Pathology review identifies frequent misdiagnoses in recurrent classic Hodgkin lymphoma in a nation-wide cohort: implications for clinical and epidemiological studies


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Pathology review identifies frequent misdiagnoses in recurrent classic Hodgkin lymphoma in a nation-wide cohort: Implications for clinical and epidemiological studies

Running title:
Misdiagnosis in recurrent classic Hodgkin lymphoma

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MB, LK, DdJ: Study design, pathology review, writing of manuscript.
MS: Study design, statistical analysis, manuscript review
AD: Study design, data collection Netherlands Cancer Registry, manuscript review
NH: immunohistochemical staining and molecular tests, manuscript review
EvdB: PALGA search, manuscript review
FvL: Study design, manuscript review
KvdO: Manuscript review
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ABSTRACT

Patients treated for classic Hodgkin lymphoma (CHL) have a reported 13-fold increased risk of developing subsequent non-Hodgkin lymphoma (NHL). In light of the growing awareness of CHL mimickers, this study re-assesses this risk based on in-depth pathology review of a nationwide cohort of patients diagnosed with CHL in the Netherlands (2006-2013) and explores the spectrum of CHL mimickers.

Among 2,669 patients with biopsy-proven CHL, 54 were registered with secondary NHL. On review, CHL was confirmed in 25/54 patients. In six of these, subsequent lymphoma concerned primary mediastinal B-cell lymphoma/mediastinal grey zone lymphoma, biologically related to CHL and 19/25 were apparently unrelated B-NHL. In 29/54 patients, CHL was reclassified as NHL, including T-cell lymphomas with secondary Hodgkin-like B-blasts (n=15), EBV+ diffuse large B-cell lymphoma (n=8), CD30+ T-cell lymphoma (n=3) and indolent B-cell proliferations (n=3). Higher age, disseminated disease at presentation, extensive B-cell marker expression and EBV-association were identified as markers to alert for CHL mimickers. Based on these data, risk to develop NHL after CHL treatment was re-calculated to 3.6-fold (standardized incidence ratio 3.61; CI 2.29-5.42). In addition, this study highlights the clinicopathological pitfalls leading to misinterpretation of CHL and consequences for individual patient care, interpretation of trials and epidemiological assessments.
INTRODUCTION

Treatment success in classic Hodgkin lymphoma (CHL) patients has resulted in a high long-term survival rate. On the downside, these patients also have a high risk of developing treatment-related secondary cancers. As part of a large epidemiological cohort study, Schaanveld et al. showed that patients treated for CHL who survived for five years or longer have a 13-fold increased risk of secondary non-Hodgkin lymphoma (NHL). A very recent study based on SEER data further showed an increased bidirectional risk of NHL and CHL, especially between CHL and peripheral T-cell lymphoma (PTCL) and between CHL and diffuse large B-cell lymphoma (DLBCL). The increased risk of secondary NHL in CHL patients may be explained by (late) treatment-related toxicity, genetic predisposition, or coincidental (low-grade) NHL diagnosed due to routine long-term follow-up in these patients. More focus has been given to the complex differential diagnosis of CHL in the past few years, and evolving insights may likely impact epidemiological aspects such as secondary cancer risk.

The diagnosis of CHL is defined by a set of typical clinical, morphological, and immunophenotypical criteria. In contrast to various other types of malignant lymphoma, the criteria for the diagnosis of CHL have largely remained unchanged since its introduction in the REAL classification in 1994 up to the latest World Health Organization (WHO) classification. Over the past years, the spectrum of grey zones and mimickers surrounding CHL has become better recognized, leading to a refinement of the diagnostic category of true CHL. This has consequences for routine patient management and clinical trials and the interpretation of previously published data on epidemiology, such as secondary NHL risk.

