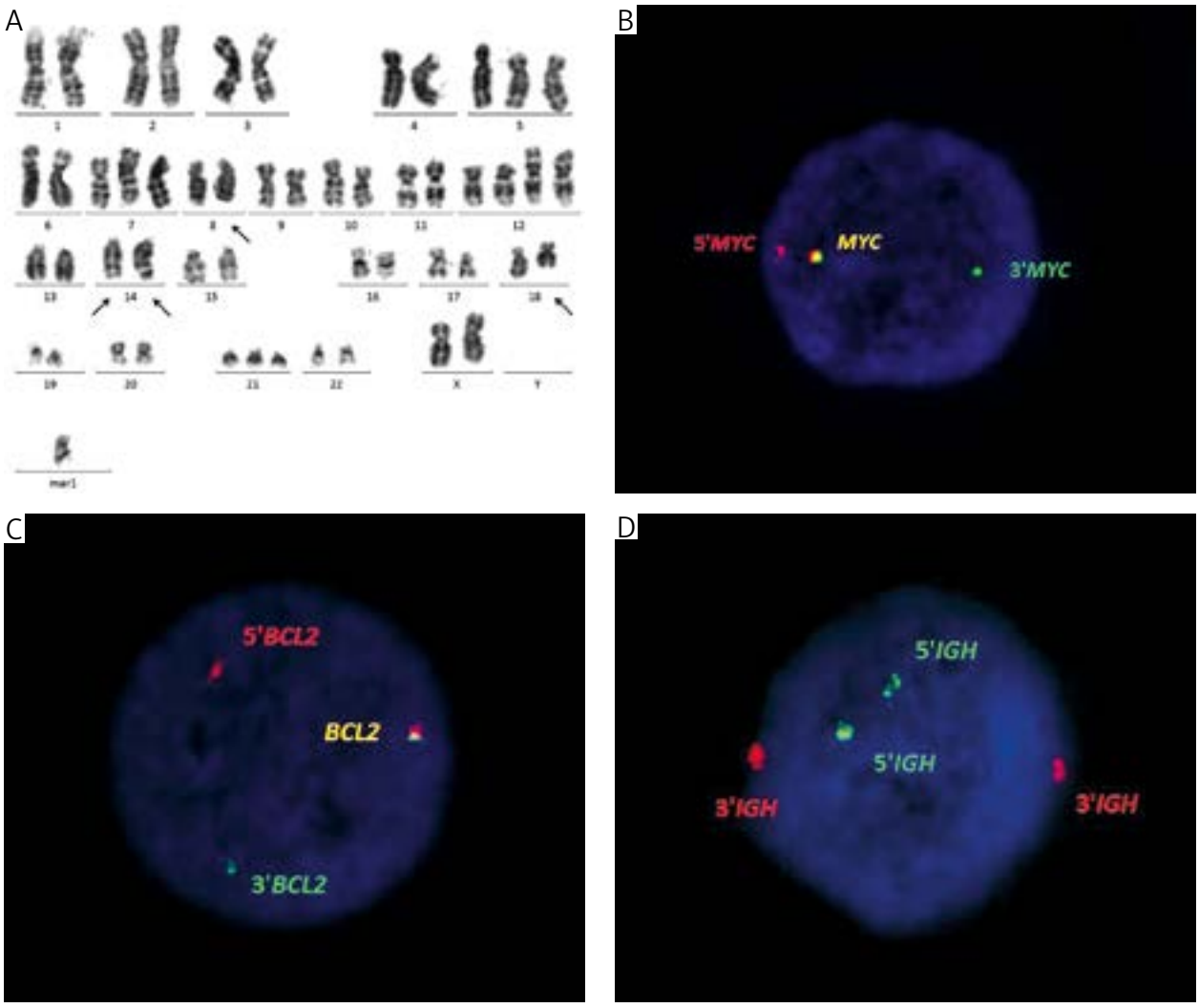


Fig. 3. A) Conventional cytogenetic diagnosis of double-hit lymphoma (DHL). Complex karyotype presenting DHL with diffuse large B-cell lymphoma morphology: 52,XX,+5,+7,t(8;14)(q24;q32),+der(12),t(6;12)(p11.2;p11.2) x2,t(14;18)(q32;q21),+21,+mar, arrows indicate *MYC*, *BCL2* and both *IGH* copies rearrangements. B) FISH with *MYC* break apart probe, split signal shows rearrangement of one copy of *MYC* gene in interphase nucleus. C) FISH with *BCL2* break apart probe, split signal indicates rearrangement of one copy of *BCL2* gene in interphase nucleus. D) FISH with *IGH* break apart probe, two split signals display rearrangement of two copies of *IGH* gene in interphase nucleus

tem (DLBCL, CNS). PMBL cells frequently express PDL1/PDL2 markers (rearrangement in about 20% of cases), CD30 and CD23, and usually are negative for IG and HLA class I and II antigens [53]. Primary mediastinal lymphoma has a specific gene expression profile that can be useful in differential diagnosis of PMBL and DLBCL, NOS involving mediastinum or other outside thorax locations. Differences between PMBL and DLBCL at the molecular level relate to *CIITA* rearrangements (38% vs. rarely respectively), which leads to the activation of NFκB and JAK/STAT signalling pathways and the reduction of the antigen expression of the major histocompatibility complex of class II [5, 54, 55].

