E8. **DIFFUSE LARGE B-CELL LYMPHOMA – UNITY IN HETEROGENEITY: CLINICO-PATHOLOGIC ENTITIES, ETIOPATOGENETIC, DIAGNOSTIC AND THERAPEUTIC ASPECTS.**

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Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of NHL, reprezenting up to 40% of cases, with an increasing occurrence. The incidence increases with age. It has an aggressive natural history, and it may present in lymph nodes or in extranodal sites (including bone, skin, gastro-intestinal tract, thyroid, lung etc). The etiology of DLBCL is unknown. **Factors that confer increased risk** include immunosuppression (including AIDS, autoimmune diseases or iatrogenic etiologies in the setting of transplantation), radiation, diet, pesticides, hair dyes. A subset of DLBCL, including immunoblastic and primary CNS lymphoma, is highly associated with the EBV virus; the concept of antigen-driven lymphomagenesis is less developed in DLBCL. DLBCL usually develops de novo, but can also arise from transformation of an indolent lymphoma.

DLBCL represents one of the most **heterogeneous** categories in WHO classification. The disease, that is seen as a „unity" in the aggressive lymphoma group, is clearly heterogeneous at a clinical, pathological, cytogenetic and molecular level. Morphologically, DLBCL is composed of large B cells with a high proliferation index resembling germinal centroblasts. There are several **morphologic variants** that include centroblastic, immunoblastic, plasmablastic, T-cell/histiocyte-rich, and anaplastic (usually ALK+) subtypes. The neoplastic **cells of DLBCL express** pan B-cell markers, including CD19, CD20, CD79a, CD45RA, and the nuclear transcription factor PAX5. Germinal centre-associated markers CD10 and Bcl-6 are expressed in aproximately 30-40% and 60%, respectively. Translocation of BCL2 gene (a hallmark of follicular lymphoma) is present in 20-30% of cases; CD5 is expressed only in 10% of DLBCL. Aproximately 10% of DLBCL cases harbor a t(8;14) MYC translocation (that confers a worse prognosis).

On the basis of **gene expression profiling (GEP)** and genes signatures, DLBCL can be divided into at least three different subtypes: 1. germinal centre B-cell (GCB) – like; 2. activated B-cell (ABC)-like; 3. primary mediastinal B-cell lymphoma (PMBL), each with significant differences in terms of prognosis, PFS, and OS following immunochemotherapy. Distinct **cytogenetic abnormalities** have been described in DLBCL subtypes. In GCB-DLBCL the most common are t(14;18) with rearrangements of BCL2 and IGH chain genes, and translocation leading to rearrangement of MYC gene. In ABC-DLBCL the most common are translocation involving BCL6 gene, and trisomy 3; deletion of tumor suppressor gene P53 is observed in 15-20% of cases. In PMBL a gain of long arm of chromosome 9 is reported (50%), with up-regulation of the JAK2 gene.

Subtypes of DLBCL arise from **genetic alterations occurring during the proces of B-cell differentiation /maturation** and, in general, are characterized by a blockage of the programmed cell death process, an increase in cell profiferation, or impaired terminal differentiation. Several oncogenic pathways have been identified in DLBCL: B-cell receptor signaling pathway, constitutive activation of NFkB activity pathways, and deregulation of the Bcl-6/apoptosis pathway.

Diagnosis of DLBCL should be made on the basis of a surgical specimen/excisional lymph node or extranodale tissue biopsy. Minimal immuno-histochemistry (CD45, CD20, and CD3) is mandatory. Molecular characterization is recommended although GEP remains investigational. The staging is established according to the Ann Arbor system. For prognostic purposes, IPI and age-adjusted IPI (aa-IPI) should be calculated. For staging are required: a complete blood count, routine blood chemistry including LDH and uric acid as well as a screening for HIV and hepatitis B and C; CT scan of the chest and abdomen; a bone marrow aspirate and biopsy; performance status and cardiac function (ejection fraction). A diagnostic spinal tap should be considered in high-risk patients.

The **clinical presentation** of DLBCL is variable and depends on histology, age, and immune status.Tipically presents with lymphadenopaty, from asymptomatic to causing pain or organ compression (ureteral, spinal cord). The involvement of bone marrow is present in approximately 20% of cases. Constitutional manifestations or „B" symptoms (weight loss, malaise, fevers, night sweats, loss of appetite) may be present as a consequance of production, by the lymphoma cells or host tissue, of inflammatory molecules and of other cytokines and chemokines.