Mediastinal grey zone lymphoma (MGZL) is now recognized as biologically related to both CHL and primary mediastinal B-cell lymphoma (PMBL); they share morphological and immunophenotypical features with both CHL and PMBL and form a disease spectrum. Relapse of CHL within this biological spectrum may account for at least a proportion of secondary NHL.
Various other NHL are increasingly recognized as CHL mimickers, especially EBV+ proliferations with Hodgkin-like cells that typically have varying expression of B-cell markers. This is most widely described in angioimmunoblastic T-cell lymphoma (AITL) and the related peripheral T-cell lymphomas with follicular T-helper cell phenotype (PTCL-TFH), while immunodeficiency related B-lymphoproliferative disorders (ID-BLPD) across various immunodeficiency settings may likewise deceptively mimic CHL. Increased awareness and recognition of these entities underscore the challenging differential diagnosis of CHL, especially in EBV-positive cases. As a result, cases diagnosed as CHL in the past may be interpreted differently today.

This study reports the spectrum and incidence of secondary NHL in patients treated for CHL, based on a nationwide, population-based cohort of CHL patients diagnosed in the Netherlands between 2006 and 2013. We re-assessed the risk of secondary NHL after pathology review and suggest clinicopathological clues that may help avoid misdiagnosis in challenging cases.

METHODS

Study design and patients

To collect an unbiased, population-based cohort of CHL patients with sufficient follow-up time for developing subsequent NHL and covering CHL patients with relevant CHL treatment and “modern” diagnostic criteria for CHL, all patients diagnosed with Hodgkin lymphoma between 2006 and 2013 in the Netherlands were identified in the Netherlands Cancer Registry (NCR) and linked to the nationwide network and registry of histo- and cytopathology (PALGA). Both the NCR and PALGA have a nationwide coverage of all cancer diagnoses and pathology reports issued in the Netherlands. Next, all pathology reports on these patients filed between 1989 and September 2019 and listing any lymphoma (differential) diagnosis were retrieved and manually curated to select primary diagnoses of CHL between 2006-2013 only. As this study focuses on diagnostic problems and secondary cancer risk in CHL, patients with an initial pathology diagnosis of nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) were excluded. All patients with one or more reported NHL diagnoses after a
reported CHL diagnosis were identified and available pathology material of both episodes was requested from the original pathology laboratories for central pathology review. The study protocol was approved by the medical ethical review committee of the VU medical center (METc 2018.556) and the PALGA Scientific Committee to comply with the EU General Data Protection Regulation (GDPR).

**Pathology review**

Both CHL and NHL diagnoses were reviewed by three hematopathologists (MB, LK, DdJ) according to a previously reported algorithm (Figure 1). In case of discrepancy between the reviewers, the case was discussed and a consensus diagnosis was reached. In brief, CHL was considered confirmed in cases with a fully consistent clinical presentation, morphology and immunophenotype. In cases of deviation in any primary criterion, additional studies for pertinent differential diagnoses were performed (Table S1), including immunohistochemistry (IHC), T-cell receptor beta and gamma (TCR) and/or immunoglobulin heavy (IGH) and kappa (IGK) light chain rearrangement assays (BIOMED-2; InVivoScribe, San Diego, USA). Next, in those cases suspected for AITL or PTCL-TFH without conclusive clonal TCR rearrangement, targeted panel next-generation sequencing (NGS) including RHOA, TET2, DNMT3A, IDH2 and CD28 was performed using Ion Torrent (ion Ampliseq™; Thermo Fisher Scientific, Waltham, USA) as used for routine diagnostic purposes in our laboratory. If specific diagnostic criteria for CHL according to the 2016 WHO classification were not met, the original CHL diagnosis was rejected in favor of an alternative diagnosis. Those cases that were highly suspicious for a diagnosis other than CHL, but in which tissue exhaustion or poor DNA quality precluded interpretation of additional studies, were classified as "highly suspicious" for this diagnosis. In the remaining cases, a CHL diagnosis was maintained.

Secondary NHL diagnoses of all patients were reviewed according to WHO Classification 2016 criteria. In cases where a relationship between the primary and secondary lymphoma diagnoses was
suspected, additional IHC, in-situ hybridisation or molecular studies were performed to either substantiate or disprove such a relationship.

