

Gray-zone lymphomas – Diagnosis

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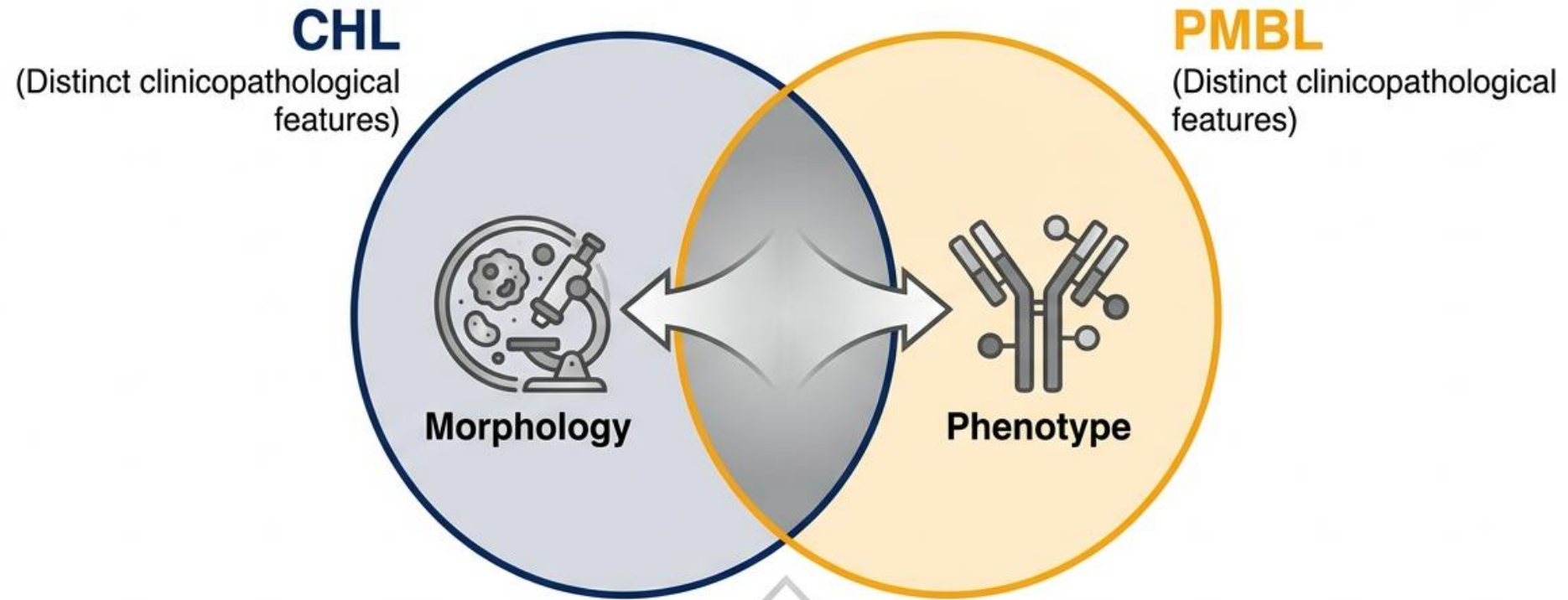
October 15-16, 2026

Hotel nhow Roma

Disclosure: Claudio Agostinelli

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

Biological Overlap Creates a Diagnostic Blind Spot



MGZL is a rare, highly heterogeneous haematological malignancy developing in the mediastinal niche. Its defining hallmark is a pathological dissociation between morphology and phenotype, exhibiting hybrid features that fail to fulfil the strict criteria of either parent disease.

2005

First formalized descriptions of lymphomas with hybrid features of CHL and PMBL developing in the mediastinal niche.

2008: WHO

Formal introduction of Grey Zone Lymphoma (GZL) as a defined entity.

Often historically used as a rescue category for hard-to-classify intermediate cases.

2022-24: WHO/ICC

Definition highly restricted to MGZL (Mediastinal only), driving the explicit need for retrospective review.

What Changed in Modern Classifications?

Location Rules: Primary extramediastinal presentations are now definitively excluded (reclassified primarily as DLBCL-NOS).

Viral Rules: Tumors must be EBV-negative. EBV+ cases prompt alternative diagnoses.



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The Location Rule: Mediastinal vs. Extramediastinal

The Golden Rule

Anatomy dictates biology. Under the new WHO-HAEM5 / ICC classifications, a diagnosis of Gray Zone Lymphoma is strictly restricted to tumors arising in the mediastinum (MGZL).

The Biological Divide

Lymphomas presenting with GZL-like morphology outside the thymic niche are biologically distinct, exhibiting different clinical courses and genetic driver mutations. They must not be grouped with MGZL.

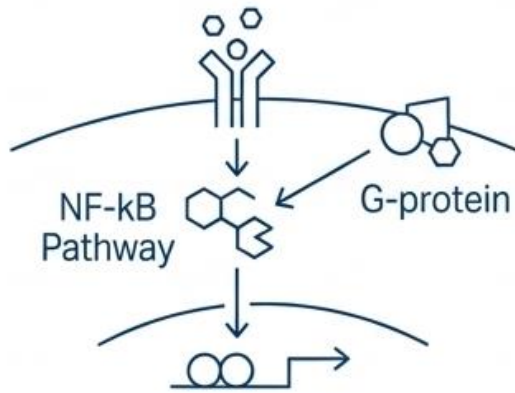
True Mediastinal GZL	Extramediastinal GZL-Like
Younger (avg ~40 years).	Older (avg ~58 years).
Localized, lower stages (I-II).	Advanced stages (IV), extranodal involvement.

Thymic and Non-Thymic GZL represent divergent mutational landscapes

	Thymic	Non-Thymic
Mutational Burden	HIGH (3.6 mut/Mb)	LOW (1.25 mut/Mb)
Coding Mutations (Average)	16.4	8.3
Dominant Pathway	NF-kB / JAK-STAT	Apoptosis Defects
Key Driver Genes	SOCS1 (45%), B2M (45%), TNFAIP3 (35%), NFKBIA (29%)	TP53 (39%), BCL2 (28%), CREBBP (28%)
Rearrangements	Rare (BCL2/BCL6 virtually absent)	Frequent (BCL2/BCL6 structural variations enriched)

Sarkozy C *et al* Blood Advances, 2020; Campo & Jaffe Blood 2021; Sarkozy Blood 2021,

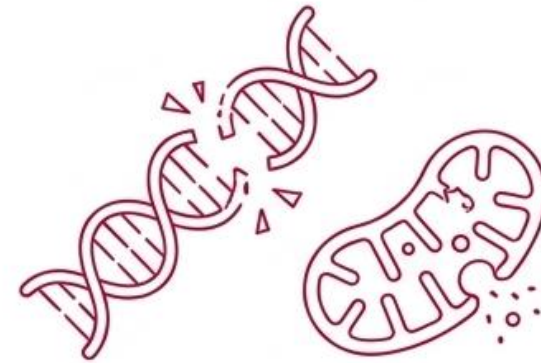
Thymic tumors evade immunity; Non-Thymic tumors refuse to die



Thymic / Immune Evasion

- High enrichment in NF-κB pathway: TNFAIP3, NFKBIE, NFKBIA, ITPKB.
- G-protein signaling: GNA13.

