MY APPROACH

Nodal aggressive B-cell lymphomas: a diagnostic approach

Sonam Prakash, Steven H Swerdlow

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The diffuse aggressive B-cell lymphomas, as recognised in the 2001 WHO classification, represent a clinically and biologically heterogeneous group of neoplasms that require very different therapeutic approaches and have very different outcomes. They should be diagnosed using a multiparameter approach that emphasises morphological and immunophenotypic studies, and in at least some cases, relies on cytogenetic and/or genotypic studies. Incorporation of clinical data may be important as well. There is also current interest in going beyond the basic diagnosis and providing pathological prognostic information when possible. Whereas the diagnosis of some cases will be relatively easy, the differential diagnosis in others is very difficult, with some cases in a grey zone between two different well defined categories.

Whereas in the past a discussion about how to approach a subset of lymphomas would require a lengthy discourse on the great attributes of one's classification of choice, today those contentious times are behind us. Built on the legacies of classifications from Rappaport[1] to REAL,[2] and organised first by cell type as introduced by the Lukes/Collins classification[3] of 1974 and an integral part of the Kiel classification,[4–6] the WHO classification of 2001 stands alone in terms of the options available in 2007.[7] A revised edition of the WHO Monograph on Tumours of Haematopoietic and Lymphoid Tissues is in preparation and currently scheduled to be available in 2008. The WHO classification first divides the non-Hodgkin lymphomas (NHL) into those of B-cell, T-cell or natural killer (NK) cell origin. Each of these categories are then further subdivided based on whether the lymphoma is composed of precursor cells (lymphoblasts) or mature lymphoid (or plasmacytic) cells, with the latter cases then divided into one of the numerous types of mature lymphomas. Many of the B-cell lymphomas can be related to one of the major B-cell subsets (fig 1); nevertheless, only distinct clinicopathological entities are separately designated. Each type of lymphoma is identified based on its morphological, phenotypic, genotypic/cytogenetic, and clinical features, although diagnoses can often be rendered without a full evaluation of each of these parameters.

This review aims to cover our approach to the diffuse aggressive B-cell lymphomas, concentrating on those with lymph node involvement, and is not intended to supplant the very useful WHO monograph (box 1).[7] The specific goals of this discussion are to explain the multiparameter approach we use for lymph node biopsies, to briefly review the aggressive B-cell lymphomas, to explain how we approach making those diagnoses and to look at selected potentially problematic differential diagnoses.

MULTIPARAMETER APPROACH FOR DIAGNOSTIC LYMPH NODE BIOPSIES

Lymph node biopsies should be received fresh and sectioned perpendicular to their long axis. Any focal lesions should be sampled and, if otherwise homogeneous, the ends of the lymph node used for ancillary studies. Both air dried (useful for a variety of stains/FISH studies) and fixed and stained touch imprints should be made. Well fixed sections are critical, and multicolour flow cytometric studies very useful to provide a complete immunophenotype with detection of even small populations with "aberrant" antigenic expression and evaluation of surface immunoglobulin (SIg) expression and intensity.[8] Material should be snap frozen for possible molecular testing and, depending on local resources, appropriate cases may be sent for classical cytogenetic studies. The latter studies would be considered the least critical component of this protocol.[9] If desired, one can also extract and store DNA and RNA for potential additional testing.

DIFFUSE AGGRESSIVE B-CELL LYMPHOMAS

Precursor B-lymphoblastic leukaemia/lymphoma

B-lymphoblastic leukaemia/lymphoma (B-LBicL) usually presents as a leukaemia but can present as a lymphoma at nodal or extranodal sites.[10] Lymph nodes show a diffuse infiltrate of monotonous cells with round to irregular nuclear contours, finely dispersed chromatin and generally inconspicuous nucleoli, that not infrequently spare islands of normal lymphoid tissue (fig 2). The neoplastic cells are positive for the B-cell associated antigens CD79a, PAX-5 and sometimes CD20 as well as for TdT and sometimes CD34. A significant proportion express the common ALL antigen, CD10. Beware, neuroendocrine neoplasms that can mimic B-LBicL can also be PAX-5 positive.[11]

Mantle cell lymphoma

Mantle cell lymphomas (MCL) have a survival similar to the most aggressive NHL, even though the cells are often small and some more indolent forms are now recognised.[12–15] MCL are usually composed of monotonous relatively small lymphocytes with variably angulated/clefted non-transformed nuclei that can have a mantle zone, vaguely follicular, diffuse or rarely very follicular growth pattern.[12] Although centroblasts are essentially absent, a blastoid variant is recognised as

See end of article for authors' affiliations

Correspondence to: Dr Steven H Swerdlow, Division of Hematopathology, University of Pittsburgh School of Medicine, UPMC-Presbyterian, Room C606, 200 Lothrop Street, Pittsburgh, PA 15213, USA; swerdlowsh@upmc.edu

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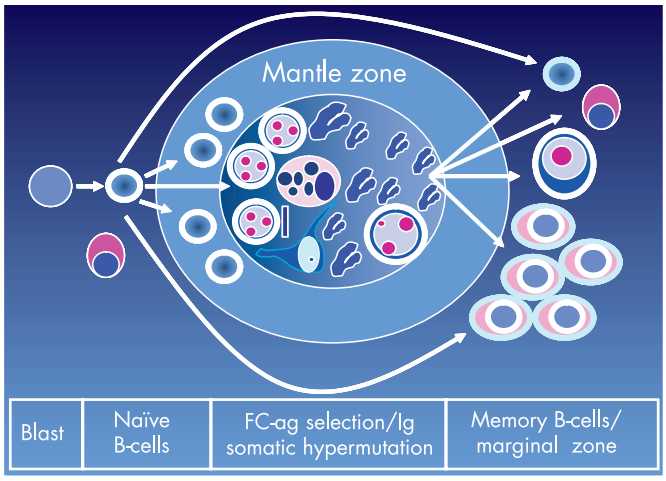


