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**76 Molecular Genetics/Immunophenotype in New Entities of the
2008 WHO Classification of Hematopoietic Neoplasms**

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76 Molecular Genetics/Immunophenotype in New Entities of the 2008 WHO Classification of Hematopoietic Neoplasms

This session will present eight unknown cases to illustrate new entities in the 2008 WHO classification. Major emphasis will be placed on molecular genetics and immunophenotyping, including a testing algorithm for cost effective work-up of these lesions.

- Use molecular genetics and immunophenotyping to diagnose new entities in the 2008 WHO classification of hematopoietic neoplasms.
- Evaluate the clinical significance of the new concepts.
- Facilitate the communication with clinicians in new terminology.

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**MOLECULAR GENETICS/IMMUNOPHENOTYPE IN NEW
ENTITIES OF THE 2008 WHO CLASSIFICATION OF
HEMATOLOGIC NEOPLASMS**

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Introduction

For many years, a diagnosis of hematopoietic neoplasm was equivalent to a death sentence to the patient. However, due to the progress of diagnostic techniques and development of new therapeutic regimens, the outlook of a patient with leukemia and lymphoma has been much improved. In fact, even with the most aggressive tumors, such as Burkitt lymphoma, a patient can be cured nowadays if he is diagnosed in the early stage and treated with the most advanced therapeutic protocol. The current classification of hematopoietic neoplasms deserves some credit in the improvement of the prospect of these patients. The World Health Organization (WHO) Classification of Tumors of Hematopoietic and Lymphoid Tissues is instrumental by providing guidelines for the diagnosis of well-defined neoplasms with the current modalities so that these tumors can be diagnosed accurately and frequently at their early stage, therefore, appropriate treatment can be applied with ideal effects. The 2008 WHO classification further expands the scope of the hematopoietic neoplasms. For instance, in the 2001 WHO classification, there were 6 morphologic variants and 4 subtypes of diffuse large B-cell lymphoma (DLBCL), but the 2008 WHO scheme recognizes 12 types of DLBCL in addition to the novel borderline category. The new classification also further defines new entities on the basis of cytogenetic karyotype, immunophenotype, age of patients, tumor locations, and infectious agent association.

These new divisions are of clinical significance, not only in terms of clinical presentation and prognosis, but also leading to new strategy of treatment. For instance, the 2008 classification redesignates chronic myeloproliferative diseases to myeloproliferative neoplasms because of the discovery of genetic mutations in these diseases. As a result, tyrosine kinase inhibitors have been used successively in treating these neoplasms. Another example is defining gray zone lymphomas based on the discrepancy between morphology and immunophenotype. The treatment and prognosis of these gray zone lymphomas are greatly different from those of the individual components. The new classification recognizes that pediatric patients differ much from adult patients for the same tumor in terms of clinical presentation, treatment and prognosis; that cutaneous follicular lymphoma differ from nodal based follicular lymphoma; and that EBV-associated DLBCL and T-cell lymphoma differ from the same tumor entity without EBV association. Among all these influencing factors, molecular genetics and immunophenotype are the most important, therefore, we have selected 8 clinical cases to illustrate the usefulness of using these parameters to help the diagnosis and guide the treatment of these patients.

Case 1

B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL)

Synonyms

- Burkitt-like lymphoma, atypical BL, gray-zone lymphoma
- High-grade B-cell lymphoma, Burkitt-like (Revised European American Lymphoma [REAL] classification)
- Small non-cleaved cell lymphoma, non-Burkitt (Working Formulation)

Introduction

The border between (nonendemic) Burkitt's lymphoma (BL) and other mature aggressive B-cell lymphomas (notable DLBCL) has been a field of diagnostic uncertainty. Indeed, classical morphologic and immunophenotypic features, but also recent gene expression studies, might suggest that BL and DLBCL are both extremes of a continuum of mature aggressive B-cell lymphoma. The clinical impact on the differentiation between BL and DLBCL nowadays heavily depends on the age groups of the patients. In children, high cure rates are achieved with related or identical treatment strategies in both BL and DLBCL. In adults, some evidence suggests that patients with BL might benefit from more intensive chemotherapy regimens than those applied to DLBCL.

Definition

The updated WHO classification of Tumors of the Hematopoietic and Lymphoid Tissues from 2008 proposes to assign aggressive B-cell lymphomas "that have morphological and genetic features of both DLBCL and BL, but for biologic and clinical reasons should not be included in these categories" to a provisional category called B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL.

This lymphoma type is defined as aggressive B-cell lymphoma with morphological and genetic features of both DLBCL and BL. This is a heterogeneous category and not a distinct entity. This category also includes "double-hit" or "triple-hit" lymphomas (cases with translocations involving *MYC* and *BCL2*, and/or *BCL6* genes).

CLINICAL ISSUES

Epidemiology

These lymphomas are uncommon, but true frequency is not yet known. Overall frequency of these lymphomas as well as the number of the potential subgroups lumped together in this provisional entity increases with patient age. They are more frequent in adults, median age 6th decade and more frequent in males than females.

Presentation

More than 50% of patients present with disseminated disease with lymphadenopathies and/or extranodal masses. Extranodal sites are commonly involved and include bone marrow (~ 50%) and brain (~ 33%). Approximately, 10-20% of patients have history of follicular lymphoma. B-type symptoms are common and leukemic presentation is uncommon but can occur (elevated leukocyte count with lymphoma cells). The serum levels of lactate dehydrogenase &/or β -2-microglobulin are frequently high.

Prognosis

DLBCL/BL is heterogeneous and therefore prognosis is also heterogeneous. A large subset of patients have poor clinical outcome despite intensive chemotherapy regimen.

PATHOLOGIC FEATURES

Histologic Features

DLBCL/BL are typically composed of a diffuse proliferation of intermediate-sized cells or spectrum of intermediate sized and large cells with high mitotic and apoptotic rates and relatively few admixed small reactive lymphocytes. A "starry sky" is common and sclerosis is uncommon.

According to the WHO classification, 4 possible scenarios for diagnosis are possible:

- o Neoplasm resembles BL but too much variation in cell size and nuclear contours
- o Neoplasm resembles BL but atypical immunophenotype &/or genetic findings
- o Neoplasm has cells intermediate between DLBCL and BL with immunophenotype typical of BL
- o Neoplasms with blastic chromatin with lymphoblastic lymphoma-like appearance.

“Double-hit” or “triple-hit” lymphomas are classified as DLBCL/BL. However, it is important to remember that double-hit or triple-hit lymphomas can morphologically resemble DLBCL, BL, or have intermediate cytologic appearance.

Immunohistochemistry

These lymphomas express pan-B-cell antigens, TCL1+/-, IRF-4/MUM1+/-, variable but usually high Ki-67, CD43+/-, and negative for pan-T-cell antigens. Some cases have an immunophenotype consistent with BL: CD10+, Bcl-6+, IRF-4/MUM1-, Bcl-2-, and

TCL1+. In double-hit lymphomas, Bcl-2 is usually strongly positive, Ki-67 can range from ~ 70-100% and surface immunoglobulins are frequently negative. Lymphomas morphologically defined as BL with BCL2 positivity can also be placed in this category.

These lymphomas are usually EBER negative.

Cytogenetics and Molecular Genetics

These lymphomas have frequently complex karyotypes (> 3 abnormalities) with multiple abnormalities in comparison with classical BL. *MYC* translocations are detected in up to 50% of the cases:

- o ~ 60% of cases with 8q24/*MYC*, translocations involve *Ig* gene loci
- o ~ 40% of cases with 8q24/*MYC*, other translocation partners are involved

Approximately 15% of these lymphomas bear *BCL2* translocations sometimes together with *MYC* rearrangements (double-hit lymphomas). Presence of *BCL6* translocations is less frequent but it exists (along with *BCL2* translocations –define triple-hit lymphomas).