Cr. aberration 9p24.1(JA2/CD274/PDCD1LG)



Non-Thymic / Apoptotic Failure

- Cell cycle/Apoptosis regulation: TP53, BCL2, BIRC6.
- Epigenetic modification: CREBBP.

True MGZL

Classic PMBL Features

A single, clonal tumor
exhibiting a biological
continuum and
intermediate/transitional
features.

Classic CHL Features

Composite Lymphoma

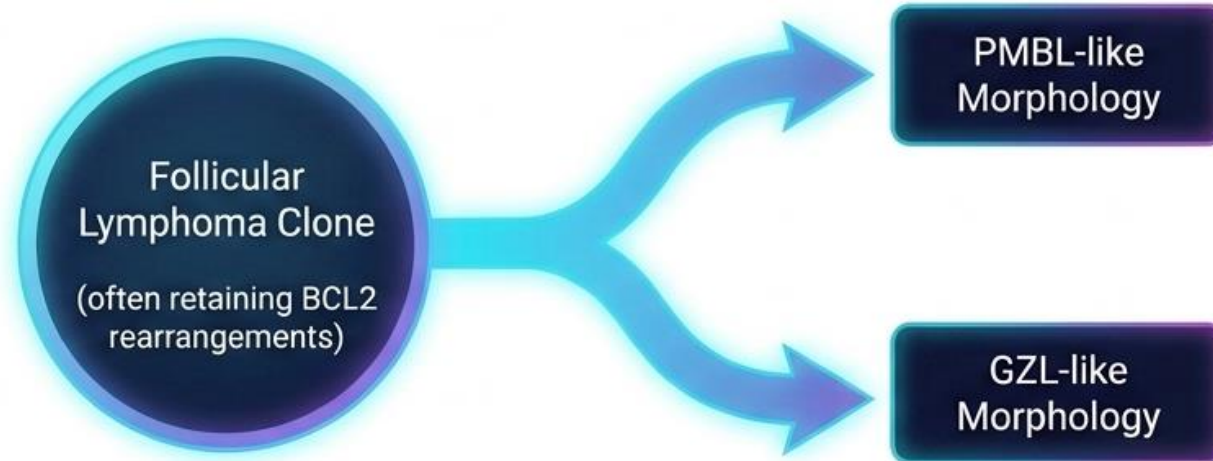
Distinct
CHL
Zone

Distinct
PMBL/DLBCL
Zone

The co-occurrence of **CHL** and **PMBL/DLBCL**
in the **same** or **separate tissues**, ,
and, **lacking transitional zones**.
(Can be synchronous or sequential).

Terminology Warning: The term “Composite GZL” is unofficial and invalid. If distinct CHL and PMBL zones exist without transition, it is a composite lymphoma, not a gray zone.

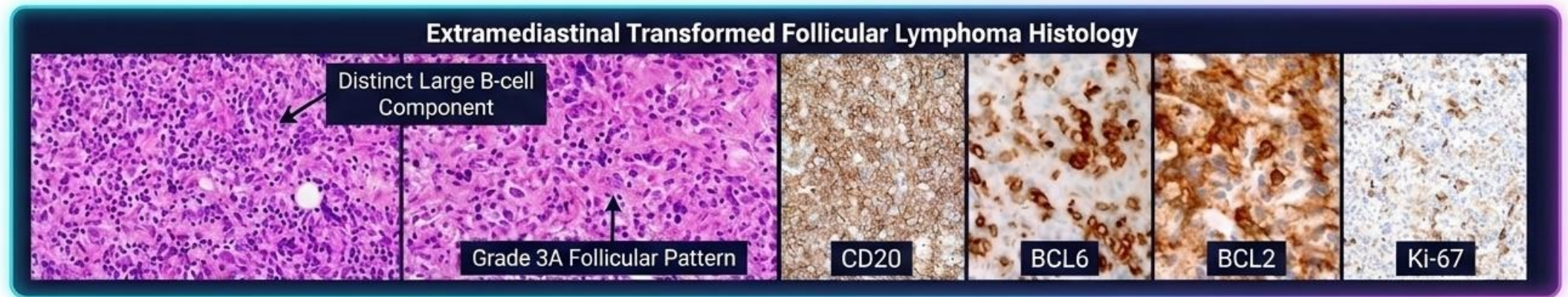
GZL-LIKE TRANSFORMATION OF FOLLICULAR LYMPHOMA



Key Insight

PMBL-like and GZL-like lymphomas can, in rare instances, arise as transformation events from antecedent Follicular Lymphoma.

EAHP workshop 20° meeting



Clinicopathologic consensus study of gray zone lymphoma with features intermediate between DLBCL and classical HL



Monika Pilichowska,¹ Stefania Pittaluga,² Judith A. Ferry,³ Jessica Hemminger,⁴ Hong Chang,^{5,6} Jennifer A. Kanakry,⁵ Laurie H. Sehn,⁷ Tatyana Feldman,⁸ Jeremy S. Abramson,⁹ Athena Kritharis,⁹ Francisco J. Hernandez-Ilizaliturri,¹⁰ Izidore S. Lossos,¹¹ Oliver W. Press,¹² Timothy S. Fenske,¹³ Jonathan W. Friedberg,¹⁴ Julie M. Vose,¹⁵ Kristie A. Blum,¹⁶ Deepa Jagadeesh,¹⁷ Bruce Woda,¹⁸ Gaurav K. Gupta,¹ Randy D. Gascoyne,⁷ Elaine S. Jaffe,² and Andrew M. Evens¹⁹

French group/LYSA

Retrospective analysis of 252 “GZL” from the LYMPHOPATH French registry

Criteria established from previous peer reviewed article descriptions of GZL.

6/12 hematopathologists agreement for consensus.

139/252 (55%) GZL confirmed

Boston/NIH

Retrospective 68 “GZL” from 15 institutions
WHO 2016 based criteria

5/5 hematopathologists agreement for consensus.

