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

Max V. Boot,[1,2] Michael Schaapveld,[3] Esther C. van den Broek,[4] the PALGA Group[4,] Nathalie J. Hijmering,[1] Kimberly van der Oord,[5] Flora E. van Leeuwen,[3] Avinash G. Dinmohamed,[6,7,8] Lianne Koens[1] and Daphne de Jong[1]

[1]Amsterdam UMC, Department of Pathology, Amsterdam; [2]Reinier Haga MDC, Department of Pathology, Delft; [3]Department of Epidemiology, Netherlands Cancer Institute, Amsterdam; [4]Dutch Pathology Registry (PALGA), Houten; [5]Laboratorium Pathologie Oost-Nederland (LABPON), Hengelo; [6]Department of Research and Development, Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht; [7]Department of Public Health, Erasmus MC, Erasmus University Medical Center, Rotterdam and [8]Amsterdam UMC, Department of Hematology, University of Amsterdam, Cancer Center Amsterdam, Amsterdam, the Netherlands

Correspondence: M.V. Boot
max.boot@reinier-mdc.nl

Received: March 4, 2022.
Accepted: July 14, 2022.
Early view: October 20, 2022.

https://doi.org/10.3324/haematol.2022.280840

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 an 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, the subsequent lymphoma was a primary mediastinal B-cell lymphoma/mediastinal gray zone lymphoma, biologically related to CHL and 19/25 were apparently unrelated B-cell NHL. In 29/54 patients, CHL was reclassified as NHL, including T-cell lymphomas with secondary Hodgkin-like B-blasts (n=15), Epstein Barr virus-positive 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 association with Epstein-Barr virus were identified as markers to alert for CHL mimickers. Based on these data, the risk of developing NHL after CHL treatment was re-calculated to 3.6-fold (standardized incidence ratio 3.61; confidence interval: 2.29-5.42). In addition, this study highlights the clinicopathological pitfalls leading to misinterpretation of CHL and consequences for the care of individual patients, interpretation of trials and epidemiological assessments.

Introduction

Successes in the treatment of patients with classic Hodgkin lymphoma (CHL) have resulted in a high long-term survival rate.[1] On the downside, these patients also have a high risk of developing treatment-related secondary cancers.[2-4] As part of a large epidemiological cohort study, Schaapveld *et al.* showed that patients treated for CHL who survived for 5 years or longer had a 13-fold increased risk of secondary non-Hodgkin lymphoma (NHL).[2] A very recent study based on Surveillance Epidemiology and End Results (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).[5] 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 have an impact on epidemiological aspects such as the risk of secondary cancer.

The diagnosis of CHL is defined by a set of typical clinical, morphological, and immunophenotypic criteria.[6] In contrast to various other types of malignant lymphoma, the criteria for the diagnosis of CHL have largely remained unchanged since the introduction of this lymphoma entity

in the revised European-American lymphoma (REAL) classification in 1994 up to the latest World Health Organization (WHO) classification.[6,7] Over the past years, the spectrum of gray 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 risk of secondary NHL.

Mediastinal gray zone lymphoma (MGZL) is now recognized as biologically related to both CHL and primary mediastinal B-cell lymphoma (PMBL); it shares morphological and immunophenotypic features with both CHL and PMBL and, together, these entities form a disease spectrum.[8-10] Relapse of CHL within this biological spectrum may account for at least a proportion of cases of secondary NHL. Various other NHL are increasingly recognized as CHL mimickers, especially Epstein-Barr virus (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 across various immunodeficiency settings may likewise deceptively mimic CHL.[6,11-17] Increased awareness and recognition of these entities underscore the challenging differential diagnosis of CHL, especially in EBV$^+$ cases.[18] 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 to have developed subsequent NHL and cover 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 Dutch network and registry of histo- and cytopathology (PALGA).[19] 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 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 European Union General Data Protection Regulation.

