

XI. Distinguishing Burkitt lymphoma from diffuse large B-cell lymphoma

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Burkitt lymphoma as a pathological entity

The description 50 years ago of ‘a sarcoma affecting the jaws of African children’ later renamed Burkitt lymphoma (BL) was a landmark in haematopathology. Subsequent studies of this tumour have provided important insights into the role of balanced translocations, the need for deregulation of several complementary cellular pathways to produce a clinically apparent tumour, the relationship between chronic immune stimulation and oncogenesis and the effects of latent Epstein–Barr virus (EBV) infection on B cells. This has set the agenda for contemporary research into the pathogenesis of haematological malignancies.

BL is defined in the WHO classification as a single pathological entity divided into three types on the basis of geography and HIV status. However, there are clearly major differences in the pathogenesis of the three sub-entities including the role of EBV infection, the age of patients and anatomical distribution of tumours, the role of immune stimulation in African and HIV+ patients and the molecular anatomy of the MYC rearrangement, which differs between endemic and non-endemic BL. Although collecting outcome data is difficult some African children respond well to simple chemotherapy [1] while the opposite is the case for patients in non-endemic areas. There have always been problems in defining the boundary of non-endemic BL from other B-cell lymphomas leading to the use of the clinically unhelpful term Burkitt-like lymphoma. The availability of effective therapy for patients with non-endemic BL means that more objective criteria are now required.

why is it important to accurately diagnose non-endemic BL?

A diagnosis of BL will generally lead to treatment with intensive chemotherapy such as CODOX-M/IVAC whereas most patients with diffuse large B-cell lymphoma (DLBCL) will receive R-CHOP as first-line therapy. This divergence of treatment approach was based on a number of lines of circumstantial evidence rather than a direct comparison within a clinical trial. A major advance was the demonstration in children with non-cleaved-cell lymphoma (the working formulation category that included BL) that short intensive chemotherapy with

CNS-directed components could cure their disease [2]. This approach was subsequently extended to adult patients and has become standard practice [2]. More recent studies have highlighted the adverse effect of a rearrangement of MYC on the outcome of patients with aggressive B-cell lymphomas treated with R-CHOP. This group of germinal centre-derived B-cell lymphomas containing a MYC rearrangement is highly heterogeneous and other balanced translocation or numerical chromosomal abnormalities are often present. Although the adverse prognostic effect may be mitigated to some extent by age most of these patients have a very poor clinical outcome with a median survival of a few months [4]. Within the group of MYC-rearranged tumours, only the BL subgroup have been shown to respond favourably to intensive chemotherapy [5].

Although there are ongoing clinical trials, the evidence that other patients with aggressive B-cell lymphoma benefit from more intensive chemotherapy is sparse, suggesting that response to this type of therapy in patient who would otherwise be refractory to R-CHOP may be a defining characteristic of BL. Given the toxicity and considerable financial cost of intensive therapy, it is important to develop an accurate definition of the patient group that will benefit and a sensitive practical strategy to distinguish them from the much larger group of patients with other aggressive B-cell malignancies.

the diagnosis of non-endemic BL is really about identifying patients with aggressive B-cell lymphomas for whom intensive therapy can be justified

Each of the individual pathological features used to define non-endemic BL are found in other types of B-cell malignancy and the distinction between BL and DLBCL has always been problematic. This is recognized in the WHO classification with the introduction of ‘B-cell lymphoma with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma’. However, the need to choose between two alternative therapies makes this approach less tenable. Instead, an operational definition of BL is needed that focuses on identifying the key diagnostic criteria that co-segregate with the indication for intensive chemotherapy.