Figure 1 Normal B-cell development (oversimplified). B-lineage lymphoblasts mature to naïve B-cells that circulate and also populate primary follicles (not illustrated) and the mantle zone of follicles. Some may differentiate to plasma cells and others to monocytoid B-cells (not illustrated). With antigenic stimulation, naïve B-cells enter follicular/germinal centres (FC) where they undergo blast transformation to centroblasts (shown with their often nuclear membrane-associated nucleoli) and, if selected as a part of the immunoglobulin gene (Ig) somatic hypermutation/antigenic (ag) selection process in FC, they mature to centrocytes (cleaved cells) that accumulate along with some possibly post-selection transformed cells in the pale zone (on the right). Follicular dendritic cells, mitotic figures and tingible body macrophages along with T-cells (latter not illustrated) are also found in the follicular centres. Cells leaving the FC may differentiate to small memory type B-cells, plasma cells, large immunoblasts with prominent nucleoli or marginal zone B-cells with their more abundant cytoplasm. As shown, some memory-type and marginal zone B-cells do not show the hallmark somatically mutated immunoglobulin genes indicative of having been through a FC. Also not illustrated is the possibility that memory B-cells may reenter the FC.

composed either of cells having very dispersed chromatin and a high mitotic rate resembling lymphoblasts (classic type) (fig 3A) or of larger and sometimes pleomorphic cells often with nucleoli, resembling the cells of a diffuse large B-cell lymphoma (DLBCL) (pleomorphic type) (fig 3B).[12 16] Although a high mitotic rate (>10/10 high power field (hpf) and usually >20–30/hpf) and relatively high proportion of Ki-67 positive cells are expected in blastoid MCL, there are no standard criteria, so that recognition of the blastoid variant of MCL remains a subjective morphological judgment. The most typical MCL phenotype is CD20+, CD5+, CD10−, bcl-6−, CD43+, CD23−, FMC7+, SIg+ and cyclin D1+.[17–19] Most but not all cases demonstrate a t(11;14)(q13;q32) cyclin D1 gene (*CCND1*)/immunoglobulin heavy chain (IgH) gene translocation.[20]

Diffuse large B-cell lymphoma

DLBCL is "a diffuse proliferation of large neoplastic B lymphoid cells with nuclear size equal to or exceeding normal macrophage nuclei or more than twice the size of a normal lymphocyte".[21] Unfortunately, there are other types of lymphoma that would also fulfil this definition and some DLBCL that do not. DLBCL is a heterogeneous entity that includes a number of different variants and subtypes, with current interest in identifying additional prognostically meaningful subsets.

Major morphological variants

There are four major morphological variants of DLBCL that are well described in the WHO monograph: centroblastic (fig 4), immunoblastic (fig 5), T-cell/histiocyte rich (T/HRBCL) and anaplastic.[21] These variants are not considered to be of tremendous clinical significance although the centroblastic

Box 1 WHO classification of B-cell lymphomas and B-cell proliferations of uncertain malignant potential[7]

Precursor B-cell neoplasm

- Precursor B-lymphoblastic leukaemia/lymphoma (precursor B-cell ALL)*

Mature B-cell neoplasms

- Chronic lymphocytic leukaemia/small lymphocytic lymphoma
- B-cell prolymphocytic leukaemia
- Lymphoplasmacytic lymphoma
- Splenic marginal zone lymphoma
- Hairy cell leukaemia
- Plasma cell myeloma
- Solitary plasmacytoma of bone
- Extraosseous plasmacytoma
- Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT-lymphoma)
- Nodal marginal zone B-cell lymphoma
- Follicular lymphoma
- Mantle cell lymphoma*
- Diffuse large B-cell lymphoma*
- Mediastinal (thymic) large B-cell lymphoma*
- Intravascular large B-cell lymphoma
- Primary effusion lymphoma
- Burkitt lymphoma/leukaemia*

B-cell proliferations of uncertain malignant potential

- Lymphomatoid granulomatosis
- Post-transplant lymphoproliferative disorder, polymorphic

*These lymphomas will be discussed.

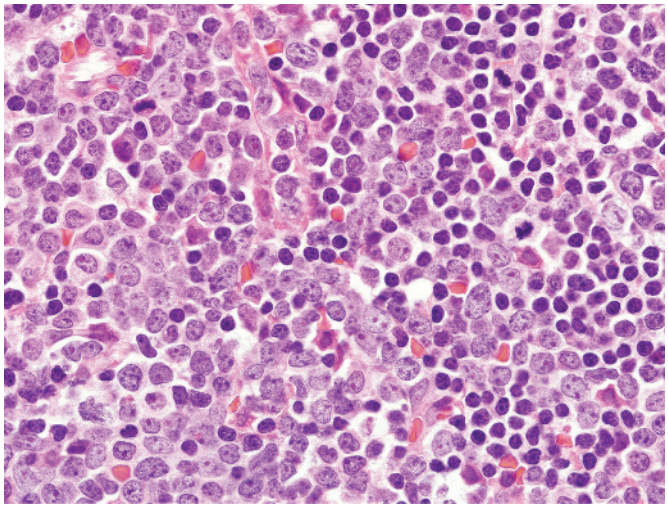


Figure 2 Precursor B-lymphoblastic leukaemia/lymphoma. The lymphoblasts are larger than the small lymphocytes seen in the disrupted mantle zone on the right and they have much more dispersed chromatin without very prominent nucleoli. The upper right corner includes the edge of a follicular centre.

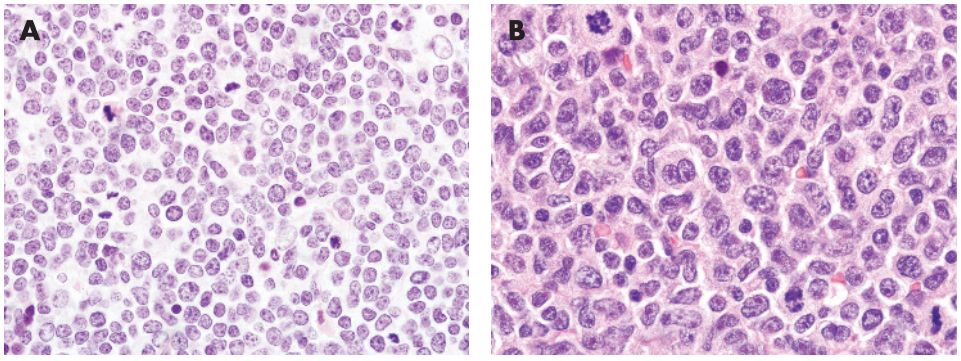


Figure 3 Blastoid mantle cell lymphoma variants. (A) The classic blastoid variant resembles a lymphoblastic lymphoma, including the presence of numerous mitotic figures. (B) The pleomorphic variant includes larger cells, some with prominent nucleoli and even a binucleate cell.

