A clinician’s guide to the updated REAL/WHO classification of non-Hodgkin’s lymphoma: part II (aggressive lymphomas)

GÖRGÜN AKPEK¹, ERIC J. SEIFTER¹, MICHAEL J. BOROWITZ²

Departments of ¹Hematology/Oncology and ²Pathology, Johns Hopkins Oncology Center, Baltimore-USA

Approximately two-third of non-Hodgkin’s lymphoma seen in the United States and Europe are clinically aggressive lymphomas. This second part of the review contains updates on the definitions, terminology, diagnostic criteria and treatment approaches for these lymphomas. [Turk J Cancer 2000;30(2):53-67]

Key words: Non-Hodgkin’s lymphoma, REAL/WHO classification

AGGRESSIVE LYMPHOMAS

Diffuse large B-cell lymphoma (DLBCL) is defined as a neoplasm of large, transformed B cells with prominent nucleoli and basophilic cytoplasm, with a diffuse growth pattern and a high (>40%) proliferation fraction (1). This is the most common form of the non-Hodgkin's lymphomas, comprising 30% of newly-diagnosed cases (2). Most patients present with rapidly enlarging masses, often with symptoms both locally and systemically (designated B symptoms with fever, recurrent night sweats or weight loss). Localized (stage I or II) extranodal disease occurs in up to 30%; bone marrow involvement is seen in only 16% (2). DLBCLs are composed of large cells that resemble centroblasts or immunoblasts, most often with a mixture of the two. About 10% of the cases have over 90% immunoblasts; these cases are more common in immunosuppressed patients and may have a worse prognosis (3).

Primary mediastinal (thymic) large B-cell lymphoma is a subset of diffuse large cell lymphoma characterized by significant fibrosis on histology (4). It comprised 8% of diffuse large B-cell lymphoma in the international REAL classification study. Patients are usually female and young (median age 30-40). Patients present with a locally invasive anterior mediastinal mass originating in the thymus which may cause airway compromise or superior vena cava syndrome. Relapses tend to be extranodal, including liver, GI tract, kidneys, ovaries and CNS (1). Therapy and prognosis are the same as for other comparably-staged patients with diffuse large cell lymphoma, except for
advanced-stage patients with a pleural effusion, who have an extremely poor prognosis (progression-free survival is <20%) whether the effusion is cytologically positive or negative (4-7).

Some cases of large B-cell lymphoma have a prominent background of reactive T cells and often of histiocytes, so-called T-cell/histiocyte-rich large B-cell lymphoma. They may resemble Hodgkin’s disease of either lymphocyte predominance or mixed cellularity type. These tumors have a very aggressive clinical course with frequent liver, spleen and bone marrow involvement (8).

Lymphomatoid granulomatosis is an EBV positive large B-cell lymphoma with a predominant T-cell background (9, 10). The histology shows association with angioinvasion and vasculitis, usually manifesting as pulmonary lesions or paranasal sinus involvement. Patients are managed like others with DLBCL, requiring doxorubicin-based combination chemotherapy.

Intravascular lymphomatosis is characterized by large cell lymphoma confined to the intravascular lumen; with the use of aggressive combination chemotherapy, the prognosis is similar to more conventional presentations (11). The brain, kidneys, lungs and skin are the organs most likely affected by intravascular lymphomatosis.

The vast majority of patients with localized DLBCL are curable with combined modality therapy (12). For patients with advanced stage disease, 40% of presenting patients are cured with doxorubicin-based combination chemotherapy (13). An international index for aggressive NHL (diffuse large cell lymphoma) identifies 5 significant risk factors prognostic of overall survival: age (<60 years of age versus >60 years of age), serum lactate dehydrogenase (normal versus elevated), performance status (0 or 1 versus 2-4), stage (I or II versus III or IV) and extranodal site involvement (0 or 1 versus 2-4) (14). Patients with 2 or more risk factors have less than a 50% chance of relapse-free and overall survival at five years. This study also identifies patients at high risk of relapse based on specific sites of involvement, including bone marrow, central nervous system (CNS), liver, lung and spleen. Patients at high risk of relapse may be considered for clinical trials including upfront autologous stem cell transplantation (15). Bcl-2 protein is expressed in up to 50% of the cases of DLBCL and does not correlate with bcl-2 gene rearrangement. Expression of bcl-2 protein appears to predict a higher relapse rate (16). CNS prophylaxis (usually with 4-6 injections of methotrexate intrathecally) is recommended for patients with paranasal sinus or testicular involvement. Some clinicians are employing high-dose intravenous methotrexate (usually 4 doses) as an alternative to intrathecal therapy because drug delivery is improved and patient morbidity is decreased (17). CNS prophylaxis for bone marrow involvement is controversial; some investigators recommend it, others do not (13). A retrospective analysis of 605 patients with diffuse large cell lymphoma who did not receive prophylactic intrathecal therapy identified an elevated serum lactate dehydrogenase and more than one extranodal site as independent risk factors for CNS recurrence. Patients with both risk factors have a 17% probability of CNS recurrence at one year after diagnosis versus 2.8% for the remaining patients (18).

