Novel lymphoid neoplasms – the borderland between diffuse large B-cell lymphoma and Burkitt's lymphoma

Daphne de Jong
The Netherlands Cancer Institute, Department of Pathology, Amsterdam, The Netherlands.
E-mail: d.d.jong@nki.nl. *doi:10.3324/haematol.2009.008128*

Tumor classification systems provide a means of communication between pathology, clinical medicine and basic research to support clinical decision-making, comparison of therapeutic protocols and development of new biological insights. Since the introduction of the Kiel classification in the 1970s, the lymphoma world has been very fortunate as compared to many other tumor systems. The paradigm of a biological foundation based on a *normal counterpart* hypothesis immediately propelled this classification beyond a mere morphological description. Since then, this principle has been further developed into classifications of *lymphoma entities* based on integrated morphological, immunohistochemical, genetic and clinical criteria. Some classes, such as classical Hodgkin's lymphoma, marginal B-cell lymphoma, MALT-type and mantle cell lymphoma, are very well delineated. Others are more complex and pose a larger problem in terms of biologically and clinically relevant categorization. Overlapping features between classes may be caused by a true biological continuum between two entity definitions, by an unusual feature within one class that may mimic another class or by subclonal alterations within a single entity resulting in marked heterogeneity. This can easily lead to much confusion and a nomenclature that is hampered by "-oid"s, "like"s and "borderlines". Since clinicians prefer to treat patients according to clear protocols for defined diseases, this is obviously unattractive and quite frustrating.

The most recent update of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (2008),[1] concentrated specifically on overlapping areas and differential diagnoses. An approach was taken to deliberately keep set entities as well-defined and as *clean* as possible and to separate off borderline situations into provisional classes awaiting further insights that will allow evidence-based classification in the future. The border between Burkitt's lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL) is such an area of diagnostic overlap and will be discussed in this review.

The differential diagnosis of diffuse large B-cell lymphoma and Burkitt's lymphoma

In the previous version of the WHO classification (2001),[2] epidemiological variants of endemic BL, sporadic BL and immunodeficiency-associated BL as well as morphological variants of BL with plasmacytoid differentiation, atypical BL/BL-like were recognized. As a result of the strategy to create maximally homogeneous classes, the morphological variants are no longer separately defined in the present classification. Results from translocation studies and especially gene-expression analysis have lent sufficient support to expand the morphological spectrum of BL allowing greater nuclear polymorphism.[3,4] The definition of the immunophenotype is very strict, however, with strong CD10 and bcl-6 expression in the absence of bcl-2 expression and with a very high proliferation fraction with (almost) 100% KI-67 positive tumor cells. *MYC* translocation to either immunoglobulin (*IG*) heavy chain genes or light chain genes is uniformly found and generally in the context of a simple karyotype with only very few other genetic alterations.

Problems of classification arise in tumors that show the morphological features of BL but whose immunophenotype differs from that defined for BL, e.g. strong bcl-2 positivity or absence of CD10. Absence of a *MYC* translocation or a complex karyotype results in a similar dilemma. Gene-expression studies by two independent teams have shown that BL has a distinct molecular signature that sets it apart from DLBCL.[3,4] Moreover, the molecular signature supports the notion that a rare subset of tumors with otherwise fully characteristic features of BL may lack *MYC* translocations. Other mechanisms of *MYC* deregulation have been suggested for these cases.[5] In practice, this diagnosis is only made in the context of a completely fitting morphology and immunophenotype and preferably also a simple karyotype. In part based on the same gene-expression studies, weak bcl-2 expression and a somewhat lower proliferation index do fit the signature of BL and cases with these features should be diagnosed as BL. Strong bcl-2 expression and a proliferation index below 95% preclude a diagnosis of BL.

Taken together, a diagnosis of BL should only be made in the presence of a single discordant diagnostic feature when all other diagnostic criteria are indisputable and completely fitting. In practice, this may require extensive assessment that includes a broad spectrum of immunohistochemical studies, evaluation of multiple translocations and a search for a complex karyotype.