type is among the less aggressive and the immunoblastic and T/HRBCL types among the more aggressive DLBCL.[21 22] The T/HRBCL cases have <10% large neoplastic B-cells, few if any small B-cells and an absence of follicular dendritic cell (FDC) networks (as documented, for example using an anti-CD21 immunostain) (fig 6). The neoplastic cells may resemble L&H cells of nodular lymphocyte predominant (NLP) Hodgkin's lymphoma (HL), centroblasts, immunoblasts or even more classic Reed–Sternberg cells.[23 24] The anaplastic variant, composed of large anaplastic neoplastic cells that can grow in sinuses and be CD30/Ki-1+, is mostly important to know about to avoid the misdiagnosis of an anaplastic large cell lymphoma (ALCL), since by definition the latter is not of B-cell origin.

Although not recognised in the WHO monograph, another important morphological variant of DLBCL is the type composed of often intermediate sized very blastoid-appearing cells (fig 7).[25] These lymphomas should not be misinterpreted as being composed of centrocytes just because the cells are relatively small and nucleoli not very prominent.

Phenotypic studies

Phenotypic studies are used in the diagnosis of DLBCL mostly to prove that a ''large cell'' neoplasm is of B-cell origin and as an aid in further classification/prognostication. Sometimes, however they may be required to help establish the neoplastic nature of a large lymphoid cell proliferation. Most but not all DLBCL express the B-cell-associated, but not B-cell-specific, CD20 antigen. This is important to document because of the widespread use of therapeutic anti-CD20 antibodies. CD20− cases are recognised using other pan-B cell antibodies that detect CD79a, CD22, PAX-5 or CD19, or in some plasmacytic cases, by using antibodies that detect plasma-cell associated antigens such as CD138 or cytoplasmic immunoglobulins.[26–28] It should be noted that CD138 is present on many epithelial neoplasms and even some mesenchymal neoplasms.[28] SIg, if present, is monoclonal but it may be non-detectable. Use of a cyclin D1 antibody is important to help exclude a blastoid MCL and, in some circumstances, especially if SIg−, demonstrating negativity for TdT may be appropriate to help exclude a B-LBicL. Genotypic studies to document B-cell monoclonality are not usually required.

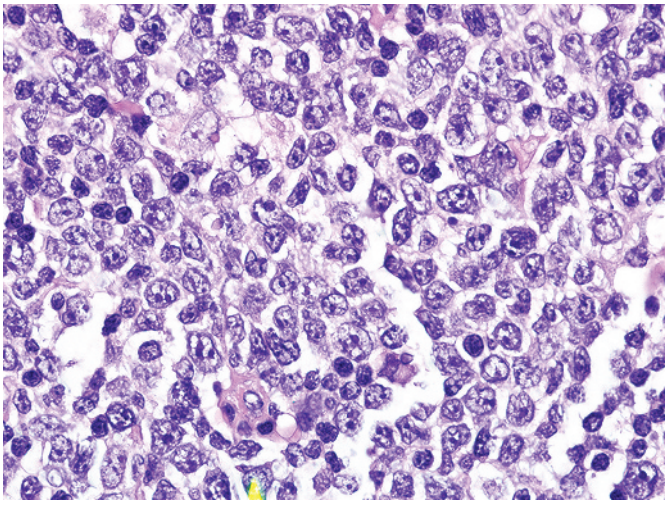


Figure 4 Diffuse large B-cell lymphoma, centroblastic. Note the numerous transformed cells, many of which have nucleoli along the nuclear membrane.

The detailed phenotype of DLBCL is variable.[21 29–32] Some cases mark like cells in a follicular lymphoma (CD10+, bcl-6+, bcl-2+) and others more like later B-cells with loss of follicular centre cell markers (CD10, bcl-6) and acquisition of transitional (MUM-1) and overt plasma cell-associated markers (CD138). Some cases express the CD5 T-cell associated antigen.[33] It is particularly important in these latter cases to exclude the possibility of a MCL or transformation of chronic lymphocytic leukaemia/small lymphocytic lymphoma.

Immunophenotypic studies have also been used in the prognostication of DLBCL and in predicting which patients will gain a therapeutic advantage from the use of rituximab. The current paradigm is that DLBCL of germinal centre (GC) type, as defined by gene profiling studies, are associated with a better prognosis than other DLBCL.[34] Immunohistochemical studies may also play an important role here, although an agreed on algorithm is lacking and reproducibility in the performance and interpretation of the stains is also problematic. Hans *et al* found that DLBCL of GC type could be recognised when >30% of cells are CD10+ or bcl-6+/Mum-1−, whereas the more aggressive cases of non-GC type were CD10− and either bcl-6+/Mum-1+ or bcl-6−.[35] Others have suggested the use of different algorithms and sometimes using additional antibodies.[36] Some discourage the use of any of these algorithms due to the many remaining uncertainties. Bcl-2 has also been reported to be an adverse prognostic indicator in many but not all studies, at least for subsets of DLBCL (for example, those of non-GC type) and it has been reported that, while rituximab may be of some benefit in many patients with DLBCL, only those with bcl-2+ disease show a survival advantage.[21 22 37–46] Others, however, have found that only patients with bcl-6− DLBCL show a significant survival advantage when rituximab is added to their chemotherapy.[47]

Chromosomal abnormalities

DLBCL demonstrate *BCL-2* rearrangements in 20–30% of cases and *BCL-6* rearrangements in up to 30%.[48 49] A small proportion have *MYC* translocations that, especially when also present with