Statistical analysis

For risk calculations, calculation of the expected incidence of NHL was based on age-, sex-, and calendar period-specific cancer incidence rates in the Dutch population, multiplied by the corresponding number of person-years at risk during follow-up. Standard methods were used to compute the standardized incidence ratios (SIRs) and corresponding 95% confidence intervals with correction for the duration of follow-up. Relation between review diagnosis category, age at diagnosis and disease stage were tested with ANOVA and Fisher exact tests, respectively, using SPSS (IBM, version 27).

RESULTS

Study population

In the NCR, 2,969 patients were identified with a primary HL diagnosis between 2006 and 2013. Linkage to the PALGA database was successful in 99.7% (2,959 patients) and a total of 12,923 complete pathology reports were manually curated. Of these, a CHL diagnosis was listed in 2,669/2,959 (90.2%) patients. The remaining 290/2,959 (9.8%) patients were excluded due to the lack of a confirmed CHL diagnosis (Figure 2). In 54/2,669 CHL patients (2.0%), an NHL diagnosis after CHL was listed, with subsequent NHL recurrence or transformation/progression in eleven of these (Figure 2). The cohort of 2,615 CHL without subsequent NHL served as a control for clinical-pathological and risk assessment evaluations.

Both pathology slides and sufficient FFPE material were available for 43/54 CHL cases and 46/54 subsequent NHL cases. For 6/54 primary CHL and 5/54 of subsequent NHL, only pathology slides were available. For the remaining 5/54 primary CHL and 3/54 subsequent NHL, no slides or FFPE tissue was available and review was based on detailed pathology reports only.
Pathology review

Clinical features and pathology characteristics at review are listed in tables 1 and S1. In 25/54 cases (46%), the primary CHL diagnosis was confirmed. In 24/54 cases (44%), criteria were met for another diagnosis and a diagnosis of CHL was rejected (Figure 3). Indeed, 10 of these were recognized as part of expert consultation at the time of initial diagnosis, but after start of treatment (n=2) or at retrospective review as part of the diagnostic workup at the time of subsequent NHL diagnosis (n=8). In 5/54 cases (9%), the primary diagnosis was highly suspicious for NHL; however, no definite immunohistochemical or molecular criteria could be added to refine the diagnosis, mainly due to exhaustion of FFPE tissue or poor DNA quality leading to unreliable clonality or NGS results. These were classified as highly suspicious for NHL, and in three of these cases, the likelihood that the original diagnosis was NHL was already recognized during follow-up after CHL treatment.

The spectrum of CHL and PMBL

Six patients covered the spectrum of CHL-MGZL-PMBL with five PMBL and one MGZL “relapse” with an interval of 6 to 70 months after initial CHL. CHL in this group was marked by varying strong and/or heterogeneous expression of CD20 and/or CD79a in Hodgkin-type cells. Clonal relation could be confirmed in one case using immunoglobulin rearrangement assays (#34). Case 35 showed a first relapse as MGZL (interval 64 months) and a second relapse as CHL (interval 21 months). Reversal of EBV-status from EBV+ CHL to EBV- PMBL was observed in case 33 (interval 6 months).

CHL with secondary B-cell lymphoma

Eighteen patients with confirmed CHL diagnosis developed secondary B-cell lymphomas other than PMCL or MGZL. These included plasmacytoma (n=2), small B-cell lymphoma with plasmacytoid differentiation including nodal marginal zone lymphoma (NMZL) and lymphoplasmacytic lymphoma (LPL; n=3), primary cutaneous follicle center cell lymphoma (n=1), follicular lymphoma (FL; n=5),
DLBCL, NOS (EBV negative, n=5), high-grade B-cell lymphoma with MYC, BCL2 and BCL6 translocation (HGBCL, TH; n=1), and B-cell acute lymphoblastic leukemia (B-ALL; n=1).

In three of these cases, the indolent B-cell lymphoma could be recognized in retrospect as a composite lymphoma in the primary CHL presentation (#37, #38, #39).

Patient #36 underpins the complex disease course observed in some of these patients. Twelve years after EBV+ CHL, this patient presented with EBV+ mononucleosis-like lymphoid hyperplasia, followed by EBV+ DLBCL (marked by sheets and individual dispersed strong and uniform CD20+ large cells with varying features of Hodgkin-like cells and proven clonal IGH-rearrangement) one year later.

