

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Clinicopathologic borderline cases in Hodgkin lymphoma

by Bálint Virga, MD

Supervisor: Árpád Illés, MD, PhD, DSc



UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF CLINICAL MEDICINE

DEBRECEN, 2025.

Clinicopathologic borderline cases in Hodgkin lymphoma

By Bálint Virga, MD

Supervisor: Árpád Illés, MD, PhD, DSc

Doctoral School of Clinical Medicine, University of Debrecen

Head of the Defense Committee: Gabriella Szűcs, MD, PhD, DSc

Reviewers: Anikó Ujfalusi, MD, PhD
László Szerafin, MD, PhD

Members of the Defense Committee: István Szegedi, MD, PhD
László Rejtő, MD, PhD

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, at 13 a.m, on 09 of April 2026.

1 Introduction

Hodgkin's lymphoma (HL) is a malignant lymphoproliferative disease of monoclonal B-cell origin most commonly presenting as painless lymph node enlargement in the neck, supraclavicular and mediastinal regions, less commonly in the subdiaphragmatic region. Majority of patients are cured thanks to modern risk and response-adapted treatment. However, about 10% of cases still have a poor prognosis, relapse early or refractory to therapy. Analysis of relapsed/refractory cases may reveal reasons for treatment failure, which may help to optimise therapeutic options.

In Hodgkin lymphoma, the second malignancy has an increased risk compared to the general population. When analyzing the therapeutic difficulties of refractory/relapsed (r/r) cases, second hematologic malignancy, as well as gray zone lymphoma, also present diagnostic and therapeutic challenges. The occurrence of second non-Hodgkin lymphomas (NHL) is rare, mostly aggressive B cell lymphomas. Their development may be explained by transformation, or by parallel differentiation of a common neoplastic progenitor cell clone, or by a completely independent oncogenesis from the primary tumour. Although their occurrence is rare, their importance is paramount as treatment of secondary NHLs is often unsuccessful. It is questionable whether the reason for the difficulty of treatment is the late recognition of NHL or the chemorefractoriness caused by the heavily pretreated transforming neoplastic clone. For these reasons, clarification of pathogenesis is also very important.

Furthermore, in refractory and early relapsing cases with unusual clinical behaviour, we should also consider transitional lymphoma. Grey zone lymphoma (GZL) is a transition between primary mediastinal B-cell lymphoma (PMBL) and nodular sclerosis type classical Hodgkin lymphoma (NS cHL), but GZL is a very rare entity with a distinct clinicopathological behaviour from the previously listed lymphomas.

The timely detection and screening of secondary NLH and grey zone lymphoma among otherwise well-cured Hodgkin lymphoma patients is crucial, as both grey zone lymphoma and NHL require treatment regimens that are quite different from those used in cHL. All these considerations highlight the importance of histological revision and rebiopsy sampling in r/r cases. The aim of our work was to search for cases of second NHL and GZL, to learn more about their pathogenesis and course, and to analyse the effectiveness of the treatment strategies used, since the available literature and experience is very poor due to the low number of cases.

In our work, we reviewed the data of cHL patients treated in our centre and collected those who were diagnosed with NHL during the course of the disease. We also reviewed patients treated for GZL at our institution. We present their pathological and clinical features, the treatment modalities used and the results of our molecular genetic studies aimed at identifying the common root of the diseases.

2 Literature review

2.1 Incidence, international data

The increased risk of developing a second malignancy in cHL patients has long been known. The cumulative incidence of secondary NHL in cHL patients treated between 1992-2009 was 2.5% (95% CI: 2.1-2.89) according to the US SEER (Surveillance Epidemiology and End Results) database. In the GHSG (German Hodgkin Study Group) between 1993-2008, the 10-year cumulative incidence of secondary NHL was 1.5 (95% CI: 1.3-1.7).

The US and German study groups detailed above excluded synchronous lymphomas in their work, and investigated secondary NHL cases that occurred sequentially. Treatment of patients with synchronous/simultaneous lymphomas is reported in the literature in the form of case reports, but precise incidence data are not available.

