9 Gray Zones and Double Hits: Distinguishing "True" Burkitt Lymphoma from Other High-Grade B-Cell Lymphomas

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While the diagnosis of Burkitt lymphoma (BL) is often straightforward, it has long been recognized that some cases resembling BL may have atypical morphologic and/or immunophenotypic features that overlap with other types of high-grade B-cell lymphoma, particularly diffuse large B-cell lymphoma (DLBCL). Such cases, originally termed atypical Burkitt lymphoma or high-grade Burkitt-like B-cell lymphoma, are particularly vexing because the treatment for BL and DLBCL is quite different, and simple techniques to resolve such "gray zone" lymphomas into either "true" BL or DLBCL (e.g., by measuring the proliferation index) have proven unsatisfactory. The 2008 WHO classification places such cases in a category called B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL, which has recently been shown to include cases with translocations of both MYC and genes such as BCL2 and BCL6, which are more typically translocated in lymphomas other than BL. Such lymphomas (termed "double-hit lymphomas") have an extremely poor prognosis, and may represent an entity distinct from BL and DLBCL. Using recommendations from the WHO and other authorities, this course will present an algorithm that guides one to the proper diagnosis for cases in which BL is in the differential diagnosis, using techniques commonly available to practicing pathologists.

- Describe the prototypic morphologic appearance and immunophenotype of Burkitt lymphoma, as well as the variations from this norm that are permissible and impermissible for that diagnosis according to published guidelines.
- Describe the clinical, morphologic, immunophenotypic, and genetic features of double-hit lymphomas, including those that distinguish them from "true" Burkitt lymphoma.
- Produce an algorithm detailing how to approach the diagnosis of cases resembling Burkitt lymphoma, to include circumstances in which rearrangements for MYC, BCL2, and BCL6 genes should be sought using fluorescense in situ hybridization or other methods.

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Gray Zones and Double-Hits: Distinguishing "True" Burkitt Lymphoma from Other High-Grade B-Cell Lymphomas

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Non-Burkitt Lymphomas with Burkitt-Like Features: Early History

Burkitt lymphoma (BL) has been recognized as a distinct pathologic entity since it was first described by Dennis Burkitt in 1958.(1) It accounts for about 40% of all childhood non-Hodgkin lymphoma, but less than 5% of cases in adults (2), and is separated into three clinical variants based on epidemiologic grounds: 1) endemic BL, which affects mainly children in areas of equatorial Africa and New Guinea where falciparum malaria is endemic; 2) sporadic BL, which affects children and young adults, often of low socioeconomic status; and 3) immunodeficiency-associated BL, which affects manly HIV+ adults.(3) BL has a characteristic morphology, being composed of diffuse sheets of monomorphic medium-sized lymphoid cells with round nuclei, variably clumped chromatin, multiple distinct nucleoli, and scant basophilic cytoplasm. This aggressive lymphoma shows high rates of mitosis and apoptosis, and often displays a "starry sky" pattern due to infiltration of pale staining macrophages that have ingested apoptotic debris.(3-4) It characteristically displays the CD10+ BCL6+ BCL2- immunophenotype of a germinal center B-cell, and typically shows translocation of the *MYC* gene on chromosome 8 to the immunoglobulin heavy chain gene (*IGH*) on chromosome 14 or, less commonly, to the immunoglobulin light chain genes (*IGL*) on chromosomes 2 or 22.(3-5)

For nearly as long, however, it has been recognized that there are malignant lymphomas that generally resemble BL, but which either display more cellular pleomorphism or contain more large cells than do typical BL cases. Such lymphomas were first described as the non-Burkitt's type of undifferentiated lymphoma in a 1974 revision of the Rappaport classification (6-7), and were included together with Burkitt's lymphoma in the small non-cleaved cell lymphoma category of the 1974 Luke-Collins classification (8) and 1982 Working Formulation.(9) The 1994 REAL classification created a provisional category termed high-grade B-cell lymphoma, Burkitt-like, specifically to recognize such cases as possibly biologically distinct from BL.(4) Although the authors of the REAL conceded that precise features to permit distinction of such cases from true (or "classical") BL and/or diffuse large B-cell lymphoma (DLBCL) were poorly defined, they speculated that at least some such tumors were unrelated to true BL because they lacked *MYC* rearrangements and often had *BCL2* gene rearrangements, both of which were unusual in BL.(4, 10)