Anaplastic large T-cell lymphoma (ALCL), primary systemic type is defined as a neoplasm of large lymphoid cells with pleomorphic or multiple
nuclei and abundant cytoplasm, a cohesive growth pattern and sinusoidal spread in lymph nodes (1). It may be confused with carcinomas and are associated with the Ki-1 (CD30) antigen. These lymphomas are usually of T-cell origin and often present with extranodal disease, including skin, but is not localized to the skin. Tumors that present with systemic disease (with or without skin involvement) have a bimodal age distribution in children and adults. Patients may present with isolated lymphadenopathy or with extranodal disease in any site, including GI tract and bone (19). ALCL in children is characterized by frequent high stage but a good response to therapy, with overall excellent survival (19,20). In adults the tumor is aggressive but potentially curable, similar to other aggressive lymphomas. Patients with these lymphomas are generally treated the same as those with diffuse, large cell lymphomas and have as good a prognosis as comparably-staged patients (21,22).

The tumor cells of ALCL are CD30+ CD25+, EMA+ CD45+ and CD15-. About 60% express one or more T-cell-associated antigens and many express cytotoxic granule proteins (23). The majority of the cases have T-cell receptor (TCR) genes rearranged; 20-30% have no rearrangement of TCR or Ig genes. There is a rare entity called B-cell anaplastic large cell lymphoma (B-ALCL) in which the lymphoma cells are identical to those of T/null anaplastic large cell lymphoma (ALCL) and strongly express CD30 (Ki-1). However, they do not have the same distinctive clinical or genetic features of T/null ALCL and in the REAL classification are considered a morphologic variant of large B-cell lymphoma. Between 20 and 50% of primary systemic ALCL have a t(2;5) which results in a fusion of the nucleophosmin gene (NPM) on chromosome 5 to a novel tyrosine kinase gene on chromosome 2, called ALK (anaplastic lymphoma kinase) (24). The anaplastic lymphoma kinase (ALK) protein is detected in 40-60% of the cases using the ALK1 monoclonal antibody, with both nuclear and cytoplasmic staining. ALK+ cases are more common in children and have a better prognosis than ALK- cases (25-27).

Primary cutaneous anaplastic large-cell lymphoma (ALCL) is defined as an ALCL that presents in the skin only with no pre-existing lymphoproliferative disease and no extracutaneous sites of involvement (1). Primary cutaneous ALCL occurs in adults (cutaneous lesion in children are usually manifestations of systemic disease). These patients encompass a spectrum ranging from lymphomatoid papulosis at the clinically benign end, marked by localized nodules which may regress spontaneously, to a progressive and systemic disease requiring aggressive doxorubicin-based combination chemotherapy in 25% of cases (28). Cytologic features are similar to those of systemic ALCL. Most cases are nonepidermotrophic. The neoplastic cells express T-cell antigens and CD30, and are usually CD4-positive. They are negative for ALK and epithelial membrane antigen (EMA), in contrast to systemic ALCL. About 50% express the cutaneous lymphocyte antigen (HECA-452) (28). ALCL presenting in the skin that is either ALK+ or shows a t(2;5) should be regarded as a likely manifestation of systemic disease.