26 / 68 GZL (38%) confirmed

Gray-zone Lymphoma Between cHL and Large B-Cell Lymphoma

A Histopathologic Series From the LYSA

Clémentine Sarkozy, MD,*†‡ Christiane Copie-Bergman, MD, PhD,§ Diane Damotte, MD,||
Susana Ben-Neriah, PhD,‡ Barbara Burroni, MD, PhD,|| Jérôme Cornillon, MD,¶
Richard Lemal, MD, PhD,# Camille Golfier, MD,** Bettina Fabiani, MD,††
Catherine Chassagne-Clément, MD,‡‡ Marie Parrens, MD,§§ Charles Herbaux, MD,|||
Luc Xerri, MD, PhD,¶¶ Celine Bossard, MD, PhD,## Camille Laurent, MD, PhD,***
Morgane Cheminant, MD, PhD,††† Guillaume Cartron, MD, PhD,‡‡‡ Jose Cabecadas, MD,§§§
Thierry Molina, MD, PhD,|||| Gilles Salles, MD, PhD,*† Christian Steidl, MD,‡
Hervé Ghesquières, MD, PhD,*† Anja Mottok, MD, PhD,‡¶¶¶
and Alexandra Traverse-Glehen, MD, PhD†###

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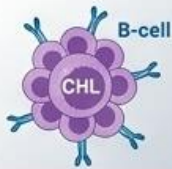
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Deconstructing MGZL: The LYSA Morphologic Continuum

Group 0: The CHL Border

Classic Hodgkin Lymphoma morphology combined with full, strong expression of all B-cell markers.



Group 1: The Transition

CHL-like architecture intermixed with transitional PMBL-like areas; variable expression of B-cell markers.



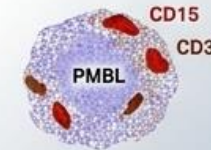
Group 2: The PMBL Border

Predominant PMBL-like morphology, but anomalously rich in inflammatory cells and Hodgkin/Reed-Sternberg (HRS) cells.



Group 3: The Extreme Edge

Substantial morphologic overlap with PMBL, featuring severe immunophenotypic anomalies (diffuse/strong CD15 and/or CD30).



Mediastinal grey zone lymphomas: Results of the expert pathological review analysis of a case series enrolled in the multicentre BIOGZL-2020 study in Italy

Elena Sabattini¹ | Stefano Ascani² | Sabino Ciavarella³ | Arianna Di Napoli⁴ |
Fabio Facchetti⁵ | Stefano Lazzi⁶ | Lorenzo Leoncini⁶ | Luisa Lorenzi⁵ |
Marco Lucioni⁷ | Giovanna Motta¹ | Nassi Luca⁸ | Alice Parisi⁹ | Marco Paulli⁷ |
Stefano Pileri¹⁰ | Maurilio Ponzoni¹¹ | Claudio Tripodo¹² | Simona Rigbi¹³ |
Claudio Agostinelli^{1,13}