2.2 Association forms between the cHL and NHL

The association of cHL and NHL can be synchronous or sequential based on the temporal appearance of lymphomas. Synchronous/simultaneous lymphoma is the simultaneous appearance of at least two different types of lymphoma in a patient's body at the same time. In the case of sequential lymphomas, the different types of lymphoma appear in succession as a second or multiple malignancy, separated in time. In the case of synchronous/simultaneous lymphomas, their spatial presentation may be referred to as composite or spatially heterogeneous discordant lymphoma. In composite lymphoma, two different types of lymphoma are detected simultaneously in the same anatomical structure. In discordant lymphoma, several types of lymphoma are present simultaneously (at the same time) in different localisations in the same patient.

2.3 Etiology and pathogenesis of Secondary Non-Hodgkin Lymphoma (NHL)

The etiology of secondary non-Hodgkin lymphoma (NHL) remains unknown; however, it is presumed to be multifactorial in origin. Potential contributing factors include genetic predisposition, environmental exposures, and treatment-related toxicity. In addition, immunodeficiency associated with classical Hodgkin lymphoma (cHL) itself and/or resulting from its treatment may also play a provocative role. While treatment-related toxicity has become a less prominent factor in the development of secondary NHL with therapeutic approaches employed over recent decades, emerging evidence suggests a distinct pattern: whereas conventional chemotherapy is not typically associated with clonal evolution of cHL, such evolution has been observed even after sustained remission in patients treated with immune checkpoint inhibitors. This phenomenon is likely attributable to the immunomodulatory effects of these agents.

The occurrence of secondary NHL is rare; however, sequential development of non-Hodgkin lymphoma following classical Hodgkin lymphoma (cHL) is more commonly observed, while the reverse—development of cHL after NHL—is significantly less frequent. Among secondary NHLs, aggressive B-cell lymphomas, particularly diffuse large B-cell lymphoma (DLBCL), are most commonly identified. Sequentially occurring NHLs frequently lead to therapeutic failure and patient loss. Their manageability is adversely affected by the advanced age of patients and diminished treatment tolerance resulting from prior therapies.

In terms of pathogenesis, secondary NHLs may be considered as second primary malignancies developing independently of the initial cHL. However, in cases of synchronous/simultaneous presentation of NHL and cHL, questions regarding shared pathogenesis are warranted. Several theories offer explanations. Parallel oncogenesis may play a role, with both malignancies developing independently but concurrently. Alternatively, a common neoplastic progenitor may give rise to divergent processes early in oncogenesis, which, with the accumulation of additional somatic mutations, results in a composite lymphoma comprising both cHL and NHL components. The possibility of histologic transformation cannot be excluded either.

2.4 Recognition of Gray Zone Lymphoma (GZL) as a Distinct Entity: Lymphoma Between cHL and DLBCL

Following the discussion of cHL-NHL associations, it is important to highlight gray zone lymphoma (GZL), a rare disease with complex pathogenesis. The morphological and immunohistochemical characteristics of mediastinal GZL were first described by Traverse-Glehen in 2005. The World Health Organization (WHO) provisionally recognized it in 2008 as

a separate entity: "B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma (cHL)." The name reflects its diagnostic ambiguity, marking it as a transitional entity between cHL and DLBCL.

In the 2008 WHO classification, GZL was divided into two clinically distinct groups: primarily mediastinal presentations (mediastinal GZL, MGZL) and disseminated forms with or without mediastinal involvement (non-mediastinal GZL, NMGZL). MGZL typically affects younger individuals in early stages and often presents with bulky disease. NMGZL more often occurs in older patients and is characterized by bone marrow and (multiple) extranodal involvement.

2.5 MGZL as a Separate Entity: Lymphoma Between PMBL and NS cHL – WHO 2022 Classification

The 2022 WHO classification introduced important changes. Based on epigenetic profiling and DNA methylation and mutational analyses of tumor cells, a new definition was established. The previously used GZL category was discontinued, and MGZL was defined as a distinct group. Cases previously classified as NMGZL are now considered diffuse large B-cell lymphomas, not otherwise specified (DLBCL-NOS).