**T-CELL LYMPHOMA WITH SECONDARY EBV-POSITIVE HODGKIN-LIKE CELLS**

In eleven patients, CHL diagnosis could in retrospect be recognized unequivocally as T-cell lymphoma with Hodgkin-like cells, mostly EBV+ and were classified as AITL (n=7), PTCL-TFH (n=1) and PTCL-NOS (n=3). Additionally, in patient #12, a primary diagnosis of CHL could unequivocally be refuted. EBV+ DLBCL was diagnosed with a dense T-cell infiltrate highly suspicious for underlying T-cell lymphoma, which could not be unequivocally substantiated. Review diagnoses were based on standard morphological and immunohistochemical criteria, including aberrant T-cell marker loss (n=3), clonal TCR rearrangement (n=6) or both (n=2). In total, eight of 12 patients relapsed as T-cell lymphoma, four developed subsequent EBV+ DLBCL and one EBV- DLBCL.

**Highly likely review diagnosis of T-cell lymphoma**

In three additional patients, the primary CHL diagnosis was highly suspicious for T-cell lymphoma based on clinical, morphological and immunohistochemical criteria. However, insufficient quality or unavailable biopsy material precluded further IHC or molecular studies for a definite diagnosis and these cases were termed equivocal between CHL and PTCL with a preference for PTCL.
**EBV-POSITIVE DLBCL MIMICKING CHL**

At review, six primary CHL diagnoses were unequivocally recognized as EBV+ DLBCL according to the current WHO Classification and further specified according to EAHP-SH 2015 nomenclature.\(^{15}\) Review diagnosis of EBV+ DLBCL was based on a polymorphous population of small EBER+ lymphoid cells and EBER+ Hodgkin-like cells with a complete B-cell immunophenotype and/or light chain expression (n=5), an overt immunodeficiency setting (HIV, methotrexate) as listed in the primary pathology reports (n=1), or both (n=1). There was no clonal TCR rearrangement or T-cell marker loss in any of these. Four patients later developed recurrence, one HIV+ patient (#17) developed subsequent EBV-DLBCL, likely also immunodeficiency related\(^{6}\) and one patient (#19) developed an EBV-negative indolent B-cell lymphoma (differential diagnosis NMZL/LPL).

**Equivocal review diagnosis of EBV+ DLBCL**

In two cases, primary CHL diagnosis was highly suspicious for EBV+ DLBCL. Patient #22 presented with isolated cerebral localization with EBER+ Hodgkin-like cells nine months after the initial disease episode. No tissue was available for additional studies. Patient #23 showed an EBER+ Hodgkin-like proliferation with complete B-cell phenotype but lacking information on CD79a, while subsequent EBV+ DLBCL diagnosis could unequivocally be made.

**CD30+ T-CELL LYMPHOMA MIMICKING CHL**

In three patients, the primary CHL diagnosis was recognized as CD30+ T-cell lymphoma on review, lacking defining B-cell lineage markers while expression of T-cell markers was confirmed. Two cases were classified as ALCL, ALK-, and one case could be recognized as regional lymph node involvement of mycosis fungoides (MF), histologically confirmed in a skin lesion biopsy 83 months later.
IMMUNOBLASTS MISTAKENLY INTERPRETED AS HODGKIN-CELLS

In three patients, CD30+ reactive immunoblasts were likely misinterpreted as CHL in cases of B-cell chronic lymphocytic leukemia (B-CLL, #27) and FL (#28), relapsing as such. Case #29 presented with reactive plasma cell hyperplasia and subsequent presentation as indolent B-cell lymphoma (differential diagnosis NMZL/LPL) 13 months later.

IMMUNOPHENOTYPE OF HODGKIN(-LIKE) CELLS

Compared to Hodgkin-type cells in confirmed CHL diagnoses, in CHL cases that on review were recognized as NHL, Hodgkin-like cells were significantly more frequently positive for CD20 (16/29 vs 3/24; p=0.001) and EBER (20/28 vs 4/22; p<.001) and significantly less often positive for CD15 (10/27 vs 19/23; p=0.002). Differences in CD79a expression were not significant (14/27 vs 7/23) and PAX-5 was positive in all cases with varying expression (excluding CD30+ T-cell lymphomas). Details regarding staining intensity are shown in table S1.