70 Historical Cases

Collected 2008–2020



14 Italian Centers



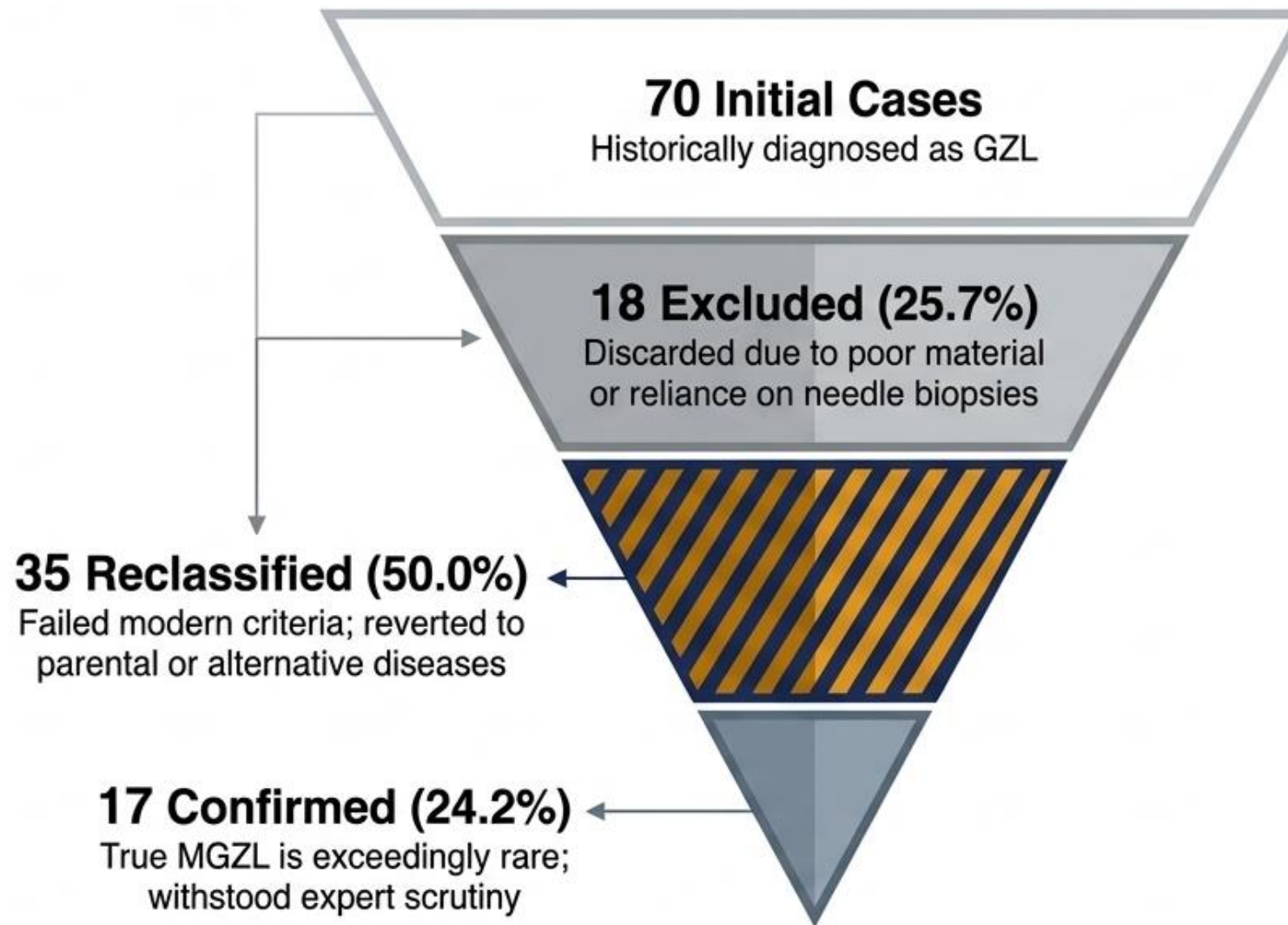
13 Expert Hematopathologists

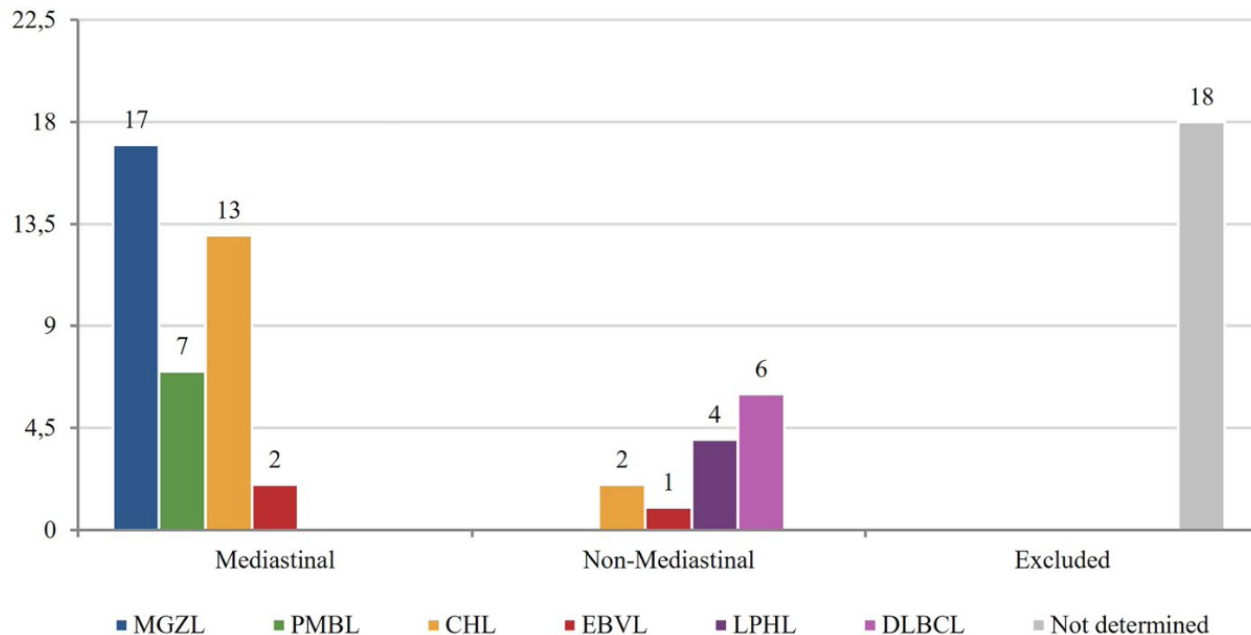
FIL Pathology Committee

The Objective: Assess reproducibility against modern WHO-HEMA5 and ICC classification standards.

The Method: Blinded, virtual live remote video review evaluating strict morphological and immunohistochemistry (IHC) parameters.

PARAMETER	EVALUATION / SCORING
GROWTH PATTERN	Nodular vs. Diffuse
FIBROSIS	Absent / Present (Fine vs. Coarse Sclerosis)
NECROSIS	Absent / Present
IHC EVALUATED ANTIGENS	CD20, CD79a, PAX5, CD30, CD15, MUM1, BCL6, CD10, CD23, BCL2, EBV-EBER, CD3, CD5
SCORING METHODOLOGY	Percentage of positive tumor cells (0-100%) or Intensity (Weak, Moderate, Strong)





17 (24.28%) cases confirmed as MGZL (mediastinal/EBV negative)

MGZL-CHL (cases n. 1–4)	4 (23.52%)
MGZL-PMBL (cases n. 5–13*)	9 (52.94%)
Intermediate [#] (cases n. 14–17)	4 (17.64%)

35 (50%) cases reclassified

Mediastinal/EBV negative (20 cases; 57.14%)

PMBL (cases n. 18–24)	7
CHL (cases n. 25–37*)	13

Non-mediastinal-EBV negative (12 cases; 34.28%)