Follicular lymphoma (Grade 3): The natural history of Grade 3 FL (follicular large cell lymphoma) remains controversial (29). While there is agreement about the significant number of long-term disease-free survivors with early-stage disease, the curability of patients with advanced disease (stage III
or IV) remains uncertain. Some groups report a continuous relapse rate similar to the other follicular lymphomas (a pattern of indolent lymphoma) (30). Other investigators report a plateau in freedom from progression at levels expected for an aggressive lymphoma (40% at 10 years) (31,32). This discrepancy may be due to variations in histologic classification between institutions and to the rarity of patients with follicular large cell lymphoma. Treatment of these patients is more like treatment of aggressive NHL than of indolent NHL. In support of this approach, treatment with high-dose chemotherapy and autologous hematopoietic peripheral stem cell transplantation shows the same curative potential in patients with follicular large cell lymphoma who relapse as it does in patients with diffuse large cell lymphoma who relapse (33).

Mantle cell lymphoma is defined as a neoplasm of monomorphous small to medium-sized B cells with irregular nuclei, which resemble the cleaved cells (centrocytes) of germinal centers. Neoplastic transformed cells with basophilic cytoplasms (centroblasts or immunoblasts) are absent (1). Lymph nodes, spleen, bone marrow (>60%), blood (up to 50%) and sometimes the gastrointestinal system (lymphomatous polyposis) are involved (34,35). It is characterized by CD5+ CD23- follicular mantle B cells, a translocation of chromosomes 11 and 14, and an overexpression of PRAD1 or cyclin D1 gene (36). The product of the cyclin D1 gene can be detected in the nuclei of neoplastic mantle cells in paraffin embedded tissue sections with the immunoperoxidase technique and is useful in distinguishing MCL from other low-grade B-cell lymphomas (37). The protein is overexpressed even in cases lacking the t(11;14), suggesting that point mutations may also result in overexpression (38). Overexpression of this protein may explain the often high mitotic index and the aggressive clinical course of this histologically low-grade lymphoma. Like the low-grade lymphomas, mantle cell lymphoma appears incurable with anthracycline-based chemotherapy and occurs in older patients with a marked male predominance (75%) and generally asymptomatic advanced-stage disease (70%) (39,40). However, the median survival is significantly shorter (three years) than that of other lymphomas and failure-free survival is around one year. Therefore, MCL is now considered to be an aggressive lymphoma (39-41). A diffuse pattern and the blastoid variant have an aggressive course with shorter survival, while the mantle zone type may have a more indolent course (39,40,42). It is unclear which chemotherapeutic approach offers the best long-term survival in this clinicopathologic entity; refractoriness to chemotherapy is a usual feature (35,41,43-45). Many investigators are exploring high-dose therapy with stem cell/marrow support or the use of interferon or anti-CD20 antibodies after CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy (35,45-48). Fludarabine is reported to be less effective in MCL than in CLL and follicular lymphoma (49).

Peripheral T-cell lymphoma (PTCL), unspecified is a group of predominantly nodal T-cell lymphomas with a variable morphologic appearance. Unlike the T-cell lymphomas mentioned above, this probably does not represent a single entity (1). Patients with PTCL have diffuse large cell or diffuse mixed lymphoma which expresses a cell surface phenotype of a post-thymic (or peripheral) T-cell expressing CD4 or CD8, but not both together. B-cell associated antigens are lacking (50,51). The median age was in the
seventh decade and 65% of the patients had stage IV disease. Most patients had elevated LDH and involvement of extranodal sites was seen in 82%. The clinical course is aggressive and relapses are more common than in large B-cell lymphoma. In the international REAL classification study, this group had one of the lowest overall and failure-free survival rates (2,51-53). Therapy involves doxorubicin-based combination chemotherapy as for B-cell diffuse large cell lymphoma.

**Adult T-cell lymphoma/leukemia (HTLV1+)** is caused by infection with the retrovirus human T-cell lymphotropic virus type 1 and is frequently associated with hypercalcemia, circulating leukemic cells, bony and skin involvement, a rapidly progressive course and poor response to chemotherapy (54). The combination of zidovudine and interferon alpha has activity against adult T-cell leukemia/lymphoma, even for patients who failed prior cytotoxic therapy. Durable remissions are seen in two-thirds of presenting patients with this combination, but long-term disease-free survival rates are not yet available (55,56).