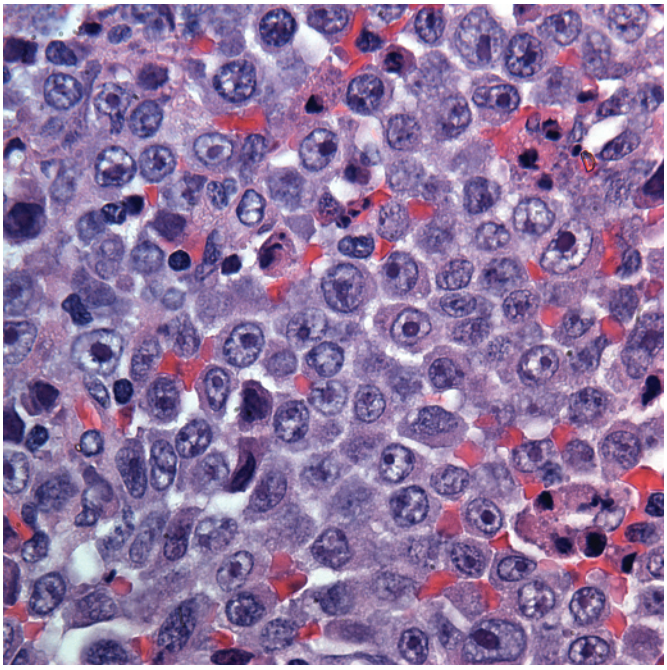


Figure 5 Diffuse large B-cell lymphoma, immunoblastic. There are numerous transformed cells with prominent single nucleoli and a moderate amount of often eccentrically placed cytoplasm.

a *BCL-2* translocation, are an adverse prognostic indicator.[50–52] In our experience, classical cytogenetic studies are successful in about 70% of DLBCL, with >90% of these studies showing an abnormal karyotype.[9]

Other variants/subtypes of DLBCL

Plasmablastic lymphomas

DLBCL with plasmablastic features/plasmablastic lymphomas (PBL) are a heterogeneous group of lymphomas, most of which are recognised as CD20−, CD45− neoplasms with a plasmacytic phenotype.[53–55] PBL of ''oral mucosa type'' is found mostly in HIV+ patients and is frequently EBV+ but HHV8−.[53 55–57] The neoplastic cells look like transformed lymphoid cells but they have a plasmacytic phenotype and must be distinguished from plasmablastic myeloma. Distinction of some plasmablastic lymphomas from extramedullary involvement by myeloma

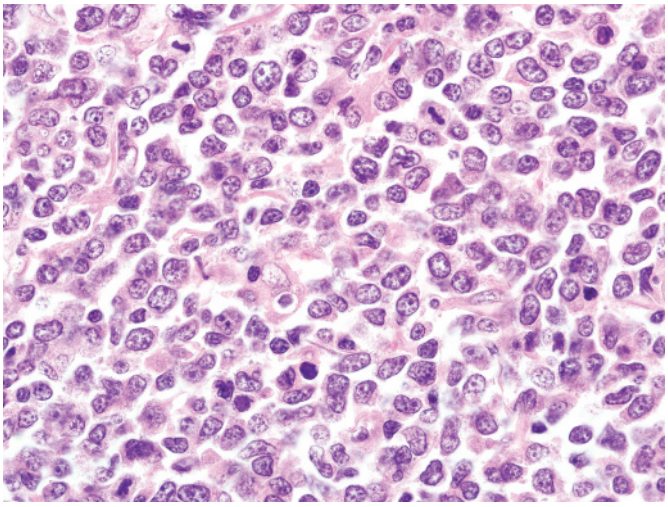


Figure 7 Diffuse large B-cell lymphoma, blastoid. Note the blastoid cells with dispersed chromatin and the mitotic figures. Most of the cells are not very large and lack prominent nucleoli. Although resembling lymphoblasts and associated with 80–90% Ki-67 positivity, these CD20+, CD5−, CD10−, bcl-2+ cells are TdT and cyclin D1 negative.

can be very difficult. Some of the findings that can be helpful include the clinical setting and laboratory findings (for example, a prior history of myeloma), and some phenotypic/cytogenetic findings, such as CCND1 (cyclin D1) translocation or cyclin D1 expression which is found in some myelomas but not in the plasmablastic lymphomas. Although not resembling a plasmablastic lymphoma, MCL with plasmacytic differentiation and a CCND1 translocation in both components has been reported.[58] CD56 expression is also more commonly found in myeloma but is present in a moderate number of plasmablastic lymphomas as well.[53] Although Epstein-Barr virus (EBV) has been reported in plasmablastic neoplasms secondary to plasma cell neoplasms, its presence, in general, would not favour the diagnosis of myeloma.[53]

The CD20− group of PBL also includes cases with more overt plasmacytic differentiation (some of which are associated with plasma cell neoplasms), HHV8+ primary effusion lymphomas (which can form solid masses), some HHV8+ plasmablastic lymphomas associated with multicentric Castleman disease and ALK+ DLBCL. Other cases are CD20+ but show morphologic

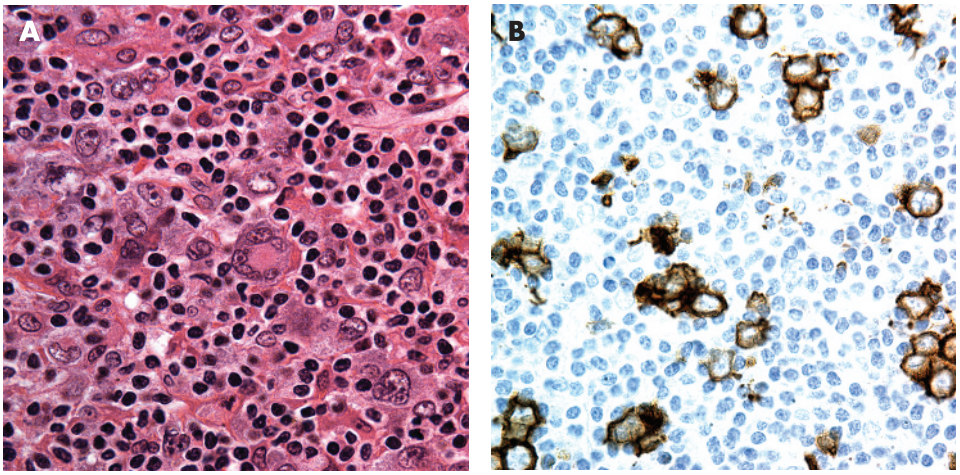


Figure 6 T-cell/histiocyte rich large B-cell lymphoma. (A) There are scattered large neoplastic cells, some of which resemble Reed–Sternberg cells, in a background of small lymphocytes and occasional histiocytes. (B) The CD20 immunohistochemical stain highlights the scattered large neoplastic B-cells and only rare small B-cells.