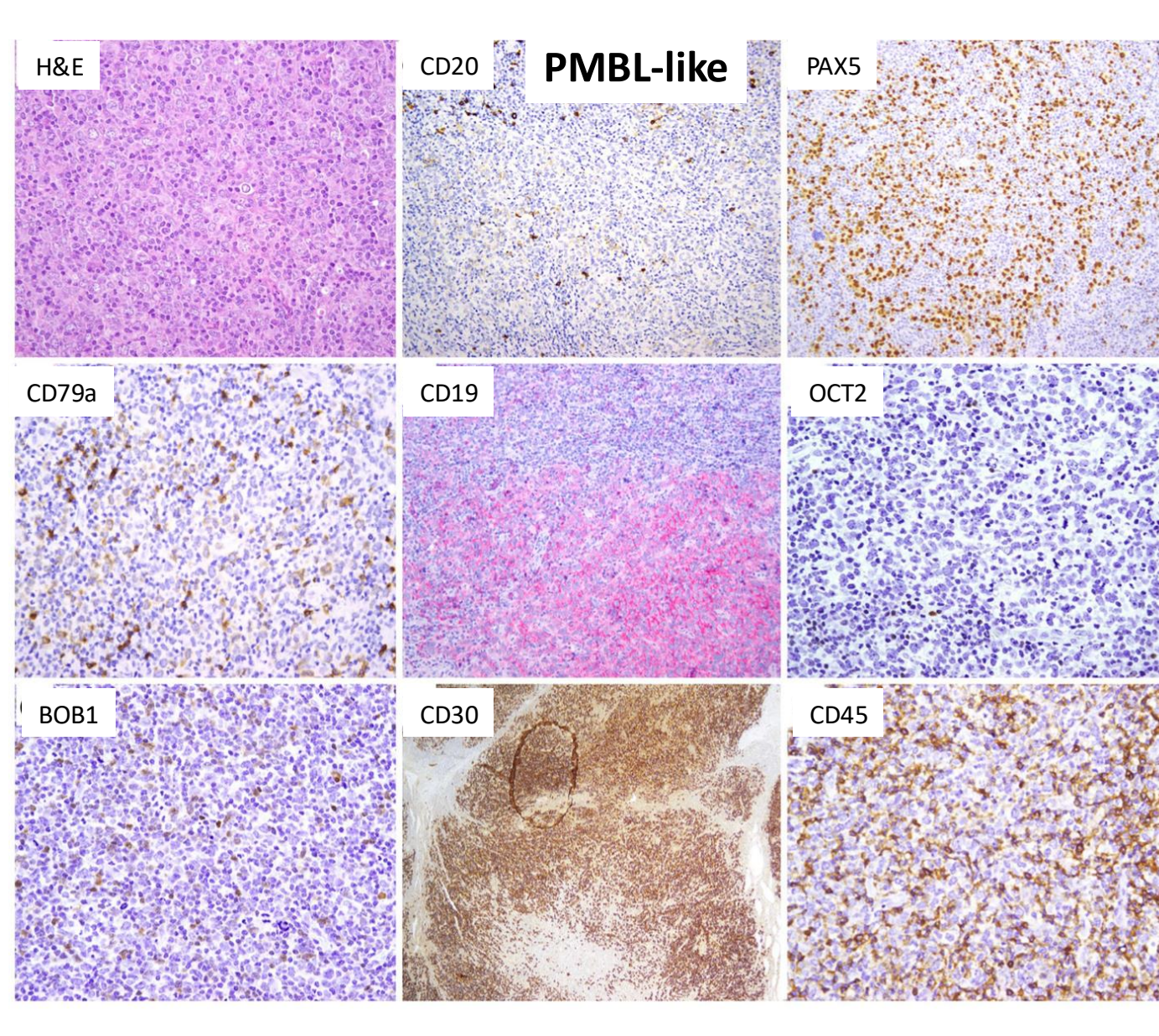
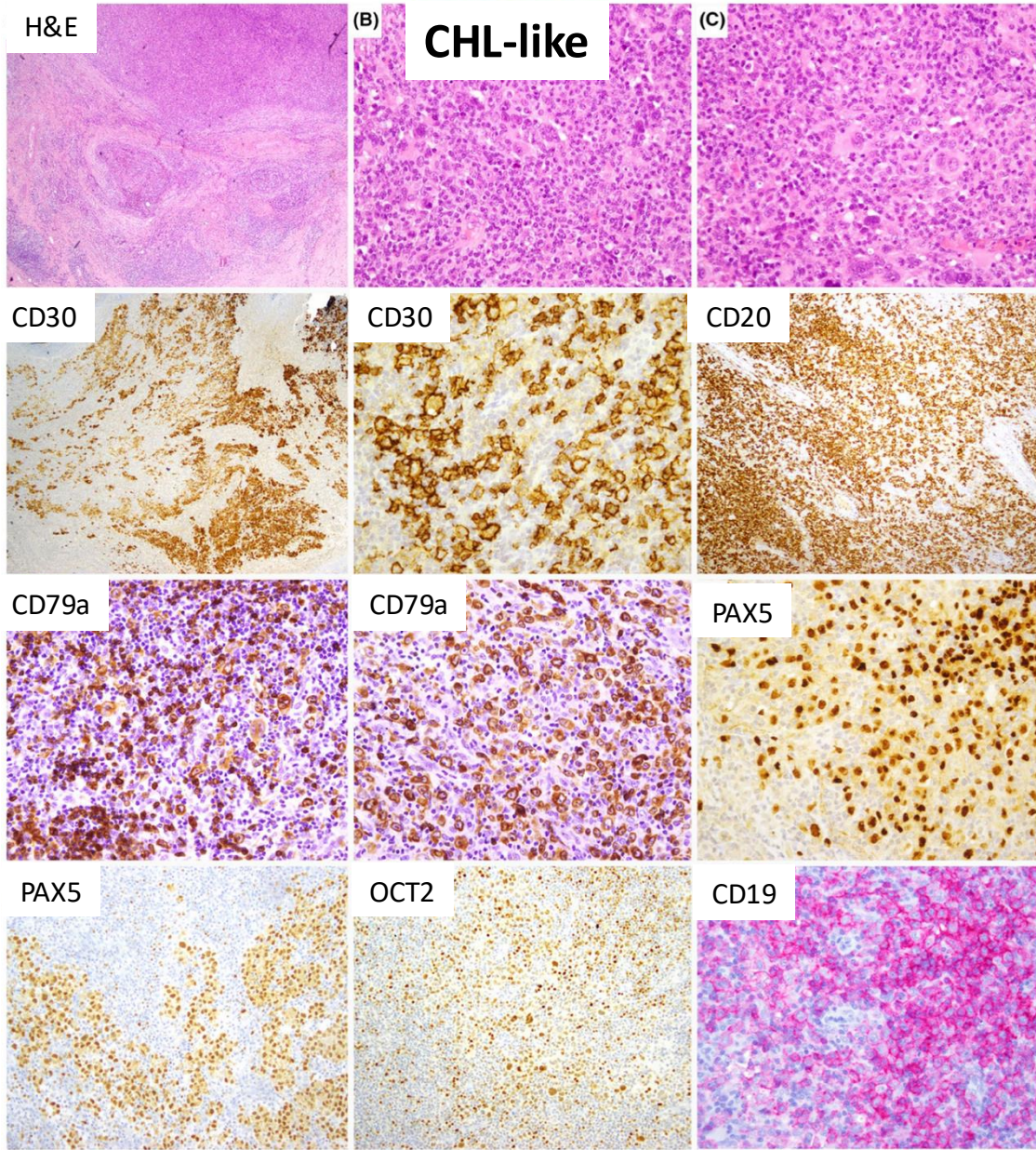
DLBCL (cases n. 38–42)	5
B-cell lymphoma related to LPHL (cases n. 43–46**)	4
CHL (cases n. 47–48)	2
LBCL with GZL-like features in Human Immunodeficiency Virus (HIV) (case n.49)	1