The essential element in the diagnosis of BL is the identification of a translocation of MYC involving the

immunoglobulin heavy or light chain loci. Only a minority of haematopathology laboratories perform this investigation routinely on all cases of aggressive B-cell lymphoma and where this is not done an appropriate screening test is needed to identify biopsies that require cytogenetic investigation. It has become clear that examination of routinely stained sections is neither a specific nor a sensitive test and while a high cell cycle fraction is an invariant feature of BL the majority of tumours with a Ki67 fraction of >95% will be DLBCL. In the UK/NCRI LY10 trial a screening test was piloted that included high Ki67, a germinal centre phenotype with co-expression of CD10 and BCL-6, absence of BCL-2 expression and expression of TP53 in the absence of expression of P21. All biopsies examined in the study subsequently diagnosed as BL in adults had this profile. About one-third of tumours with this profile did not have a MYC rearrangement [5]. This is an important group in that DLBCL with a germinal centre phenotype and absence of BCL-2 expression should have an excellent prognosis and intensive therapy is not indicated [6]. However, it is possible that contained within this set of cases are a small number of 'true' BL with activation of MYC by a mechanism other than translocation.

These simple screening criteria are robust and useful in practice. However, there are a number of markers that have the potential to increase the sensitivity of immunocytochemistry. The expression of SOX11 is used frequently in diagnosis of mantle cell lymphoma, but expression of this transcription factor is seen in BL [7]. Absence of BCL-2 expression is a key feature of BL but in a number of cases of both follicular lymphoma and DLBCL expression of BCL-2 is present but not detected by the standard antibodies because of mutation of the gene. These cases can now be detected using the E17 antibody. The VpreB3 protein appears to be strongly associated with the presence of a MYC rearrangement [8] and recently an effective antibody for the direct detection of MYC has been described. These antibodies may improve the effectiveness of immunocytochemical prescreening. Flow-cytometric approaches have also been described.

Based on the immunophenotype further molecular cytogenetic investigations will be necessary in ~10% of cases of aggressive B-cell lymphomas. The role of conventional metaphase cytogenetic techniques is limited by the tendency of unfixed samples of BL to deteriorate rapidly and the technique of choice is interface FISH performed on formalin-fixed paraffin-embedded sections, imprint preparations or bone marrow aspirate or blood smears. This is now a highly reliable method that should be part of the standard test repertoire of all haematopathology laboratories. An effective approach is to screen samples using a breakapart probe set covering the MYC locus on chromosome 8; a split signal suggests the presence of MYC translocation. There are rare exceptions to this where breakpoints outside the MYC locus may be detected [9]. A putative translocation detected by a breakapart probe set should be confirmed to demonstrate co-localization of the MYC-specific probe with IgH and in the small proportion of cases where this is not found additional tests using MYC in combination with κ and λ light chain probes should be carried out.

The final step in the diagnosis of BL is the exclusion of other genetic abnormalities. It is standard practice to carry out

appropriate FISH tests to exclude the presence of t(14;18) and 3q27 rearrangements involving BCL-6. It is not yet clear whether excluding other genetic abnormalities could contribute to more closely defining the population of patients who could benefit from intensive therapy. The presence of aneuploidy may be apparent from routine FISH studies but the availability of array comparative genomic hybridization (CGH) studies or single-nucleotide polymorphism (SNP) arrays now makes it feasible to perform whole genome studies. A recently reported study using high-resolution CGH has confirmed that BL has relatively few copy number abnormalities although there is a lack of proper comparative data with non-Burkitt-type aggressive B-cell lymphoma [10]. This study also identified recurrent abnormalities of TP53, which would be expected, and gains of the microRNA 17-92 cluster. This microRNA cluster is under the transcriptional control of MYC and overexpression of these microRNAs accelerates the development of MYC-related lymphomas. As yet there are no data on the treatment outcomes of patients investigated using these techniques. The role of NF κ B deregulation in the pathogenesis of B-cell lymphomas is now recognized as being of central importance. Several mutations of NF κ B pathway components or elements of closely related signalling pathways have been described. While these are predominately found in ABC-type DLBCL these abnormalities may also be found in a proportion of tumours with a germinal centre phenotype. The incidence of these abnormalities in BL is not known with certainty. However, this is an important question since suppression of NF κ B by MYC appears to be a key element in the pathogenesis of experimental MYC-driven tumours and re-expression of NF κ B would be expected to considerably modify the clinical behaviour of the tumour through decreased apoptosis [11]. As well as presence of mutation of key pathway components NF κ B activation and the up-regulation of other apoptosis-suppressing pathways could also occur as a result of EBV infection through the action of LMP1 and LMP2A [12]. EBV is not usually associated with non-endemic BL but is found in some cases in all age groups. Again it is unclear how this affects clinical behaviour.