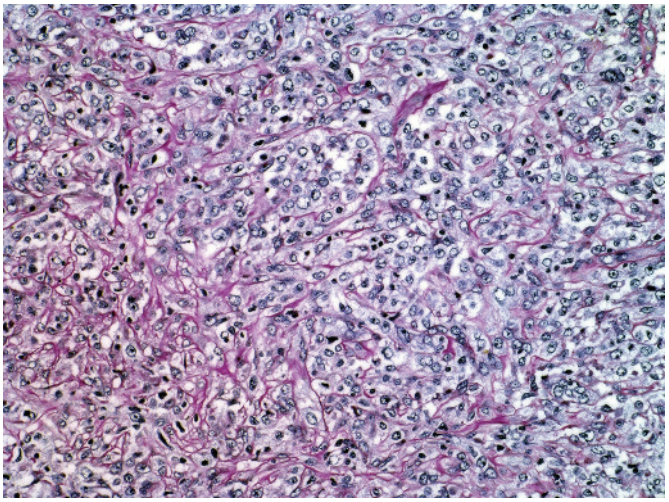


Figure 8 Mediastinal large B-cell lymphoma. This somewhat lower magnification image of a PAS stain from a very typical case shows the diffuse proliferation of intermediate-sized to large lymphoid cells with moderately abundant pale cytoplasm and the associated fine sclerosis.

plasmacytic/plasmacytoid differentiation and have cytoplasmic immunoglobulin that is usually IgM.[59] The expression of CD20 is generally not expected in the vast majority of true plasmablastic lymphomas, and with the exception of the plasmablastic proliferations associated with multicentric Castleman disease,[57] its presence should strongly favour one of the non-plasmablastic B-cell lymphomas.[53] It has been specifically suggested that CD20 positive lymphomas resembling a plasmablastic lymphoma are better considered as immunoblastic DLBCL.[53] Remember, however, that even in multiple myeloma the plasma cells can be CD20+.

DLBCL with expression of anaplastic lymphoma kinase

Lymph node biopsies with anaplastic lymphoma kinase (ALK) positive DLBCL demonstrate a diffuse and intrasinus proliferation of large immunoblastic/plasmablastic-appearing cells that may include pleomorphic forms and Reed–Sternberg-like cells.[60 61] Necrosis is frequent. The neoplastic cells in most cases lack pan-B-cell markers, but express plasma cell-associated markers like CD138 and monoclonal cytoplasmic immunoglobulin that is often IgA. ALK+ DLBCL are EMA+ and sometimes have little or no CD45 expression, so they can be confused with metastatic carcinomas. Conversely, metastatic carcinomas may be CD138 positive, potentially causing confusion in the opposite direction as well. Most ALK+ DLBCL lack CD30 and, except for many cases with CD4 and CD57 expression, they lack T-cell associated antigens. It is now recognised that ALK+ DLBCL usually have a t(2;17)(p23;q23) translocation involving the ALK and clathrin genes, and show granular cytoplasmic ALK positivity.[62 63] Rare ALK+ DLBCL with the classic ALCL-associated t(2;5) translocation has also been reported.[64]

Mediastinal large B-cell lymphoma

Mediastinal large B-cell lymphoma (MLBCL) is a DLBCL that most typically presents with an infiltrative anterior mediastinal mass but without disseminated disease.[65 66] Although often characterised by a diffuse proliferation of relatively large lymphoid cells with moderately abundant pale cytoplasm and associated diffuse sclerosis (fig 8), there is wide cytological variation, including some cases that will even raise the possibility of a marginal zone B-cell lymphoma.[67] MLBCL are CD5−, CD10−/+, frequently CD23+ (unlike most DLBCL), often CD30+, SIg− and CD20+.[66 68 69] Other features, including some shared with a subset of classical HL, of importance, but not currently of great diagnostic utility, are discussed elsewhere.[70–75]

Other large B-cell lymphoproliferative disorders

Intravascular large B-cell lymphomas grow within small vascular channels at extranodal sites and can easily be missed.[76] Primary effusion lymphoma occurs mostly in HIV+ patients with neoplastic large B-cells that are usually HHV8+ and EBV+.[77] Another sometimes aggressive extranodal lymphoproliferative disorder, considered a lesion of uncertain malignant potential, is lymphomatoid granulomatosis, a usually EBV+ angiocentric lymphoproliferative disorder that has variable numbers of large monoclonal B-cells admixed with reactive elements.[78]

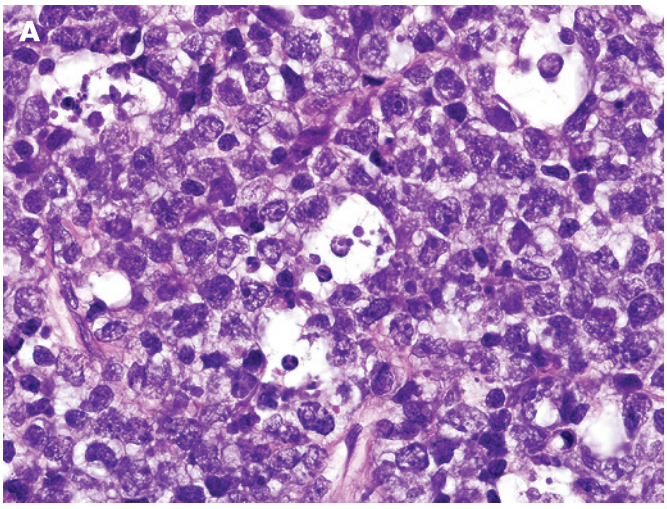
It should also be remembered that grade 3 follicular lymphomas belong among the non-indolent B-cell lymphomas.[79 80] These are divided into those with a mix of centrocytes and centroblasts (grade 3a) and those composed of pure centroblasts (grade 3b). The latter are more likely to have a pathogenesis like DLBCL and some would argue that they belong in that category.[81 82] When follicular lymphomas are identified in which there are diffuse areas fulfilling the criteria for a DLBCL, both diagnoses need to be reported and the approximate area of each component should be noted.[80] Although a complete discussion is beyond the scope of this review, it has been reported that grade 3 FL with a diffuse component of >50% have a worse outcome compared to those with less of a diffuse component.[79]