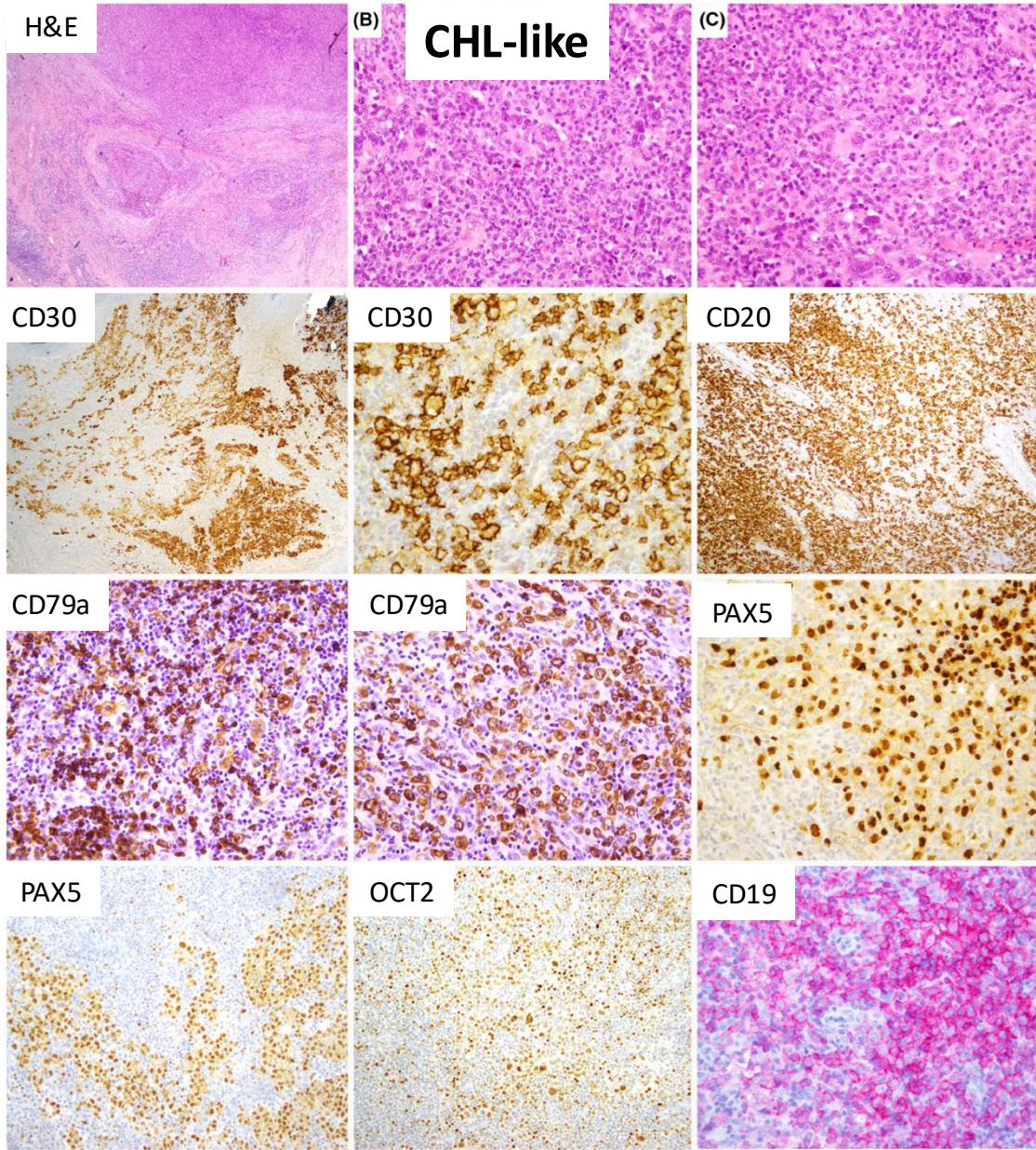
EBV positive (3 cases; 8.5%)

Mediastinal/LBCL (cases n. 50–51)	2
Non-mediastinal/LBCL (case n. 52)	1

18 (25.71%) cases excluded from evaluation

Suboptimal/poor material (cases n. 53–67)	15
Only needle biopsies available (cases n. 68–70)	3





MGZL-CHL (HL-like) & Intermediate



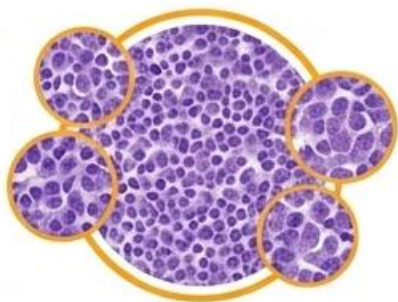
Growth: Vaguely nodular

Cytology: Abundant Hodgkin-Reed-Sternberg (HRS)-like cells

Environment: Moderate to abundant inflammatory background; fibrosis common

Phenotype: Expresses ≥ 4 B-cell markers (Strong CD20, CD79a, OCT2; Moderate PAX5). CD23 detected; BCL6 mostly negative.

MGZL-PMBL (PMBL-like)