FISH assays are very useful for detecting *MYC* translocations, generally using a *MYC* break-apart probe. FISH probes are also available to detect *Ig* genes that are translocation partners of *MYC* as well as *IgH-BCL2* and *BCL6* gene rearrangements.

Gene expression profile is either intermediate between DLBCL and BL or very similar to BL.

Top Differential Diagnoses

- Burkitt lymphoma
- Diffuse large B-cell lymphoma
- Lymphoblastic leukemia/lymphoma
- Mantle cell lymphoma, blastoid variant

Burkitt Lymphoma (BL)

Three variants of BL are recognized: endemic BL, sporadic BL, and immunodeficiency-associated BL. The sporadic variant of BL accounts for 1% to 2% of patients with lymphoma in western countries. It occurs mainly in children with a strong bias toward the male sex. The median age of adult patients lies in the third decade of life, with a still slight predominance among males. Recent studies have shown that considering cytogenetic and gene expression data, sporadic BL in children and adults is biologically almost identical. Morphologically, the tumor cells are intermediate-sized

cells with "squared-off" cytoplasmic borders, thick nuclear membranes, and multiple (2-4) nucleoli. They show a diffuse and monotonous pattern of growth.

The classical immunophenotype of BL:

- o Surface Ig+, IgM+, CD10+, CD20+, Bcl-6+, and Ki-67 positive virtually in every cells
- o BCL2- and TdT-

MYC/8q24 translocations are characteristic but not exclusive to this disease. They juxtapose the *MYC* gene and one of the three *IgG* loci (ie, *IgH* in 14q32, *IgL* in 22q11, and *IgK* in 2p12). These *Ig-MYC* fusions result in deregulated expression of the intact *MYC* oncogene. In BL, the *Ig-MYC* fusion mostly is part of a simple karyotype with secondary aberrations being singular events. On the cytogenetic level, around 70% of BL have no or only one secondary alteration.

Using a gene-expression profiling approach, independent groups have been able to recognize specific gene expression signatures for BL that identify almost the very same lymphomas. The cases that carry this signature are described as molecular BL (mBL) and be clearly differentiated from non-mBL, a group mainly composed of DLBCL.

Diffuse Large B-cell Lymphoma

DLBCLs are a group of morphologically, immunohistochemically and clinically heterogeneous tumors rather than one single entity. Morphologically the tumor cells are large. The neoplastic cells usually express CD19, CD20, CD22, and CD79a. Surface and/or cytoplasmic immunoglobulin can be demonstrated. CD10, BCL2 and BCL6 can be positive or negative. The proliferation rate is usually lower than 90%.

Chromosomal translocations affecting *BCL6* are one of the most common genetic abnormalities in DLBCL (30%). Translocations of the *BCL2* gene next to the *IgH* gene through t(14;18)(q32;a21) is detected in 30 to 40% of DLBCL (those of germinal center type, whereas it is absent in DLBCL of activated B-cell type). *MYC* translocation occurs in ~ 10% of classical DLBCL cases.

Important to remember that some double-hit lymphomas closely resemble DLBCL.

T- or B-Lymphoblastic Leukemia/Lymphoma

Morphologically, the tumor cells are small- to medium-sized blasts with "dusty"

chromatin. The immunophenotype would support immature lymphoid lineage with positivity for TdT and/or CD34.

Mantle Cell Lymphoma (MCL), Blastoid Variant

The blastoid variant of MCL can have a prominent starry sky pattern and is composed of intermediate-sized cells with dispersed nuclear chromatin. The positivity for CD5 and cyclin D1 supports the diagnosis of MCL. The chromosomal translocation t(11;14)(q13;q32) is characteristic and support the diagnosis. Some of these cases may also have translocations involving *MYC* gene.

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Case 2

A 39-year-old man presented with progressive skin lesions on his trunk associated with constitutional symptoms for six months. He had arthralgias, bone pain in the ankles and wrists, fatigue, fever, and a 20-pound weight loss over a period of six months. He was treated with a short course of antibiotics without benefit. Physical examination revealed diffuse subcutaneous nodules of varying sizes over his trunk. There were no lymphadenopathy and organomegaly. Hematology workup showed no cytopenia or

leukocytosis.

A skin biopsy showed extensive tumor cell infiltration in the dermis and subcutis without epidermic involvement. Immunohistochemical stain of the biopsy showed that the tumor cells were positive for CD4, CD56, and CD68, but were negative for CD3, CD5, CD8, CD20, myeloperoxidase (MPO) and lysozyme. Flow cytometry showed positive reactions to CD2, CD4, CD7, CD45, CD56, and HLA-DR, but negative reactions to surface CD3, CD8, CD10, CD13, CD19, CD33, CD34, CD117, terminal deoxynucleotidyl transferase (TdT) and MPO.

A bone marrow biopsy also revealed infiltration by tumor cells. Flow cytometry showed positive reactions to CD2, CD4, CD7, CD13, CD33, and CD56, but negative reactions to CD3, CD8, CD11c, CD14, CD19, CD20, CD57, kappa, lambda and TdT. T-cell receptor gene rearrangement revealed a germline pattern.

The patient received 3 cycles of induction chemotherapy with an acute lymphoblastic leukemia regimen. As a result, he had a complete resolution of his skin lesions and systemic symptoms. A second bone marrow biopsy was found to be negative for leukemic cells by both morphology and flow cytometry. He subsequently received an autologous stem cell transplant. However, a third bone marrow biopsy 8 months later showed evidence of relapse. A new chemotherapeutic regimen was started with anticipation of a second bone marrow transplantation.

History

In 1995, Brody et al described a case of purportedly CD56+ natural killer (NK) cell leukemia, which differed from the classical NK cell neoplasm in its agranular morphology, CD4+ (instead of CD8+) immunophenotype and an initial skin presentation. The 2001 WHO classification designated this tumor as blastic NK-cell lymphoma. The term CD4+/CD56+ hematodermic neoplasm was adopted by the WHO/European Organization for Research and Treatment of Cancer (EORTC) Classification for Cutaneous Lymphomas in 2005. However, recent immunophenotypic and functional studies indicate that this tumor is of plasmacytoid dendritic cell (PDC) origin and the 2008 WHO classification designates it as blastic plasmacytoid dendritic cell neoplasm (BPDCN).

Morphology

The morphologic description of the BPDCN cells in the literature is greatly variable but one consistent feature is that the tumor cells are blast-like with immature chromatin and with or without the presence of nucleoli. Cytoplasmic granules are characteristically absent. The cell size ranges from small to large, but medium-sized cells are most frequently encountered. The nuclei are usually round or oval but irregular

configuration (notched, folded or bilobed) has also been reported. The cytoplasm can be scanty or abundant with gray-blue color.

The specific features described in several studies are the presence of peripheral microvacuoles in the cytoplasm (pearl necklace appearance) and pseudopod-like extensions, recapitulating the pinocytotic process in normal PDCs. However, these features are not present in most reported cases.

In the skin biopsy, dermis is extensively infiltrated with frequent extension into the subcutis, while epidermis is usually not involved. In the early lesion, there is a perivascular and periadnexal infiltration, but later lesion may show destruction of the skin appendages. The degree of infiltration in the bone marrow depends on the stage of the disease. In the late stage, the normal hematopoietic cells can be largely replaced. Lymph node involvement often presents as leukemic type infiltration in the interfollicular area, but effacement of nodal architecture may be demonstrated in the later stage.

Immunophenotype

The immunophenotype for initial diagnosis of BPDCN is the CD4+, CD56+, cell lineage associated marker negative profile. Myelomonocytic markers (CD13, CD33, CD11c, CD14, CD64, MPO, chloroacetate esterase, butyrate esterase, and lysozyme), B cell (CD19, CD20, CD79a, PAX5) and T cell (CD1a, CD2, CD3, CD5, CD7 and CD8) lineage-associated markers, NK cell-associated antigens (CD16, CD57, T-cell intracellular antigen 1 and perforin), progenitor cell markers (CD34 and CD117), and lymphoid activation markers (CD25, CD30, and CD71) are usually absent. CD21, CD35, CD36, CD10, S100 and Epstein-Barr virus antigen are also not present in this tumor. However, a few cell lineage markers may be present occasionally.