Burkitt's/atypical Burkitt's lymphoma

Burkitt's lymphoma (BL) are diffuse, and much less often in part follicular, proliferations of relatively small transformed cells with amphophilic cytoplasm (fig 9A).[83] They have an extremely high proliferation fraction, prominent apoptosis and a ''starry sky'' appearance. Some cases, usually found in immunodeficient patients, have plasmacytoid features. The current convention is that Burkitt's and atypical Burkitt's (Burkitt-like) lymphomas represent the same entity but with the latter showing more cytological variation. The terms atypical Burkitt's or Burkitt-like lymphoma are not to be used simply because the cells of a DLBCL are on the smaller side. Most BL are SIg+, CD10+, and bcl-2− with virtually 100% of their nuclei Ki-67+, a phenotype shared with some DLBCL. Cytogenetic studies show a translocation involving *MYC* at chromosome 8q24 and usually either the IgH locus at 14q32 or less commonly either the kappa or lambda light chain loci at 2q11 or 22q11, respectively (fig 9B). There are usually few other abnormalities.[84] It is critical to realise that a *MYC* translocation is not specific for BL, so that if one is going to use FISH to help document the diagnosis of BL, one should also look for *BCL-2* and *BCL-6* translocations that usually ought to be absent.

OUR APPROACH TO THE DIAGNOSIS OF THE NODE-BASED AGGRESSIVE B-CELL LYMPHOMAS

Our multistep diagnostic algorithm relies on morphological evaluation, summarised in fig 10, followed by further diagnostic refinement using immunophenotypic studies, as summarised in fig 11. Caution is always advised as there are exceptions to all of the phenotypic profiles indicated in the figure. We use flow cytometric methods whenever possible for the reasons discussed earlier. Detection of light chain class restricted SIg favours the presence of a mature (non-lymphoblastic) B-cell neoplasm most likely not of MLBCL type. If there is any chance of a MCL, we perform a cyclin D1 stain since MCL can mimic almost any other type of B-cell lymphoma and not all are CD5+. Classical cytogenetic studies can also be helpful, but they are not widely utilised for lymph node biopsies.[9] Cytogenetic FISH studies can also be used to help resolve



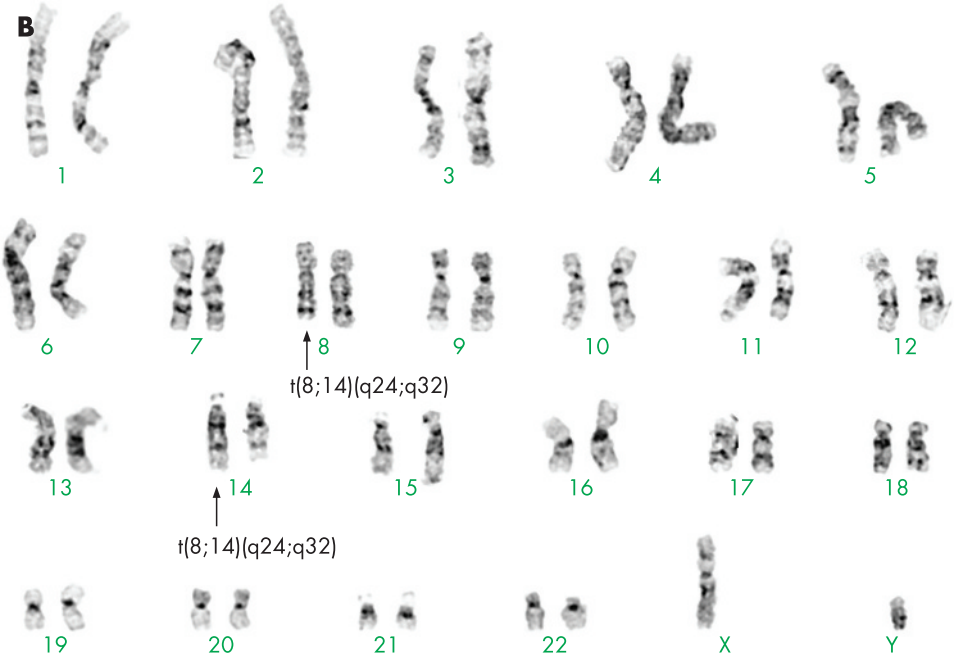


Figure 9 Burkitt's lymphoma. (A) There is a diffuse proliferation of intermediate sized transformed lymphoid cells and associated tingible body macrophages, creating a starry sky appearance. The degree of cytological variation is acceptable for a Burkitt's/atypical Burkitt's lymphoma. (B) Classical cytogenetic studies in this case showed an isolated t(8;14)(q24;q32), further supporting the diagnosis.

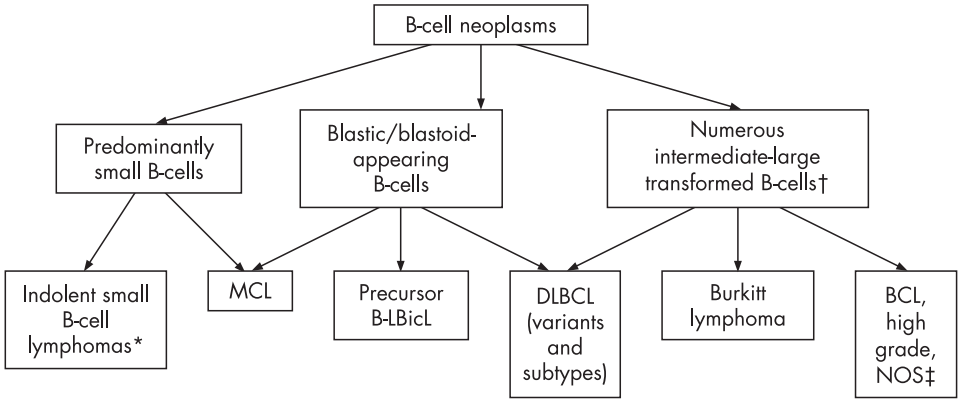


Figure 10 Morphological-based decision tree for major B-cell lymphomas. *These are not discussed in this manuscript. They include chronic lymphocytic leukaemia/small lymphocytic lymphoma, follicular lymphoma (except for grade 3), marginal zone B-cell lymphomas, and lymphoplasmacytic lymphoma. †T-cell/histiocyte rich large B-cell lymphoma will have <10% large B-cells and predominantly small T-cells. ‡This is not currently a recognised diagnosis in the WHO classification. It can be used descriptively for cases not classifiable into one of the better defined entities (see text). MCL, mantle cell lymphoma; B-LBicL, B-lymphoblastic leukaemia/lymphoma; DLBCL, diffuse large B-cell lymphoma; BCL, B-cell lymphoma; NOS, not otherwise specified.