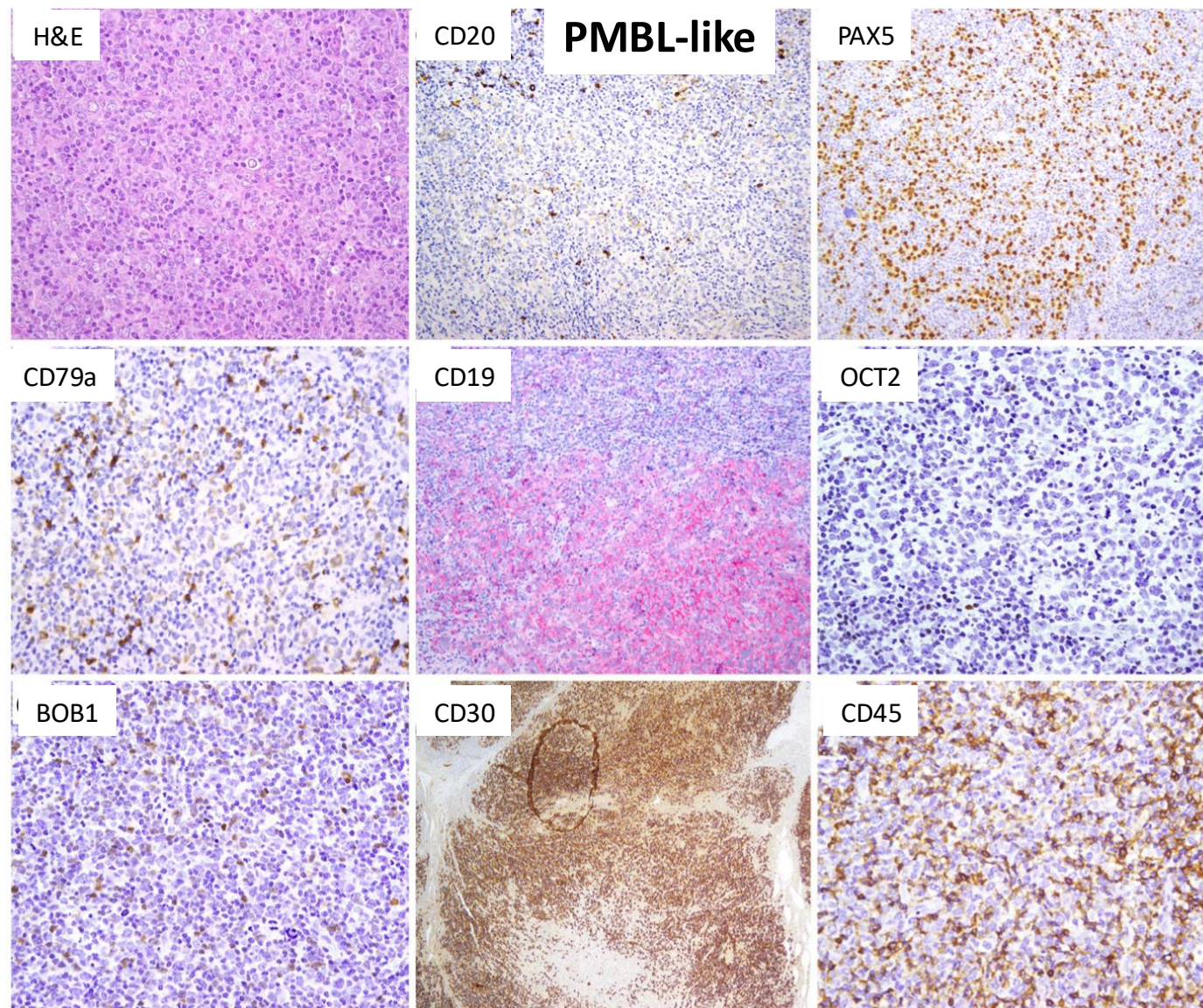


Growth: Diffuse

Cytology: Sheetting mononucleated centroblast-like cells

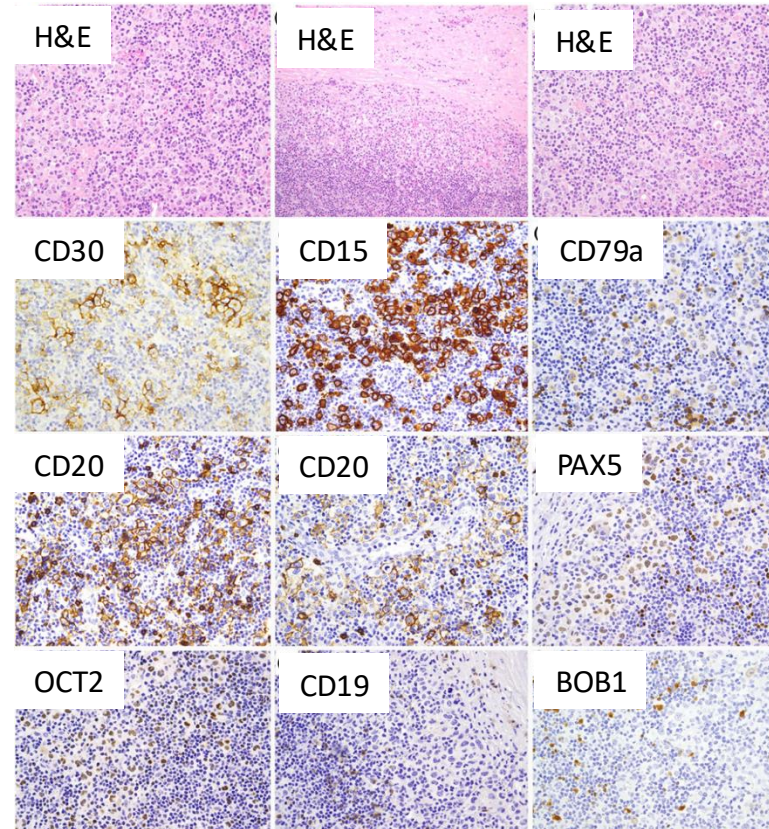
Environment: Mild to moderate inflammatory cells

Phenotype: Defective B-cell antigens (Average 3.4 markers per case). Variable intensity. Weak/moderate PAX5. CD23 and BCL6 frequently negative.



False MGZL: The Limits of Atypical Hodgkin Lymphoma

A strong isolated CD20 reactivity is NOT sufficient for the diagnosis of MGZL in the presence of CHL morphology.



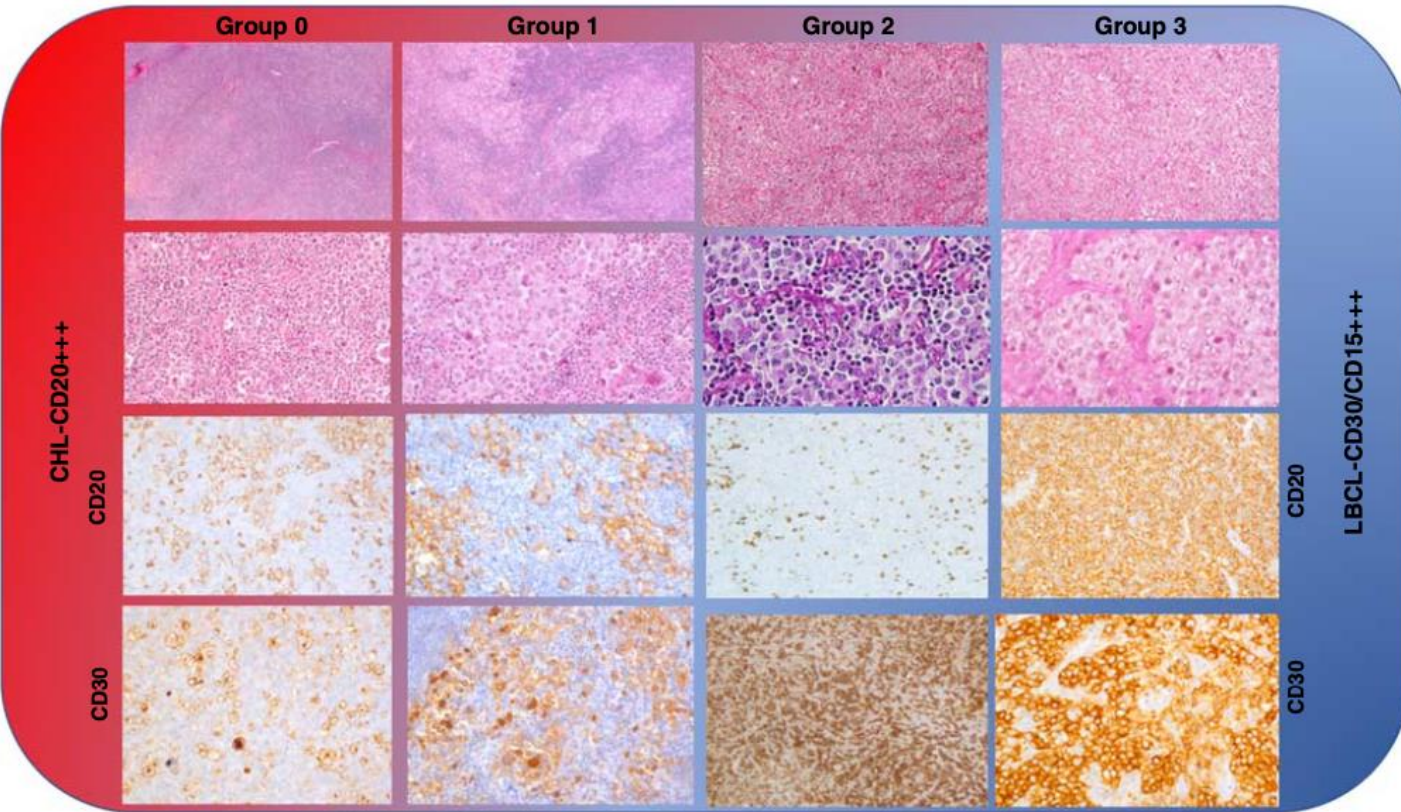
The Pitfall: Syncytial CHL lacks typical Nodular Sclerosis architecture and grows cohesively, mimicking MGZL.