CLINICAL-PATHOLOGICAL CORRELATIONS

Patients recognized as AITL/PTCL were significantly older at initial lymphoma presentation with significantly more advanced disease stage compared to those with confirmed CHL and those without secondary NHL (median 49 vs 36 years, p=0.032; 80% stage III/IV vs 41.7% stage III/IV; p=0.003). This was not the case in patients recognized as EBV+ DLBCL (median 39.5 years, p=0.473; 38% stage III/IV, p=0.483; table 1). Of note, patients without subsequent NHL, but with relapsing CHL (n=289) also presented significantly more often with advanced disease stages (62% (177/289) stage III/IV) compared to patients without relapse (39% (908/2326) stage III/IV; p<0.01).

Interestingly, the initial CHL diagnosis was more often of the mixed cellularity subtype in cases recognized on review as misdiagnosis (28% (8/29) vs. 10% (256/2640) of confirmed/unreviewed CHL diagnoses). The same held true for the lymphocyte rich subtype (10% (3/29) vs. 3% (88/2640)). The nodular sclerosis subtype however was more prevalent in the group of confirmed/unreviewed
diagnoses with 59% (1531/2640) vs. 28% (8/29) in cases recognized as misdiagnosis. These findings were statistically significant (p<0.01). The remaining cases were either classified as “not otherwise specified” or lacked subclassification with 34% in misdiagnoses (10/29) vs 28% in unchanged CHL diagnoses (727/2640; not significant).

Cases recognized in retrospect as mimickers were evenly spread throughout the period of primary CHL diagnosis (2006-2013). No significant differences were noted in the interval time between the primary and secondary lymphoma episodes for confirmed CHL and mimickers.

**RISK FOR SECONDARY NHL AFTER CHL**

Based on the present selection of cases and original pathology diagnoses, risk calculations show a SIR of developing NHL after CHL of 7.79 (5.78-10.3). Based on diagnoses after pathology review, SIR was significantly lower at 4.39 (CI 2.92-6.35; p=0.015) when still including the equivocal cases (highly likely misdiagnoses) as CHL, and 3.61 (CI 2.29-5.42; p=0.002) when also excluding these equivocal cases. In these calculations, the three patients with composite CHL/NHL and recurring NHL were not included as CHL patients with subsequent NHL. It should be noted that the 2615 CHL patients without subsequent CHL diagnosis were not subjected to in-depth pathology review. As the *a priori* rate of misinterpretation is deemed very low, any misdiagnoses in these patients would therefore result in potential minor underestimation of SIRs.24

**DISCUSSION**

The WHO Classification of lymphoma is dynamic and continuously incorporates novel insights into lymphoma biology, which in turn impacts classification. As a result, various cases that may have previously fulfilled the diagnostic criteria for CHL may be diagnosed differently today.

We initiated this study to evaluate whether the previously reported 13-fold increased risk of NHL arising as a second malignancy in patients treated for Hodgkin lymphoma, could be substantiated based on the most current WHO classification.2 The present study found that in patients diagnosed
with CHL between 2006-2013 with reported secondary NHL, 44-54% of CHL diagnoses were classified as NHL according to current WHO criteria. Next, these patients actually presented with relapse or transformation of this NHL in the second episode. As a consequence of reclassification in the present study, the previously reported 13-fold risk to develop NHL as a secondary malignancy dropped significantly to a SIR of 3.61-4.39. Although general expert pathology review is reported to show 6.7% reclassification of CHL by various national and regional pathology review facilities, in specific populations such as relapsed or primary therapy-refractive CHL patients, this problem may be significantly larger at a reported 12%.\textsuperscript{20,24} In light of the relatively low \textit{a priori} incidence of NHL, the absolute risk for secondary NHL is thereby very low. This revised view sheds a quite different light on CHL risk assessments and underscores the importance of pathology review in epidemiological studies.\textsuperscript{2–4}

