NEW ENTITIES IN AGGRESSIVE B CELL LYMPHOMA

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# Historical background of Lymphoma classification

<table>
<thead>
<tr>
<th>Classification</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rappaport classification</td>
<td>1957</td>
</tr>
<tr>
<td>Kiel classification</td>
<td>1974</td>
</tr>
<tr>
<td>Working formulation</td>
<td>1982</td>
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<tr>
<td>Kiel update</td>
<td>1988</td>
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<tr>
<td>REAL classification</td>
<td>1994</td>
</tr>
<tr>
<td>WHO classification</td>
<td>2001</td>
</tr>
<tr>
<td>WHO classification</td>
<td>2008</td>
</tr>
</tbody>
</table>

*REAL-WHO classification (1994-2008)*

- Morphology
- Phenotype
- Genotype
- Clinical course
- Evidence based
- Entity

- morphology
- morphology/clinical
- immunohistochemistry
- cytogenetics, FISH
- molecular biology
- expression profile
**REAL classification**

B-Cell Neoplasms

I. Precursor B-cell neoplasm: Precursor B-lymphoblastic leukemia/lymphoma

II. Peripheral B-cell neoplasms

1. B-cell chronic lymphocytic leukemia/prolymphocytic leukemia/small lymphocytic lymphoma
2. Lymphoplasmacytoid lymphoma/immunocytoma
3. Mantle cell lymphoma
4. Follicle center lymphoma, follicular
   Provisional cytologic grades: I (small cell), II (mixed small and large cell), III (large cell)
   Provisional subtype: diffuse, predominantly small cell type
5. Marginal zone B-cell lymphoma
   Extranodal (MALT-type +/− monocytoid B cells)
   Provisional subtype: Nodal (+/− monocytoid B cells)
6. Provisional entity: Splenic marginal zone lymphoma (+/− villous lymphocytes)
7. Hairy cell leukemia
8. Plasmacytoma/plasma cell myeloma
9. **Diffuse Large B-cell lymphoma***
   Subtype: Primary mediastinal (thymic) B-cell lymphoma
10. Burkitt’s lymphoma
11. Provisional entity: High-grade B-cell lymphoma, Burkitt-like*

**WHO classification**

- Diffuse large B-cell lymphoma, not otherwise specified
  - Primary DLBCL of the CNS
  - Primary cutaneous DLBCL, leg type
  - T cell/histiocyte rich large B-cell lymphoma
  - EBV+ DLBCL of the elderly*
- DLBCL associated with chronic inflammation
- Primary mediastinal (thymic) large B-cell lymphoma
- Intravascular large B-cell lymphoma
- ALK-positive large B-cell lymphoma
- Plasmablastic lymphoma
- Primary effusion lymphoma
- Large B-cell lymphoma arising in HHV-8–associated multicentric Castleman Disease
- Burkitt lymphoma
- B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma
- B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma
I. Primary DLBCL of the CNS

- Primary DLBCL of the central nervous system comprises all primary CNS or intraocular (PCNSL + PIOL) DLBCL.
- Diagnosis should be made after exclusion of systemic primary lymphoma and do not apply to patients with immunodeficiency conditions.
- PCNSL accounts for 2% to 3% of all primary brain tumors and usually affects elderly patients with a slight male predominance.
- PCNSL can present as solitary (75%) or multiple (25%) masses in the brain parenchyma; approximately 1/5 of the patients go on to develop ocular lesions, and conversely, the majority of patients with PIOL ultimately develop brain lesions.
- Relapses typically remain confined to the CNS but may occasionally occur outside the CNS, especially in the testis or breast.
- The prognosis is poorer than that of DLBCL, NOS outside the CNS.
The tumor cells usually infiltrate peri-vascular space and adjacent brain parenchyma. CD20 highlights the peri-vascular distribution of the tumor cells which show nuclear expression of MUM-1.
In addition to CD20, the tumor cells are positive for BCL6 (60-80%), and almost all cases are MUM-1 positive, whereas CD10 expression is infrequent. 

PCNSL carries a high load of somatic hypermutations in the IG variable region genes which suggest the derivation of PCNSL from late GC B cells. 


Chromosomal translocations involving BCL6 (3q27) are frequent (17-47% of cases), often involve non-IG partners, and are associated with poor outcome. Conversely, rearrangements of BCL2 (18q21) and cMYC (8q24) are virtually never found in PCNSL. 


By gene expression profiling, the molecular signature of PCNSL includes both the GCB and the ABC subtypes; PCNSL shows a gene expression pattern that is distinct from non-CNS DLBCL, but the identified ‘CNS signature’ might in fact reflect the non-neoplastic brain microenvironment.