Pathology review

Both CHL and NHL diagnoses were reviewed by three hematopathologists (MB, LK, DdJ) according to a previously reported algorithm (Figure 1).[20] When there was a discrepancy between the diagnoses of 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.[6] In cases of deviation in any primary criterion, additional studies for pertinent differential diagnoses were performed (*Online Supplementary Table S1*), including immunohistochemistry, T-cell receptor beta and gamma (TCR) and/or immunoglobulin heavy (IGH) and kappa (IGK) light chain rearrangement assays (BIOMED-2; InVivoScribe, San Diego, CA, USA).[21] Next, in those cases suspected to be AITL or PTCL-TFH without a conclusive clonal TCR rearrangement, targeted panel next-generation sequencing including *RHOA*, *TET2*, *DNMT3A*, *IDH2* and *CD28* was performed using IonTorrent (Ion Ampliseq™; Thermo Fisher Scientific, Waltham, MA, USA) as used for routine diagnostic purposes in our laboratory.[22]

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.

The diagnoses of secondary NHL in all patients were reviewed according to WHO Classification 2016 criteria. In cases in which a relationship between the primary and secondary lymphoma was suspected, additional immunohistochemistry, *in situ* hybridization or molecular studies were performed to either substantiate or disprove such a relationship.

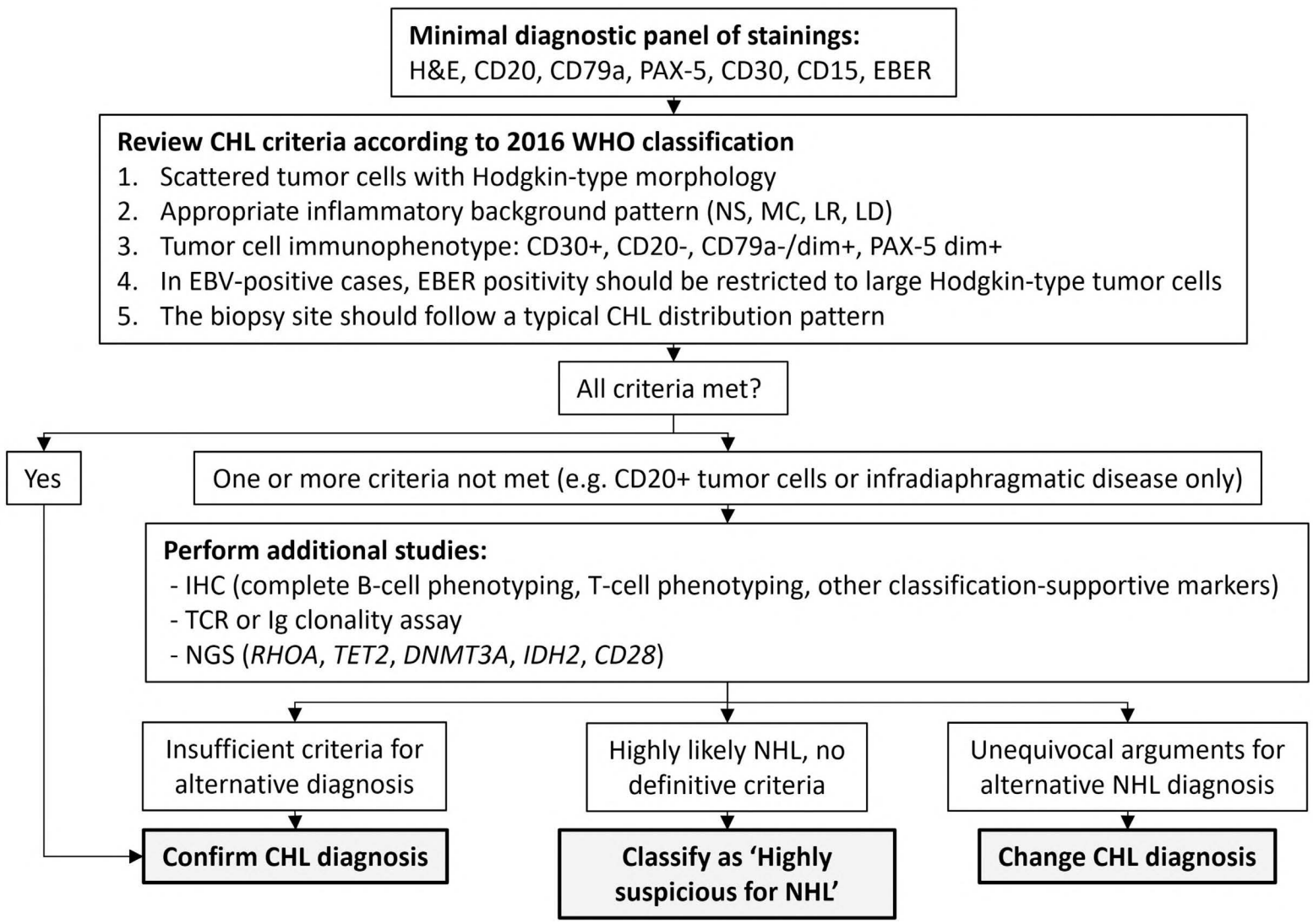

Figure 1. Diagnostic algorithm for the central pathology review of reported diagnoses of classic Hodgkin lymphoma with subsequent non-Hodgkin lymphoma. All 54 cases of primary classic Hodgkin lymphoma with a report of subsequent non-Hodgkin lymphoma were reviewed according to this algorithm. CHL: classic Hodgkin lymphoma; EBV: Epstein-Barr virus; LD: lymphocyte-depleted; LR: lymphocyte-rich; Ig: immunoglobulin; EBER: Epstein-Barr virus-encoded RNA; H&E: hematoxylin and eosin; IHC: immunohistochemistry; MC: mixed cellularity; NGS: next-generation sequencing; NHL: non-Hodgkin lymphoma; NS: nodular sclerosis; TCR: T-cell receptor; WHO: World Health Organization.