**Angioimmunoblastic T-cell lymphoma (AIL-TCL), formerly called angioimmunoblastic lymphadenopathy with dysproteinemia (AILD)** is defined as a lymphoma of mature T-cells, characterized by a polymorphous infiltrate of reactive T and B cells, with clusters of atypical T-cells with pale or "clear" cytoplasm and prominent proliferation of both high endothelial venules and follicular dendritic cells (1). The morphology is distinctive and the diagnosis can often be made without immunophenotype or clinical data. This is a clonal disease that has been shown by T-cell receptor gene rearrangement studies (57). Patients present with generalized lymphadenopathy, fever, night sweats, weight loss, skin rash, a positive Coomb’s test and polyclonal hypergammaglobulinemia. Opportunistic infections are frequent due to an underlying immune deficiency. The course is moderately aggressive, with occasional spontaneous remissions and is not reliably predicted by the histologic appearance. About 30% of patients may experience initial remission on steroid alone, but most require some form of chemotherapy. Doxorubicin-based combination chemotherapy is recommended as for other aggressive lymphomas (1,58). Median survival ranges from 15 to 24 months and the rate of cure has not been well established. A few patients may progress to an Epstein-Barr virus (EBV) + diffuse large B-cell lymphoma (1,59).

**Extranodal NK/T-cell lymphoma, nasal type (formerly angiocentric lymphoma)** is defined as an extranodal lymphoma, usually with NK-cell phenotype, with a broad morphologic spectrum. This is an aggressive lymphoma marked by extensive necrosis and angioinvasion, most often presenting in extranodal sites, in particular the nasal or paranasal sinus region. Other extranodal sites include the palate, trachea, skin and gastrointestinal tract (1,60-62). Hemophagocytic syndrome may occur; historically these tumors were considered part of "lethal midline granuloma". In most cases, EBV genomes are detectable in the tumor cells by in situ hybridization for EBER-1. The atypical cells in most cases are CD2+ CD56+, surface CD3- and cytoplasmic CD3+. They are typically CD4- CD8- but may express CD4 and/or CD7. Most cases express cytotoxic granule proteins such as granzyme B, perforin and TIA-1 (63-65). Cases with blood and marrow involvement are considered NK-cell
leukemia. In addition to doxorubicin-based combination chemotherapy, the increased risk of CNS involvement and of local recurrence has led to recommendations for radiation therapy locally and for intrathecal prophylaxis and/or prophylactic cranial irradiation (61). The highly aggressive course, with poor response and short survival with standard therapies, has led some investigators to recommend bone marrow or peripheral stem cell transplantation consolidation (62).

**Enteropathy-type intestinal T-cell lymphoma** is defined as a tumor of intraepithelial T-lymphocytes and involves the small bowel of patients with gluten-sensitive enteropathy (celiac sprue) (1,66). Since a gluten-free diet prevents the development of lymphoma, patients diagnosed with celiac sprue in childhood rarely develop lymphoma. This disease occurs in adults, typically with a rather brief history of gluten-sensitive enteropathy. Patients present with abdominal pain, often associated with jejunal perforation. Stomach and colon are affected less often and the tumor may involve liver, spleen, lymph nodes and other viscera such as gall bladder. Tumor cells are T cells expressing pan-T antigens (CD3+ CD7+), usually CD8+ CD4-, and express mucosal lymphoid antigen CD103 (67). The diagnosis of celiac disease is usually made by finding villous atrophy in the resected intestine. The course is aggressive and death usually occurs from multifocal intestinal perforation due to refractory malignant ulcers. Surgery is often required for diagnosis and to avoid perforation during therapy. Therapy is with doxorubicin-based combination chemotherapy, but relapse rates appear higher than for comparably staged DLBCL.

**Hepatosplenic T-cell lymphoma** is defined as a neoplasm of T cells with sinusoidal infiltration of spleen, liver and bone marrow (1,68). The tumor cells are CD2+ and CD3+, CD5-, CD4-, CD8-, CD16+, CD56+/- and lack the T-cell receptor protein, expressing instead the complex. The cells are EBV negative. Isochromosome 7q has been reported in the majority of cases (1). The clinical syndrome is an important part of the diagnosis. Patients are predominantly adolescent and young adult males, who present with marked hepatosplenomegaly. This is a rare and aggressive tumor. Although there is often an initial response to chemotherapy, relapse and death are common (68).