Elimination of the Gray Zone between Burkitt Lymphoma and Diffuse Large B-Cell Lymphoma: The 2001 WHO Classification

The creation of a borderline category between BL and DLBCL by the REAL classification created problems for both pathologists and clinicians. Pathologists did not like rendering a diagnosis that their clinical colleagues often regarded as a hedge. It addition, they found it difficult to distinguish the new provisional entity of high-grade Burkitt-like lymphoma (BLL) from classic BL and/or DLBCL because the criteria were vague, with low rates of consensus for the diagnosis of BLL found even among expert hematopathologists.(11-14) Oncologists were unsure what therapy to employ for a lymphoma that straddled the line between the Working Formulation categories of intermediate and high grade, and seemed biologically heterogeneous. (15-16) While

DLBCL often responds well to standard “intermediate-grade” CHOP-based chemotherapeutic regimens, BL does not, and instead requires intensive chemotherapeutic regimens that include high-dose methotrexate and CNS prophylaxis if there is to be a chance of cure.(17-18)

To address these concerns, pathologists meeting to develop the 2001 WHO classification decided to essentially eliminate the BLL category by splitting it down the middle.(15) In a 1999 “sneak preview” of what was to become the 2001 WHO, it was stated that BLL cases with the t(8;14) of classic Burkitt, or the t(2;8) or t(8;22) light chain variants, would be placed in the category of *bona fide* BL (the definition of which was modified to require the presence of such *MYC* translocations) as the “Burkitt-like” variant of BL (the full diagnosis of which would thus incongruously read “Burkitt lymphoma, Burkitt-like variant”).(15) BLL cases lacking *MYC* translocations would be placed in the category of DLBCL.(15) Because pathologists often received biopsy material fixed in formalin, which precluded use of the standard methods of cytogenetics and Southern blotting to detect *MYC* rearrangements, and because fluorescence in situ hybridization (FISH) to detect *MYC* rearrangements was not widely available at that time, the tumor’s proliferation fraction (as measured by immunohistochemical staining for Ki-67 or MIB-1) was chosen to serve as “the most reasonable surrogate for cMYC rearrangement.”(15) The developers of the WHO then set a high bar for a proliferation fraction indicative of a *MYC* rearrangement, declaring “*the definition of Burkitt-like lymphoma is a lymphoma that morphologically resembles Burkitt’s lymphoma, but has more pleomorphism or large cells than classical Burkitt’s lymphoma, and has a proliferation fraction of >99%.*”(15) This was an *ad hoc* decision, rather than one based on empirical data actually showing that a very high proliferation fraction predicts *MYC* translocation (14), and would cause further problems down the road, as we shall soon see. The same publication also reported that the term “atypical Burkitt’s lymphoma” had been proposed for this variant, but it was felt that name assumed a relationship to classical Burkitt’s lymphoma that was not known to exist in all cases, hence the preference for the term BLL.(15) Although the final version of the WHO classification published in 2001 regarded atypical BL and BLL to be essentially synonyms, one can still find that earlier distinction reflected in recent publications that consider atypical BL to be a form of “true” BL, varying from classical BL only in its morphology, but BLL to be a likely distinct biological entity based on the presence of immunophenotypic or genetic features unacceptable for a diagnosis of BL.(12, 19-20)

In its final published form, the 2001 WHO classification also changed several of the criteria required for the diagnosis of the atypical BL/BLL variant of BL, making them somewhat less clear than in the 1999 sneak preview cited above. The proliferation fraction required for the diagnosis was changed from >99% to “nearly 100%” (21), leading to confusion among later authors, who advocated as appropriate thresholds a variety of lower rates, including 95% (22-23), 90% (12, 19, 22, 24), and even 80% (25). In addition, the final 2001 WHO changed the unequivocal requirement of a *MYC* translocation to the rather cryptic statement that the diagnosis was “reserved for cases with proven or with a strong presumptive evidence of *MYC* translocation.” (21) What such presumptive evidence might be was not explicitly stated, although many assumed it to be a high proliferation fraction.(14)

The Distinctive Nature of Many Burkitt-Like Lymphoma Cases, Including Double-hit Lymphomas