Activation of the interleukin-4 pathway in endothelial cells may account for the angiotropism typically encountered in PCNSL.
2. Primary cutaneous DLBCL, leg type

- Accounts for about one-fifth of primary cutaneous B-cell lymphomas.
- This is a tumor-forming non-epidermotropic neoplasm that preferentially affects the *lower limb* but may occur elsewhere.
- It usually affects *elderly women*.
- The tumor cells express CD20, BCL6, and MUM1 but are usually negative for CD10.
- BCL2 is overexpressed in most cases as a consequence of *BCL2* amplification.
- The gene expression profile is that of the *ABC subgroup*.
- The disease frequently disseminates to non-cutaneous sites and has an *aggressive course*. 
3. T-cell/histiocyte-rich Large B-cell Lymphoma (THRLBCL)

(A) THRLBCL involving the spleen as multiple cellular nodules. (B) There are few large neoplastic cells, scattered in a background of histiocytes and small lymphocytes. (C) CD20 highlights few large neoplastic cells and no small cells. (D) CD5 stains most of the background small lymphocytes.
THRLBCL usually presents in middle-aged or older male adults, often with advanced-stage disease and frequent involvement of *spleen, liver, and bone marrow*.

The neoplastic cells may resemble centroblasts, immunoblasts, lymphocyte-predominant Hodgkin cells, or *classic RS cells*.

They express CD45 and B-cell antigens, are strongly positive for BCL6, variably express BCL2 and EMA, are *negative for CD30 and CD15*, and do not harbor EB virus. 

*Small B cells are virtually absent*, and T cells with a follicular helper T-cell phenotype (CD57+ and/or PD1+) – not like NLPHL.

Because of the **scarcity of neoplastic cells**, THRLBCL may be mistaken as a reactive granulomatous process, especially in cases of liver or lung biopsies.

Conversely, THRLBCL may develop an increasing density of neoplastic cells with progression or relapse and exhibit an appearance indistinguishable from DLBCL, NOS.

**THRLBCL and NLPHEL are similar** each other with regard to morphology and immunophenotype but have different clinical courses.

It is currently recommended that the diagnosis of THRLBCL be restricted to de novo cases and **not** be applied in patients with a history of NLPHEL.

Some studies have reported that THRLBCL follows a more **aggressive clinical course** than DLBCL, NOS, other reports indicate a similar prognosis.


4. EBV positive DLBCL of the elderly

- Occurs in elderly patients (with a median age in the eighth decade) without HIV infection and without any known immunodeficiency syndrome.
- Other EBV-related DLBCL entities should be excluded.
- The disease often involves extranodal sites (skin, tonsil, lung, stomach), with or without lymph node involvement.
- Large cell infiltrate with inflammatory background – like to THRLBCL.
- RS-like cells may be numerous, raising the differential diagnosis with classical Hodgkin lymphoma.
- The tumor cells express CD20 and CD79a, are positive for EBER, LMP-1, and EBV nuclear antigen (EBNA)-2, and variably express CD30, but are negative for CD15.
- Poorer prognosis than that of EBV-negative DLBCLs; the presence of B symptoms and age more than 70 years are additional adverse prognostic factors.
(A) Tumor of large cells, some of which resemble RS cells, in a background rich in histiocytes. (B) CD20 highlights numerous large B cells. (C) Many of the tumor cells are positive for EBER by in situ hybridization.
EBV-positive large B-cell lymphoid proliferations

<table>
<thead>
<tr>
<th>Type of Lymphoid Proliferation</th>
<th>Clinical Setting</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV+ DLBCL</td>
<td>Associated with primary immune disorders&lt;br&gt;Associated with HIV infection&lt;br&gt;Post-transplant-associated&lt;br&gt;Iatrogenic immunodeficiency-associated (methotrexate, TNFα antagonists)&lt;br&gt;With no known predisposing conditions, &lt;50 years old</td>
<td>Nodal or extranodal</td>
</tr>
<tr>
<td>Plasmablastic lymphoma</td>
<td>HIV infection&lt;br&gt;Less often: HIV-negative individuals</td>
<td>Extranodal (oral cavity or other), less commonly nodal</td>
</tr>
<tr>
<td>Primary effusion lymphoma a</td>
<td>HIV infection&lt;br&gt;Less often: post-transplant, elderly Mediterranean individuals</td>
<td>Body cavity effusion&lt;br&gt;Solid extracavitary</td>
</tr>
<tr>
<td>Lymphomatoid granulomatosis</td>
<td>Congenital immunodeficiency syndromes&lt;br&gt;HIV infection&lt;br&gt;Post-transplant</td>
<td>Lung, brain, skin; often multifocal involvement</td>
</tr>
<tr>
<td>DLBCL associated with chronic inflammation</td>
<td>Chronic pyothorax or chronic infection</td>
<td>Pleural-based mass, bone, or joint tumor</td>
</tr>
<tr>
<td>EBV+ DLBCL of the elderly</td>
<td>&gt;50 years old, immune senescence</td>
<td>Extranodal</td>
</tr>
<tr>
<td>Germinotrophic lymphoproliferative disorder a/b</td>
<td>Immunocompetent individuals</td>
<td>Germinal center of lymph nodes</td>
</tr>
</tbody>
</table>