The primary DLBCL, CNS constitutes less than 1% of non-Hodgkin's lymphomas and approximately 2.4-3% of all brain tumours. It has separate biological features related to the immunologically privileged location in which it develops and the lack of expression of HLA class I and II proteins, which allow tumour cells to avoid immune control. It should be differentiated with other large cell lymphomas occurring in the CNS, particularly with those associated with immunosuppression [56]; they are characterised with deletions and loss of gene expression within the HLA system and *MYD88 L265P* [> 50%], *CD79B* [20%], and *CARD11* [16%] mutations are often observed, which may have potential therapeutic significance [57, 58].

B-cell lymphomas with terminal B-cell differentiation include a heterogeneous group of aggressive lymphomas characterised by immunoblastic or plasmablastic cell morphology, a plasma cell phenotype with no or reduced expression of B-cell markers

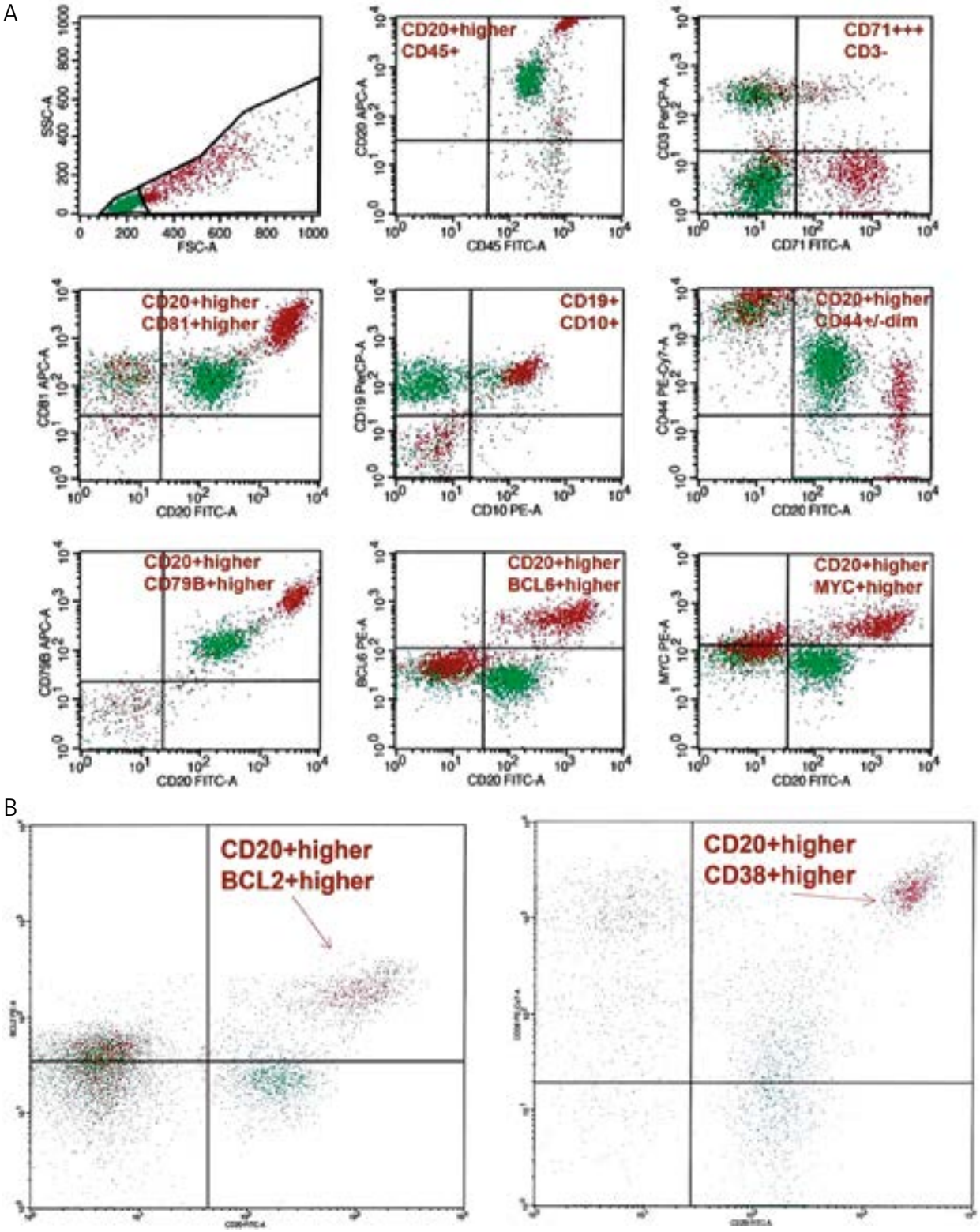


Fig. 4. Flow cytometry [FCM] of high grade B-cell lymphoma, triple hit [HGBL, TH]. A) FNAB/FCM analysis case with *MYC*/*BCL2*/*BCL6* rearrangements. Forward scatter/side scatter picture showing the larger HGBL lymphoma cells (red cells) coexisting with of small normal T/B lymphocytes (green cells). HGBL express: CD45/ CD20+higher/ CD19+(CD20>CD19) / CD71+++/ CD81+higher / CD10+/ CD44+/–dim/ CD79β+higher / BCL6+ higher / MYC+ higher / BCL2+ higher, and CD38+ higher ["higher" or "dim" means higher or more heterogeneous (from very weak to high) expression compared to expression on normal (green cells) B lymphocytes, respectively]. B) Higher level of BCL2 and CD38 expression (marked by an arrow) than in normal T/B lymphocytes correlates with *BCL2* and *MYC* rearrangements, respectively. Dot-plots

Table III. Characteristics of high-grade B-cell lymphomas

	High grade B-cell lymphomas	
Presentation	**HGBL, R**	**HGBL, NOS**
Clinical	Elderly patients, the 6th - 7th decade; M:F (with slight male predominance); Advanced disease (in 70-100% patients Ann Arbor IV stage) More than one extranodal localisation including bone marrow and CNS High IPI Elevated LDH HGBL DH more often in patients with diagnosed DLBCL who do not respond to R-CHOP regimen or who have early relapses after complete remission	Elderly patients Still too few epidemiological data