In part to clarify the issue of *MYC* translocation in BLL and its relation to proliferation fraction, McClure et al. at the Mayo Clinic in 2005 reported a study of 31 cases of Burkitt-like lymphoma in adults (>18 years) and compared them to control groups of childhood BL and adult DLBCL, analyzing each for translocations of *MYC* to *IG* heavy or light chain genes by FISH and for expression of various markers by immunohistochemistry, including the proliferation marker MIB-1.(14) They found no significant difference in the proliferation fraction assessed by MIB-1 staining between BLL cases with *IG-MYC* fusion (mean 74%, range 46-91%) and those without (mean 62%, range 34-89%), showing proliferation fraction to be worthless as a surrogate marker of *IG-MYC* translocation. Moreover, the proliferation fraction for control DLBCL cases often reached the high 90s (range 30-97%) and for classic childhood BL sometimes dipped into the 80s (range 87-100%), although the median for childhood BL was quite high at 98%. Their study also revealed a number of other interesting findings. Every case of childhood BL displayed exactly the same CD10+ BCL6+ BCL2- CD43+ p53+ immunophenotype characteristic of classical BL, while expression of these markers in adult BLL cases was highly variable, with not a single adult case having an immunophenotype completely identical to childhood BL. In addition, while their intent had been to study all available adult lymphomas with features of BL, including both classic and Burkitt-like morphology, they found that essentially all of such lymphomas in adults had at least some areas that fit the WHO morphologic criteria for BLL. In the FISH studies, each of the childhood BLs had a single balanced *IG-MYC* translocation as the only detectable genetic abnormality. In contrast, the adult cases often showed complex FISH patterns indicating complex chromosomal abnormalities, including multiple *IG-MYC* signals, extra *MYC* signals (not explained by chromosome 8 ploidy), or *IG-MYC* plus extra *MYC* signals. These findings caused the authors to question whether there even was a true adult equivalent of childhood BL.(14)

Overall, this group of Mayo Clinic investigators found *IG-MYC* breakpoints in 11/27 (41%) of adult BLL cases.(14) But because the 1992 study that provided the original inspiration for the category of BLL in the REAL classification had found such cases to sometimes have translocations of *BCL2* instead of *MYC* (10), these investigators looked for *BCL2* translocations as well. By FISH, they were able to demonstrate *IG-BCL2* translocations (corresponding to the t(14;18) found often in follicular lymphoma and in some cases of DLBCL) in 7/27 (26%) of adult BLL; three of these cases (11%) overlapped with the *MYC*-positive group, having both *IG-MYC* and *IG-BCL2* translocations in the same neoplastic cells.(14) Such cases, which were soon to be termed “double-hit” lymphomas in the 2008 WHO classification (26), were found to be associated with a particularly poor prognosis. These findings by McClure et al. mirrored those of MacPherson and colleagues, who had previously found that BLL patients with double-hit lymphomas were older, presented at a more advanced stage, and had significantly worse survival than BLL cases with only a *MYC* translocation.(16) Later reports by Kanungo et al.(27) and Snuderl et al.(28) similarly found a worse prognosis to be associated with dual translocations of both *MYC* and *BCL2* in lymphomas resembling BL and/or BLL. Like

McClure et al., both of these later reports found double-hit lymphomas to have complex karyotypes, and to express BCL-2 by immunohistochemistry in virtually all cases, providing a potentially useful marker to exclude such cases and from the category of classical BL.(27-28) Snuderl et al. also found MUM1 expression to be significantly more common in double-hit lymphoma than in Burkitt lymphoma, although some double-hit lymphomas were MUM1-negative.(28)

Double-hit lymphomas featuring *MYC* and *BCL2* translocations are not restricted to cases of BL or BLL, but have also been reported in some cases of DLBCL and B-lymphoblastic leukemia/lymphoma, as well as rare cases of plasmablastic myeloma and low-grade follicular lymphoma (10, 27-29), making the point that double-hit lymphoma is not a specific histopathologic entity, but rather a genotype that can be observed in variety of mainly high-grade B-cell lymphomas, although cases with Burkitt or Burkitt-like features are certainly among the most common.