T-cell lymphomas with admixed Hodgkin-like B-cells, especially those with follicular-T-helper phenotype such as AITL and PTCL-TFH, are increasingly recognized as a diagnostic pitfall. These Hodgkin-like B-cells display varying phenotypes with a spectrum ranging from CHL to DLBCL immunophenotype and are most often EBER-positive (Figure 4). Thereby, subsequent “overgrowth” of this population at relapse, resulting in EBV+ DLBCL, may not be unexpected and is observed in 4 of 15 AITL/PTCL cases in our series. This aspect also contributes to difficulties in differentiating between these entities.\textsuperscript{25,26}

Likewise, CHL-like B-cell proliferations are part of the spectrum of ID-BLPD. In cases of overt immunodeficiency settings such as HIV or post-solid organ transplantation settings, this may not pose a major differential diagnostic problem. In elderly patients with presumed immune senescence, this may be more controversial.\textsuperscript{15}

In addition to reducing the risk of subsequent NHL in CHL patients, this study highlights several clues that help to alert pathologists to avoid pitfalls in CHL diagnosis. Advanced age, generalized lymphadenopathy at presentation (stage III/IV disease) and EBV-association should raise awareness of CHL mimickers. CHL is characterized by a defective B-cell program and loss of mature B-cell
markers. While varying and generally weak expression of CD20 and CD79a may be fully acceptable in CHL, strong expression should raise suspicion and justifies in-depth studies to exclude alternative options such as AITL/PTCL or ID-BLPD, as was apparent in our series. In such cases, correlation with clinical information, including clinical staging, disease distribution (lack of mediastinal involvement, exclusively infradiaphragmatic lymphadenopathy) and potential immunodeficiency settings (previous medical history, medication, age) are paramount to establish the most appropriate diagnosis.

In the 25 cases in which CHL was confirmed, various types of secondary NHL were observed that bear different relationships to the initial CHL. Extensive clinical and molecular evidence supports that CHL, PMBL and MGZL belong to a single biological disease spectrum. Therefore, PMBL and MGZL after CHL may rather be considered a form of relapse than a second malignancy. This may be different for other types of subsequent indolent and aggressive B-cell lymphoma classes that in our study included DLBCL (EBV-negative, n=5), HGBCL TH (n=1), B-ALL (n=1) and various types of indolent B-cell lymphoma (n=11). While rare cases are reported in which a common clonal origin of synchronous and metachronous CHL and NHL are reported, it is currently unknown whether this is a universal phenomenon or rather the exception.

At the individual patient level, an adequate diagnosis is obviously required to determine appropriate treatment strategies and guide communication on the expected outcome. Moreover, the problem of misdiagnosis also impacts the interpretation of clinical trials in CHL patients, especially for high stage and relapsed/refractory disease, as was recently shown. Both pathologists and treating physicians should be perceptive concerning pitfalls surrounding the diagnosis of CHL. Close interaction between pathologists and hemato-oncologists in multidisciplinary tumor boards is therefore key to optimal patient management in these settings.

This study may have various limitations. Most importantly, the interpretation of diagnostic criteria of CHL and its mimickers are to a certain level subjective and highly complex. We based our review on a combination of morphological, immunohistochemical and molecular findings in all cases. Various cases represent complex differential diagnostic problems in the spectrum of lymphocyte-rich CHL,
AITL/PTCL-TFH and EBV+ DLBCL. While we have, to the best of our ability, set objective criteria for each of these options, a certain level of subjective interpretation remains in which other experts might make different choices. Therefore, we have refrained from subjective conclusions in cases where unequivocal interpretations were not justified.

In conclusion, this study demonstrates that the risk of subsequent NHL in patients treated for CHL is significantly lower than was previously reported and underscores the need for pathology review in epidemiological studies regarding recurring lymphoma patients. Furthermore, this study shows both underrated and well-known pitfalls in the pathology diagnosis of CHL and their impact on both daily practice and epidemiological descriptions. We emphasize the importance of close interaction between pathologist and hemato-oncologist in establishing a CHL diagnosis, exploring its differential diagnosis, and parameters that may serve to avoid such pitfalls.
REFERENCES


Table 1. Clinical characteristics of all patients with reported CHL and immunohistochemical features of all cases with reported subsequent NHL.