DLBCL incorporates clinical and/or pathological distinct **subtypes and variants**, and also new entities based on unique clinical features, age or anatomic site, viral pathogenesis, or distinctive pathological features:

1. **DLBCL, not otherwise specified**; morphologic: centroblastic, immunoblastic, anaplastic; molecular subgroups: GCB, ABC; immunohistochemical subgroups: CD5+, GCB, non-GCB.
2. **T-cell or histiocyte-rich LBCL**; often presents in younger patients, with advanced stage, BM, liver and spleen involvement, and aggressive clinical behavior.

3. **Primary mediastinal B-cell Lymphoma (PMBCL)**. Commonly presents in young women and usually remains localized to the mediastinum with frequent superior vena caval syndrome. Regional lymph nodes may be involved, but spread to distant nodal sites is uncommon. Frequent extranodal sites, particularly at relapse, include the liver, kidneys, adrenal glands, ovaries, gastrointestinal tract, and CNS. An origin from medullary thymic B-cells has been proposed. GEP studies have found that PMBL shares features of classical Hodgkin lymphoma.
4. **Primary DLBCL of the CNS**. Less than1% of NHL and 2-3% of brain tumours; may be present in immunocompetent or immunodeficient (HIV) patients. Clinical features: neurological deficits (50-80%), neuropschiatric symptoms, headache, asymmetric cranial neuropathies, blurred vision and floaters. It has some distinctive features on GEP and shares some similarities with DLBCL arising in other immune privileged sites such as the testis. Poor prognosis ameliorated by novel chemotherapeutic protocols.
5. **Primary cutaneous DLBCL, leg type (PCLBCL)**. Tipically occurs in elderly patiens, in particular in women, preferentially affects the lower legs, with red or bluish-red tumours; frequently disseminates to extracutaneous sites. PCLBCL has a GEP resembling the ABC type of DLBCL, and it has an unfavourable prognosis.
6. **EBV positive DLBCL of the elderly**. Occurs in patients >50 years and without any known immunodeficiency or prior lymphoma, as a consequence of decreased immune surveillance as a part of the aging process. 70% of patients present with extranodal disease (skin, lung, tonsil, stomach), with or without lymph node involvement. The clinical course is aggressive, with a median survival of about two years. Lymphomatoid granulomatosis may progress to an EBV+ DLBCL.
7. **DLBCL associated with chronic inflammation**. It occurs in the context of long-standing chronic inflammation (over 10 years), it is associated with EBV-driven large B-cell proliferation. Most cases involve body cavities or narrow spaces (most often pleural cavity, but also bone, joint and periarticular soft tissue). Pyothorax-associated lymphoma (PAL) develops in the pleural cavity of patients with long-standing phyotorax, predominantly in men, with a median age around 65-70 years. The tumour mass (in the pleura and/or lung) is larger than 10 cm; patients present with chest pain, back pain, fever, cough, hemopthysis or dyspnea. Good prognosis if successfully resected.
8. **Intravascular large B-cell lymphoma (IVLBCL)**. It is a rare type of extranodal DLBCL, with selective growth of lymphoma cells within the lumina of vessels, particularly capillaries. IVLBCL occurs in adults, it is usually widely disseminated in extranodal sites. The symptoms are predominantly neurological or cutaneous (Western form), or patients present with multiorgan failure, hepatosplenomegaly, pancytopenia and hemophagocytic syndrome (Asian form). This is an aggressive lymphoma which responds poorly to chemotherapy.
9. **ALK-positive large B-cell lymphoma.** It is a neoplasm of ALK+ monomorphic large immunoblast-like B cells, sometimes with plasmablastic differentiation. It involves lymph nodes or presents as a mediastinal mass, affect older individuals, most patients present with advanced stages; the prognosis is very poor.
10. **Plasmablastic lymphoma (PBL)**. PBL is a diffuse proliferation of cells with immunophenotype of plasma cells (CD138, CD38), and high incidence of MYC translocations. It has higher incidence in HIV-positive patients, is usually positive for EBV; most often presents extranodal (especially oral cavity). Clinical course is very aggressive.
11. **Primary effusion lymphoma (PEL)**. PEL arises in HIV-infected patients, it is universally associated with HHV8, the cells are coinfected with EBV, with immunophenotype resembling plasmablastic cells. Usually presents with serous effusions (pleural, pericardial, peritoneal), in the absence of lymphadenopathy or organomegaly. Clinical course is extremely unfavourable (survival < 6 months).
12. **Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease (MCD)**. Profiferation of HHV8-infected lymphoid cells resembling plasmablasts expressing IgM, and arising in HIV-infected patients who have developed HHV8 MCD. Characteristically involved lymph nodes and spleen, but can disseminate (including leukemic aspect). Very aggressive entity, survival a few months.
13. **Borderline lymphomas**: **a**. B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma; **b**. B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Hodgkin lymphoma.