2.6 Epidemiology

Due to its rarity, the precise incidence of gray zone lymphoma is difficult to estimate. At the time of writing of this thesis, no accurate epidemiological data are available for MGZL, owing to the novelty of its classification and the rarity of the disease. However, epidemiological features suggest that MGZL primarily affects young males and children, with a male-to-female ratio of 1.4:1, differing significantly from PMBL and nodular sclerosis-type cHL (NS cHL), which show a female predominance. The mean age at diagnosis ranges from 32 to 37 years, similar to cHL and PMBL.

2.7 Treatment of MGZL

No standardized treatment guidelines exist for MGZL. Literature suggests that first-line treatment protocols used for DLBCL are more effective than the cHL standard ABVD regimen (doxorubicin, bleomycin, vinblastine, and dacarbazine). Dose-intensified protocols may further

improve outcomes. Nonetheless, treatment results remain inferior compared to those seen in PMBL and cHL. For relapsed/refractory (r/r) patients, high-dose chemotherapy followed by autologous hematopoietic stem cell transplantation (AHSCT) offers the best outcomes. Based on dominant tumor antigen expression, monoclonal antibody and immunotherapy targeting CD20, CD79, CD30, or PD-1 are gaining increasing support.

3. Objectives

1. To investigate the relationship between cHL and NHL, and the patterns of association observed in patients under our care, with the goal of deepening our understanding of disease progression and evaluating diagnostic and therapeutic approaches.
2. To assess the incidence, histological types, and prognosis of synchronous and sequential NHLs among patients treated for cHL.
3. To collect clinicopathological data indicating possible transformation of classical Hodgkin lymphoma into non-Hodgkin lymphoma.
4. To identify all cases of gray zone lymphoma treated at our institution, evaluate the outcomes of applied treatment strategies, develop best clinical practices, and define the role of novel immunotherapies in the management of GZL.

4. Patients and Methods

We retrospectively reviewed the records of patients diagnosed with Hodgkin lymphoma at the Department of Hematology, University of Debrecen, between January 2011 and December 2020, using the e-MedSolution medical database. We identified cases where NHL developed synchronously or sequentially in relation to a prior diagnosis of cHL. Clinical characteristics, disease course, treatment protocols, and outcomes were analyzed. All available histological samples were re-evaluated by an experienced hematopathology team at the Department of Pathology, University of Debrecen. In cases where sufficient tissue was available, clonality and lymphoid next-generation sequencing (NGS) studies were performed. The lymphoid NGS analysis utilized the Archer FusionPlex Lymphoma Panel, a targeted NGS approach designed to detect 125 gene fusions, point mutations, and gene expression levels commonly associated with lymphoid malignancies, using Anchored Multiplex PCR (AMP™) enrichment. Genomic RNA was isolated from formalin-fixed, paraffin-embedded (FFPE) tissue samples, and sequencing was conducted using the Illumina platform. Data analysis was performed using Archer's analysis software (Archer, Boulder, CO). Clonality testing included IgHV

rearrangement analysis across three framework regions (FR1, FR2, FR3), based on the Biomed II protocol.

To identify cases of gray zone lymphoma, we reviewed the institutional registries of Hodgkin lymphoma and DLBCL patients treated between 2008 January 1 and 2018 December 31 applying the 2008 WHO classification criteria. We identified nine patients who fulfilled the histopathological definition of GZL and had complete clinical and tissue data. Two additional cases were excluded due to insufficient tissue or incomplete clinical records. Both primary and relapse-diagnosed GZL cases were included. GZL diagnosis was established using morphological and immunophenotypic criteria outlined in the 2008 WHO classification.

The study was retrospective and based on clinical data obtained during standard patient care and diagnostic procedures. Histological analyses were conducted at the University of Debrecen's Department of Pathology using FFPE tissue from excisional or core needle biopsies. Immunohistochemistry included markers relevant to Hodgkin and B-cell non-Hodgkin lymphomas: CD3, CD4, CD8, CD15, CD20, CD30, LCA, LMP1, Mib-1, MUM1, OCT-2, PAX-5, and PD-L1. All procedures followed standard protocols using a Leica BondMax automated system.