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<th>Interval CHL-NHL diagnosis in months</th>
<th>Immunophenotype HR(like) cells in primary diagnosis percentage (positive/ tested)</th>
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FIGURE AND TABLE LEGENDS

Table 1. Clinical characteristics of all patients with reported CHL and immunohistochemical features of all cases with reported subsequent NHL.


Figure 1. Diagnostic algorithm for central pathology review of reported CHL diagnoses with subsequent NHL.

All 54 cases of primary CHL with a report of subsequent NHL were reviewed according to this algorithm.

CHL: Classical Hodgkin lymphoma; LD: Lymphocyte depleted; LR: Lymphocyte-rich; Ig: Immunoglobulin; EBER: EBV encoded RNA; IHC: immunohistochemistry; MC: Mixed cellularity; NGS: Next-generation Sequencing; NHL: Non-Hodgkin lymphoma; NS: Nodular sclerosis; TCR: T-cell receptor

Figure 2. Selection of CHL patients with reported subsequent NHL for pathology review.

**Figure 3.** Overview of subsequent diagnosis in patients with confirmed CHL diagnosis on review and of alternative diagnoses in patients where CHL diagnosis was not confirmed.

*: one case was highly likely an alternative diagnosis though lacking unequivocal arguments such as marker loss in absence of sufficient DNA for molecular testing; †: two cases were highly likely EBV+ DLBCL, though no unequivocal arguments could be found in absence of sufficient material for additional diagnostic testing; AITL: Angio-immunoblastic T-cell lymphoma; ALCL: Anaplastic large cell lymphoma; B-ALL: B-cell acute lymphoblastic leukemia; CHL: classical Hodgkin lymphoma; CLL: Chronic lymphoid leukemia; DLBCL: Diffuse large B-cell lymphoma; HGBL, TH: High grade large B-cell lymphoma, triple hit; ID-LBCL: Immunodeficiency-related large B-cell lymphoma; LPL: Lymphoplasmacytic lymphoma; MGZL: Mediastinal grey zone lymphoma; Mono-like hyperplasia: Mononucleosis-like lymphoid hyperplasia; NHL: non-Hodgkin lymphoma; NMZL: Nodal marginal zone lymphoma; PCFLCL: Primary cutaneous follicle center cell lymphoma; PMBCL: Primary mediastinal large B-cell lymphoma; PTCL, NOS: Peripheral T-cell lymphoma not otherwise specified; PTCL, TFH: Peripheral T-cell lymphoma, T follicular helper cell phenotype.

**Figure 4.** Morphological and immunohistochemical features of CHL and AITL and EBV+ DLBCL mimicking CHL.

A and B (case #52) concern a case of confirmed CHL from a cervical lymph node with CD20-, EBER+ (not shown) Hodgkin-type cells all with similar size (B, illustrated by PAX-5); C and D (case #17) show a case of EBV+ DLBCL from an axillary lymph node in an HIV+ patient with CD20+/EBER+ Hodgkin-like cells, EBER demonstrates a variation in size and morphology of tumor cells. E and F (case #5) demonstrate a case of AITL from an axillary lymph node with CD20-/EBER+ Hodgkin-like cells, also showing a variable size and morphology of tumor cells. Further details regarding histology are noted in table S1. AITL: Angio-immunoblastic T-cell lymphoma; CHL: classical Hodgkin lymphoma; DLBCL: Diffuse large B-cell lymphoma; EBER: EBV-encoded RNA in-situ hybridization. Scale bars: A, C, E: 20µm; B, D, F: 40µm.
Minimal diagnostic panel of stainings:
H&E, CD20, CD79a, PAX-5, CD30, CD15, EBER

Review CHL criteria according to 2016 WHO classification
1. Scattered tumor cells with Hodgkin-type morphology
2. Appropriate inflammatory background pattern (NS, MC, LR, LD)
3. Tumor cell immunophenotype: CD30+, CD20-, CD79a-/dim+, PAX-5 dim+
4. In EBV-positive cases, EBER positivity should be restricted to large Hodgkin-type tumor cells
5. The biopsy site should follow a typical CHL distribution pattern

All criteria met?