Treatment of DLBCL. The mainstay of treatment is systemic chemotherapy (current standard is R-CHOP); other therapies include radiation (with no proven clear benefit), stem cell transplantants (ASCT), and other chemotherapy. Treatment strategies should be stratified according to age, aa-IPI and feasibility of dose-intensified approaches. In cases with high tumor load, precautions (prednison administering) are required to avoid tumor lysis syndrome. Prophylactic use of hematopoietic growth factors to prevent febrile neutropenia is justified in patients treated with curative intent and in all elderly patients.

1. In young patients (<60 years), ***IPI low-risk***

without bulky disease, R-CHOP 21 x 6 is the current standard; consolidation by radiotherapy to initial sites has proven no clear benefit. In cases ***IPI low-risk with bulk or IPI low-intermediate-risk*** the treatment is R-CHOP 21 x 6 with radiotherapy to the site of previous bulky disease, or the intensified regimen R-ACVBP. In ***IPI intermediate-high risk or IPI high-risk*** patients, 6-8 cycles R-CHOP are most frequent applied; intensified treatment with R-ACVBP or R-CHOEP; high-dose chemotherapy (HDC) with ASCT as consolidation treatment after immunochemotherapy has shown promising results; HDC with ASCT may be suggested for selective high-risk patients.

2. In elderly patients (>60 years), 8 cycles of R-CHOP 21 is the current standard. In patients with localized disease, consolidation by radiotherapy has proven no benefit. In patients aged >80 years without cardiac dysfunction, R-miniCHOP combination could be used; in patients with cardiac dysfunction can be considered doxorubicin substitution with etoposide, mitoxantrone or liposomal doxorubicin – R-C(X)OP 21 x 6, or palliative care.

3. CNS profilaxis should be recommended for patients with high-intermediate and high-risk IPI, especially those with more than one extranodal site or elevated LDH, who are at higher risk of CNS relapse. Can be used intrathecal methrotexate or intravenous high-dose methrotexate associate with efficient disease control. Testicular lymphoma and other specific involvement sites (paranasal sinus, upper neck or bone marrow) must receive CNS prophylaxis.

4. Some extranodal DLBCL require special consideration. Treatment of **primary DLBCL of the CNS** must contain high-dose of methotrexate and of cytarabine; CNS irradiation is usually administered as consolidation. **Primary DLBCL of the testis (PTL)** is characterized by an increased risk of extranodal, CNS, and contralateral testis recurrence with poor outcome. The standard treatment of PTL is R-CHOP21 with CNS prophylaxis (intrathecal and intravenous) and contralateral testis irradiation. In **PMBCL** the R-CHOP 21 is not established as the definitive treatment option and radiotherapy remains controversial.

5. **Response evaluation and follow-up**. Abnormal radiological tests at baseline should be repeted after 3-4 cycles and after the last cycle of treatment. Bone marrow aspirate and biopsy should be only repeted at the end of treatment if initially involved. PET is highly recommended for the post-treatment assessment. A CT scan is recommended at 6, 12, and 24 months after the end of treatment; a blood count and LDH at 3, 6, 12, and 24 months need to be evaluated.

6. **Relapsed and refractory DLBCL**. Histological verification should be obtained whenever possible, and is mandatory in relapse >12 months after the initial diagnosis, especially in order to ensure CD20 positivity. ***In suitable patients*** with adequate performance status, salvage regimen (R-DHAP, R-ICE) followed in responsive patients by high-dose regimen (BEAM) with stem-cell support is recommended. ASCT following chemotherapy should be considered in patients with refractory disease, early relapse or relapse after ASCT. **Patients not suitable** for HDC may be treated with the same or other salvage regimens (R-GEMOX), which may be combined with involved-field radiotherapy or preferentially be enrolled in clinical trials.

Conclusions. Despite its initial appreciation as a single entity, DLBCL is in reality very heterogeneous at a clinical, pathological, cytogenetic and molecular level. DLBCL incorporates distinct subtypes and variants, based on unique clinical feature, age or anatomic site, viral pathogenesis, or distinctive pathological features. DLBCL are aggressive but potentialy curable with multi-agent chemotherapy. R-CHOP regimen remains a standard therapeutic approach for most patients with DLBCL. The future optimal therapy will incorpotare molecular information (particularly from gene espression analyses) for appropriate risk-adapted therapy, and novel agents (including epigenetic targets) will be included in treatment regimens.

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