5. Results

5.1 Findings in cHL patients with secondary NHL

Between 2011 and 2020, 164 patients were treated for cHL at our institution. Among them, secondary NHL was diagnosed in 6 patients (3.65%). The median age was 43.5 years (range: 20–68), and two-thirds were female. Histologically, one case was classified as high-grade B-cell lymphoma (HGBCL), and the remaining five as post-germinal center origin DLBCL. In three patients, the NHL diagnosis was made following successful treatment and at least 18 months of complete remission from cHL. The other three patients had primary refractory disease. Two clinical patterns emerged: one group consisted of relapsed patients following initial remission, including two early (within 1 year) and one late (nearly 5 years post-treatment) relapse. The other group comprised patients with primary refractory disease, who never achieved remission; persistent tumor mass was radiologically detectable throughout treatment. Re-biopsy confirmed NHL in these refractory cases.

5.1.1 Clonality Study Results

PCR-based IGH gene rearrangement testing was performed in one patient (68 year old woman), revealing identical patterns in both cHL and DLBCL, indicating clonal relationship. In other cases, NGS analysis was conducted, but insufficient tissue precluded PCR confirmation.

NGS was evaluable in 4 out of 5 tested patients. Clonality was established in one patient (20 year old woman), not between cHL and HGBCL samples, but between an initial reactive lymph node (later reclassified as cHL) and HGBCL tissue, both showing NOTCH2 and CCND3 mutations. The reclassified lymph node biopsy also exhibited HRS cells, though no additional cHL-confirming sample was available. Overall, a clonal relationship between cHL and HGBCL is likely in this case.

5.2 Gray Zone Lymphoma Findings

Between January 1 of 2008, and December 31 of 2018, nine GZL cases were identified through histologic review or primary diagnosis. During this period, 257 cHL and 423 DLBCL patients were newly diagnosed at our center. Six patients were female, three male, with a median age of 50.2 years (range: 17–80). Seven patients presented at advanced stage; five had bone marrow involvement at diagnosis. Pure mediastinal localization was not observed. Among the two early-stage cases, one had both mediastinal and cervical involvement; the other exhibited widespread supradiaphragmatic disease in addition to mediastinal involvement. These were classified as MGZL; the others were NMZL. B symptoms were common, while bulky disease and extranodal involvement were less frequent. Diagnosis was based on HRS-like morphology and immunoreactivity for CD30 and MUM1 (IRF4). Immunophenotypes were heterogeneous. In three patients, GZL was diagnosed at presentation; in three others, diagnosis followed histological revision prompted by unusual clinical behavior. The remaining three were diagnosed upon re-biopsy during disease progression or relapse. In one primary refractory case initially diagnosed as DLBCL, and in two relapsing cHL patients (relapse at 78 and 35 months, respectively), repeat biopsy revealed features intermediate between cHL and DLBCL. Immunohistochemical analysis showed CD20-negativity in 3/9 cases; CD15 and/or CD30 and MUM1 positivity were universal. EBV association (LMP1 and EBER in situ hybridization) was negative in all samples. PD-L1 expression was strongly positive in 6/9 cases, and negative in 3/9.

Initial treatment included R-CHOP in six cases and ABVD in three. In one ABVD case, treatment was switched to R-CHOP after the first cycle due to histologic revision. In two cases, ABVD was completed as originally planned; initial diagnosis was cHL, with GZL only identified upon relapse. Six patients received rituximab in combination with CHOP, and one received brentuximab vedotin with CHOP due to CD30 positivity and unfavorable prognosis. This patient achieved complete metabolic remission after three cycles, confirmed by PET/CT at treatment completion, and continues on brentuximab vedotin maintenance in full remission. Initial treatment responses in cHL-diagnosed patients were complete, but remission was less durable (relapse at 7 and 39 months). Six patients had primary chemo-refractory disease. Most relapsed/refractory patients were treated with R-DHAP salvage therapy, followed by R-BEAM conditioning and AHST (5/9 patients). One transplant patient died due to comorbidities and poor clinical status. One relapsed post-AHST and received local irradiation. An elderly, transplant-ineligible female patient received rituximab plus gemcitabine. One young male, refractory to DHAP, was treated with brentuximab vedotin and bendamustine without response and ultimately died despite nivolumab therapy. The median overall survival for the entire cohort was 60.5 months (range: 8–138); among transplanted patients, it was 96.2 months (range: 21–138).

