Histopathology in the light of molecular profiling

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The 2001 WHO classification distinguishes five variants (centroblastic, immunoblastic, plasmablastic, anaplastic and T-cell rich) and three subtypes (primary mediastinal, intravascular and primary effusion large B-cell lymphoma) of diffuse large B-cell lymphomas (DLBCLs). The recognition of the three subtypes as distinct disease entities can be considered as an advance in our understanding of these tumours. However, the variants of DLBCLs, which significantly outnumber the subtypes in frequency, represent an unresolved area. Gene expression profiling (GEP) of the variants led to a discrepancy in results and produced more questions than answers. The authors, therefore, initiated a multi-institutional collaborative research project in Germany aimed at a subtle morphologic, genomic and transcriptional characterisation of DLBCLs and Burkitt lymphoma (BL). We included BL in our study for two reasons: (1) it belongs to aggressive B-NHLs; and (2) at present, there are no reliable criteria that can be applied to distinguish BL from DLBCL. The GEPs derived from 200 patient samples were correlated with reviewed histology, the degree of genetic imbalances and clinical features. The results of this approach show that: (i) the DLBCL can be divided into more than four molecular groups; and (ii) the BL cases, identified by the consensus of five out of six lymphoma expert pathologists, displayed a genomic and gene expression profile that was clearly distinct from those of most DLBCLs. The group of DLBCLs that resembled BL in their GEP had a remarkably good prognosis, whereas those that differed in their GEP from the consensus BLs had unfavourable survival rates. In conclusion, combined application of genomic and gene expression profiling in conjunction with consensus reviewed histology and clinical features, appears to be a reliable approach that enables a reproducible and clinically meaningful characterisation of mature aggressive B-NHLs.

Key words: Large B-cell lymphoma, gene expression profiling, Burkitt lymphoma, survival rates

Among the diffuse large B-cell lymphomas (DLBCLs), the WHO classification distinguishes several variants. The most frequent variants are centroblastic and immunoblastic, followed by variants which are T-cell rich, plasmablastic and anaplastic. The WHO classification also regards three DLBCL subtypes—primary mediastinal, intravascular and effusion—as distinct disease entities.

The problem with this subclassification is overlaps between the mentioned categories meaning that the current subclassification of DLBCL is imprecise. Many patients have, within the same tumour lesion, neoplastic cells with characteristics of both centroblasts and immunoblasts. There is also a morphological overlap between plasmablastic and immunoblastic variants, and between centroblastic and mediastinal categories.

The distinction between Burkitt lymphoma and DLBCL is also imprecise by the use of current diagnostic criteria. A study on the reproducibility of lymphoma type diagnosis by morphology revealed that the agreement between expert haematopathologists about the distinction of Burkitt lymphoma from DLBCL is lower than 55%.

To overcome the imprecision of the current subclassification, DLBCLs were subjected to gene expression profiling. These studies have confirmed that mediastinal DLBCL represent a real distinct disease entity. These studies also provided evidence the most common variants of DLBCL, i.e. the centroblastic and immunoblastic variant, are composed of two main subgroups, one carrying the signature of germinal centre B cells and the other of activated B cells [1].

In their 2002 paper in the *New England Journal of Medicine*, Rosenwald et al. [1] reported that patients with a DLBCL of germinal centre B-cell (GCB) type and patients with a DLBCL of an activated B-cell (ABC) type (identified by using the Lymphochip) differed significantly in survival. However, Shipp et al.’s team, using Affymetrix technology to identify GCB and ABC subgroups, threw us into confusion since the finding by Rosenwald et al. was not, or not fully, confirmed [2].

A significant amount of uncertainty has now been resolved by Wright et al. from Staudt’s group [3]. They demonstrated that the removal of patients whose gene expression profile is intermediate in the continuum between GCB and ABC (DLBCL with the borderline characteristic is now termed ‘type 3’) leads to a sharp border between GCB and ABC-type DLBCLs. If this method is applied in conjunction with the GCB/ABC classifier designed by Wright et al. to cases studied

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by Shipp’s team, the difference in survival between the two groups is restored [3].

We applied the Wright classifier to our own collection of DLBCL and also found a clear difference in gene expression (Figure 1A) and in overall survival between the GCB and ABC type (Figure 1B). However, if we restricted the analysis to the centroblastic variant of DLBCL cases the difference in survival disappeared (Figure 1C). This confirms earlier studies claiming that DLBCLs of immunoblastic variant have a significantly worse prognosis when compared with the centroblastic variant of DLBCL. This finding is also in line with recent results of the German prospective study of high-grade B-NHL. The histological subcategorisation of the DLBCL was performed by an individual review of six internationally well known experts of haematopathology using H&E and Giemsa-stained sections. This subtle and careful morphological review might be the reason for the difference to reports with other results.

The distinction between GCB and ACB types among DLBCL constitutes progress, but is certainly—especially in the light of the above—not the final answer. An interesting question that remains is whether the DLBCL of centroblastic and immunoblastic variant are composed of only two groups (i.e. GCB and ABC types) or whether there are more than two groups. The impact of morphology also remains to be clarified. A further question is whether the so-called type 3 represents real borderline cases or a mixture of distinct disease entities that cannot be identified by present gene expression profiling methods. In 1997 my own research team described a group of patients suffering from DLBCLs with a special immunophenotype and clinical features [4]. We termed this subtype plasmablastic lymphoma because of its plasma-cell-like immunophenotype. We recommended its distinction from other types of DLBCL since patients with plasmablastic lymphoma require—because of their very unfavourable outcome—a totally different treatment when compared with the non-plasmablastic subtypes. Rituximab will probably be of no help as the tumour cells of most plasmablastic lymphomas do not express CD20. We strongly believe that a molecular signature of plasmablastic lymphoma

![Figure 1.](image-url)
and additional other subtypes will be identified among DLBCLs, especially if gene expression profiling is applied in conjunction with other molecular techniques, e.g. genome-wide genetic methods. With such an approach we have already identified a molecular signature of Burkitt lymphoma, which can be used for a more precise diagnosis of this distinct aggressive B-NHL.

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references