There is evidence to suggest that in double-hit lymphoma, the *MYC* translocation may be the second hit, which serves to kick the lymphoma into high gear. A number of case reports describe acquisition of *MYC* translocation by low-grade follicular lymphoma already possessing a *BCL2* translocation to be associated with transformation to an aggressive high-grade B-cell lymphoma, with some of these high-grade tumors resembling BL or BLL (10, 30), but others resembling diffuse large B-cell lymphoma or even TdT-positive B-lymphoblastic leukemia/lymphoma. (10, 28, 31-33) (It is tempting to speculate that the cases reported by Natkunam et al. in 2000 as “blastic/blastoid transformation of follicular lymphoma” could represent further examples of this phenomenon, although these investigators did not examine their cases for *MYC* translocation.(34)) Further evidence for the *MYC* translocation being the second hit comes from the 2009 study by Boerma et al.(35) These investigators found the *IG-MYC* translocations in Burkitt-like double-hit lymphoma to more commonly involve the Ig light chain genes on chromosomes 2 and 22 than does single-hit classical BL; this supports the hypothesis that the *IG-MYC* translocation in such cases is a secondary event, as one *IGH* locus is already occupied by the primary translocation, i.e., the t(14;18), and the other *IGH* allele needs to remain functionally rearranged to permit B-cell receptor signaling.(35) In addition, several of the double-hit lymphoma patients reported by Snuderl and colleagues had pre-existent low-grade follicular lymphoma, from which the high-grade neoplasm could have arisen.(28) The majority of double-hit lymphomas, however, appear to arise *de novo*, that is, in patients with no evidence of a pre-existent low-grade lymphoma.(14, 27-28)

Some authors expand the definition of double-hit lymphoma to include lymphomas with any kind of *MYC* translocation plus any translocation involving the *BCL2* gene, the *BCL6* gene, or the *CCND1* gene that encodes cyclin D1.(35) The 2008 WHO classification does not go as far, but accepts as double-hit lymphoma cases having translocations of *MYC* plus either *BCL2* or *BCL6*, and cheekily refers to rare cases having all three as “triple-hit” lymphomas.(26) Like *BCL2*, *BCL6* is translocated in a significant proportion of cases of both DLBCL (7-31%)(36-38) and follicular lymphoma (10%).(39) The unifying theme behind the concept of double-hit lymphoma in the 2008 WHO thus appears to be a

MYC translocation combined with a translocation common in DLBCL or follicular lymphoma.

The proportion of double-hit lymphomas appears to increase with age, and may exceed 30% in elderly patients.(26). Some authors have speculated that double-hit lymphomas could explain the inferior survival rates in adult BL and BLL, which like childhood BL, shows an excellent initial response to appropriate therapy (17-18), but has poor long-term survival rates of 15-25% compared with the 70-80% seen in pediatric cases.(40) But interestingly, Boerma at al. did find among adult BL a number of cases showing the same low cytogenetic complexity that typifies childhood disease (35), suggesting that true BL does exist among adults. Given the excellent response of classical BL to appropriate therapy, and the very poor prognosis associated with double-hit lymphomas even when treated with intensive chemotherapeutic regimens (28), it is important for pathologists to distinguish such cases if at all possible. To a first approximation, this can be done by FISH, with *BLC2* and *BCL6* breakpoints serving as markers of high genetic complexity.(24)

Clues to Distinguishing Classical Burkitt and Burkitt-Like Lymphoma from Studies of Gene Expression Profiling

Clues to additional features that could be useful to diagnostic pathologists in distinguishing true BL from histopathologic imitators were provided by two studies published in 2006, each of which sought to establish a molecular definition of BL based on gene expression profiling.(19, 41) Both of these reports were able to identify a so-called molecular signature for BL based on a core group of well-characterized classical BL cases. This signature proved to be distinct from the molecular signature of most DLBLC cases, providing hope for a molecular method to establish the diagnosis of BL.