The PDC specific markers, including CD123, blood dendritic cell antigen 2 (BDCA2), BDCA4, CD2-associated adaptor protein (CD2AP), T-cell leukemia/lymphoma 1 (TCL1), and cutaneous lymphocyte-associated antigen (CLA), are most useful in the differential diagnosis. BDCA2 and CD2AP seem to be most specific. However, some of these markers may also be present in other hematologic neoplasms. For instance, CD123 can be demonstrated in acute basophilic leukemia, Langerhans cell histiocytosis, histiocytic sarcoma and hairy cell leukemia as well as in many acute leukemias. TCL1 can be present in T-cell prolymphocytic leukemia, T-cell acute lymphoblastic leukemia, adult T-cell lymphoma/leukemia and some B-cell lymphomas. CLA is also detected in leukemia cutis secondary to acute myeloid leukemia or chronic myelomonocytic leukemia.

Differential Diagnosis

The major differential diagnosis includes cutaneous T-cell lymphoma, extranodal

NK/T-cell lymphoma, and leukemia cutis secondary to chronic myelomonocytic leukemia or acute monocytic leukemia. Cutaneous T-cell lymphoma may share with BPDCN the expression of CD4 and CLA, but not CD56, CD123, and BDCA2. NK cells are similar to BPDCN in expressing CD56 and in absence of surface CD3. However, NK tumors are usually positive for EBV and cytotoxic proteins but do not express all the PDC markers and CD4. The most difficult differential diagnosis is cutaneous infiltration by myelomonocytic or monocytic leukemia, which may express CD4, CD56, CD68 and in some cases, CD123. Leukemia cutis also shows negative reactions to T- and B-cell lineage antigens. However, myelomonocytic/monocytic leukemia always expresses myelomonocytic markers, but not BDCA2 and CD2AP. The distinction between BPDCN and cutaneous myelomonocytic leukemia is listed in Table 2-1.

	BPDCN	CutMML
CD4	+	+
CD56	+	+
CD123	+	±
BDCA2	+	–
BDCA4	+	–
CD2AP	+	–
TCL1	+	–
CLA	+	+
CD68	+	+
CD45RA	+	–
CD11c	–	+
CD13	–	+
CD33	–	+
CD64	–	+
Myeloperoxidase	–	+
Lysozyme	–	+
B-cell markers	–	–
TCR gene rearrangement	–	–

Molecular Genetics

Immunoglobulin heavy chain gene and T-cell receptor gene rearrangement usually show a germline pattern. Cytogenetic abnormalities have been identified in about two thirds of BPDCN patients, but specific karyotypic aberrations have not been found. Gene expression profiling may demonstrate high expression of various PDC-related genes in BPDCN cases and may distinguish BPDCN from cutaneous myelomonocytic leukemia.

Clinical Features

Clinically, PBDCN is an aggressive neoplasm usually seen in elderly patient with a median age of about 65 years. The initial presentation usually involves the skin with solitary or multifocal lesions. It may appear from small bruise-like area to violaceous patches, nodules, and ulcerated masses. Extracutaneous involvement at presentation is rare and is mostly seen in draining lymph nodes, followed by spleen, liver and tonsil. Central nervous system is seldom involved at presentation but it is not unusual in cases of relapse. Bone marrow or blood involvement may be existent at a low level in the early stage, but overt leukemia is usually seen in advanced stage or relapse after therapy. However, cytopenia, particularly thrombocytopenia is a frequent clinical presentation. B-symptoms are uncommon.

BPDCN has been reported to be associated with precedent, concurrent, or subsequent myelomonocytic leukemia. Whether this phenomenon represents direct transformation of BPDCN to myelomonocytic leukemia or vice versa or merely coexistence of two separate diseases has not been settled. However, nodules composed of PDCs have been frequently found in lymph nodes, skin, spleen or bone marrow of patients with chronic myelomonocytic leukemia or other monocytic neoplasms and some studies found that these PDC nodules have a clonal relationship with the leukemias.

Prognosis

Patients usually respond well to radiotherapy and/or chemotherapy initially, but relapse often occurs weeks or months later. The median survival period is approximately 12-14 months. Long-lasting remission is usually seen in patients who received an acute lymphoblastic leukemia-type induction therapy followed by allogeneic stem cell transplantation in first complete remission. Pediatric cases usually respond well to chemotherapy with an overall survival rate of 72% in one study of 25 patients.

Summary

In summary, PBDCN is an aggressive neoplasm with an initial cutaneous presentation followed by lymph node or bone marrow involvement. The cytology and histology are variable. The preliminary immunophenotype is CD4+ CD56+ with negative lineage markers of myelomonocytic and lymphoid series. The diagnosis should be confirmed by the detection of one or more of the following markers: CD123, BDCA2, BDCA4, CD2AP, TCL1 and CLA. No specific molecular genetic markers are available.

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Case 3

Primary cutaneous diffuse large B-cell lymphoma, leg type (PCDLBCL-LT)

Synonyms

- Primary cutaneous large B-cell lymphoma, leg type
- Primary cutaneous diffuse large B-cell lymphoma

Introduction

The 2005 WHO-EORTC classification of cutaneous lymphomas divided primary cutaneous B-cell lymphomas into 3 main groups: primary cutaneous marginal zone B-cell lymphoma, primary cutaneous follicles center cell lymphoma and primary cutaneous diffuse large B-cell lymphoma, leg type. Whereas the first 2 groups had been identified long before and studies in numerous large reports the third group is less well characterized.

Definition

PCDLBCL-LT is a primary cutaneous diffuse large B-cell lymphoma composed exclusively of large transformed B cells without significant admixture of centrocytes, most commonly arising on the lower leg. These lymphomas can arise at other skin sites.

Primary defined on the basis of morphologic features by the presence of confluent sheets of large cell with round nuclei, ie, centroblasts and immunoblasts (large cleaved cells are not allowed). For some authors this lymphoma needs to be BCL2 positive.

CLINICAL ISSUES

Epidemiology

PCDLBCL-LT is a rare tumor, represents 4% of all cutaneous lymphomas and 20% of primary cutaneous B-cell lymphomas. It occurs late in life, with more than 80% of cases occurring in patients older than 70 years and is more frequent in women (male to female ratio: 1:1.6; as high as 1:4 in some studies).

Presentation

PCDLBCL-LT usually present with red or blue-red lesions on skin, often with ulceration. Most cases, ~ 85% of all cases, arise in skin of lower leg; one or both legs. A subset of cases, ~ 15% of cases, arises in skin of other sites (trunk, arms, head and neck). The neoplasm can present with single or multiple lesions (~20%). B symptoms are present in 10-20% of patients.

Prognosis

Large studies of 100 or more patients have reported that DLBCL involving the lower extremity has a less favorable prognosis than DLBCL arising at other cutaneous sites. Relapse is common. This is the form of cutaneous B-cell lymphoma associated with the worst prognosis, with a 5-year disease survival rate of 50%.

In cutaneous lymphoma, leg location is an adverse prognostic factor.

PATHOLOGIC FEATURES

Histologic Features

PCDLBCL-LT is composed of a monomorphic population of large lymphoid cells with vesicular nuclear chromatin and round nuclei resembling centroblasts and immunoblasts. The neoplasm has a diffuse pattern of growth and the infiltrate can be deep. Mitotic figures are usually numerous and there are few small reactive T cells in the background and no epidermotropism.

Important features are the monomorphism of the infiltrate and the lack of background inflammatory cells.