a, co-infection by HHV8 and EBV.
5. DLBCL associated with chronic inflammation

- Disease associated with chronic inflammation and related with *EBV*, of which *pyothorax-associated lymphoma* (PAL) is the prototypic form.
- This rare disease develops in the pleural cavity of patients with a history of longstanding (>10 years) pyothorax, usually resulting from artificial pneumothorax for treatment of tuberculosis.
- The disease affects elderly patients and shows a strong male predominance.
<table>
<thead>
<tr>
<th>Clinical setting</th>
<th>DLBCL ass. C. chronic inflammation</th>
<th>Primary effusion lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>presentation</td>
<td>Tumor mass</td>
<td>effusion ± mass</td>
</tr>
<tr>
<td>HHV8</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>EBV</td>
<td>EBNA-2 +</td>
<td>EBNA-2 -</td>
</tr>
<tr>
<td>CD79a</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Genetic alteration</td>
<td>P53 mutation (70%)</td>
<td>Gain in chromosome 12 &amp; X</td>
</tr>
<tr>
<td>Gene expression profile</td>
<td>Distinct from nodal DLBCLs, different expression of interferon response genes</td>
<td>Features of plasma cells and EBV-transformed lymphoblastoid cell lines</td>
</tr>
<tr>
<td>Cell of origin</td>
<td>Late GC/post-GC B cell</td>
<td>Post-GC B cell</td>
</tr>
<tr>
<td>Prognosis</td>
<td>OS 20-35% in 5 yr.</td>
<td>OS &lt; 6 months, median</td>
</tr>
</tbody>
</table>
### 6. Primary mediastinal large B-cell lymphoma (PMLBCL)

- PMLBCL is a distinct DLBCL entity arising in the mediastinum from putative thymic B cells, and comprises 2% to 4% of all NHLs.

<table>
<thead>
<tr>
<th></th>
<th>DLBCL, NOS</th>
<th>PMLBCL</th>
</tr>
</thead>
</table>
| **Clinical features** | Adults, M > F  
Stages I-II: 50%  
Bulky mass occasional | Young women  
Stages I-II > 50%  
Bulky mass frequent |
| **Immunophenotype** | Common to both: CD45+, CD20+, CD79a+, PAX5+, BOB2+, Oct1+  
slg + | slg –  
CD30 +/-  
CD23 +  
MAL +  
TRAF1 +/-c-REL + (50%) |
| **Genetics** | Rearranged BCL6: 30%  
Rearranged BCL2: 20% | Gains at 2p15: cREL, BCL11 (50%)  
Gains at 9p24: JAK (75%) |
| **Molecular signature** | GCB and ABC subtypes | PMLBCL signature  
Down regulation:  
BCR signaling pathway  
Overexpression:  
Extracellular matrix components  
Cytokines/JAK/STAT |
- PMLBCL tends to occur in young patients (median age, about 35 yrs) and affects women more commonly than men.
- The disease is usually localized at presentation, but progression can be characterized by dissemination to other extranodal sites, including lung, liver, kidney, adrenals, ovary, brain, and the GI tract.
- Although PMLBCL was initially believed to carry an adverse prognosis, recent studies have shown an OS similar or superior to that of DLBCL, NOS.


- The tumor cells express CD19, CD20, and CD79a but often lack surface Ig.
- Most cases express BCL6, MUM1/IRF4, BCL2, and CD23; in contrast to cHL, the CD30 expression is usually weak.
- Expression of MAL, FIG1 (the product of the IL-4-induced gene 1), and tumor necrosis factor (TNF)-receptor-associated factor 1 (TRAF-1) is characteristic of PMLBCL.
The most frequent genetic abnormalities are gains in chromosome 9 p24 (including JAK2 locus) in up to 75% of cases and gain of REL on chromosome 2p in about 50% of cases.