Yes

One or more criteria not met (e.g. CD20+ tumor cells or infradiaphragmatic disease only)

Perform additional studies:
- IHC (complete B-cell phenotyping, T-cell phenotyping, other classification-supportive markers)
- TCR or Ig clonality assay
- NGS (RHOA, TET2, DNMT3A, IDH2, CD28)

Insufficient criteria for alternative diagnosis
Confirms CHL diagnosis

Highly likely NHL, no definitive criteria
Classify as ‘Highly suspicious for NHL’

Unequivocal arguments for alternative NHL diagnosis
Change CHL diagnosis
NCR Query (2006-2013):
- All patients with HL diagnosis (n=2969)

PALGA-linkage successful (n=2959)
- 12923 pathology reports

Excluded (n=10)
- PALGA linkage failed (n=10)

Verification of CHL diagnosis and subsequent NHL diagnoses in reports
- Reported CHL diagnosis (n=2669)
- Reported NHL diagnosis after CHL (n=54)

Excluded (n=289)
- NLPHL diagnosis (n=246)
- NHL, no CHL diagnosis (n=36)
- No lymphoma diagnosis (n=6)

Tissue availability original CHL diagnosis
- FFPE (+/- slides) (n=43)
- Slides only (n=6)
- Pathology report only (n=5)

Tissue availability subsequent NHL diagnosis
- FFPE (+/- slides) (n=46)
- Slides only (n=5)
- Pathology report only (n=3)
CHL diagnosis confirmed on review?

Yes (n=25)
Subsequent NHL diagnosis:

- **Spectrum CHL-PMBL** n=6
  - PMBL n=5
  - MGZL n=1

- **Other NHL** n=18
  - DLBCL/HGBCL, TH n=6
  - B-ALL n=1
  - Plasmocytoma/NMZL/LPL n=5
  - Follicular lymphoma n=5
  - PCFCL n=1

No (n=29)
Primary lymphoma after review:

- **EBV+ Hodgkin-like cells** n=23
  - AITL n=8*
  - PTCL, TFH n=2*
  - PTCL, NOS n=5*

- **EBV+ DLBCL / ID-LBCL** n=8+

- **CD30+ T-cell lymphoma** n=3
  - ALCL ALK- n=2
  - Mycosis Fungoides n=1

- **Misinterpreted CD30+ B-cells** n=3
  - CLL n=1
  - Follicular lymphoma n=1
  - Reactive hyperplasia n=1

No subsequent NHL n=1
Mono-like hyperplasia EBV+ n=1
**SUPPLEMENTARY TABLE LEGEND** (see table in the excel file)

**Table S1:** Detailed information regarding reviewed cases.

Table showing detailed information on clinical parameters, material availability and diagnostic arguments and decisions of all cases with reported non-Hodgkin lymphoma subsequent to classic Hodgkin lymphoma.

- : no expression, +: strong expression; AITL: Angio-immunoblastic T-cell lymphoma; ALCL: Anaplastic large cell lymphoma; B-ALL: B-cell acute lymphoblastic leukemia; CHL: classical Hodgkin lymphoma; CLL: Chronic lymphoid leukemia; dim+: weak expression; DLBCL: Diffuse large B-cell lymphoma; Equivocal: Highly likely diagnosis but cannot be further proven due to lack of sufficient material and/or clinical information; HGBCL, TH: High grade large B-cell lymphoma, triple hit; ID-LBCL: Immunodeficiency-related large B-cell lymphoma; LPL: lymphoplasmacytic lymphoma; MGZL: Mediastinal grey zone lymphoma; Mono-like hyperplasia: Mononucleosis-like lymphoid hyperplasia; NHL: non-Hodgkin lymphoma; n/a: data not available; NMZL: Nodal marginal zone lymphoma; PCFLCL: Primary cutaneous follicle center cell lymphoma; PMBCL: Primary mediastinal large B-cell lymphoma; PTCL, NOS: Peripheral T-cell lymphoma not otherwise specified; PTCL, TFH: Peripheral T-cell lymphoma, T-follicular helper cell phenotype; Unequivocal: diagnosis fully supported by objective criteria.