Immunohistochemistry

These lymphomas express pan-B-cell antigens and are cytoplasmic IgM+, IgD+/-, Bcl-2+ (strong), IRF-4/MUM1+, FOXP1+, Bcl-6+ and CD10-. No follicular dendritic cell (FDC) meshworks are usually seen. The intensity of the BCL2 staining may exceed that of the T-cells. They have a high proliferation index.

In Situ Hybridization

EBV small-encoded RNA (EBER) is negative

Molecular Genetics

Monoclonal *IgH* gene rearrangements are frequently detected. Specific cytogenetic abnormalities have not been identified. The translocation *IgH-BCL-2/t(14;18)* is absent. Gene expression studies identified a profile consistent with activated B-cell phenotype.

DIFFERENTIAL DIAGNOSIS

Primary Cutaneous Follicle Center Cell Lymphoma (PCFCL)

PCFCL is defined as a neoplastic proliferation of large and small centrocytes and centroblasts, cases composed exclusively of centroblasts are excluded. Note that areas of follicular pattern can be predominant, focal, or absent and that stromal reaction with fibrosis and sclerosis is common.

Most PCFCL have follicular pattern and can therefore be distinguished from PCDLBCL-LT. However, PCFCL cases with diffuse pattern and predominance of large centrocytes or centroblasts are challenging. PCFCL is confined to the skin and has a good prognosis.

- Sites of skin involvement:
 - o Mostly in head and neck, trunk, back, arms
 - o Some cases of PCFCL can present on leg

Patients with PCFCL on leg often have worse prognosis than patients with PCFCL at other sites. Prognosis of PCFCL of leg is similar to, or slightly better than, PCDLBCL-LT

The tumor cells are usually positive for BCL6, and CD10, and are negative or weakly positive for BCL2 and negative for IRF-4/MUM1 and FOXP1. Meshworks of follicular dendritic cells can be seen.

Systemic DLBCL Involving Skin

Systemic DLBCL can involve skin and can be difficult to distinguish from

PCDLBCL-LT based on morphology and immunophenotype. Clinical history of systemic disease is key for differential diagnosis.

Plasmablastic Lymphoma Involving Skin

Usually occurs in the setting of immunosuppression. Typically present in extranodal and mucosa-associated sites and most patients have high-stage disease at time of presentation. Any skin site can be involved but leg is uncommon.

These tumors are composed of cohesive sheets of monomorphic plasmablasts can closely mimic PCDLBCL-LT. The tumor cells are positive for CD138, CD38, IRF-4/MUM1 and cytoplasmic monotypic Ig light chain and negative for CD20. EBER is also positive.

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Case 4

A 71-year-old man was admitted for splenectomy. He was diagnosed polycythemia vera 5 years ago. Physical examination revealed splenomegaly and fluid wave in the abdomen. Laboratory studies demonstrated a leukocyte count of 16,500/ μ l, Hb 11.8 g/dL, Hct 35%, platelet count 325,000/ μ l and LDH 690 U/L. No immature myelomonocytic cells or nucleated erythrocytes were shown in the peripheral blood. The splenectomy specimen showed extramedullary hematopoiesis. A bone marrow biopsy confirmed myelofibrosis with megakaryocytic hyperplasia. Karyotyping on both peripheral blood and bone marrow specimens showed a normal male karyotype 46,XY. Fluorescence in situ hybridization for BCR-ABL1 fusion product on bone marrow was negative. About one month later, the patient had left upper quadrant pain and a CT scan revealed portal vein thrombosis. Because of several episodes of cytopenia, the need of parenteral nutrition and wound infections, the patient stayed in the hospital for nearly 3 months and was finally transferred to a nursing home.

Definition

One of the major revisions in the 2008 WHO classification is the group of chronic myeloproliferative disorders and this group is now redesignated as myeloproliferative neoplasms (MPN) because of the recent discoveries of various mutations in these diseases, so that, by definition, they are clonal or neoplastic disorders. The currently known MPN-associated mutations involve mainly JAK2V617F, JAK2 exon 12 mutation and MPL W515L/K, but several other mutations, such as TET2, ASXL1, IDH1, IDH2, CBL, IKZF1, LNK and EZH2, have also been reported recently. These mutations help to confirm the diagnosis of MPNs and also lead to the possible targeted treatment, such as the experimental treatment with JAK2 inhibitors.

The so-called BCR-ABL1 negative MPN, including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), have drawn most attention, because they have to be distinguished from many reactive hematologic

disorders with clinical presentations of erythrocytosis, thrombocytosis and myelofibrosis. Each entity has its distinct clinical features, but there are also many overlapped clinical presentations among them. For instance, PV and PMF may have thrombocytosis and both PV and ET may have myelofibrosis. Most importantly, post-PV and post-ET myelofibrosis are clinically indistinguishable from PMF. JAK2 mutations can be demonstrated in each of these entities. Therefore, the differential diagnosis of MPN is sometimes very difficult. However, the treatment of these entities is different. PV and ET can be managed with low-dose aspirin, phlebotomy or hydroxyurea, but the treatment of PMF is much more complicated and unsatisfactory.

Diagnostic Criteria

Peripheral blood examination is effective in screening of MPN, but bone marrow examination is the key in distinguishing the three BCR-ABL 1 negative MPN. It is important to differentiate MPN from reactive hematopoietic cell hyperplasia, particularly ET from reactive thrombocytosis. The previous diagnostic threshold of platelet count for ET was 600,000/ μ l, but the 2008 WHO classification lower the threshold to 450,000/ μ l so that many prodromal ET cases will not be missed. This low threshold unavoidably includes many cases of reactive thrombocytosis, but it is important not to miss the prodromal ET cases, because severe vascular events, such as thrombosis and hemorrhage may occur in this stage. For these complicated problems, clear-cut diagnostic criteria are required. The criteria in the 2008 WHO Classification are as following.

Table 4-1 WHO Diagnostic criteria of PMF

Major	1. Megakaryocytic proliferation and atypia accompanied by either reticulin/collagen fibrosis or increased marrow cellularity, granulocytic proliferation and decreased erythropoiesis (prefibrotic PMF)
	2. Not meeting WHO criteria for CML, PV, MDS, or other myeloid neoplasm
	3. JAK2V617F or other clonal marker or no evidence of reactive myelofibrosis
Minor	1. Leukoerythroblastosis 2. Increased serum LDH level 3. Anemia 4. Splenomegaly

Due to the difficulty in distinguishing PMF and post-PV and post-ET myelofibrosis, the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) recommended the following criteria for the differential diagnosis.

Table 4-2 IWG-MRT Criteria for post PV MF

Required criteria
<ol style="list-style-type: none"> 1. Documentation of a previous diagnosis of PV as defined by WHO criteria 2. Bone marrow fibrosis grade 2-3 (on 0-3 scale) or grade 3-4 (on 0-4 scale)
Additional criteria (two are required):
<ol style="list-style-type: none"> 1. Anemia or sustained loss of requirement of either phlebotomy or cytoreductive treatment for erythrocytosis. 2. Leukoerythroblastosis 3. Increase in palpable splenomegaly of ≥ 5 cm or new appearance of splenomegaly 4. Development of one or more B-symptoms

Table 4-3 IWG-MRT Criteria for post-ET MF

Required criteria
<ol style="list-style-type: none"> 1. Documentation of a previous diagnosis of ET as defined by WHO criteria 2. Bone marrow fibrosis grade 2-3 (on 0-3 scale) or grade 3-4 (on 0-4 scale)
Additional criteria (two are required):
<ol style="list-style-type: none"> 1. Anemia and a ≥ 2 mg/ml decrease from baseline Hb level 2. Leukoerythroblastosis 3. Increase in palpable splenomegaly of ≥ 5 cm or new appearance of splenomegaly 4. Increased LDH 5. Development of one or more B-symptoms

From these criteria, it appears that clinical history and bone marrow fibrosis are most important for the secondary myelofibrosis. Myelofibrosis should be evaluated by the following grading system.