Pathological	Morphology: ~50% DLBCL, NOS (5-10% of all primary DLBCLs are double-hit lymphomas [DHL], while 30–40% are MYC/BCL2 protein co-expressing lymphomas); ~50% DLBCL/BL; Rare cases with blastoid appearance (closely mimic true lymphoblasts). Architecture: Completely diffuse; Sometimes with follicular component/fibrosis	Morphology: DLBCL/BL (most cases, mimics more closely BL); Blastoid; Usually not "classical" DLBCL; Architecture: Completely diffuse; No follicular component, stromal reaction nor fibrosis
IHC	GCB *in 100% of BCL2/MYC DHL and in 50%-80% of BCL6/MYC DHL*; CD19(+), CD20(+), CD79a(+), PAX5(+), TdT(–), sIg(–/+), CD10(+/–) i BCL6(+/–) [75-90%], IFR4/MUM1(–/+) [20%], BCL2(+) [strong cytoplasmic in contrast to absent/weaker in BL], Ki-67 variable [BL morphology: high 80-95%; morphology DLBCL may be low: even below 30%]	GCB (70%) or ABC (30%) (simultaneously with *BCL2R* and *BCL6R* or *BCL6R* or extra copies of *BCL2* and BCL6); CD20(+) [100%], BCL6(+/–), CD10(–/+)*[variable], IRF4/MUM1(–), Ki-67 [variable], *MYC* [variable, depending on *MYCR* or extra copies of *MYC*]
FCM/IHC	CD20(+) [often weaker], CD19(+) [often weaker], CD79a(+), CD79β(+), CD10(+) [75-90%], BCL6(+) [75-90%], MYC(+) [but MYC negative cases exist], CD38(+)^{higher}, BCL2(+)^{higher} [if *BCL2R*], or BCL2 (+)weak or (–) [if *BCL6*R], CD44(–) or CD44(+/–)^{dim}, MUM1(–/+) [20% if *BCL6*R], CD5(–), CD43(+/–), IgM(+), or IgM/IGD(+), TdT(–) [if blastoid morphology], CD71(+++) [100%]	CD20(+),CD19(+),CD22(+),CD79a(+),CD79β(+), CD10(+/–)*,CD43(+/–), CD81(+)^{higher} or CD81(+)*, CD44(–) or (+/–)dim or (+)*, CD5(–), CD71(+++) [100%]
Genetic	By definition: *MYC* (8q24) and *BCL2* (18q21) and/or *BCL6* (3q27) rearrangements; Complex karyotype [cases with DLBCL/BL morphology can have a low complex or simple karyotype closer to BL; D/THLs show a significantly higher genomic complexity than BL]; 60-65% *IG/MYC* (juxtaposition of *MYC* to one of the immunoglobulin loci: *IGH* at 14q32, *IGK* at 2p12, or IGL at 22q11); 35%-40% non–*IG/MYC* [partners such as at 9p13 (gene unknown), 3q27 (BCL6) or other loci]; Combination of a chromosomal rearrangement of one gene and copy-number increase or amplification of other genes [e.g. *MYC*, R(8q24) with gain or amplification *BCL2*(18q21)] are not sufficient to classify a case as DHL	By definition: *MYC* (8q24) and *BCL2* (18q21) and/or *BCL6* (3q27) rearrangements should be excluded; 20-35% *MYC*, *R* with or without increased copy numbers or, rarely, amplification of 18q21 [*BCL2*]; Still too few coherent data

