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Burkitt lymphoma versus diffuse large B-cell lymphoma

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Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL) represent distinct entities among aggressive B-cell non-Hodgkin lymphomas (B-NHLs) in the WHO classification [1]. According to the clinical setting of the occurrence, endemic, sporadic and immunodeficiency-associated BL can be distinguished as clinical variants. The diagnosis of classical BL rests on the presence of a monotonous infiltrate of medium-sized blastic lymphoid cells that show round nuclei with clumped chromatin and multiple, centrally located nucleoli. The tumor cells have a high proliferation rate and intermingled macrophages containing apoptotic debris lead to the morphological aspect of a 'starry sky' [1] pattern. Immunophenotypic features of BL include positivity of tumor cells for CD20 and CD10 (and BCL6), negativity for BCL2 and a proliferation fraction measured by Ki-67 immunohistochemistry of nearly 100%. In addition, a chromosomal rearrangement of *MYC*, usually in form of the classical translocation t(8;14)(q24;q32) should be demonstrated at the molecular level. In contrast, DLBCL is characterized by the proliferation of large neoplastic B-cells comprising centroblastic, immunoblastic, T-cell/histiocyte-rich and anaplastic morphological variants [1]. While the differential diagnosis between BL and DLBCL may appear clear-cut in theory, daily practice shows that aggressive B-NHLs are encountered that display some (but not all) morphologic, immunophenotypic and genetic features of classical BL. For these cases, the terms 'atypical BL' or 'Burkitt-like lymphomas' have been coined. According to the criteria of the WHO, the term 'atypical BL' denotes true BL with some unusual—for the most part cytological—features, while the term 'Burkitt-like lymphoma' is a non-compromising diagnosis. Hence, whether these cases are biologically and clinically closer to BL or DLBCL is currently a matter of debate.

For example, are aggressive B-NHLs with morphological features of classical BL, positivity for CD20 and CD10 and a Ki-67 index of 100% 'true' BL, when they show coexpression of BCL2? Likewise, do 'true' BL exist that lack genetic rearrangements of *MYC*?

Clinically, the diagnosis of the hematopathologist is of major importance, since treatment regimens for BL [2] and DLBCL [3] differ significantly. Specifically, adult BL are frequently treated with high-intensity chemotherapy regimens including central nervous system prophylaxis leading to an overall survival of 50–70% of these patients [2].

Classical BL, i.e. B-NHL that fulfills all of the above mentioned diagnostic criteria, can be highly reproducibly diagnosed among hematopathologists and, therefore, these cases do not represent a problem—neither with respect to their proper classification nor to the therapeutic implication of the diagnosis. However, what does the diagnosis 'atypical BL' or 'Burkitt-like lymphoma', which has an unacceptably low inter- and intra-observer reproducibility [4] imply for the treating hematologist and where should pathologists draw the line between the diagnosis of atypical BL and DLBCL?

On the genetic level, translocations involving *MYC* are a hallmark feature of classical BL. Information on the *MYC* translocation status, however, is insufficient to discriminate between BL and DLBCL, since 5–10% of DLBCLs also carry a *MYC* translocation [5].

In recent years, several groups have attempted to establish molecular categories within the gray zone between BL and DLBCL. In a study by Haralambieva and colleagues [6], two independent approaches were tested to assign gray zone lymphomas either to the BL or to the DLBCL category. One approach used only information on morphology, immunohistochemistry, age of the patient and site of involvement (without knowledge of any molecular data), whereas in an independent approach immunohistochemical and molecular markers recommended by the WHO classification (positivity for CD10, BCL6, Ki-67 > 90%, negativity for BCL2; presence of a *MYC* breakpoint, but absence of *BCL2* and/or *BCL6* breakpoints) were used as a basis for the distinction. In a control group of pediatric BLs, both algorithms were in agreement in 100% of cases. However, only 20% of the investigated gray zone cases fulfilled the criteria of both algorithms suggesting that only a minority of these cases may represent bona fide BL [6]. Nakamura and colleagues [7] investigated 18 cases of B-NHL with proven *MYC* rearrangement, which were classified as BL or DLBCL based on histological features. Although BLs were characterized by a significantly higher Ki-67 index than DLBCLs and, vice versa, DLBCLs were more frequently positive for BCL2, this study demonstrated a significant overlap between these two groups. A practical approach to the subdivision of highly proliferative B-NHL within the spectrum of BL and DLBCL was recently suggested by Cogliatti and coworkers [8]. Based on available information on morphology (categorized as classical BL, atypical BL and DLBCL), immunophenotype (CD20, CD10,