B-cell lymphoma unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt's lymphoma

The narrow definition of BL and increasing information on immunophenotypic and genotypic features in diagnostic pathology have produced the need for a class to accommodate cases that do not fulfill the criteria for BL, but do share sufficient features that would make one uneasy just to classify the case as DLBCL. *B-cell lymphoma unclassifiable with features intermediate between DLBCL and BL* should not be regarded as an entity, but rather as a quarantine to set this cases apart until future insights for better classification are available.

"Dual hit" lymphoma

One distinct class stands out as relatively well-characterized, however, and actually consists of the majority of cases within *B-cell lymphoma unclassifiable with features intermediate between DLBCL and BL*. These are the so-

called *double hit* lymphomas that are the subject of a report by Tomita *et al.* in the current issue of Haematologica.[6] These cases are characterized by both *MYC* and *BCL2* rearrangements and, more rarely, *MYC* and *BCL6* rearrangements. Also *triple hit* lymphomas bearing all three translocations have been described, including 7/27 cases in the series by Tomita *et al*. The morphological spectrum is most often reminiscent of BL with a striking starry-sky pattern, but in general these cases show greater nuclear polymorphism. Bcl-2 expression is uniformly high and this should direct pathologists to the correct diagnosis.[7,8] Most often, a complex karyotype is found. There are, therefore, a least two more reasons, apart from morphological arguments, not to consider such cases as BL. Interestingly, however, some of the few *dual hit* cases that were included in both reported gene-expression series showed a molecular signature of BL while others clustered with DLBCL.[3,4] From a biological point of view, one might consider *dual hit* lymphomas as transformed follicular lymphoma (FL). There are some cases of patients with a known history of FL prior to diagnosis of this aggressive lymphoma and the *MYC/IG* translocation can be identified as a secondary event after a prior *BCL2/IG* translocation.[9,10] In other cases, *dual hit* lymphoma is diagnosed as a first presentation and transformation of a (possibly subclinical) FL cannot be proven. The molecular structure of the *BCL2/IG* translocation is virtually always very similar to a normal VDJH complex, suggesting a very early event in lymphomagenesis in an immature precursor cell. The molecular structure of the *MYC/IGH* translocation in sporadic BL and in *dual hit* cases is rather related to class switching events. *MYC/IGL* translocations are more frequently found in *double hit* lymphoma than in BL. Both alterations are related to molecular events that take place later in B-cell development than the VDJH rearrangement, further supporting the concept that *BCL2* alterations precede *MYC* events.[11]

BCL2/IG as an early event linked to VDJH recombination may imply the presence of a FL tumor stem cell compartment with precursor B-lymphoblastic features. Indeed, a transforming *MYC* event in FL does not, in all instances, result in a lymphoma with features reminiscent of DLBCL or BL, but rather in an aggressive lymphoma with immature characteristics with TdT expression, lacking CD20 and often lacking membranous immunoglobulin.[8] These lymphomas are not included in the category of *B-cell lymphoma unclassifiable with features intermediate between DLBCL and BL* but classified as B-lymphoblastic leukemia.

Other genetic alterations involving MYC

From the discussion on *B-cell lymphoma unclassifiable with features intermediate between DLBCL and BL* it follows that *MYC* translocation may be characteristic of, but not specific, to BL. Moreover, *MYC* alterations outside the context of BL more often show unusual features. As also seen in the series reported by Tomita *et al.*, translocation to *IG* light chain partners is more often seen in *dual hit* lymphoma.[6] *MYC* alterations may be seen in approximately 20-30% of morphologically perfect DLBCL both with and without a BL immunophenotype.[12,13] Up to 40% of these cases do not have a *MYC/IG* translocation, however, but have other *MYC* partners.[3,14] Based on a similar distribution among *intermediate* and *non-molecular BL* gene-expression profiles, it is suggestive that translocation to other partners results in a similar deregulation of MYC as does the classical *MYC/IG* translocation. Proliferation rates in these lymphomas are generally very high. For rare amplifications of *MYC* which generally do not exceed ten copies, the biological similarity is more debatable. Again, the decision algorithm for classification starts with morphology. Morphology of DLBCL directs the diagnosis to DLBCL irrespective of the presence of a BL immunophenotype or a *MYC* translocation. Not all morphologies should, however, be acceptable.