**depending on GCB vs. ABC subtype*

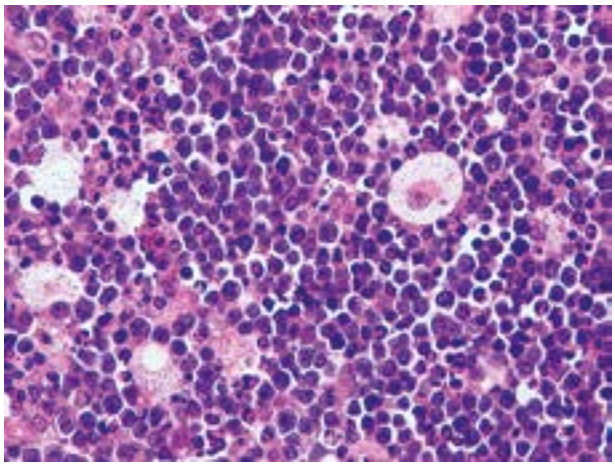


Fig. 5. Histopathological features of Burkitt-like lymphoma with 11q aberration (BLL,11q). Diffuse growth is composed of medium-sized lymphoid cells showing jigsaw puzzle effect of cytoplasmic borders with a starry pattern due to admixed macrophages (but in this case, you see a reduced phagocytosis and apoptotic bodies). The nuclei are similar in size and shape (HE, magnification 600×)

(CD20 and PAX5), and strong expression of plasma cell antigens (CD38, CD138, IRF4/MUM1, and PRDM1/BLIMP1) [1]. Highly aggressive plasmablastic lymphoma (PBL) develops in immunocompromised patients, mainly caused by HIV infection, as well as during iatrogenic immunosuppression (after transplantations, autoimmune diseases). It is usually located extranodally within the head and neck, including the oral cavity, also in the gastrointestinal tract. In majority of cases the generalised disease stage is determined at the time of diagnosis (over 75% of patients with HIV infection) [59, 60, 61]. In some cases, PBL involves bones and with its morphological and immunophenotype features overlaps with plasmablastic plasma cell myeloma. The differential diagnosis should take into account the clinical picture of PBL and immunodeficiency and EBV infection. The EBV virus in the first type of latency is present in about 70% of cases. *MYC* translocation occurs in about 50% of patients, usually with the *IG* partner [62].

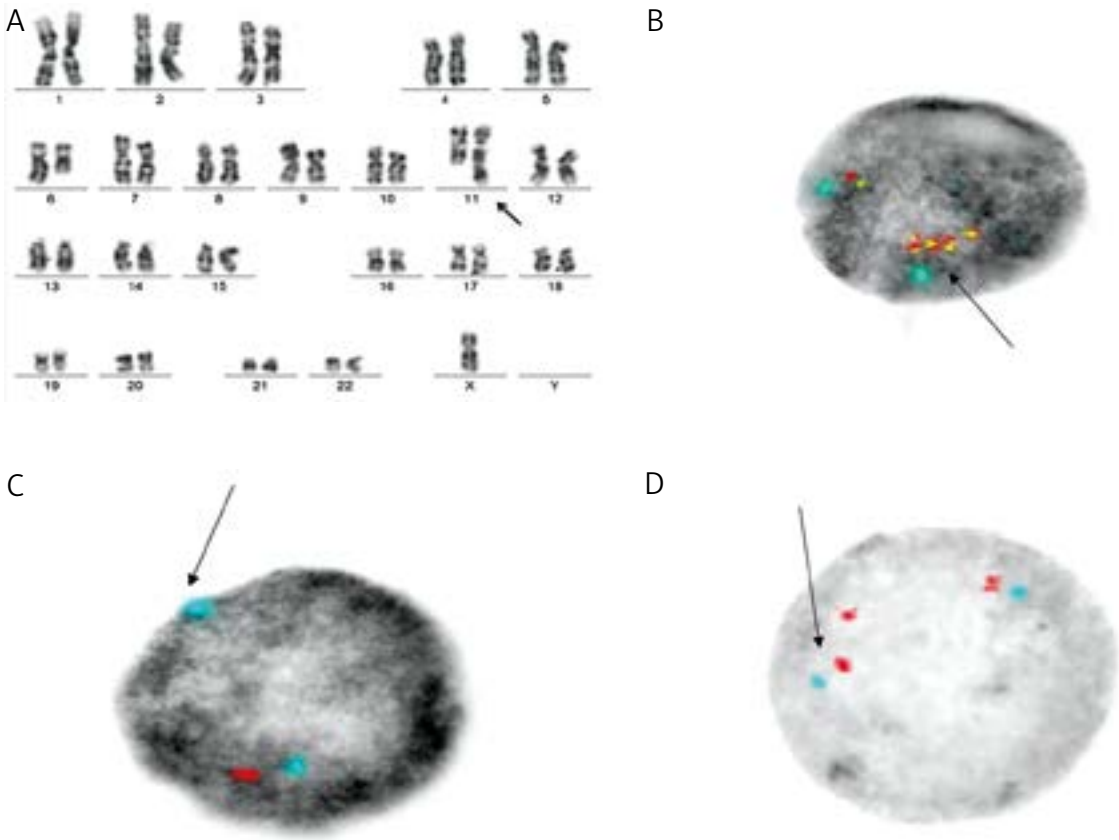


Fig. 6. Conventional cytogenetic diagnosis of Burkitt-like lymphoma with 11q aberration (BLL,11q). A) A low complexity karyotype of BLL,11q: 45,X,-Y,del(6)(q21), dup(11)(q23q21), arrow indicates 11q gain/loss. B) FISH with centromere 11 (Aqua) and *KMT2A* (break apart) probes, four red-green signals show multiplication of this region on duplicated chromosome 11 (arrow). C) FISH with centromere 11 (Aqua) and *ATM* (locus specific, red) probes, two red signals indicate a duplication in 11q aberration region (arrow). D) FISH with centromere 11 (Aqua) and telomeric 11q (red) probes, one red 11q telomeric signal indicates normal chromosome 11, lack of red signal in 11q aberration confirms terminal deletion (arrow)