The Rule: True MGZL requires diffuse positivity (>75% cells) of at least three B-cell markers (including PAX5) at strong intensity.

Another challenging issue: cases that present with massively cohesive PMBL-like morphology with variable number of HRS-like cells, minimal/absent in-inflammatory milieu and a CHL phenotype with only weak expression of PAX5. T

discordance between morphology and phenotype would well fit with the definition of MGZL, but the silent B-cell phenotype would not and would better suggest a syncytial CHL

Takeaway: In cases reclassified as CHL, the average of positive B markers was only **2.1/case**. Always apply the extended panel.



MGZL of the G0 subtype as described by Sarkozy et al

very rare cases of morphologically CHL with strong diffuse positivity of CD20,

were reported to cluster with MGZL at gene expression profiling studies /sarkozy

the significance of this bona fide subtype of MGZL is controversial at present,

further evaluation of wider series is desirable, particularly in the light of the therapeutic impact that a diagnosis of MGZL would have in the patient's management, as opposed to CHL

MGZL-PMBL cases and borders with atypical PMBL



Acceptable Deviations List

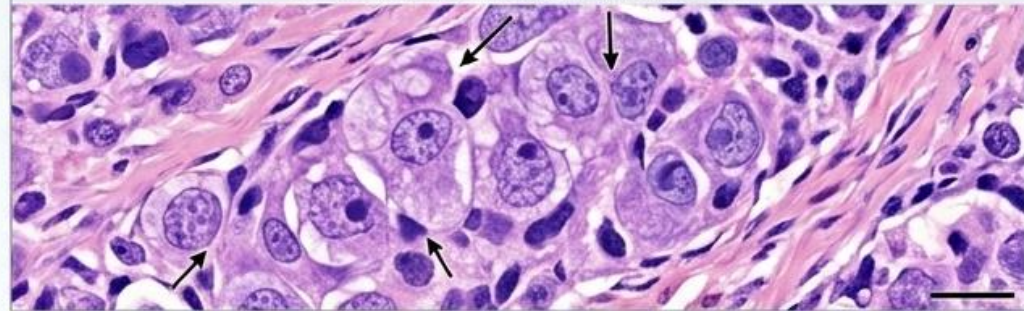
- ☐ Extensive necrosis ⚠️
- ☐ Polymorphous appearances ⚠️
- ☐ CD45 loss ⚠️
- ☐ CD20 loss (rare but documented) ⚠️
- ☐ Partial/paranuclear dot-like CD15 positivity



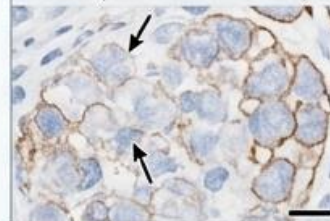
Diagnostic Confirmation Gate

When standard markers (CD30/CD20) behave aberrantly, confirm PMBL via secondary markers: CD200, MAL, PDL1/2, or Lymph3Cx gene expression assay. Do not automatically default to MGZL based on a single aberrant marker.

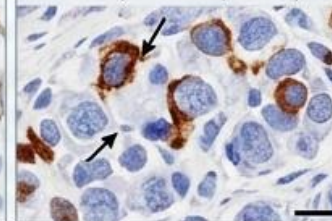
Centroblastic Cytology with Sclerosis



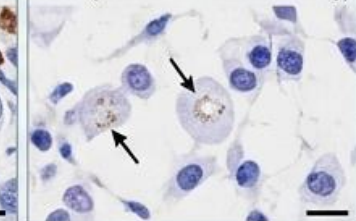
CD20 (Aberrant Loss in PMBL)



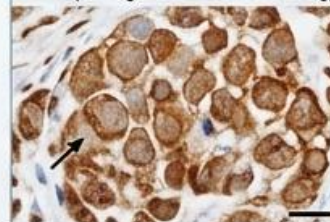
CD30 (Variable Expression)



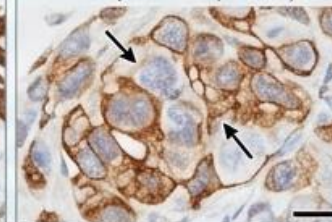
CD15 (Partial/Paranuclear Positivity)



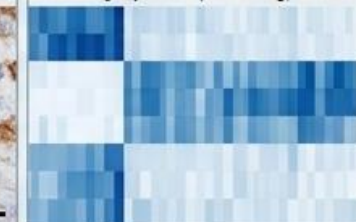
PDL1 (Strong Membranous Staining)



MAL (Positive Expression)



Lymph3Cx (PMBL-sig)



Conclusion:

- MGZL shows high variability and heterogeneity in the combination of morphology and phenotype (number of positive B- cell markers, staining intensity, percentage of positive neoplastic cells): all these features must be considered collectively, case by case,
- Apply wide set of B-cell molecules trying to adhere to the recent classifications criteria, being aware that not all cases may easily be included.
- Caution is recommended in otherwise CHL expressing only, yet strongly, CD20 or partially expressing more B antigens, as well as in syncytial/sheeting DLBCL-like lymphomas with an otherwise CHL phenotype.
- A second opinion to corroborate and/or share the diagnosis may be necessary and/or desirable.
- **Availability of adequate tissue as regards both quantity and quality is the *conditio sine qua non* for a histopathological diagnosis of MGZL: the recommendation is to refrain to make a definitive diagnosis of MGZL in small samples, requiring additional tissue and in case it is still not sufficient or not interpretable or not feasible, clearly state to resample if the clinical response is not as expected.**