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BCL6, BCL2, Ki-67; categorized as prototypic, compatible and incompatible) and genetic rearrangements of the *MYC* and *BCL2* loci, four diagnostic categories (DCs) were defined. These include DC I (classical BL), DC II (atypical BL), DC III (*MYC*-positive DLBCL) and DC IV (MYC-negative highly proliferative DLBCL). Considering the required time needed for tissue processing, immunohistochemistry and fluorescence *in situ* hybridization (FISH), the authors suggest that a reliable assignment to one of the diagnostic categories can be achieved within 1 week [8]. While, however, the assignment to groups DC I and DC IV appears straightforward, DC II and DC III may harbor molecularly heterogeneous cases including so-called 'double hit' or 'dual translocation' cases (i.e. B-NHL carrying both *MYC* and *BCL2* translocations).

The morphological feature of a 'starry sky' pattern that is considered a hallmark in BL can also be present in a subset of highly proliferative DLBCLs raising the question whether these cases may resemble BL molecularly and whether these cases should be subsequently classified as atypical BL. According to a recent study, however, highly proliferative DLBCLs with a 'starry sky' pattern are only rarely positive for CD10 and infrequently carry a *MYC* translocation [9]. Thus, BL and highly proliferative DLBCL with a 'starry sky' pattern might be reliably distinguished.

From a clinical point of view, the optimal therapeutic choice for patients with B-NHL in the gray zone between BL and DLBCL remains controversial and reliable clinical data are sparse. In a retrospective study by Sevilla and colleagues [10] only 18 of 33 highly proliferative B-NHLs with cytomorphologic, histologic and immunophenotypic features of BL carried a *MYC* translocation, and survival analysis demonstrated that adults with *MYC*-positive tumors had superior survival in comparison with patients with *MYC*-negative tumors [10]. McClure and colleagues [11] concluded from a study of 31 tumors that fulfilled the WHO criteria for atypical Burkitt/Burkitt-like lymphomas that a Burkitt-like morphology was correlated with decreased survival. Moreover, within the groups of Burkitt/Burkitt-like lymphoma and DLBCL, the presence of *MYC* translocations was associated with inferior clinical outcome. Ohshima's group [12] retrospectively analyzed clinical outcomes of high-grade mature B-cell lymphomas with Burkitt-like morphology and concluded that overall survival of patients who received aggressive short-term therapy was much better than that of patients who received cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) therapy. Whether aggressive short-term therapy is still superior when immunotherapy, e.g. anti-CD20 antibodies (rituximab), is combined with CHOP/CHOP-like regimens, remains to be tested in prospective clinical trials. One major factor emerging from these retrospective studies cited above is, again, either that morphological criteria for diagnosing BL are not well established, or that morphological differences are too subtle to allow for reliable inter-observer discrimination.

Two major DNA-microarray studies [13, 14], therefore investigated whether global gene expression profiles might help to discern BL from DLBCL on the molecular level. In an effort by the German Network Project 'Molecular Mechanisms in Malignant Lymphomas (MMML)', Hummel and associates [14] profiled 220 mature aggressive B-cell lymphomas that included classical BL, atypical BL and DLBCL. Initially, a 'core group' of eight BLs was defined, all of which fulfilled the current WHO criteria including a *MYC* translocation as detected by FISH. Using a newly developed statistical approach termed 'core group extension', gene expression profiles of the remaining cases were compared with the BL core group and each of these cases was labeled with a 'BL similarity index'. This 'BL similarity index', derived from the gene expression levels of 58 genes, classified aggressive B-NHL into molecular Burkitt lymphoma (mBL; 22%), intermediate cases (20%) and non-molecular Burkitt lymphoma (non-mBL; 58%). Array-based comparative genomic hybridization (CGH) furthermore revealed that mBL cases had very few genetic alterations in addition to the *MYC* translocation (low genetic complexity), whereas intermediate and non-mBL cases carried a higher load of chromosomal imbalances (high genetic complexity). Of importance, non-mBL (DLBCL) cases that harbored a *MYC* translocation had inferior overall survival as compared with DLBCL cases without *MYC* translocation in a retrospective analysis of patients receiving mostly a CHOP/CHOP-like therapy.