Statistical analysis

For risk calculations, the expected incidence of NHL was calculated 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 and corresponding 95% confidence intervals (95% CI) with correction for the duration of follow-up.[23] Relations between review diagnosis category, age at diagnosis and disease stage were tested with analysis of variance and Fisher exact tests, respectively, using SPSS (IBM, version 27).

Results

Study population

In the NCR, 2,969 patients were identified with a diagnosis of primary Hodgkin lymphoma between 2006 and 2013. Linkage to the PALGA database was successful in 99.7% of cases (2,959 patients) and a total of 12,923 complete pathology reports were manually curated. Among these, a CHL diagnosis was listed for 2,669/2,959 (90.2%) patients. The remaining 290/2,959 (9.8%) patients were excluded because of the lack of a confirmed CHL diagnosis (Figure 2). In 54/2,669 CHL patients (2.0%), a diagnosis of NHL after CHL was listed, with subsequent NHL recurrence or transformation/progression in 11 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 formalin-fixed paraffin-embedded 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 formalin-fixed paraffin embedded tissue was available and the review was based on detailed pathology reports only.

Pathology review

Clinical features and pathology characteristics at review are listed in Table 1 and *Online Supplementary Table 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, ten of these were recognized as part of expert consultation at the time of the initial diagnosis, but after start of treatment (n=2) or at retrospective review as part of the diagnostic workup at the time of the 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 formalin-fixed paraffin-embedded tissue or poor DNA quality leading to unreliable clonality or next-generation sequencing 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.