Table 4-4 Semiquantitative grading of bone marrow fibrosis

MF-0	Scattered linear reticulin with no intersections (cross-over), corresponding to normal bone marrow
MF-1	Loose network of reticulin with many intersections, especially in perivascular areas
MF-2	Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of collagen and/or focal osteosclerosis
MF-3	Diffuse and dense increase in reticulin with extensive intersections and coarse bundles of collagen, often associated with osteosclerosis.

The current case had a history of PV and then developed splenomegaly and hepatic cirrhosis. His bone marrow was consistent with post-PV myelofibrosis. He had characteristic complications of MPN with portal vein thrombosis and hemorrhage episodes and finally succumbed to gastric bleeding.

Summary

In summary, patients with myeloproliferative manifestations, such as erythrocytosis, increase of hemoglobin and hematocrit, thrombocytosis and/or leukocytosis should be suspicious for MPN. The cutoff for hemoglobin is 18.5 g/dL in men and 16.5 g/dL in women. The cutoff for platelets is 450,000/ μ l. If patients have clinical symptoms, particularly splenomegaly, JAK2 or MPL mutation should be investigated. JAK2-V617F has been found in 95% of PV patients and in about 50-60% ET and PMF patients. If no mutation is present, bone marrow biopsy can help for further differential diagnosis. PV may show panmyelosis, ET shows megakaryocytic hyperplasia with no atypia and PMF reveals myelofibrosis with megakaryocytic proliferation and atypia. A reliable clinical history is important to distinguish PMF from post-PV or post-ET myelofibrosis. There is no specific immunophenotype for MPN.

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Case 5

ALK-positive diffuse large B-cell lymphoma (DLBCL)

Introduction

In 1997, 7 cases of a new subtype of diffuse large B-cell lymphoma expressing the anaplastic lymphoma kinase (ALK) gene product were reported by Delsol et al. This lymphoma was identified due to its characteristic lack of CD30 expression in an otherwise large series of classical ALK-positive anaplastic large cell lymphomas. ALK-positive DLBCL display clinico-pathologic features that distinguish them from

common DLBCL.

Definition

Diffuse large B-cell lymphoma expressing ALK protein and associated with *ALK* gene abnormalities

CLINICAL ISSUES

Epidemiology

ALK-positive DLBCL is a rare tumor, represents less than 1% of all cases of DLBCL. There are approximately 80 cases reported to date. Patient median age is 43 years (range 9-85) with 30% of cases occur in the pediatric population. It is more frequent in male than female with a ratio of 5 to 1. There is no apparent ethnic predisposition. There is no association with immunosuppression.

Presentation

This neoplasm presents with high-stage nodal disease (60-70% of the cases) with widespread lymphadenopathies, systemic (B-type) symptoms and aggressive clinical course. The patients can also present with enlarged mediastinal lymph nodes and also with leukemic involvement. Extranodal sites of involvement include:

- o Bone marrow in ~ 25% of patients
- o Nasal cavity, nasopharynx, oral cavity
- o Stomach, small intestine
- o Spleen, ovary
- o Bones, soft tissues
- o Epidural mass, brain

Prognosis

The 5-year overall survival is 25% and approximately 50% of patients die within 1 year. Children appear to have better prognosis.

PATHOLOGIC FEATURES

Histologic Features

ALK-positive DLBCL shows a partial or diffuse effacement of the lymph node architecture with lymphoma cells seen infiltrating sinusoids in many cases along with focal necrosis, binucleated HRS-like cells and multinucleated giant lymphoma cells. The tumor cells are relatively monomorphic with immunoblastic or plasmablastic appearance. They can appear deceptively cohesive and thus may be misinterpreted as

carcinoma cells. Mitotic figures are easily identified. The bone marrow can show variable degree of involvement but sinusoidal involvement is uncommon at this site.

Immunohistochemistry

These lymphomas, by definition, express ALK. The pattern of ALK expression predicts *ALK* partner in fusion gene:

- Granular and cytoplasmic (most frequent pattern): *CLTC* and *SEC31A*
- Nuclear, nucleolar, and cytoplasmic: *NPM*

In addition, the tumor cells are usually positive for CD138, VS38, EMA and CD45/LCA (weak) and can be positive for CD4 (40%) and CD43 (rare). Approximately 90% of the cases express cytoplasmic Ig being IgA the most frequently detected (>95%; rare cases express IgG). The tumor cells are usually negative for CD30, although rare cases (< 5%) can show focal and weak expression of CD30 in a small subset of tumor cells. CD57 expression as well as cytokeratin expression (~10%; dot like paranuclear) can be detected in a small subset of cases. The tumor cells are usually negative for CD20 and CD79a; although they can be weakly expressed in 10% of cases and negative for IRF-4/MUM1. HHV8 and EBV-LMP are negative.

In Situ Hybridization

EBER is negative

Molecular Genetics

Upregulation of the ALK gene is mainly due to the presence of the t(2;17)(p23;q23) (70% of cases) resulting in clathrin heavy-chain gene (*CLTC*)-*ALK* fusion protein. The t(2;5)(p23;q35) and/or *NPM*-*ALK* fusion is present in occasional (10%) cases. The fusion transcripts consequence of these translocations, can be detected by reverse transcriptase (RT)-PCR. FISH studies can also be helpful to detect abnormalities involving *ALK* gene. Rare cases with cryptic insertion of 3'*ALK* gene sequences into chromosome 4q22-24 have been reported.

TOP DIFFERENTIAL DIAGNOSES

- Plasmablastic lymphoma
- Diffuse large B-cell lymphoma, immunoblastic variant
- Plasmacytoma/Plasma Cell Myeloma
- ALK-positive, anaplastic large cell lymphoma
- Poorly differentiated carcinoma

Plasmablastic Lymphoma (PBL)

PBL is a DLBCL with morphologic and immunophenotypic features of plasmablasts that often occur in the oral/nasal region. There is a morphologic and immunophenotypic overlap between PBL and ALK-positive DLBCL. Both neoplasms have immunoblastic/plasmablastic cytologic features and are positive for CD138, VS38c, and negative for CD20.

However, patients with PBL often present differently from patients with ALK-positive DLBCL. PBL is usually associated with HIV infection (most common) or with other immunodeficiency states. PBL more commonly involves extranodal sites.

There are also immunophenotypic differences that also help in the diagnosis. PBL are:

- o ALK-
- o CD79a+, in 50-85% of cases
- o IRF-4/MUM1 usually +
- o CD30+/-
- o CD4-/+ , CD57-

In addition, EBER is positive in 60-75% of cases of PBL and there are no abnormalities of *ALK* gene.

DLBCL Immunoblastic Variant

These neoplasms usually exhibit plasmacytoid differentiation that can overlap with ALK-positive DLBCL. Immunophenotypic studies are helpful to establish the diagnosis of DLBCL immunoblastic variant. DLBCL immunoblastic variant is usually strongly positive for B-cell markers such as CD20, CD79a, and pax-5 and negative for CD30 and ALK. In addition, DLBCL immunoblastic variant has no abnormalities of *ALK* gene. Note also that some DLBCL (anaplastic variant) may have a sinusoidal growth pattern.

Plasmacytoma/Plasma Cell Myeloma

A subset of ALK-positive DLBCL may exhibit marked plasmacytoid features overlapping with plasma cell tumors. Plasma cell tumors would be associated with other features such as lytic bone lesions and/or monoclonal protein in serum and/or urine. Immunophenotypic studies are helpful, plasmacytoma/plasma cell myeloma are negative for ALK, and CD45/LCA and usually express cytoplasmic IgG and may be positive for cyclin-D1.