Table IV. Characteristics of Burkitt lymphoma and Burkitt-like lymphoma with 11q aberration

	Burkitt lymphoma and Burkitt-like lymphoma with 11q aberration	
Presentation	**BL**	**BLL,11q**
Clinical	Three clinical variants are: endemic, sporadic, and immunodeficiency-associated BL; The incidence is low, accounting for only 1-2% of all lymphomas in Europe and in the USA; HIV infection positive in some cases; EBV-EBER positive [15%-30%] in some cases; Mainly children, young males but also rare cases in elderly patients are reported; Median age at time of diagnosis among adults: 30 years; M : F = 2-3 : 1; Extranodal >>Nodal involvement, often a bulky tumor; Bone marrow and cerebrospinal fluid involvement more frequently in children and HIV-positive patients	Still not very well described entity; Two clinical settings of BLL,11q are classical one and post-transplantation; The incidence is rare, probably less than 10% of all BL cases; HIV/EBV infection negative; Young males; Median age at time of diagnosis: 25 years, M : F > 10 : 1; Nodal and Tonsilar>>Extranodal, often a bulky tumor; No bone marrow nor cerebrospinal fluid involvement
Pathological	Medium-sized cells, with diffuse, monotonous growth type; Squared-off borders [cytoplasm retraction as a result of formalin fixation]; Round nuclei with numerous basophilic, medium-sized nucleoli; Basophilic cytoplasm with lipid vacuoles [visible in touch imprint slides or fine-needle aspiration cytology]; "Starry-sky" image, numerous macrophages and granulomatous background; Less often nuclear pleomorphism or features of plasma differentiation [eccentric basophilic cytoplasm, single central nucleus]; Rarely DLBCL/BL morphology	Diverse morphology – mainly BL, frequently slightly differed from classical BL features by the reduced number of macrophages and apoptotic bodies (loss of the "starry-sky" appearance), sometimes of DLBCL/BL, and sporadically DLBCL
IHC	Pan B (+), CD20(+), CD10(+), MYC(+), LMO2(–)^, BCL6(+),BCL2(–) [positive suggest HGBL], MUM1(–), CD43(+)^, CD44(–), IgM(+)[moderate to strong membrane reaction, light chain restriction],CD56(–), EBV(+/–)#,Ki-67 [high, ~100%]	Pan B (+),CD20(+), CD10(+), MYC(+), LMO2(+)^, BCL6(+), BCL2(–), MUM1(–), CD43(+/–)#, CD44(–), IgM(+), CD56(+/–)#, EBV(–),Ki-67 [high, ~100%]
FCM/IHC	CD45(+)weaker/CD38(+)higher/CD16/CD56(–)/ CD8(–) /CD43(+) or* CD43(+/–)	CD45(+)bright/CD38(+)/CD16/CD56(+) or* CD16/CD56(–) /CD8(+) or* CD8(–)/ CD43(+/–) or* CD43(+) or* CD43(–)
Genetic	The simple karyotype [40% of cases: *MYC translocation* as the sole abnormality]. Molecular hallmark is *MYC translocation* 8q24 to one of three immunoglobulin *loci*: in 85% the *IGH locus* on 14q32, in 5%, the *IGK* on 2p11 and 10% the *IGL* on 22q11; 10% cases without *MYC* rearrangement alternative mechanisms of *MYC* activation i.e. microRNA, amplification, transcriptional increase of *MYC* activity. Additional recurrent abnormalities: gains in chromosomes 1q, 7 and 12, and losses of 6q, 13q32–34 and 17p	The more- complex karyotype than BL and lack MYC rearrangement. Typical 11q aberrations: inverted duplication dup(11)(q23q13) with mono- or biallelic telomeric loss of 11q as a recurrent 11q abnormality. The most frequent additional changes comprised deletions of 6q, and trisomy 12

^*usually negative or positive, #sometimes positive, *alternative expressions are ordered from more to less frequent*