In an independent study conducted by the Lymphoma and Leukemia Molecular Profiling Project (LLMPP) [13] 303 patients with the initial diagnosis of BL, atypical BL and DLBCL were profiled using a custom-made oligonucleotide array (Affymetrix) containing 2524 genes. In a two-step procedure, aggressive B-NHL was first divided into cases in which a *MYC* target gene signature was identifiable in the global gene expression profile and those in which such a signature was not evident. In a second step, expression profiles of the cases that contained a *MYC* target gene signature were compared with the signatures of germinal center B-cell-like (GCB) DLBCL, activated B-cell-like (ABC) DLBCL and primary mediastinal lymphoma (PMBL). Only when all four pair-wise comparisons of the gene expression profiles were in favor of the diagnosis of BL, was the diagnosis 'molecular BL' assigned. This rigorous statistical approach explains the lack of an intermediate group in the LLMPP study, since, by default, all lymphomas that showed equivocal results in one of the four pair-wise statistical tests were assigned to the DLBCL group. Biological features of the gene expression-based classifier for BL include the expression of *MYC* target genes (as expected), a particular subgroup of germinal center B-cell-associated genes and a low expression of major histocompatibility (MHC) class I genes and NF-κB target genes [13].

Although the gene expression signatures of molecular BL are composed of different genes in the two studies [13, 14] both predictors remarkably identify the same cases as molecular BL in the independent data sets (R. Spang, personal communication). Both gene expression studies, remarkably, agree in their findings that a subset of aggressive B-NHL with clear-cut morphological features of DLBCL were identified that, nevertheless, showed a gene expression profile of BL ('discrepant BL'). Therefore, both studies help to sharpen the molecular distinction between BL and DLBCL, but, at the same time, extend the spectrum of molecular BL to some cases that would currently be classified as DLBCL. Should the gene expression-based molecular predictors be considered the

gold-standard for the diagnosis of BL in the future? This question will have to be answered in future molecular studies and correlations with clinical parameters. It should be emphasized, however, that there is 100% agreement between the diagnosis of classical BL (i.e. cases that fulfill all current WHO criteria) and the molecular diagnosis of these cases based on a gene expression signature. Therefore, these cases can be reliably discerned using methods that are currently applied in the state-of-the-art work-up of lymphomas in experienced diagnostic centers.

Some of the questions that will have to be addressed in the future are listed below.

(i) Most B-NHL that is currently classified as atypical BL show a molecular BL signature that is indistinguishable from that of classical BL. However, both gene expression studies [13, 14] identified a few atypical BLs that had a molecular signature closer to DLBCL. What are the underlying biological features of these 'aberrant' atypical BL?

(ii) Some of the molecular BL cases show expression of BCL2. Can future molecular and clinical studies confirm that these cases represent bona fide BL?

(iii) The study by the German Network Project identified a few molecular BL cases without any evidence of a *MYC* rearrangement. Can such cases be accepted as bona fide BL? If so, what are the molecular surrogates that compensate for the lack of a *MYC* rearrangement during the pathogenesis of BL?

(iv) Does 'discrepant BL', i.e. B-NHL with clear-cut morphological features of DLBCL, but a gene expression profile of BL, represent bona fide BL? There is clearly some doubt in a subset of these cases. First, this group harbors tumors carrying both *MYC* and *BCL2* translocations (double hit or dual translocation cases) suggesting that these cases are genetically distinct from BL. Moreover, there is accumulating evidence, also presented at this meeting, that dual translocation cases show a dismal clinical course with survival times of <1 year. Finally, patients with 'discrepant BL' are significantly older than patients with classical or atypical BL and carry an increased number of chromosomal imbalances as detected by CGH (LLMPP, unpublished).

In summary, despite recent advances in the efforts to establish a molecular diagnosis of BL, there remains a gray zone of cases that show morphological, immunophenotypic and molecular features that are intermediate between BL and DLBCL. This aspect will be reflected in the next edition of the WHO classification, in which the category 'B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL' will be suggested. It is recognized that this category will include a heterogeneous group of cases and it is therefore not considered a distinct disease entity. However, the separation of these cases from BL and DLBCL might allow more detailed molecular studies in the future and, especially, a thorough correlation with clinical features of these patients.

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