Follicular lymphoma grade 3B: is it a real disease?

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Follicular lymphoma (FL) is defined as a neoplasm of germinal center B cells, usually with a follicular growth pattern. The neoplastic population typically consists predominantly of centrocytes, the resting cells present in the light zone of normal germinal centers, and relatively few centroblasts, the more immature and rapidly proliferating cells of the germinal center dark zone. FL cells usually express Bcl6, CD10 and other proteins that are also expressed in germinal center B cells. A major difference between FL cells and germinal center B cells is the former's expression of the anti-apoptotic bcl2 protein, which is caused by the characteristic translocation t(14;18) involving the *BCL2* gene or its variants t(2;18) and t(18;22) and enables the cells to survive in the absence of antigenic stimulation. This results in accumulation of non-proliferating centrocytes with a minority of proliferating centroblasts and a low proliferation index with Ki-67 staining. According to the pathologist, this configuration is "translated" into a low histological grade of the lymphoma.

Although FL is typically widespread at the time of the diagnosis and indolent in its clinical evolution, it has long been recognized that clinical aggressiveness and risk of transformation to diffuse large B-cell lymphoma (DLBCL) increases proportionally to the numbers of large cells (centroblasts) and the proliferation fraction.[1-3] Pathological classification schemes developed largely in the USA (where FL is very common) suggested dividing FL into three subtypes (or grades), based on the number of large cells.[4] In contrast, the European Kiel classification[5] considered that cases containing both centrocytes (usually predominant) and centroblasts comprised one disease (centroblastic/centrocytic lymphoma, equivalent to the World Health Organization FL1-2, and possibly 3A), while those in which centroblasts predominated were considered a follicular variant of centroblastic lymphoma (a morphological variant of DLBCL). The WHO classifications of 2001 and 2008[6] recognized three grades - FL1-2, FL3A and FL3B (Table 1), based on the number of centroblasts present per high power field; the difference between FL3A and FL3B is the presence of a mixture of centrocytes and centroblasts in FL3A and the presence of follicles existing exclusively of centroblasts, immunoblasts, or both in FL3B. The continuation of a grading system is supported by the fact that several studies suggested that grades predict outcome, higher grades being associated with poor clinical outcome and more rapid progression to DLBCL. However, the reproducibility of grading is low, and it has not been easily replaced by immunohistochemistry for Ki67/MIB1 (proliferation index).

Several studies have tried to address the issue of whether there are biological differences between typical low-grade FL1-2 and FL with increased centroblasts (grade 3), and between FL3A and FL3B.[7-11] Several recent studies suggest that higher grade FL, in particular FL3B, are different with regards to genetic, immunophenotypic and clinical features from the prototypic FL1-2. While FL1-2 lymphomas are relatively homogeneous (expression of CD10, BCL2 and BCL6, and carrying a t(14;18) in 90% of the cases), FL3A and, in particular, FL3B are more heterogeneous with respect to immunophenotype, genetic abnormalities, and gene expression. Individual studies have taken different approaches to this issue but encountered the same problem: some cases of FL3B have many features in common with FL1-2, whereas other cases have more in common with DLBCL (Table 2).

Any study on the biology of FL3B is complicated by relatively simple factors related to the definition itself and purely histopathological aspects. First, some cases of FL contain large centrocytes (large cleaved cells) or small centroblasts that may be interpreted differently by individual pathologists, so that the border between FL3A and FL3B may be blurred. Second, some cases of FL show considerable heterogeneity from area to area. Third, poor tissue handling, fixation or technical processing problems may introduce artifacts that interfere with grading. Fourth, in many cases of FL3B the biopsy contains diffuse areas with sheets of centroblasts, consistent with DLBCL. The presence of such diffuse areas is associated with a worse outcome,[2,12] and the WHO classification requires that a separate diagnosis of DLBCL be made in such cases (Table 2). However, this interpretation is not unambiguous, and in addition, areas of DLBCL may be easily missed in small biopsies. Another problem is that in some cases of FL there are discrepancies between the cellular composition and the Ki67 proliferation index.[13,14] Some cases of FL1/2 may show a majority of cells being in cycle, whereas some FL3B cases have a relatively low proliferation index. Finally, cases of FL3B with a history of FL1-2 were sometimes included in published series.

The problems regarding the histological definition of FL3B are illustrated in the paper by Horn *et al.* published in

Table 1. Grading of follicular lymphoma (WHO 2008).[5]

Grade	Definition
Grade 1-2	0-15 centroblasts / 0.159 mm^2
Grade 1	0-5 centroblasts / 0.159 mm^2
Grade 2	5-15 centroblasts / 0.159 mm^2
Grade 3	>15 centroblasts / 0.159 mm^2
Grade 3A	centrocytes present
Grade 3B	solid sheets of centroblasts
DLBCL with follicular component	diffuse area with solid sheets of centroblasts outside histologically or immunophenotypically (CD21, CD23+ FDC) recognizable follicles

Table 2. Pathology of FL3B.