In one of these studies, by Hummel et al., lymphomas with the molecular signature of BL (termed “mBL”) were found to be much more likely than non-mBL cases to express CD10 and BCL6, lack expression of BCL2, have a proliferation fraction of ≥95%, have an *IG-MYC* translocation, lack translocations of *BCL2* and *BCL6*, and show low cytogenetic complexity.(41) Of particular note, 100% of their mBL cases were positive for CD10 and BCL6 by immunohistochemistry, suggesting these may be virtually defining markers for *bona fide* BL. In contrast, significant minorities of mBL cases expressed BCL2 (19%) or had proliferation fractions <95% (34%). These various immunophenotypic features of BL grew less common as the molecular signature of the various lymphomas grew more different from the classical BL core group. Interestingly, while most of the 41 mBL cases had a *IG-MYC* translocation and low cytogenetic complexity, one case had a translocation of *MYC* to a non-*IG* partner, and four cases (9%) lacked *MYC* translocations entirely, with all five of these genetically atypical cases appearing cytogenetically complex. All lymphomas in this study showing both the classic morphology and immunophenotype of BL had the mBL signature. However, “atypical” BL cases (which this group defined as having either BLL morphology or a “deviant immunophenotype,” but lacking “the distinctive morphologic appearance of DLBCL”) displayed the mBL signature in only 75% of cases. These findings went on to influence the revised definition of BLL in the 2008 WHO classification, as we will see below. 7% of

the lymphomas identified in this study by expert hematopathologists as DLBCL based on morphology and immuno-phenotype were identified by gene expression profiling as mBL; the significance of such cases remains unclear.

The other gene expression profiling study, by Dave et al. (19), also identified a molecular signature for BL that was present in all cases of classical BL, somewhat less common in morphologically atypical BL, and much less common in cases resembling DLBCL. Interestingly, if both the original pathologist and the expert panel of reviewing hematopathologists felt that a case represented either BL or BLL based on morphology and immunophenotype (45 cases), the molecular diagnosis was nearly always mBL. But there were 26 cases in which the original pathologist made a diagnosis of BL or BLL, but after expert review the diagnosis was changed to either DLBCL or high-grade B-cell lymphoma, NOS. But in 8 of these 26 cases (31%) the molecular gene expression pattern reversed the diagnosis again, to mBL. As diagnosed by experts, BL or BLL became a "purer" category, "contaminated" by fewer cases with a molecular signature of DLBCL. But these experts also excluded from the category of BL significant numbers of mBL cases, which in routine practice could have denied these patients the more intensive chemotherapeutic regimens that this same study proved much more effective in treating mBL cases. Lessons that emerge from this study include: 1) cases with unequivocal Burkitt-like features (i.e., resemble BL or BLL to all observers) almost always represent BL molecularly, and 2) expert hematopathologists may sometimes do more harm than good! The rare cases of double-hit lymphoma identified in this study all showed high levels of *BCL2* mRNA expression, in keeping with the above-described studies that found high levels of BCL2 protein expression in double-hit lymphoma by immunohistochemistry.

As discussed in an editorial that accompanied their publication (23), neither of these two studies provides a definitive answer to the question of whether molecular diagnosis of BL is "better" than standard morphology and immunophenotyping, particularly in terms of patient outcome (i.e., directing more patients to the most effectively therapy). In addition, gene expression profiling remains impractical at present for routine diagnosis, but the prospect of being able someday to provide a precise molecular definition of BL remains alluring.

Return of the Gray Zone between Burkitt Lymphoma and Diffuse Large B-Cell Lymphoma: The 2008 WHO Classification

The 2008 WHO classification resurrects the concept of a high-grade B-cell lymphoma occupying a gray zone between BL and DLBCL in a new category with the ungainly title of "B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL,"(26), which is commonly abbreviated DLBCL/BL.(24) DLBCL/BL is one of the two officially sanctioned "gray zone" lymphomas in the new 2008 WHO, the other being lymphomas showing features intermediate between DLBCL and classical Hodgkin lymphoma.(24, 26) This new DLBCL/BL category is similar but not identical to the earlier categories of atypical BL or BLL, with many of the changes inspired by the findings in the study by Hummel et al. discussed above.(41)