6 Discussion

6.1 Our experience with secondary non-Hodgkin lymphoma (NHL) among classical Hodgkin lymphoma (cHL) patients

We observed secondary NHL in 3.65% of our cHL patients, a frequency exceeding reported international rates. This may be explained by two factors, on one hand our intentional identification of cases where NHL diagnosis occurred during cHL treatment or early relapse, aiming to better understand clonal relationships and synchronous oncogenesis (a group often excluded from international studies), and on the other hand as a hematologic referral center, we treat numerous refractory, relapsed, and transplant-eligible patients, who are more likely to develop secondary malignancies.

In our cohort, secondary NHLs were most frequently aggressive B-cell lymphomas, predominantly DLBCL. Based on our observations, age was not a decisive factor for poor prognosis, as young patients also exhibited rapidly progressive disease. Treatment resistance and selection of therapy-resistant clones were clearly documented in our cases.

Among our six patients, three were diagnosed with secondary (sequential) NHL, all female, with only one over 65 years of age. In this elderly patient, IGH rearrangement confirmed clonality between the two lymphomas, suggesting that immune deficiency related to cHL and selection pressure from treatment together facilitated DLBCL development. Surgical excision of a solitary lesion led to long-term disease-free survival. In another 48-year-old female patient, no clonality analysis was performed, but DLBCL histology suggested transformation from cHL. She was refractory to conventional chemotherapy but responded with durable remission to the PD-1 inhibitor nivolumab. A subsequent nodal relapse, nearly two years later, confirmed DLBCL, indicating likely clonal evolution during PD-1 inhibitor therapy. In the third relapsed female patient, NHL likely represented a second independent malignancy. The other three cases appeared as synchronous/discordant lymphomas based on retrospective analysis; NHL was likely present at initial cHL diagnosis. While clonal relationships between co-occurring histological types may exist, they are not obligatory and may arise independently. In a 20-year-old female patient, synchronous disease was supported by the presence of NOTCH2 and CCND3 mutations in both the initially removed reactive lymph node (reclassified as cHL) and subsequent HGBCL tissue.

In summary, our cohort included all patterns of cHL–NHL association. We observed transformation of cHL into NHL in both synchronous and sequential presentations. Both variants proved difficult to treat, with synchronous cases associated with worse prognosis. Post-NHL survival was 2–4 months in synchronous cases, compared to 12–27 months for sequential cases. Poor tolerance due to prior therapies and intrinsic therapy resistance—potentially driven by clonal evolution and drug resistance—were likely contributors, while age was not prognostically relevant. In future, liquid biopsy approaches, such as circulating tumor DNA (ctDNA) analysis, may overcome spatial heterogeneity of lymphomas and enable earlier detection of secondary processes or clonal evolution. This requires standardization of NGS protocols in cHL and prospective studies on larger patient cohorts.

6.2 Gray Zone Lymphoma – Clinical Experience

Managing patients with gray zone lymphoma (GZL) is a true clinical challenge from diagnosis through treatment. Atypical histological features or clinical behavior must raise suspicion, prompting clinicopathological review, and where necessary, re-evaluation of diagnosis and therapy. More aggressive treatment regimens and DLBCL-based protocols appear more effective. While the efficacy of rituximab remains uncertain, it may be beneficial

in CD20-positive cases. Brentuximab vedotin (BV) may play a critical role, and its use as part of first-line therapy in CD30-positive cases should be considered. For relapsed/refractory patients, BV offers clear advantages. In patients with poor general condition, BV maintenance may deepen partial remission or help consolidate response post-AHSCT, as in cHL.