**Subcutaneous panniculitis-like T-cell lymphoma** is defined as a T-cell lymphoma that preferentially infiltrates subcutaneous tissue with atypical cells of variable size, showing prominent tumor necrosis and karyorrhexis (1). Patients present with subcutaneous nodules, which are often misdiagnosed as panniculitis. Hemophagocytic syndrome is common (69,70). This entity is treated with the same paradigm as for DLBCL.

**Precursor T-lymphoblastic lymphoma** is a very aggressive form of NHL. It often occurs in young patients, but not exclusively (71). It is commonly associated with large mediastinal masses and has a high predilection for disseminating to bone marrow and the central nervous system (CNS). Treatment is usually patterned after that for acute lymphoblastic leukemia (ALL). Intensive combination chemotherapy with or without bone marrow transplantation is the standard treatment of this aggressive histologic type of NHL (72). Irradiation of areas of bulky tumor masses is sometimes given. Since these forms of NHL tend to progress so quickly, combination chemotherapy is instituted rapidly once the diagnosis has been confirmed. Careful review of the
pathologic specimens, bone marrow aspirate and biopsy specimen, cerebrospinal fluid cytology and lymphocyte marker constitute the most important aspects of the pretreatment staging work-up.

**Burkitt's lymphoma** is defined as a B-cell lymphoma composed of monomorphic, medium-sized cells with basophilic cytoplasm and a high proliferation fraction, characterized by translocation and deregulation of the c-myc gene on chromosome 8, which is often extranodal and occurs most often in children (endemic, sporadic) and immunocompromised hosts. Cytoplasmic lipid vacuoles are usually evident on imprints or smears. There is an extremely high rate of proliferation as well as a high rate of spontaneous cell death. A "starry-sky" pattern is usually present, imparted by numbers of benign macrophages that have ingested apoptotic tumor cells. Most cases have a translocation of c-myc from chromosome 8 to either the Ig heavy chain region on chromosome 14 [t(8;14)] or light chain loci on chromosome 2 [t(2;8)] or 22 [t(8;22)] (1).

Burkitt's lymphoma typically involves younger patients and represents the most common type of pediatric non-Hodgkin's lymphoma (73). A subgroup of patients with dual translocation of c-myc and bcl-2 appear to have an extremely poor outcome despite aggressive therapy (5 month overall survival) (74). Endemic cases, usually from Africa, involve the facial bones or jaw of children. Most African cases contain Epstein-Barr virus (EBV) genomes, as do 25-40% of the cases associated with acquired immune deficiency syndrome (75). In HIV+ patients, it typically affects those with a relatively high CD4 count and no opportunistic infections. Immunodeficiency-related cases more often involve lymph nodes and may present as acute leukemia. Sporadic cases usually involve distal ileum, cecum and/or mesentery, ovaries and/or kidneys. Patients typically present with rapidly growing masses and a very high lactate dehydrogenase, but are potentially curable with intensive doxorubicin-based combination chemotherapy (1).

Although most cases present no problem in diagnosis, some cases may have larger cells and there is morphologic overlap with diffuse large B-cell lymphoma. These borderline cases are often called "non-Burkit" or "Burkitt-like" (76). In children and HIV+ patients these often have a c-myc translocation and behave similarly to typical Burkitt's lymphoma, whereas in adults, so called "Burkitt-like" lymphomas often have bcl-2 rearrangement and may represent an aggressive variant of diffuse large cell lymphoma (77). In the international study of the REAL classification Burkitt-like lymphoma was a non-reproducible category in which the pathologists agreed on the diagnosis only 50% of the time (40). Disagreement was equally split between large B-cell and Burkitt's lymphoma. The current recommendation, therefore, is that "Burkitt-like" lymphoma should be defined as a lymphoma that should be treated "like Burkitt's lymphoma".