ALK-positive Anaplastic Large Cell Lymphoma (ALCL)

Morphologic differences: hallmark cells are usually present in ALK-positive ALCL but not in ALK-positive DLBCL. Immunophenotypic differences: by definition CD30 is strongly and uniformly positive in ALK-positive ALCL. In addition, ALK-positive ALCLs are usually positive for T-cell markers (CD2 and CD43 more frequently), express cytotoxic proteins (TIA-1) and carry monoclonal T-cell receptor gene rearrangements.

Poorly Differentiated Carcinoma

ALK-positive DLBCL can be misdiagnosed as carcinoma. A small subset of cases can be positive for keratin and negative for CD45/LCA. In those cases, the detection of expression of ALK is key to correct diagnosis. Note that carcinomas can be also positive for EMA and CD138 and that the dot-like paranuclear expression of cytokeratin has also been described in myeloma and plasmablastic lymphomas.

PATHOLOGIC INTERPRETATION PEARLS

Consider performing ALK immunostaining on all tumors with immunoblastic/plasmablastic features in particular in cases not associated to HIV. ALK immunostaining may be necessary for identifying scattered cells in bone marrow.

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Case 6

A 48-year-old man presented with high fever and severe back pain for several days. He started with rigors and chills with muscle spasm of the chest, moving around to the back. The pain then descended and is localized in the back for the last 3-4 days. During the observation period, his platelets dropped rapidly from 208,000 to 30,000/ μ l in 3 weeks. He had epistaxis and oral mucosal bleeding. On admission, his total leukocyte count was 10,500/ μ l with 13% blasts in the peripheral blood. His hematocrit was 33% and hemoglobin 11.7 g/dL. Physical examination revealed no hepatosplenomegaly and no lymphadenopathy. A bone marrow biopsy demonstrated 94% blasts, which had a high nuclear to cytoplasmic ratio and a thin rim of basophilic cytoplasm that contains many vacuoles. The core biopsy showed almost total replacement of normal hematopoietic cells with undifferentiated blasts.

Flow cytometry showed a CD45 negative population with CD10 58%, CD13 37%, CD19 97%, CD34 99%, CD79a 98%, myeloperoxidase (MPO) 89%, terminal deoxynucleotidyl transferase (TdT) 97%. Other markers including CD20, kappa, lambda, CD5, CD7, CD11b, CD14, CD33, CD64 and CD117 were all negative.

Molecular genetic studies for BCR-ABL1 showed positive result by fluorescence in situ hybridization (FISH) and a minor BCR/ABL fusion transcript (bcr/abl fusion protein 190 kd) was demonstrated by reverse transcriptase-polymerase chain reaction (RT-PCR). FISH for MYC gene rearrangement was negative. RT-PCR failed to identify Fms-like tyrosine kinase 3/internal tandem duplication (FLT2/ITD) mutation.

Most cases of acute leukemia can be classified into either myeloid or lymphoid leukemia by morphology and immunophenotyping. However, there are about 5% of acute leukemia cases that are difficult to identify even using the modern techniques. These cases are designated acute undifferentiated leukemia if both morphology and phenotyping are inconclusive. Cases consisting separate populations that have different morphologic and phenotypic features are termed acute bilineal leukemia. Cases showing only a single population that coexpresses two immunophenotypes are called biphenotypic acute leukemia. The 2008 WHO classification combines the bilineal and biphenotypic leukemias into a single category, the mixed phenotype acute leukemias. (MPAL).

Definition

In the 2008 WHO classification of MPAL, there are several new changes. 1. Modification of the scoring system developed by the European Group for the Immunological Classification of Leukemia (EGIL). 2. Classifying two karyotypes as distinct entities of MPAL. 3. Exclusion of many special conditions from MPAL. As a result, MPAL has become a more uniform category with characteristic features.

Immunophenotype

The EGIL scoring system has been used for many years, but this system includes many markers, some of which are not specific and lead to misclassification. The 2008 WHO scheme restricts the use of a few markers that are more specific for the classification. In this system, MPO, CD3 and CD19 are designated as specific markers for myeloid, B-cell and T-cell lineages, respectively. For myeloid lineage, only MPO is accepted for its identification. Other myeloid markers, such as CD13, CD33, and CD117 are deemed nonspecific for lineage identity. For monocytic lineage, at least two of the following markers should be positive for its identification. These markers include nonspecific esterase, CD11c, CD14, CD64 and lysozyme. CD3, no matter whether it is cytoplasmic or surface in location, is the only acceptable marker for T-cell identity. It is cautioned that immunohistochemistry using polyclonal CD3 antibody may detect CD3 zeta chain, which is not T-cell specific (can be NK cell).

Identification of B-cell lineage requires more than one marker. If CD19 staining is strong, coexpression with either CD79a, CD10 or cytoplasmic CD22 is required. If CD19 staining is weak, coexpression with two of the above three markers is required. Flow cytometry is the preferred method for detection of these markers. Immunohistochemistry and cytochemistry are also acceptable for detection of certain markers, such as MPO and nonspecific esterase.

On the basis of immunophenotype, MPAL is further divided into B/myeloid, T/myeloid and rare type. The rare type includes mixed phenotype of T cell and B cell or trilineage phenotype (T cell, B cell and myeloid cell). Mixed phenotypes involving lymphoid/megakaryoblast or lymphoid/erythroblast have not been reported.

Molecular Genetics

Two karyotypes are singled out as distinct entities because of their high frequency. They are t(9;22)(q34;q11.2) or BCR-ABL 1, and t(v;11q23) or MLL (mixed lineage leukemia gene) rearrangement.

Differential Diagnosis

Conditions that are excluded from the diagnosis of MPAL are three karyotype-defined acute myeloid leukemia entities, i.e. t(8;21), t(15;17) and inv(16), even when multiple lymphoid markers are detected in these cases. Acute leukemia with FGFR1 mutation is also excluded. If lymphoid phenotype is expressed in chronic

myelogenous leukemia in blast crises, myelodysplastic syndrome-related acute myeloid leukemia, and therapy-related acute myeloid leukemia, the diagnosis of these entities will remain the same with a secondary notation that they have a mixed phenotype.

In a recent study of 100 MPAL cases, 59% were B/myeloid, 35% T/myeloid, 4% T/B and 2% trilineage. On the other hand, T/myeloid cases are predominant in pediatric series. Morphology does not help for the diagnosis of MPAL, because MPAL cases may show either myeloid, lymphoid or undifferentiated morphology, except for a small subset which shows a distinct dual blast population. Generally, MPAL has a poor prognosis particularly in adults and/or those with a Philadelphia chromosome. Experience in treatment is limited, but it appears that the ALL regimen is more effective than that of AML regimen. The diagnosis of MPAL is mainly based on immunophenotyping with the WHO criteria except for two entities which is based on molecular genetic studies.

The current case showed a undifferentiated leukemic feature in the bone marrow core biopsy, but the marrow aspirate revealed large blasts with vacuolated cytoplasm mimicking Burkitt leukemia. The immunophenotype showed coexpression of both myeloid (MPO, CD13) and B-cell (CD19, CD79a) markers that fulfill the diagnostic criteria of both WHO and EGIL. Karyotyping was unsuccessful due to the absence of mitosis in the cell culture. The tumor cells of aggressive leukemia may also die rapidly, leaving only the normal cell population. Therefore, several molecular genetic tests were performed. MYC arrangement was done because of suspicion for Burkitt lymphoma. BCR-ABL1 was done because of the high frequency of this aberration in MPAL. FLT3 was tested because of its high incidence in acute leukemia and its potential for therapy with inhibitors. The detection of minor BCR-ABL fusion is consistent with BCR-ABL1 positive ALL and not chronic myelogenous leukemia. The patient was treated with imitinib mesylate and ALL regimens and he was still alive three years after initial diagnosis.