Autologous stem cell transplantation (AHSCT) remains vital in relapsed/refractory cases, but its role as frontline consolidation remains under investigation. PD-1 inhibitors were used successfully in relapsed/refractory cases, including in combination with BV, with favorable outcomes. In elderly or frail patients, they may serve as alternatives to transplantation.

In conclusion, GZL treatment still presents many unanswered questions due to its rarity. Prospective, multicenter clinical studies are needed to better understand its pathobiology and clinical features, and to develop more effective treatment strategies.

7 Novel Findings

1. A clonal relationship may exist between Hodgkin lymphoma and a sequential or synchronously developing non-Hodgkin lymphoma.
2. Synchronous and sequential NHLs may develop during or after cHL treatment via transformation of the primary Hodgkin lymphoma.
3. Secondary NHLs in cHL patients are most frequently aggressive B-cell malignancies (DLBCL, HGBCL).
4. NHLs in cHL patients, particularly those resulting from synchronous oncogenesis, have poor prognosis, are refractory to combination chemotherapy, and exhibit short post-NHL survival.
5. In patients with Hodgkin lymphoma, histopathological re-evaluation or re-biopsy is warranted in cases of unusual clinical behavior and/or markedly elevated focal SUV_{max} on PET/CT imaging, in order to exclude grey zone lymphoma or a synchronous non-Hodgkin lymphoma.
6. Anti-CD30 treatment combined with CHOP-based chemotherapy in GZL patients results in effective long-term remission.
7. AHSCT in remission achieved with CHOP + anti-CD20/CD30 therapy offers a potential cure for GZL patients.

8 Acknowledgements

I would like to express my sincere gratitude to Professor Árpád Illés for introducing me to the world of hematology and scientific research, and for his patience, guidance, and motivation.

Special thanks to Professor Gábor Méhes for his contribution to the histopathological and molecular diagnostics that formed the foundation of this study.

I am deeply grateful to László Pinczés for his mentorship, support, and valuable advice from the beginning of my clinical training.

Many thanks to Zsófia Simon for her work, dedication, and patience throughout this project. I would also like to thank Zsófia Miltényi and Ferenc Magyarai for their constructive insights.

Finally, I am thankful to all my colleagues at the Department of Hematology, my co-authors, and my family for their support, understanding, and patience.

9 References



Registry number: DEENK/436/2025.PL
Subject: PhD Publication List

Candidate: Bálint Virga
Doctoral School: Doctoral School of Clinical Medicine

List of publications related to the dissertation

1. **Virga, B.**, Pinczés, L. I., Illés, Á., Miltényi, Z., Magyar, F., Méhes, G., Simon, Z.: Occurrence of Secondary Non-Hodgkin Lymphomas Among Our Classical Hodgkin Lymphoma Patients: a Single-Centre Experience.
Cureus. 16 (6), 1-10, 2024.
DOI: <http://dx.doi.org/10.7759/cureus.63307>
IF: 1.3
2. Simon, Z., **Virga, B.**, Pinczés, L. I., Méhes, G., Miltényi, Z., Barna, S., Szabó, R., Illés, Á.: Transition Between Diffuse Large B-Cell Lymphoma and Classical Hodgkin Lymphoma- Our Histopathological and Clinical Experience With Patients With Intermediate Lymphoma.
Pathol. Oncol. Res. 27, 1-7, 2021.
DOI: <http://dx.doi.org/10.3389/pore.2021.625529>
IF: 2.874

List of other publications

3. Virga, I., Páyer, E., Barna, S., Bedekovics, J., Váróczy, L., **Virga, B.**, Nagy, Z., Illés, Á., Magyar, F.: Ritka lokalizációjú plasmocytoma autológ hemopoetikus őssejtátültetést követően.
Hematol. Transzfuziol. 56 (2), 93-98, 2023.
DOI: <http://dx.doi.org/10.1556/2068.2023.00173>

Total IF of journals (all publications): 4,174

Total IF of journals (publications related to the dissertation): 4,174

The Candidate's publication data submitted to the Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

03 July, 2025