Table V. Characteristics of other aggressive B cell lymphomas

	T-cell/histiocyte-rich large B-cell lymphoma
Pathology	Scattered, single, large B cells [do not form aggregates/sheets] in background of T-cells/ histiocytes [bland = looking non-epithelioid]; Large B cells may mimic: LP cells of NLPHL, centroblasts, HRS cells; Absent meshwork of follicular dendritic cells; No eosinophils / plasma cells; Spectrum of B cell size, morphology, and distribution (clusters/sheets of medium-sized to large B cells) - consider DLBCL, NOS
IHC	PanB(+), BCL6(+), BCL2(–/+) [variable], EMA(–/+) [variable], CD15(–), CD30(–), CD138(–); the background: histiocytes with CD68(+) and CD163(+), T cells CD3(+) and CD5(+); no residual IgD-positive mantle cells; if EBV(+) cells, consider the spectrum EBV-positive DLBCL
	Diffuse large B-cell lymphoma, topographic site related
	Primary mediastinal (thymic) large B-cell lymphoma
Pathology	Variable morphology, usually diffuse pattern with fibrosis; Medium-sized to large cells, pale cytoplasm, round to ovoid nuclei; Some cases: more pleomorphic – mimic HRS cells
IHC	PanB+, slg(-/+) [commonly lacks of expression despite IgG functional rearrangement], PAX5(+), BOB1(+), OCT2(+), PU1(+), CD30(+) [> 80%, weak and heterogeneous], CD15(-/+) [rare cases], CD23(+) [70%], MAL(+) [70%], PDL1/PDL2(+) [70%], EBV(-), IFR4/MUM1(+) [75%], BCL2(+) [55-80%], BCL6(+) [45-100%], CD10(-/+) [8-32%], CD11c(–/+) [40%], MYC(–/+) [30%, independent of MYC alterations], CD54(+), FAS/CD95(+), TRAF1(+), REL(+) [nuclear expression], HLA I/II(–) [often]
	Primary diffuse large B-cell lymphoma of the central nervous system
Pathology	Highly cellular, medium-sized to large round, oval, irregular or pleomorphic nuclei and distinct nucleoli [centroblastic/immunoblastic morphology]; Rarely monomorphic cell population mixed with macrophages [mimics Burkitt lymphoma]; Central areas of geographical necrosis, perivascular infiltration, infiltration of cerebral blood vessels with fragmentation of argyrophilic fibre network
IHC	PAX5(+), CD20(+), CD22(+), CD79a(+), sIgM(+), sIgD(+), sIgG(–) [either kappa or lambda light chain restriction], BCL6(+/–) [60-80%], IRF4/MUM1(+) [90%], CD138/CD38(-), CD10(–/+) [< 10%, if positive check because it may be DLBCL with CNS dissemination], HLA-A/HLA-B/HLA-C/HLA-DR [variable], HLA I(–/+) / HLA II(–/+) [lost in 50%], BCL2(+) [82% high expression not related to t(14;18)(q32;q21) and MYC-high phenotype], Ki-67 high [> 70%, even up to > 90%], EBV(–) [positive if immunodeficiency in background]
	Primary cutaneous diffuse large B-cell lymphoma, leg type
Pathology	Monotonous, diffuse, non-epidermotropic infiltrate of confluent sheets of centroblasts/immunoblasts
IHC	sIG(+), CD20(+), CD79a(+), BCL2(+) [together with IRF4/MUM1 absent in 10%], IRF4/MUM1(+), FOXP1(+), MYC(+), cIgM(+) with coexpression of IgD(+) [50%], BCL6(+) [weak], CD10(–) [usually]
	Intravascular large B-cell lymphoma
Pathology	Large cells with prominent nucleoli in small or intermediate-sized vessels; Sinusoidal involvement in spleen, liver, and bone marrow
IHC	PanB(+), CD5(–/+) [38%], CD10(–/+) [13%], sIG(+),IRF4/MUM1(+) [almost all negative cases with CD10], CD29/integrin beta-1(–), CD54/ICAM1(–)

Table V. Cont.