In the 2008 WHO, a diagnosis of DLBCL/BL can be arrived at in two different ways. (24, 26) First, mature B-cell lymphomas can be placed in this category if they have the CD10+ BCL6+, BCL2- immunophenotype of classical BL, but show morphologic features intermediate between DLBCL and BL, with "some" cells resembling BL and "some" cells resembling DLBCL (the definition of "some" remaining frustratingly unspecified). It is noted that cases in this group should have a "high proliferation fraction," although the WHO notes (citing the McClure study among others) that "high" can be anywhere from 50 to 100%. This first DLBCL/BL group generally resembles the "Burkitt-like" categories of the 1994 REAL and 2001 WHO classification with one important exception: minor atypical cytologic features that had previously pushed cases into the Burkitt-like category, such as irregular nuclear contours and solitary prominent nucleoli, no longer exclude a diagnosis of classical BL (3); instead only marked cellular pleomorphism, specifically large cells in excess of that acceptable for classical BL, place a lymphoma otherwise typical of BL in this category.(24, 26) Second, B-cell lymphomas can be placed in the DLBCL/BL group if the morphologic is entirely typical of BL, but there are atypical immunophenotypic or genetic features that preclude a diagnosis of BL. Strong uniform expression of BCL2 is specifically mentioned as a contraindication that should suggest a possible double-hit lymphoma, which remains the best characterized category within DLBCL/BL.(26, 42)

The 2008 WHO classification specifically excludes cases of morphologically typical DLBCL from the DLBCL/BL category, even if they show rearrangement of *MYC* or have a very high proliferation fraction. It further notes that the absence of a *MYC* translocation alone does not move a case to this category from classical BL provided all other features support the diagnosis. The WHO concedes that DLBCL/BL is a heterogeneous category that is not considered a single disease entity, but can nonetheless be useful in identifying cases that should be excluded from the category of BL because of their fundamentally different biology and inferior prognosis.

Distinguishing Burkitt Lymphoma from Other Entities: State of the Art in 2011

First and most importantly, don't panic! Although the criteria for the diagnosis of DLBCL/BL provided in the 2008 WHO classification can seem frustratingly vague, most cases can be relatively easily classified into the BL, DLBCL/BL, or DLBCL categories following an algorithmic approach drawn from the WHO classification itself and commentaries published by expert hematopathologists.(3, 24, 26)

Starting first with morphology, it is important to remember that most cases with convincing BL histology will prove to be classical BL, even by molecular studies. These features include medium size lymphoid cells (nuclei similar in size to nuclei of benign histiocytes, or about twice the size of small round lymphocytes) that have round to minimally irregular nuclei, variably clumped chromatin, distinct nucleoli that are typically multiple, and scant basophilic cytoplasm (which may contain cytoplasmic lipid vacuoles in cytologic preparations), along with a high mitotic rate and, frequently but not invariably, admixed histiocytes that produce a starry-sky appearance. In such cases, demonstration of the CD10+ BCL6+ BCL2- phenotype of BL permits a diagnosis of classical BL without additional diagnostic studies, according to the WHO (26); however, given the increasing

incidence of double-hit lymphoma with age, some authorities recommend FISH testing of all adult cases to exclude translocations of *BCL2* and *BCL6*.(24) One should also keep in mind that minimal cytologic pleomorphism, such as irregular nuclear contours or prominent nucleoli, although once considered unacceptable features for classical BL, are no longer contraindications to the diagnosis in the 2008 WHO.

Certain morphologic features should, however, raise doubt as to the diagnosis of BL. If the neoplastic cells appear small rather than medium size (as occurs in some BL cases), it should prompt a consideration of mature small B-cell lymphomas, most cases of which will have few mitotic figures and proliferation fractions well under 50%, and will (with the exception of follicular lymphoma) lack the immunophenotype of BL. Follicular lymphoma, though often positive for CD10 and BCL6, can be further distinguished from BL by its characteristic centrocytic cytology and frequent follicular architecture. In addition, small B-cell lymphomas are quite uncommon in children and young adults, and thus the diagnosis should be questioned in young patients. If the neoplastic cells appear to have indistinct nucleoli and finely dispersed chromatin, a stain for TdT should be performed to exclude B-lymphoblastic leukemia/lymphoma, with surface immunoglobulin detected by flow cytometry also helpful in excluding lymphoblastic B-cell neoplasia.