**PMLBCL and nodular sclerosis HL** exhibit strikingly similar clinical presentations (young women with an anterior mediastinal mass) and some overlap in pathologic, genetic, and molecular features.

The chromosome 2p and 9p aberrations, activation of the **NFκB** pathway, altered **JAK/STAT signaling**, and activation of **PI3K/AKT** pathway are common to both PMLBCL and cHL.
7. Intravascular large B-cell lymphoma (IVLBCL)

- No extravascular tumor mass or leukemia.
- The disease is often associated with a *hemophagocytic syndrome* with pancytopenia, hepatosplenomegaly, and BM involvement. (Asian)
- It affects elderly patients and presents as *disseminated* and *aggressive* malignancies eventually resulting in mutiorgan failure.
- The dismal prognosis can be overcome with *rituximab* based chemotherapy


- The cells express CD20, with coexpression of CD5 in 1/3 patients, and negative for EBV.
- Expression of BCL6 and CD10 is infrequent, whereas MUM1/IRF4 is often positive. Many cases express BCL2, but the t(14;18) is usually absent.
- *Lack of CD29 and CD54*, that are necessary to transvascular migration, can localize tumor cells within vessels.
8. Anaplastic Lymphoma Kinase-positive large B-cell lymphoma

- Tumor cells bear chromosomal translocations affecting the anaplastic lymphoma kinase gene (ALK).
- Most cases express clathrin-ALK fusion protein and a few cases express the NPM-ALK fusion protein.
- The tumor cells are negative for CD20, CD79a, and CD30 but are positive for EMA and CD138 and express cytoplasmic IgA.
- The disease affects mostly adult males and is often disseminated at presentation, with a poor prognosis.
9. Plasmablastic lymphoma (PBL)

- PBL is a group of tumors that morphologically resemble DLBCL but have an *immunophenotypic profile of plasma cells*.
- The tumor cells strongly express CD138 and MUM-1/IRF4 but weak positive or negative for CD45 and B-cell associated antigens.
- PBL has a *highly aggressive* clinical behavior, but improved survival outcomes have been reported in *HIV-infected patients* in the era of highly active anti-retroviral therapy.
- PBL usually exhibits a *high proliferation rate* with *frequent apoptotic cells*, and a *starry-sky pattern* may be present.
- Virtually all cases are *positive for EBER* but lack the EBV-associated proteins LMP1 and EBNA. *HHV8 is consistently absent.*
The tumor consists of a diffuse proliferation of large cell with immunoblastic/plasmablastic features.
# Lymphomas with plasmablastic features

<table>
<thead>
<tr>
<th>Presentation</th>
<th>PBL</th>
<th>Myeloma</th>
<th>PEL</th>
<th>PBL multicentric Castleman dis.</th>
<th>ALK+ DLBCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral cavity, other extranodal sites</td>
<td>BM and/or extramedullary</td>
<td>Body cavity effusion</td>
<td>LN, spleen</td>
<td>LN, sinusoidal pattern</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HIV relationship</th>
<th>Yes</th>
<th>No</th>
<th>Yes</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
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<table>
<thead>
<tr>
<th>EBV</th>
<th>+</th>
<th>-</th>
<th>+</th>
<th>-</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD20</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>CD79a</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD30</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>ALK</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell origin</th>
<th>plasmablast</th>
<th>Fully diff. B cell</th>
<th>Post-GC B cell</th>
<th>Naïve B cell</th>
<th>Post-GC B cell</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Clinical course</th>
<th>aggressive</th>
<th>variable</th>
<th>Very aggressive</th>
<th>Very aggressive</th>
<th>aggressive</th>
</tr>
</thead>
</table>
10. Primary effusion lymphoma (PEL)

- PBL was originally identified in *AIDS patients* presenting as effusions in serous cavities (pleural, pericardial, or peritoneal).
- Generally, there are *no tumor masses*, but some cases may have concomitant or subsequent solid tissue involvement of adjacent structures.
- The tumor cells are typically infected by *HHV8*, which likely plays a major role in lymphomagenesis; most cases are also *coinfected with EBV*. 
# Lymphomas presenting in body cavities