	Diffuse large B-cell lymphomas, EBV related
	EBV-positive diffuse large B-cell lymphoma, not otherwise specified
Pathology	Large transformed cells/immunoblasts and HRS-like cells; Reactive background: small lymphocytes, plasma cells, histiocytes and epithelioid cells; Geographical necrosis and angioinvasion
IHC	PanB(+), IRF4/MUM1(+), CD10(–), BCL6(–), CD30(+) [frequently], CD15(–/+) [sometimes with CD30 co-expression but lacks other phenotypic features of HRS cells], EBNA2(–/+) [7-36%, type II > type III EBV latency], LMP1(+) [> 90%], PDL1/PDL2(+/–), EBER(+) [>80%, mandatory technique for diagnosis]
	Diffuse large B-cell lymphoma associated with chronic inflammation
Pathology	DLBCL, NOS phenotype; Massive necrosis and angiocentric growth
IHC	CD20(+/–), CD79a(+/–), CD138(–/+), IRF4/MUM1(–/+), CD30(–/+) [occasional], T-cell markers(–/+) [occasional, one or more; CD2, CD3, CD4 and/or CD7], LMP1(+/–), EBER(+/–)
	Lymphomatoid granulomatosis
Pathology	Angiocentric and angiodestructive polymorphous lymphoid infiltrate [small number of EBV-positive large B cells and prominent inflammatory background]
IHC	CD20(+), CD30(+/–) [variably], CD15(–), LMP1(+/–), EBER(+), the background T-cells CD3(+), CD4(+)>CD8(+)
	Large B-cell lymphomas with terminal B-cell differentiation
	Plasmablastic lymphoma
Pathology	Morphological spectrum; Diffuse and cohesive cells with immunoblastic to plasmablastic morphology;
IHC	CD38(+), CD138(+), Vs38c(+), IRF4/MUM1(+), PRDM1/BLIMP1(+), XBP1(+), CD79a(+/–) [40%], CD45(–/+) and CD20(–/+) and PAX5(–/+) [negative or weakly positive; if strongly positive the diffuse LBCL, NOS should be diagnosed], cIgG(+), light chains lambda(+) or kappa(+), CD56(–/+) [25%], EMA(+/–) and CD30(+/–) [frequently], Ki-67 high [> 90%], BCL2(–), BCL6(–), CD10(–/+) [20%], Cyclin D1(–), CD43 or CD45RO(–/+) [rare cases], EBER(+/–) [60-75%; more frequently in HIV-positive and post-transplant cases], LMP1(–/+), HHV8(–)
	ALK-positive large B-cell lymphoma
Pathology	Diffuse and a sinusoidal growth pattern; Monomorphic large immunoblast-like cells with round pale nuclei and large central nucleolus and abundant amphophilic cytoplasm
IHC	ALK(+) [granular cytoplasmic staining – expression CLTC-ALK fusion protein, cytoplasmic, nuclear and nucleolar staining – expression NPM1-ALK fusion protein, cytoplasmic staining other ALK partners], EMA(+), CD138(+), VS38(+), PRDM1/BLIMP1(+), XBP1(+), PanB(–) [positive only occasional cases], CD45(–/+), IRF4/MUM1(+), CD30(–), cIgA>cIgG(+) [with light chain restriction], cytokeratins(–/+) [single cases; be aware of carcinoma diagnosis], EBER(–), HHV8(–), CD4(–/+), CD57(–/+), CD43(–/+), perforin(–/+)
	Primary effusion lymphoma
Pathology	Large immunoblastic/plasmablastic cells to more anaplastic morphology; Variable morphology

Table V. Cont.

IHC	CD45+, PanB(–), surface/cytoplasmic Ig(–), BCL6(–), HLA-DR(+/–), CD30(+/–), CD38(+/–), VS38c(+/–), CD138(+/–), EMA(+/–), HHV8/LANA1/ORF73(+), EBER(+), LMP1(–)
	HHV8-positive diffuse large B-cell lymphoma, NOS
Pathology	Large plasmablastic cells with vesicular often eccentric nuclei with one/two nucleoli and amphophilic cytoplasm
IHC	HHV8/LANA1(+), cIgM(+) [light chain restriction], CD20(+/–), CD79a(–), CD38(–/+), CD138(–), CD27(–), EBER(–)
	Mantle cell lymphoma
Pathology	Blastoid: lymphoblasts-like with dispersed chromatin and a high mitotic rate; Pleomorphic: pleomorphic cells, large with oval to irregular nuclei, pale cytoplasm, prominent nucleoli [some of the cells]
IHC	sIgM/IgD(+) [more frequently with lambda than kappa restriction], BCL2(+), CD5(+), CD43(+), IRF4/MUM1(+/–) [sometimes positive], CD10(–), BCL6(–), CD23(–) [or weakly positive], cyclin D1(+) [> 95%], SOX11(+) [> 90%], LEF1(+) [blastoid/pleomorphic variant], CD200(+) [more frequent in leukemic non-nodal variant]
	B-cell lymphoma, unclassifiable, with features intermediated between diffuse large B-cell lymphoma and classic Hodgkin lymphoma
Pathology	Morphological spectrum – areas of classical Hodgkin lymphoma and primary mediastinal (thymic) large B-cell lymphoma Fields of pleomorphic cells mimic HRS / lacunar cells with fibrosis, inflammatory infiltrates, necrotic areas
IHC	The phenotype with intermediate features between cHL and PMBL: CD45 (+), CD20(+/–), CD79a(+/–), CD30(+), CD15(+/–), PAX-5(+/–), OCT-2(+/–), BOB.1(+/–), BCL6 (+/–) CD10(–), ALK(–), EBER(–)

In Table V pathological and immunohistochemical presentation of other aggressive B cell lymphomas were presented.

Summary

In the diagnosis of aggressive B-cell lymphomas, parallel genetic tests should be done to determine the status of the *MYC* gene; these changes are complex and, although mostly involving classic translocations with one of the *IG* partners, also translocations with non-*IG* partners, mutations, transcriptional up-regulations, amplifications, and microRNA-dependent mechanisms are possible. This has a direct impact on the amount of protein product and its expression. *BCL2* and *BCL6* rearrangement and translocation mechanisms are well established. Two basic methods include the fluorescence *in situ* technique in break-apart probe or dual-fusion; these are convenient and stable methods and can be used on formalin-fixed paraffin-embedded material. It makes these methods more available and allows the FFPE to be sent to a standardised FISH laboratory. FISH analysis can detect critical gene rearrangements or abnormalities in aggressive B-cell lymphomas. If fresh biopsy or tissue material is available karyotype analysis can reveal additional chromosome aberrations giving a more accurate picture of genetic changes clarifying the diagnosis.