Finally in the morphologic assessment of possible BL cases comes the issue of large cells, which are defined in the WHO has having nuclei larger than those of benign histiocytes, or more than twice the diameter of small lymphocytes. In addition, large lymphoid cells typically have cytoplasm that is both more abundant and more eosinophilic than in the cells of BL. As discussed above, it is important to keep in mind that lymphomas with the characteristic morphology of DLBCL, composed mainly of large cells and lacking significant populations of medium size cells resembling BL, are at present excluded from both the category of BL or the “gray zone” category of DLBCL/BL in the current WHO classification, regardless of whether they have MYC translocations, high proliferation fractions, or even show the molecular signature of BL.(24, 26) So how many large cells in a BL case should suggest an alternate diagnosis of DLBCL/BL? And conversely how many medium size BL-like cells does it take to push a DLBCL into the gray zone DLBCL/BL? This subject appears to have been left deliberately vague in the 2008 WHO, but some rules of thumb seem appropriate. If either medium size Burkitt-like cells or large lymphoid cells predominate to the point that one would be willing to publish photographs of most fields as examples of classical BL or DLBCL, then DLBCL/BL should not be considered on morphologic grounds (although it would need to be reconsidered if the immunophenotype argues against BL; see below). But if significant areas of the tumor resemble the alternate entity, or have a significant admixture of medium and large cells producing an appearance inconsistent with either classic BL or DLBCL, then a diagnosis of DLBCL/BL should be made, and FISH studies performed to exclude double-hit lymphoma. In such cases, either more or fewer immunophenotypic or genetic features of classical BL might push one towards one end or the other of the DLBCL/BL gray zone, but an unequivocal diagnosis of either DLBCL or BL is not possible. It is important however not to use the DLBCL/BL category as a wastebasket for all high-grade B-cell lymphomas that have any combination of medium and large cells, lest all of one’s high-grade B-cell lymphoma cases end up residing there!

Even in cases that show morphology compatible with classic BL, an unacceptable immunophenotype or genotype can still move the case into the DLBCL/BL category. What immunophenotypic features are unacceptable? Strong BCL2 expression is a general contraindication to the diagnosis of BL, and indeed suggests the likelihood of a double hit lymphoma. Weak BCL2 expression, however, is seen in 10-15% of classical BL cases (43), and does not exclude the diagnosis. It is not specifically stated in the 2008 WHO classification whether all cases of BL must express both CD10 and BCL6, but certainly most do (5), and given that 100% of cases showing the molecular BL signature in the gene expression profiling study by Hummel et al. (41), lack of either marker should call the diagnosis of classical BL into question.

Proliferation fraction as measured by immunohistochemical staining for Ki-67 or MIB-1 is often >90% but can be in the 80-90% range even in pediatric cases, and 50% or even lower in adult cases.(14, 43) Keep in mind that in the molecular study by Hummel et al., fully a third (33%) of mBL cases had proliferation fractions of <95% (41). Thus a threshold of 90% or 95% will exclude a significant proportion of *bona fide* BL cases, and a relatively low proliferation fraction should not stand in the way of the diagnosis in otherwise typical cases.

If FISH studies are performed, translocations involving the *BCL2* or *BCL6* genes exclude the diagnosis of classical BL. But the lack of a *MYC* rearrangement does not exclude BL, as the 2008 WHO permits the diagnosis of BL in *MYC*-negative cases if all other features are entirely typical. *MYC* translocations are neither sensitive nor specific for BL. They are found in 7-15% of DLBCL cases.(36-38) The have also been reported to be absent in up 45% of otherwise classical BL cases in one study (44), which found no difference in response to therapy between *MYC*-positive and *MYC*-negative BL patients. However, because *MYC*-negative BL appears to show some distinctive genetic features, this group appears deserving of further study.(35) Other cytogenetic features that argue against classical BL and in favor or DLBCL/BL include MYC translocations to non-Ig partners and highly complex karyotypes.(26, 35, 41)

In summary, the diagnosis of BL should be restricted to B-cell lymphomas with BL or BLL morphology that express CD10 and BCL6 but show absent (or at most very weak) expression of BCL2, have a relatively high proliferation fractions of at least 50%, and lack evidence of translocations involving the *BCL2* or *BCL6* genes, although FISH testing for *BCL2* or *BCL6* is not essential if both the morphology and immunophenotype features are classic, at least in pediatric cases.(3, 24, 26) All other mature B-cell lymphomas with BL or BLL morphology should be placed in the new WHO category of DLBCL/BL, and mature B-cell lymphomas with DLBCL morphology should be placed in the category of either DLBCL or pleomorphic mantle cell lymphoma, depending on whether or not BCL-1 expression or the t(11;14) translocation characteristic of mantle cell lymphoma is present. Although most BL cases have them, the lack of a demonstrable *MYC* translocation at present does not exclude the diagnosis of BL provided all other features are typical.(3)

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