<table>
<thead>
<tr>
<th></th>
<th>DLBCL assoc. with chronic inflammation</th>
<th>Primary effusion lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical setting</strong></td>
<td>Elderly men, chronic pyothorax</td>
<td>AIDS, post-transplant, elderly Mediterranean (HHV8 endemic)</td>
</tr>
<tr>
<td><strong>Presentation</strong></td>
<td>Tumor mass</td>
<td>Effusion</td>
</tr>
<tr>
<td><strong>HHV8</strong></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>CD20</strong></td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td><strong>CD79a</strong></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>CD30</strong></td>
<td>-/+</td>
<td>-</td>
</tr>
<tr>
<td><strong>EMA</strong></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>TCR gene rearrangement</strong></td>
<td>polyclonal</td>
<td>monoclonal</td>
</tr>
<tr>
<td><strong>Genetic alterations</strong></td>
<td>P53 mutations (70%)</td>
<td>Gains in chromosome 12 &amp; X</td>
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<tr>
<td><strong>Cell origin</strong></td>
<td>Late GC/post-GC B cell</td>
<td>Post-GC B cell</td>
</tr>
<tr>
<td><strong>Prognosis</strong></td>
<td>Heterogenous, OS5yr: 20-35%</td>
<td>Poor, OS &lt; 6 months</td>
</tr>
</tbody>
</table>
11. DLBCL arising in multicentric Castleman disease

- This lymphoma is composed of monoclonal *HHV8-positive, EBV-negative* large B cells.
- The majority of cases occur in *HIV-infected patients*.
- The disease involves LN, spleen, can evolve toward a *leukemic phase*, and has a *short median survival*.
- The neoplastic cells are *naïve, unmutated B cells* (CD20+ CD138-).
- Precursor lesions to this lymphoma can manifest as clusters or aggregates of large IgM lambda-positive, *HHV8-positive* B cells with a plasmablastic appearance in the mantle zones of LN.
- This pattern of involvement has been called “microlymphoma”. 
### 12. B-cell lymphoma unclassified, with features intermediate between DLBCL and Burkitt lymphoma (DLBCL/BL)

<table>
<thead>
<tr>
<th>Expected BL finding</th>
<th>Relative contraindication for a Dx of BL</th>
<th>Absolute contraindication for a Dx of BL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uniform, medium-sized cells</td>
<td>Mild or moderate cellular pleomorphism</td>
<td>Blastic morphology, large cell size or marked cellular pleomorphism</td>
</tr>
<tr>
<td>CD10 strong positive</td>
<td>CD10 negative</td>
<td>NA</td>
</tr>
<tr>
<td>BCL6 positive</td>
<td>BCL negative</td>
<td>NA</td>
</tr>
<tr>
<td>BCL2 negative</td>
<td>NA</td>
<td>BCL2 strong positive</td>
</tr>
<tr>
<td>Ki67</td>
<td>NA</td>
<td>&lt;90% proliferation index</td>
</tr>
<tr>
<td>MYC rearrangement with LG locus</td>
<td>Absent or shown to be with a non-LG locus</td>
<td>NA</td>
</tr>
<tr>
<td>Simple karyotype</td>
<td>Complex karyotype (3 or more abnormalities in addition to 8q24)</td>
<td>NA</td>
</tr>
<tr>
<td>No BCL6 rearrangement</td>
<td>NA</td>
<td>BCL6 rearrangement present</td>
</tr>
<tr>
<td>No BCL2 rearrangement</td>
<td>NA</td>
<td>BCL2 rearrangement present</td>
</tr>
</tbody>
</table>
Molecular profiling studies have shown that 17-34% of cases that had a molecular signature of BL were not classified as BL; conversely, cases diagnosed as BL almost always displayed a BL molecular signature.

DLBCL/BL is unlikely to represent a single biologic disease entity but rather comprises a heterogenous group of cases that have some morphologic, immunophenotypic, or genetic features resembling BL and others that resemble DLBCL.

Strong BCL2 staining and/or a Ki67 proliferation index of less than 90% should be placed in the DLBCL/BL category even if they have BL morphology.

DLBCL/BL may or may not contain an MYC rearrangement.

“Double-hit” lymphoma (MYC and BCL2 rearrangement) comprise a large and relatively well-characterized subset of DLBCL/BL.
13. B-cell lymphoma unclassified, with features intermediate between DLBCL and classical Hodgkin lymphoma (cHL)

- An overlap in biologic and clinical features has been identified among cHL, nodular sclerosis, and primary mediastinal B-cell lymphoma (PMBL).
- This “gray zone lymphoma (GZL)” is thought to overlap with what had been termed Hodgkin-like anaplastic large-cell lymphoma in the older literature.
- Most, but not all, of these cases present with mediastinal disease, although in contrast to the parent entities of PMBL and cHL, a male predominance is seen.
- The immunophenotypic features and methylation profiling results are also intermediate between PMBL and cHL.
- Poor prognosis can be partially overcome with rituximab based combination chemotherapy.
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