the role of gene expression profiling in diagnosis of BL

The approach to diagnosis outlined above is clearly complex. If the objective is to dichotomize patients into those who should be treated with intensive chemotherapy and those who receive standard therapy this approach is difficult to implement and a technique based on a single diagnostic platform is clearly desirable. The only approach available currently that has the potential to do this is gene expression profiling, which permits individual patients to be assigned to a particular diagnostic category. Bayesian statistics can be used to assign a probability that the diagnosis is correct and this is potentially an important tool in clinical decision making. The feasibility of this approach was demonstrated by two studies published in 2008 that described molecular classifiers that distinguished tumours diagnosed as non-endemic BL, using current gold standard approaches, from other types of DLBCL [13, 14]. Recently, this approach has been confirmed and extended to include both

HIV-associated and endemic BL. In this study all three types clustered separately from other types of lymphoma and demonstrated a close similarity with germinal centre B cells confirming the putative cell of origin. There were, however, important differences in the patterns of gene expression between the three groups, most notably the involvement of BCR signalling and NF κ B pathway genes in the endemic group, presumably reflecting the distinctive pathogenesis of this type of tumour. Although the total number of cases included in these three studies is relatively small the evidence would suggest that gene expression profiling has the potential to be a highly specific diagnostic test for non-endemic BL. However, a more important issue is the question of sensitivity. All cases diagnosed by a combination of FISH and immunocytochemistry are captured by this technique there are additional tumours that have a molecular profile of BL but do not have the classical features including absence of a MYC rearrangement. Including these molecular BLs would potentially increase the total number of patients by ~10%–15%. The key question is whether this group of patients require intensive chemotherapy and the evidence for this is sparse but suggestive that this may be the case.

If it emerges that the 'molecular' BL requires intensive chemotherapy then there would be a case for regarding gene expression profiling as the definitive diagnostic test that should be employed routinely. The feasibility in doing this depends on a number of factors. All of the data published so far have used stored frozen tissue and the Affymetrix platform. To be applicable to routine cases formalin-fixed paraffin-embedded tissue would have to be used. Extraction of good quality RNA from these blocks is straightforward and the Illumina DASL platform or similar system can be used but separate validation of the molecular classifier for these platforms is required. This may amount to more than simply cross-platform validation. BL tissue is extremely labile after removal from the patient and it is possible that cases where good quality fresh material was available for study may themselves be atypical. The cost of performing a whole genome expression array has fallen very sharply and is only slightly more expensive than an extended FISH panel and much less than a combination of FISH and SNP array analysis. The cost effectiveness depends on how many patients with aggressive lymphoma would have to be screened to identify all cases. If prescreening with immunophenotyping is possible using current methods this would be ~10% of all cases and this would be cost effective given that even in the largest centres the total cost would still only be a small fraction of the cost of treating a single patient. This could be improved further if more targeted expression analysis could be performed that obviated the need for high levels of multiplexing to ensure maximum cost effectiveness.

conclusion

Studies of BL have provided many of the key insights that have informed current understanding of the pathogenesis and epidemiology of haematological malignancies. The latest stage in this process may be the transition from traditional pathology-based lymphoma classification to diagnostic approaches based on broad categories subdivided using

biomarkers linked to specific treatment pathways. Although BL may serve as a paradigm for this approach the relatively low incidence means that it will always be difficult to perform definitive clinical trials and some of the evidence will remain circumstantial. In larger patient populations biomarker validation can be incorporated into the design of large phase III clinical trials as shown by the current UK/NCRI REMoDL-B trial where gene expression profiling-based signatures are being evaluated for their ability to predict preferential response to R-CHOP–bortezomib in DLBCL. Future biomarkers in haematological oncology will be based on advanced flow cytometry, molecular cytogenetics, gene expression profiling and next-generation sequencing. Although often portrayed as expensive options this is no longer always the case and the very high cost of some therapies will make these approaches increasingly cost effective when viewed as part of the whole patient pathway. This will have major implications for the organization of haematopathology services.

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