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Case 7

B-CELL LYMPHOMA, UNCLASSIFIABLE, WITH FEATURES INTERMEDIATE BETWEEN DIFFUSE LARGE B-CELL LYMPHOMA AND CLASSICAL HODGKIN LYMPHOMA

Synonyms

- Gray zone lymphoma
- Mediastinal gray zone lymphoma
- Large B-cell lymphoma with Hodgkin features
- Hodgkin-like anaplastic large cell lymphoma

Introduction

In recent years, overlap in biologic and morphologic features has been identified between classic Hodgkin lymphoma (cHL) particularly nodular sclerosis CHL (NSCHL) and primary mediastinal large B-cell lymphoma (PMLBCL). These observations suggested that a true biologic gray zone between these 2 entities could exist. The 2008 WHO classification incorporates these new ideas and recognizes a provisional category of B-cell neoplasms with features intermediate between DLBCL and CHL. The category does not include the composite or sequential cases of both neoplasms. Other intermediate forms between CHL and DLBCL, as may be seen with EBV transformation, represent a different biologic phenomenon. With the use of this concept it appears that these tumors occur predominantly in young men and have more aggressive behavior than either PMLBCL or NSCHL.

Definitions

Lymphoma with clinical, morphologic, &/or immunophenotypic features between diffuse large B-cell lymphoma (DLBCL) and classical Hodgkin lymphoma (CHL)

CLINICAL ISSUES

Epidemiology

These lymphomas are more frequent in young patients, 20-40 years or age (range: 13-70 years). There is a male predominance and these lymphomas are most common in western countries and less common in Asians and African Americans.

Presentation

Most frequently patients present with anterior mediastinal mass with often direct extension into lungs and advanced clinical stage (III or IV). Supraclavicular lymph nodes can be involved; however, other peripheral lymph node groups are rarely

involved

Treatment

No consensus on optimum treatment protocol. Some patients treated with CHL protocols have failed to respond. Some groups have recommended treating DLBCL/CHL cases as aggressive DLBCL.

Prognosis

Patients have aggressive clinical course and poorer outcome than patients with either CHL or primary mediastinal B-cell lymphoma

PATHOLOGY FEATURES

Histologic Features

Some tumors show areas of confluent sheets of pleomorphic large tumor cells resembling DLBCL with other areas showing scattered large cells, resembling Hodgkin and Reed-Sternberg (HRS) cells in CHL. The neoplastic cells may have broad spectrum of cytologic appearance including centroblasts, immunoblasts, and/or HRS-like cells. Cells with cytoplasmic retraction, resembling lacunar cells, can be seen and mummified cells (apoptotic large cells) are frequent. There is a variable inflammatory infiltrate in the background with mild stromal fibrosis and focal necrosis. When necrosis is seen, it is not usually associated with neutrophils (as seen CHL). Non-necrotizing granulomas and histiocytes can be seen.

Immunohistochemistry

DLBCL/CHL has a "mixed immunophenotype"

with expression of common markers of classical HL:

- CD30+ all cases) &/or CD15+ in most cases
- pax-5+ and IRF-4/MUM-1+

And expression of markers usually absent in CHL:

- CD45/LCA+, CD20 +; uniformly strong, and CD79a+
- OCT2+, BOB1+

The tumor cells with this "mixed immunophenotype" constitute predominant neoplastic cell population. These neoplasms have usually high proliferation rate, as measured by MIB-1 (Ki-67).

Other makers:

- MAL+ in ~ 60% of cases
- Bcl-6+ variable, CD10 is usually negative
- Negative for T-cell markers and ALK
- Some cases reported were EBV+; EBER &/or LMP1

Like CHL, the lymphoid infiltrate in the background is predominantly composed of T cells, positive for CD3 and CD4.

DLBCL/CHL can present with a PMLBCL-like morphology but with a CHL phenotype with expression of CD30 and CD15 and loss of CD20 and CD79a. Alternatively, the lymphoma can present with CHL morphology but with a phenotype of PMBL (CD20 strongly positive and CD15 negative).

Molecular Genetics

Studies have shown similarity between CHL and PMLBCL. A number of common genetic aberrations in PMLBCL and CHL further underscore their close relationship. Most cases have monoclonal *IgH* gene rearrangement. Few cases have rearrangements involving *BCL6* and most cases lack t(14;18)(q32;q21). In almost all cases assessed, *P53* was in germline configuration.

TOP DIFFERENTIAL DIAGNOSIS

- PMLBCL
- Nodular sclerosis CHL
- DLBCL

Primary Mediastinal (Thymic) Large B-cell Lymphoma (PMLBCL)

PMLBCL usually present in young women with a rapidly progressive anterosuperior mediastinal mass. Patients can have extrathoracic disease, but this is rare at the time of diagnosis and more common at time of relapse and usually in extranodal sites such as CNS, liver, adrenals, ovaries, and kidneys (lymph nodes are often not involved at relapse).

The neoplasm has a diffuse growth pattern with large cells with pale cytoplasm (often is retraction artifact) and sclerosis that often compartmentalizes tumor cells mimicking cohesive clusters. Reed-Sternberg-like or Hodgkin-like cells can be present.

The tumor cells are positive for common pan B-cell markers (CD20, CD79a, pax-5),

CD45/LCA, and IRF-4/MUM-1. CD30 is positive in 80% of the cases and is usually weak &/or focal. CD23 and MAL are positive in 70% of the cases. Often surface immunoglobulins are negative; best shown by flow cytometry. The tumor cells are negative for CD10 and CD15. EBV is usually negative.

Nodular Sclerosis Classical Hodgkin Lymphoma

NSCHL usually presents as a mediastinal mass (~80%) in young patients with a slight female predominance. The neoplasm has a nodular growth pattern with fibrosis and dense collagenous bands surround nodules (collagenous bands are polarizable). There is a variable number of large HRS cells. Many histological variants of nodular sclerosis CHL have been described based on number of neoplastic cells, extent and nature of fibrosis, and inflammatory background. Of these, syncytial variant is particularly relevant.

Syncytial variant:

- Sheets of large tumor cells that can mimic DLBCL
- Often large areas of necrosis
- Immunophenotype is typical of CHL

The tumor cells in NSCHL are positive for CD30, CD15 (in most cases) and pax-5+ with characteristic weaker (dimmer) expression than reactive B-cells. CD20 is weakly &/or variably (+) in ~ 20% of cases. Small subset (~ 5%) of CHL can express T-cell antigens. These cases also express pax-5 or other B-cell antigens. The tumor cells are negative for CD45/LCA and EMA.

Diffuse Large B-cell Lymphoma

DLBCL is a neoplasm of older adults, but also occurs in children and young adults. The neoplasm has a diffuse growth pattern with large neoplastic cells (centroblasts &/or immunoblasts). Large anaplastic cells can be present; known as anaplastic variant. These neoplasms may have intrasinusoidal growth pattern and CD30 is often focally positive.

The tumor cells are positive for pan-B cell markers, CD20, CD22, CD79a, pax-5, OCT2, and BOB1. CD10 and Bcl-6 are positive in a variable proportion of cases. When CD30 is positive is usually weak and focal. CD45/LCA is usually positive and CD15 is negative.

The t(14;18)(q32;q21)/*IgH-BCL2* is detected in ~ 20-30% of the cases and *BCL6*

rearrangements in ~ 10-20%.

PATHOLOGY INTERPRETATION PEARLS

DLBCL/CHL is more frequent in young men and usually present with large anterior mediastinal mass. In general, there is discordance between morphologic features and immunophenotype.

In cases that morphologically resemble CHL: Uniform and strong expression of B-cell markers and absence of CD15 suggest DLBCL/CHL

In cases that morphologically resemble DLBCL: Positivity for CD15, EBV, &/or negativity for CD20 suggest DLBCL/CHL

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Case 8

A 70-year-old man presented with a two-week history of chest pain. He also had low grade fever and night sweat. Physical examination revealed no lymphadenopathy and/or organomegaly. Peripheral blood examination showed a total leukocyte count of 14,500/ μ l with 77.5% neutrophils, 12.2% lymphocytes, 9.1% monocytes, 1.2% eosinophils and 0.2% basophils. His hemoglobin was 15.7 g/dL and platelets 279,000/ μ l. His blood chemistry panel was unremarkable except for an elevated level of lactate dehydrogenase (182 IU/L). While in the emergency department, the patient was found to have a right hilar fullness on chest x-ray. Subsequent CT scan revealed a large 11 x 5 cm mass in the posterior mediastinal space with compression of subclavian vein.