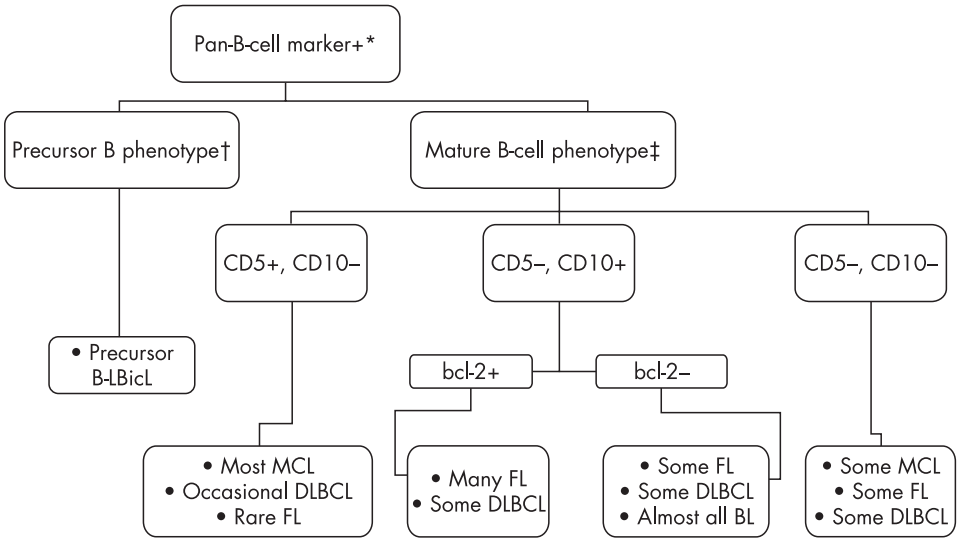


Figure 11 Basic immunophenotypic evaluation of non-indolent B-cell lymphomas. *Pan-B-cell markers include antibodies to CD19, CD20, CD22, CD79a and PAX-5. Some may be negative in selected cases. Use of all of these antibodies is almost never required. †TdT+, SIg−, CD10+/−, CD20 usually weak to negative. ‡TdT−, SIg+/−, usually CD20+. SIg, surface immunoglobulin; FL, follicular lymphoma; MCL, mantle cell lymphoma; B-LBicL, B-lymphoblastic leukaemia/lymphoma; DLBCL, diffuse large B-cell lymphoma; BL, Burkitt's lymphoma.

selected differential diagnoses. Selection of appropriate FISH panels is important, since, for example, isolated *MYC* translocations have different implications than those associated with another lymphoma-associated translocation. Genotypic studies can be used to document a clonal B-cell population or selected chromosomal translocations; however, they have their own limitations and we utilise them only infrequently in dealing with potential aggressive B-cell lymphomas.

Potentially problematic differential diagnoses

BL versus DLBCL

Although it is considered very important to distinguish BL from DLBCL to prevent overtreatment of DLBCL and undertreatment of BL, it can be very difficult, especially in adults, and gold standard criteria are lacking.[84–89] The problematic cases usually have many Burkitt-like morphological features but either the phenotype/genotype/karyotype is too "atypical", the cytological appearance is beyond that described for "atypical" BL, or both. Depressingly, Stein and Hummel wrote just last year that "at present, there are no reliable criteria that can be applied to distinguish BL from DLBCL".[90] Studies that have addressed this problem have found only a minority of cases that fulfil all of the typical BL criteria described above; one study found no adult cases.[85 87 91]

Recent gene profiling studies have demonstrated the existence of a molecularly homogeneous group of BL that are morphologically, phenotypically and even cytogenetically heterogeneous.[84 88 89] While this suggests that the criteria we use to diagnose BL cannot always be relied on, gene profiling studies are not standardised and not currently a clinical laboratory test. Furthermore, consistent with our daily experience, one of the major gene profiling studies also demonstrated the presence of cases that were "intermediate" between BL and DLBCL, substantiating the existence of a true grey zone.[92] Our current imperfect modus operandi is to diagnose BL when the morphological appearance is acceptable (but not necessarily typical), the phenotype is typical and a *MYC* but not a *BCL-2* or *BCL-6* translocation is present. Most other cases require a more descriptive diagnosis and use of a term like "high grade B-cell lymphoma, not otherwise specified" or DLBCL with "high

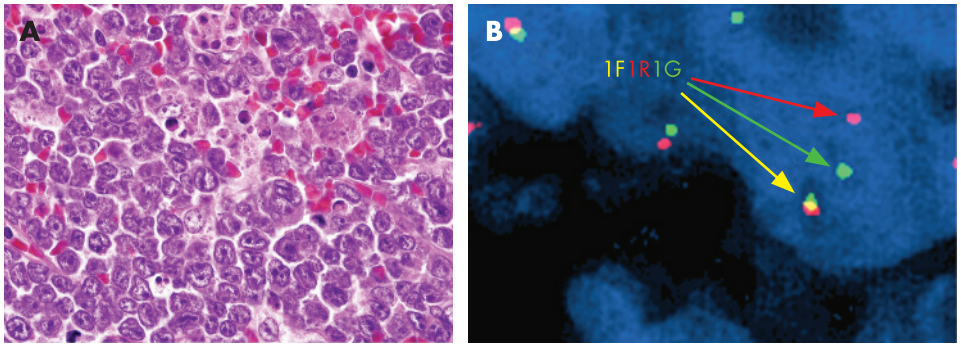


Figure 12 High grade B-cell lymphoma, not otherwise specified/diffuse large B-cell lymphoma with high grade features, CD10+/− (negative by flow cytometry), bcl-2+, MUM-1+, Ki-67 almost 100% positive (A) Note the deep cellular clefts in some of the intermediate sized transformed cells and a large multinucleate cell. (B) This case showed both a *MYC* and *BCL-6* translocation, based on cytogenetic FISH studies using break-apart probes performed on paraffin embedded material. The illustrated cell demonstrates one normal fused signal for *MYC* (F, red/orange and green with yellow where they seem to overlap), and one red/orange (R) and one green signal (G), indicating splitting of the 8q24 region consistent with a *MYC* translocation. Classical cytogenetic studies yielded no metaphases. Despite significant Burkitt-like features, especially in some areas of the histological sections, the atypical morphological features, atypical phenotype and atypical cytogenetic findings would preclude our making the diagnosis of a Burkitt's lymphoma in this case. Nevertheless, we would not want to lump cases such as this with not otherwise specified diffuse large B-cell lymphomas.