Clinical consequences

The principle of treatment of BL is to interfere with the very high doubling time of the tumor of 24 to 48 h. Therefore, intensive multi-agent chemotherapeutic regimens are considered to be most appropriate for BL, as they maintain effective serum drug concentrations for at least 48-72 h. Within that period of time, almost all tumor cells will have passed through the cell cycle and consequently be targeted by the cytostatic drugs.

Table. Diagnostic features of DLBCL, BL and B-cell lymphoma unclassifiable with features intermediate between DLBCL and BL ("gray zone").

	DLBCL	gray zone	BL
Morphology	Variable, ranging from medium-sized to large, pleiomorphic nuclei with a large morphological range	BL-like morphology wit or without large cells	Cohesive, starry-sky, medium-sized, round nuclei with multiple small nucleoli
Immunophenotype	BCL-2, CD10, BCL-6, MUM-1 highly variable Ki-67 30->95%	BCL-2 often strong, CD10 mostly+, BCL-6 mostly +, MUM-1 variable Ki-67 50->95%	BCL-2-, CD10+, BCL-6+, MUM-1 variable Ki-67 >95%
MYC translocation	5-15%	80%	90-100%
Non*IG* partner in *MYC* translocation	40%	40%	None
BCL2 translocation	20-30%	45%	No
BCL6 translocation	30%	9%	No
Simple karyotype	Rarely	Rarely	Typical
Complex karyotype	Generally	Generally	No

Furthermore, the tumor outgrowth and repopulation between cycles and possibly the development of drug resistance is kept as low as possible by keeping the intervals between the chemotherapy courses as short as possible and by using combinations of non-cross-resistant agents. Moreover, based on knowledge on the natural behavior of the disease, prophylactic central nervous system treatment is included. With these regimens, 2-year survival rates of 60-80% in high-risk pediatric patients and even up to 100% in low-risk patients can be reached.[15] Application of these regimens in adults patients results in lower, but still very good survival rates.[16]

The principle of high dose-high density treatment does not apply to the majority of DLBCL and such treatment would be excessive in most patients, with high treatment-related mortality and morbidity but without a survival benefit. Especially since the introduction of immunotherapy (R-CHOP), survival rates are rather good to excellent for patients with a good-to-intermediate risk score according to the International Prognostic Index (IPI). For the patients with a DLBCL with a poor risk according to the IPI score, the treatment requires improvement, but whether or not this shoud be achieved with dose intensification is a matter of considerable debate, especially with regards to the elderly population of patients. It should be noted that series of adult patients diagnosed as having BL inevitably include patients who would now be diagnosed as in the *gray zone* and *vice versa.* Such cases are also present in series treated as DLBCL, albeit with a lower frequency. Results from these studies cannot, therefore, be extrapolated to draw conclusions on the optimal treatment of *gray zone* patients.

Of the whole group of *gray zone* lymphomas, the dual translocation lymphomas bearing both a *BCL2* or *BCL6* and a *MYC* translocation are best defined and recognized. As described by Tomita *et al.* in the current issue of Haematologica, as well as in other series, the outcome of these patients is dismal irrespective of standard R-CHOP or intensified treatment including bone marrow transplantation.[17] While in elderly patients, who actually form the majority of this group, this may be sufficient grounds to refrain from aggressive treatment, this policy may not be acceptable for younger and fit patients. In these patients, treatment with BL regimens may therefore be preferred over standard R-CHOP, which will certainly not be sufficient to control the disease. This choice is not evidence-based, however, and preliminary results in small series do not give much grounds for optimism.[18,19] In ideal situations, a clinical trial for *gray zone* patients would shed more light on optimal treatment. In view of the rareness of the disease, poor recognition and the complex diagnostic procedures needed, such trials are very unlikely to be successful. Development of new treatment methods based on biological insights of relevant genes, molecular pathways and interactions may be a more successful approach in the future.

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Dr. Daphne de Jong is a staff pathologist with a special focus on hematopathology at the Netherlands Cancer Institute in Amsterdam, The Netherlands.

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