After admission, an endobronchial biopsy of the right lower lobe of the lung showed no diagnostic abnormality. A mediastinal biopsy finally demonstrated a malignant lymphoma. Immunohistochemical stain of the mediastinal lymph node showed that a few scattered large tumor cells were positive for CD20, but were negative for CD15 and CD30. CD3 and CD68 stains demonstrated numerous small lymphocytes and histiocytes, respectively. No follicular dendritic cell meshwork was identified by CD21 stain. Subsequently the patient received 5 cycles of elective chemotherapy. The patient responded well to chemotherapy at the beginning, but he had multiple medical problems, including hypertension, aortic aneurysm, acute renal insufficiency and fungemia. He died 15 months after the initial diagnosis.

Definition

The current case is a T-cell/histiocyte-rich large B-cell lymphoma (THRBCL), which was classified as a morphologic variant of diffuse large B-cell lymphoma in the 2001 WHO scheme. In the 2008 WHO classification, THRBCL is categorized as a distinct pathologic entity. As THRBCL shares several morphological and immunophenotypic features with nodular lymphocyte predominant Hodgkin lymphoma (NLPHL), cases with overlapping features between these two entities are called gray zone lymphoma, similar to the other two unclassifiable B-cell lymphomas presented in this workshop.

Morphology

THRBCL is characterized by the effacement of the normal lymph node architecture by extensive histiocyte and small lymphocyte infiltration with scattered large tumor cells that are fewer than 10% of the total population. In a minority of cases, a vaguely

nodular pattern may be discernable. In some cases, the tumor cells may be so inconspicuous that immunohistochemistry is required for their recognition. The morphology of tumor cells differs from case to case; they may be manifested as centroblasts, immunoblasts, Reed-Sternberg-like cells, or lymphocyte predominant cells (LP cells or popcorn cells), or the combination of two or more cell types. The background cells, though not neoplastic, are important in the differential diagnosis by their immunophenotype and are also a major contributing component in the gene expression profiling of THRBCL. The lack of eosinophils, neutrophils and plasma cells helps distinguish THRBCL from Hodgkin lymphomas.

THRBCL is also characterized by its frequent extranodal distribution, particularly the spleen, liver and bone marrow. Increasing numbers of primary splenic THRBCL cases, which are characterized by a micronodular pattern, have been reported. The tumor nodules are composed of histiocytes and small lymphocytes with rare large tumor cells. The bone marrow also shows a nodular pattern with the same composition as that in the spleen.

Differential Diagnosis

THRBCL is very similar to NLPHL morphologically and immunologically. The tumor cells of both entities have nearly identical immunophenotype. The prominent nodular pattern and the cellular milieu in NLPHL may help to distinguish it from THRBCL, but it is the immunophenotype of the background cells that can provide a definitive distinction. These two entities not only show overlapped features, but THRBCL can be coexistent with or subsequent to NLPHL in the clinical course. Some authors believe that these cases represent transformation from NLPHL to THRBCL.

THRBCL may also mimic classical Hodgkin lymphoma (cHL) for its scarcity of tumor cells and an inflammatory background. As mentioned before, THRBCL may also show Reed-Sternberg-like cells. However, cHL also contains other tumor cells, such as lacunar cells, mononucleated and multinucleated Hodgkin cells as well as mummified cells. Eosinophils, neutrophils and plasma cells are also seen in cHL and not in THRBCL. Finally, the immunophenotype of both tumor cells and background cells are quite different between these two entities.

When the rare tumor cells are missed or not demonstrated by immunophenotyping, THRBCL may be misdiagnosed as inflammatory reaction or peripheral T-cell lymphoma. Indeed, THRBCL is frequently mistaken as other lymphomas or reactive conditions by the primary pathologists; one study showed that the initial diagnoses were incorrect in as high as 82% of the cases studied.

Immunophenotype

Immunophenotyping is the mainstay for the diagnosis of THRBCL. The tumor cells can be readily identified by immunohistochemical staining of CD20, and they are also consistently positive for the B-cell transcription factors, including PAX5/BSAP, OCT2 and BOB1. One transcription factor, PU.1, is usually negative in THRBCL cases and is thus useful for distinguishing THRBCL from NLPHL, which is positive for this marker. IgD is also negative for THRBCL and positive for NLPHL. To distinguish from cHL, THRBCL is CD45 positive and CD15 negative, but some cases can be weakly positive for CD30. CD79a and BCL6 are demonstrated in most cases of THRBCL, while CD10 is only present in the minority of cases. BCL-2 expression is variable. EBV is seldom positive in THRBCL. If it is positive, EBV positive diffuse large B-cell lymphoma should be considered.

Unlike other lymphomas, the immunophenotype of the background lymphocytes is instrumental for the distinction between THRBCL and NLPHL. The T lymphocytes in THRBCL are CD3 positive and some studies also show a CD8 positive cytotoxic T-cell phenotype. They do not form rosettes around the tumor cells. On the other hand, the background T lymphocytes in NLPHL are CD3+ CD57+ PD1+, forming rosettes around the LP cells. The background in NLPHL is also rich in B lymphocytes with a CD21+/CD23+ meshwork of follicular dendritic cells.

Molecular Genetics

Immunoglobulin heavy chain gene rearrangement of THRBCL cases has demonstrated clonal gene rearrangement carrying high numbers of somatic mutations and intraclonal diversity, characteristic of germinal center cells. Gene expression profiling studies showed that NLPHL clustered with THRBCL and classical Hodgkin lymphoma, but differed from germinal center reactive cells and all other common types of B-cell lymphomas (follicular lymphoma, Burkitt lymphoma, and diffuse large B-cell lymphoma, NOS). There is no specific karyotype for THRBCL.

The current case shows a few scattered large tumor cells, which are strongly positive for CD20 and negative for CD15 and CD30. The background cells are composed of large numbers of CD3 positive T lymphocytes and CD68 positive histiocytes. No B-lymphocytes and CD21 positive follicular dendritic cells are demonstrated in the cellular milieu. No nodular histologic pattern is present. The morphology and limited immunohistochemical profile substantiate the diagnosis of THRBCL and exclude the possibility of cHL and NLPHL. However, in borderline cases, a large immunophenotypic panel is required to distinguish THRBCL, NLPHL and cHL. The differences between these three entities are enlisted in Table 8-1

Immunophenotype	THRBCL	NLPHL	cHL
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Tumor cells			
CD45	Positive	Positive	Negative
CD20	Positive	Positive	Negative or positive
CD79a	Positive	Positive	Negative
BCL-6	Positive	Positive	Negative or positive
PAX5/BSAP	Positive	Positive	Positive
BOB1	Positive	Positive	Negative
OCT2	Positive	Positive	Negative
PU.1	Negative	Positive	Negative
CD10	Variable	Negative	Negative
BCL-2	Variable	Negative	Negative
CD15	Negative	Negative	Positive
CD30	Rare	Negative	Positive
EBV	Negative	Negative	Variable
EMA	Variable	Positive	Rare
J-chain	Positive	Positive	Negative
sIg	Positive	Positive	Negative
IgD	Negative	Positive	Negative
Background cells			
CD3	Positive	Positive	Positive
CD57	Negative	Positive	Negative
CD20	Negative	Positive	Negative
CD21/CD23	Negative	Positive	Positive
CD68	Positive	Rare	Variable
PD1	Negative	Positive	Negative

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