grade'' features, with the cases having the fewest discrepancies from the typical constellation of BL findings being considered closer to a ''real'' BL.[87] In addition, although not a quantitative science and beyond this brief review, the reality is that some discrepancies also carry less weight than others, especially if the finding is not entirely convincing, such as possible weak bcl-2 protein expression in an otherwise typical BL. Once one is dealing with a case with BL at least in the differential, reliance solely on ''biological markers'' (Ki-67 >90%, CD10+, bcl-6+, bcl-2−, *MYC* translocated but not *BCL-2* or *BCL-6*) seems to identify a more homogeneous population of patients, than use of a consensus histological/immunophenotypic diagnosis.[85] However, many of the lymphomas in the BL/DLBCL grey zone region that are not felt to fulfil the criteria for a BL, still have ''a phenotypic and/or genetic shift to BL''. We would specifically designate cases with coexistent *MYC* and *BCL-2* and/or *BCL-6* translocations as at least those with dual *MYC* and *BCL-2* translocations are known to be extremely aggressive (fig 12).[50 93 94] Although *BCL-2* translocations are rarely found in molecularly-defined BL, Hummel *et al* found them more commonly in their intermediate or non-BL groups and *BCL-6* translocations were never found in BL.[84] The best therapy for the DLBCL with some BL-like features remains to be defined, although some report that cases with some of the BL features do not do well when they receive more typical DLBCL therapy.[95 96]

T/HRBCL versus NLPHL or T-cell NHL with large B-cells

The distinction of T/HRBCL from nodular lymphocyte predominant Hodgkin's lymphoma (NLPHL) is important since these entities are usually treated very differently and because T/HRBCL is a more aggressive neoplasm. This differential diagnosis, however, can be difficult because diffuse areas of NLPHL can be indistinguishable from T/HRBCL and some NLPHL are very rich in small T-cells.[97] Two major criteria that favour a diagnosis of T/HRBCL over NLPHL include absence of a nodular growth pattern as documented with an immunostain for FDC and a paucity of small B-cells. The differential diagnosis may also include peripheral T-cell lymphoma (PTCL) with admixed large B-cells or Reed–Sternberg-like cells.[98 99] The presence of significant cytological atypia and immunophenotypic aberrancy in the T-cells as well as genotypic support of a clonal T-cell population would favour the diagnosis of a PTCL, even though some small lymphoid atypia can be seen in NLPHL.

DLBCL versus classical HL

Some cases of DLBCL, especially T/HRBCL, may resemble classical HL with scattered Reed–Sternberg-like cells in a background of small lymphoid cells. Conversely, some classical HL can have numerous large transformed-appearing cells and resemble a DLBCL (or other type of NHL). The presence of uniform strong staining for pan B-cell markers such as CD20 and CD79a, positivity for both Oct-2 and BOB.1, positivity for CD45 and lack of staining for CD15, although not specific, all support the diagnosis of DLBCL. However, there are a group of ''grey zone'' lymphomas, often within the mediastinum.[100 101] Some of these cases demonstrate morphological features of classical HL and immunophenotypic characteristics of DLBCL or vice versa. Other cases demonstrate both morphological and immunophenotypic features intermediate between classical HL and DLBCL, being CD30+, CD15+ and strongly CD20+.[100] While it is worth favouring one diagnosis over the other, in some cases that may be impossible, and the concept of ''grey zone'' lymphomas should be discussed. It is probably wise to err on the side of a NHL and to treat as such, but also to acknowledge the diagnostic difficulties and the importance of keeping an open mind in cases refractory to therapy.[101]

Take-home messages

- Diagnosis of the aggressive B-cell lymphomas requires a multiparameter approach incorporating morphological, immunophenotypic, clinical and in certain instances cytogenetic and other genotypic features.
- The aggressive B-cell lymphomas should be classified as accurately as possible using the WHO classification.
- There is growing interest in the evaluation of pathological features that may have prognostic implications; however, as therapies change so can prognostic markers, and standardisation in the performance and interpretation of these tests is often lacking.
- Even using the most sophisticated tools, some cases will fall in a grey zone between two entities. These cases should be acknowledged as such and not forced into a category they might not truly belong in.
- Pathologists should keep up with the current literature as even this publication will be out of date in a depressingly short period of time.

Blastoid MCL versus DLBCL or B-LBicL

Blastoid MCL can closely resemble DLBCL or B-LBicL so that we advise liberal use of a stain for cyclin D1 which is almost always positive in MCL but not in DLBCL or B-LBicL. Furthermore, only B-LBicL will be TdT+. Myeloma and hairy cell leukaemia (HCL) can also be cyclin D1+, but other morphological and phenotypic differences usually help exclude those entities and HCL does not have CCND1 translocations. Whether infrequent non-MCL cyclin D1+ DLBCL exist remains to be determined.

DLBCL versus diffuse small B-cell lymphomas with increased transformed cells

Some small B-cell lymphomas, not discussed above, may also raise the question of a DLBCL. Lymph nodes with chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL) may have very prominent proliferation centres that can become partially confluent in the absence of classic transformation. It is important to review the peripheral blood smears in these cases that can begin to resemble a DLBCL. Ultimately there may be a sheet of paraimmunoblasts with prolymphocytic/prolymphocytoid transformation of CLL/SLL. The correct diagnosis can usually be reached by correlating the clinical, pathological and immunophenotypic features. Lymphoplasmacytic lymphoma and extranodal, nodal and splenic marginal zone B-cell lymphomas can also have a moderate number of transformed cells in the absence of transformation to a higher grade neoplasm.[102–108] In at least some of these situations, however, the increased numbers of transformed cells may be associated with an adverse prognosis. At the current time, other than adding a comment to their report, pathologists should be conservative in these circumstances unless sheets of transformed cells are found, in which case the diagnosis of a DLBCL should be given.

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Authors' affiliations

Sonam Prakash, Steven H Swerdlow, Division of Hematopathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

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