	N.	CD10 (%)	Bcl6 (%)	MUM1 (%)	Bcl2 (%)	*BCL2* breakpoint (%)	*BCL6* breakpoint (%)	*MYC* breakpoint (%)	Gene expression	Ref.
Bosga -Bouwer *	21	43	100	nd	67	33	33	14	nd	[9;10]
Katzenberger	5	60	60	bd	60	0	0	20	nd	[7]
Horn	23	43	nd	42	45	9	17	22	nd	[15]
Piccaluga	4	nd	nd	nd	100	nd	nd	nd	Distinct from but closer to FL1-3A than to DLBCL	[24]
Guo	14	57	79	nd	71	43	36	nd	nd	[11]

** Cases with antecedent FL1-2 and concomitant DLBCL omitted.*

this issue of Haematologica.[15] These authors collected a large series of 23 cases of FL3B and compared the immunophenotypic and genetic features of these cases with other cases of FL, including FL3A, FL3B with DLBCL, cases in which a distinction between FL3A and FL3B was not possible (FL3U), cases of FL consisting of large centrocytes or having a high proliferation fraction, and cases of typical FL1-2. Interestingly, even after introducing these six different categories, it appeared that while FL1-2 was very homogeneous, all other categories, including FL3A and FL3B, remained heterogeneous with regards to most features analyzed. Half of the FL3B cases expressed the germinal center marker CD10, as FL1-2 and FL3A do, while half lacked CD10 and expressed MUM1/IRF4, a post-germinal center marker not seen in FL1-3A. Only 9% of FL3B contained a *BCL2* break, in contrast to 88% of FL1-2; 17% of FL3B cases had a *BCL6* break and 22% a *MYC* break, which were not seen in FL1-2. However, FL3A was also not homogenous; only 58% had a *BCL2* break and 22% had a *BCL6* break, the latter being similar to the frequency in FL3B. FL3U, in which stratification as 3A or 3B was not possible by morphological criteria, was not surprisingly heterogeneous, with some cases resembling FL1-2 and others FL3B. Both FL3A and FL3B differed importantly from DLBCL with a component of FL3B, which rarely expressed CD10, often expressed MUM1/IRF4, and had a *BCL6* break in 50% of cases, similar to DLBCL without a partially follicular pattern. Interestingly, in all categories, including those of FL1-2 with a high proliferation fraction, when *MYC* breaks occurred, they were often seen together with *BCL2* or less often, *BCL6* breaks, suggesting that these are events associated with progression or transformation to a higher-grade process.

What can we conclude for clinical practice from this study? Unfortunately clinical follow-up data are not available on the patients studied. The biological heterogeneity of all categories of FL3 suggests that a simple system of subclassification of this category may not be possible. No clinical differences have been found to date between cases of FL3A and FL3B without a component of DLBCL treated with current anthracycline-containing regimens, so that the clinical relevance of this distinction is not evident.[15,16] In the absence of clinical follow-up data from the series reported by Horn *et al.*, it is not possible to determine whether separating FL1-2 with a high proliferation index or large centrocytes from typical FL1-2 is clinically relevant. It is also not possible to determine from this study whether FL3A (or for that matter FL3B) with a FL1-2 biological profile (CD10$^+$, t(14;18)$^+$) should be folded in with FL1-2 or kept as FL3, because the presence of numerous centroblasts may still predict a more aggressive course than that associated with FL1-2.

An important question is whether the histological grading system could be replaced or supplemented by a genetically oriented classification system, for instance by separating t(14;18)-positive FL from translocation-negative ones. Such an approach is appealing and has been followed in several studies.[11,17-24] However, while FL that lack a t(14;18) have clinical and pathological differences from those with the translocation, the category of t(14;18)-negative FL is not homogeneous. Nonetheless, it might be useful to identify such cases for further study or individualized treatment. Importantly, even within the category of t(14;18)-positive FL, a grading system might still be desirable to identify cases with increased centroblasts (FL3A or B) or a high proliferation index, which may still have a more aggressive clinical behavior than grade 1-2 cases. Thus, stratification of FL based solely on genetic features does not seem practical at present.

In summary, the study by Horn *et al.* has demonstrated the relative (and not unexpected) immunophenotypic and genetic homogeneity of typical FL1-2, including those cases with a high proliferation fraction or large centrocytes. It is also clear that FL3B with a component of DLBCL is genetically and immunophenotypically similar to DLBCL, and distinct from any of the FL categories. FL3, regardless of the exact proportion of centroblasts, is heterogeneous. While the majority of cases of FL3A have a germinal center B-cell immunophenotype, similar to FL1-2, 40-50% lack *BCL2* rearrangement or expression. While only rare cases of FL3B have a *BCL2* break, expression of CD10 or Bcl2 was seen in about 40%. Assessment of *BCL2*, *BCL6*, and *MYC* breaks as well as expression of CD10 and Mum1/IRF4 could be used to identify FL3 cases that are biologically closer to FL1-2 and those that are distinct; however, clinical data to support the need for this distinction in practice are lacking.

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Can multiple myeloma become a curable disease?

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For decades, multiple myeloma (MM) has been considered a disease of the elderly, with few therapeutic options apart from alkylators and corticosteroids. The treatment goal was disease control, with response rates of 50%, with occasional complete responses (CRs) and median survival of 2-3 years.[1] In fact, a cure was considered unattainable. It is possible that this state of affairs, which has lasted for more than 30 years, is the reason why the myeloma community has developed a rather conservative outlook.

The introduction of high-dose therapy followed by autologous stem cell support (HDT/ASCT) produced three important changes in the myeloma landscape: i) CR in 15-30% of patients; ii) the possibility of long treatment-free periods with excellent quality of life (QoL); and iii) prolongation of survival by one year.[2] Nevertheless, the greatest change has occurred in the last decade with the discovery of novel agents such as immunomodulatory drugs (thalidomide and lenalidomide) and proteasome inhibitors (bortezomib). These have contributed to doubling survival in myeloma patients as compared to the 1990s when only chemotherapy was used.[3,4]

Despite the fact that, until recently, MM was considered incurable, the introduction of HDT and novel drugs has