In the comprehensive histopathological report, the following components should be determined:
- cell morphology {blastoid, DLBCL, BL, DLBCL/BL};
- immunophenotype with reference to origin according to Hans's algorithm {GCB vs. non-GCB subtype};
- MYC and BCL2 immunohistochemistry {double expressor vs. non-double expressor};
- rearrangement of *MYC, BCL2, BCL6* genes {presence or absence of *MYC* and *BCL2* and/or *BCL6* rearrangements}.

A summary of all diagnostic procedures in aggressive B-cell lymphomas is shown in Fig. 7.

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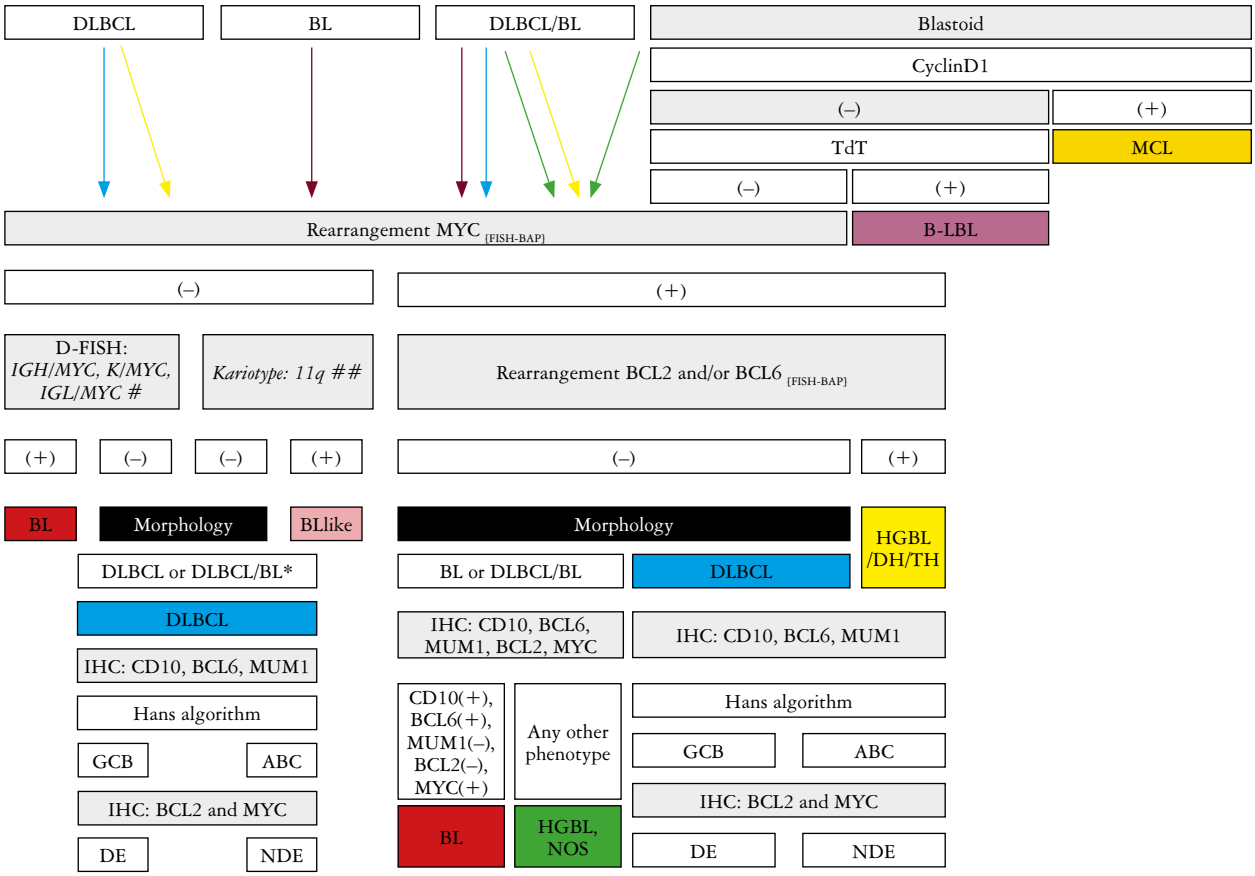
The authors declare no conflict of interest.



DLBCL – diffuse large B-cell lymphoma; BL – Burkitt lymphoma; MCL – cell lymphoma mantle; B-LBL – B-cell lymphoblastic leukaemia/lymphoma, HGBL,NOS – high-grade B-cell lymphoma, not otherwise specified; DH – double hit; TH – triple hit; DE – double expressor; NDE – not a double expressor; FISH-BAP – break-apart FISH probe; D-FISH – dual fusion FISH probe
 # additional evaluation, to be considered for the availability of the technique in correlation with the morphological image and clinical presentation
additional evaluation, to be considered for the availability of the technique in correlation with the morphological image and clinical presentation
** morphology corresponding to small centroblasts or similar to lymphoblasts*

Fig. 7. Diagnostic algorithm for aggressive B-cell lymphomas

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