The tumor cells in Burkitt's lymphoma remain constantly in cycle as a consequence of c-myc deregulation. Unfortunately, detection of c-myc translocation is not practical in all clinical specimens for technical reasons. The best practical surrogate for c-myc deregulation is proliferation fraction. In a tumor with c-myc deregulation, 100% of viable cells should be in cycle, and should express Ki-67, which is a proliferation marker. Cases with morphologic
features that are borderline between Burkitt's lymphoma and large B-cell lymphoma should be classified as Burkitt's lymphoma if the Ki-67+ fraction of viable tumor cells is close to 100% and bcl-2 protein expression is absent (1). Burkitt's lymphoma cells express IgM and B-cell associated antigens and CD10. They lack CD5, CD23 (78), and bcl-2 protein (79).

Treatment of Burkitt's lymphoma involves aggressive multidrug regimens similar to those used for the advanced-stage aggressive lymphomas (diffuse large cell) (80,81). An intensive clinical trial using aggressive combination chemotherapy patterned after that used in childhood Burkitt's lymphoma has been described and has been very successful for adult patients, with more than 60% of advanced stage patients free of disease at 5 years (82-84). Adverse prognostic factors include bulky abdominal disease and high serum lactate dehydrogenase. In some institutions, treatment includes the use of consolidative bone marrow transplantation (85). Patients with Burkitt's lymphoma have a 20% to 30% life-time risk of CNS involvement. Prophylaxis with intrathecal chemotherapy is required as part of induction therapy.

Post-transplantation lymphoproliferative disorder (PTLD): Patients who undergo transplantation of the heart, lung, liver, kidney, or pancreas usually require life-long immunosuppression. This may result in PTLD in 1% to 3% of recipients, which appears as an aggressive lymphoma (86). Pathologists can distinguish a polyclonal B-cell hyperplasia from a monoclonal B-cell lymphoma; both are almost always associated with EBV (87). In some cases, usually for the polyclonal forms of the disease, withdrawal of immunosuppression results in eradication of the lymphoma (88). When this is unsuccessful or not feasible, a combination of acyclovir and interferon alpha has been used (86, 89). If these measures fail, doxorubicin-based combination chemotherapy is then recommended (90). Localized presentations can be controlled with surgery or radiation therapy alone. These localized mass lesions, which may grow over a period of months, are often phenotypically polyclonal and tend to occur within weeks or a few months after transplantation (87). Multifocal, rapidly progressive disease occurs late after transplantation and is usually phenotypically monoclonal. EBV-negative post-transplantation lymphoproliferative disorders occur late (median 5 years post-transplant) and have a particularly poor prognosis (91). Clinical trials are underway looking at withdrawal of immunosuppression, treatment with acyclovir and interferon alfa, and combination chemotherapy when the previous modalities fail (92). A sustained clinical response after failure from chemotherapy was attained using an immunotoxin (anti-CD22 B-cell surface antigen antibody linked with ricin) (93).

True histiocytic lymphomas are very rare tumors which show histiocytic differentiation and express histiocytic markers in the absence of B-cell or T-cell lineage-specific immunologic markers (94). Many of these are derived from dendritic cells. Care must be taken with immunophenotypic tests to exclude anaplastic large cell lymphoma or hemophagocytic syndromes due to viral infections, especially EBV. Therapy is modelled after the treatment of comparably staged diffuse large cell lymphomas, but the optimal approach remains to be defined.

Primary effusion lymphoma presents exclusively or mainly in the pleural, pericardial or abdominal cavities in the absence of an identifiable tumor mass.
Patients are usually HIV seropositive, and the tumor usually contains Kaposi's sarcoma-associated herpes virus/human herpes virus 8. Therapy is usually modelled after the treatment of comparably staged diffuse large cell lymphomas, but the prognosis is extremely poor (95).

References


15. Canellos GP. CHOP may have been part of the beginning but certainly not the end: issues in risk-related therapy of large-cell lymphoma. J Clin Oncol 1997;15:1713-6.


nodal anaplastic large cell lymphoma of T-cell or null cell phenotype. Histopathology 1993;23:127-35.


44. Teodorovic I, Pittaluga S, Kluin-Nelemans JC, et al. Efficacy of four different regimens in 64 mantle lymphoma cases: clinicopathologic comparison


92. Swinnen LJ, Southwest Oncology Group: Phase II Study of Sequential Therapy Consisting of Immunosuppression, Interferon Alfa, and Chemotherapy in Patients with Lymphoproliferation Following Organ Transplantation (Summary Last Modified 04/1999), SWOG-9239, clinical trial, active, 05/04/1995.