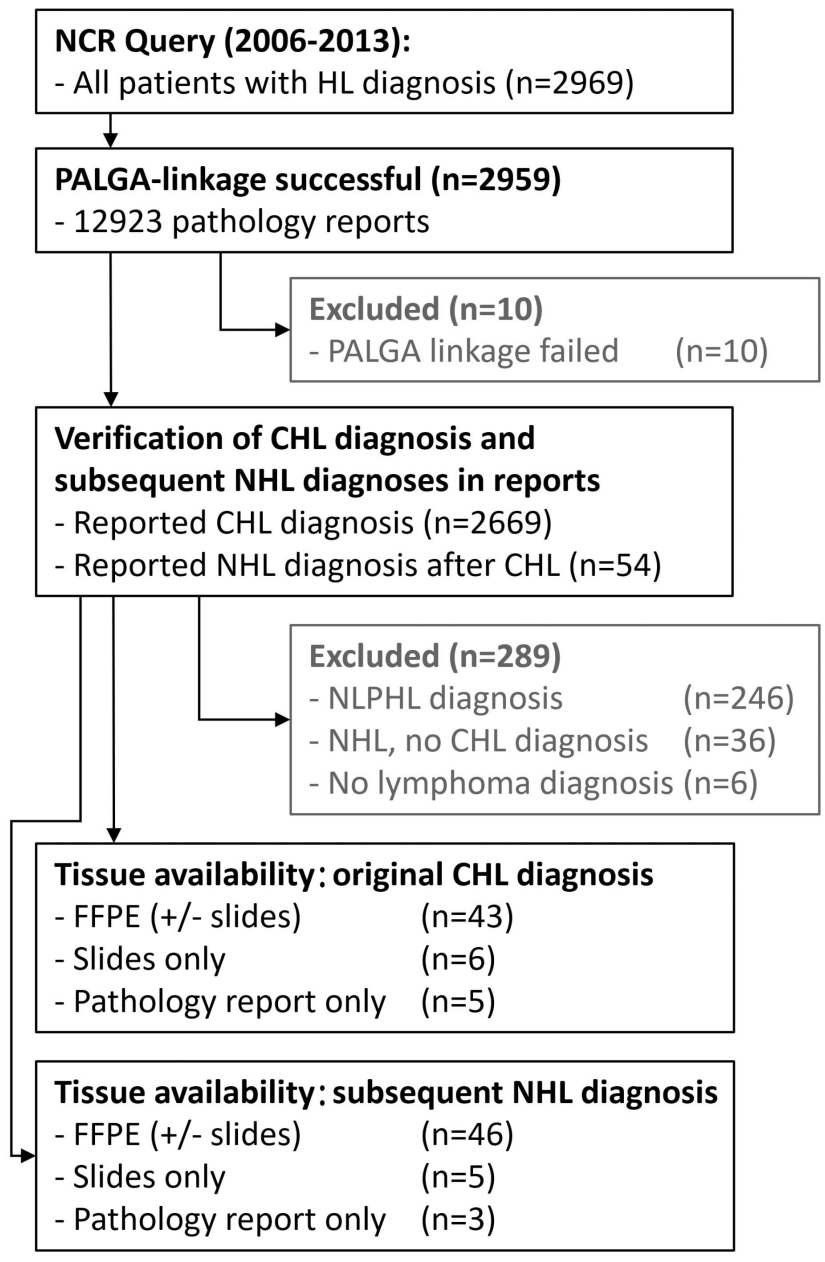

Figure 2. Selection of patients with classic Hodgkin lymphoma with a reported subsequent non-Hodgkin lymphoma for pathology review. CHL: classical Hodgkin lymphoma; FFPE: formalin-fixed paraffin-embedded tissue material; HL: Hodgkin lymphoma; NCR: Netherlands Cancer Registry; NHL: non-Hodgkin lymphoma; NLPHL: nodular lymphocyte-predominant Hodgkin lymphoma; PALGA: Dutch pathology registry.

The spectrum of classic Hodgkin lymphoma and primary mediastinal B-cell lymphoma

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

Classic Hodgkin lymphoma with secondary B-cell lymphoma

Eighteen patients with a confirmed diagnosis of CHL 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 and lymphoplasmacytic lymphoma (n=3), primary cutaneous follicle center cell lymphoma (n=1), follicular lymphoma (n=5), DLBCL, not otherwise specified (EBV^-, n=5), high-grade B-cell lymphoma with *MYC*, *BCL2* and *BCL6* translocation (n=1), and B-cell acute lymphoblastic leukemia (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 exemplifies the complex disease course observed in some of these patients. Twelve years after being diagnosed with EBV^+ CHL, this patient presented with EBV^+ mononucleosis-like lymphoid hyperplasia, followed 1 year later 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).

T-cell lymphoma with secondary Epstein-Barr virus-positive Hodgkin-like cells

In 11 patients, the initially diagnosed CHL could in retrospect be unequivocally recognized as T-cell lymphoma with Hodgkin-like cells, mostly EBV^+ and were classified as AITL (n=7), PTCL-TFH (n=1) and PTCL-not otherwise specified (n=3). Additionally, in patient #12, a primary diagnosis of CHL could be unequivocally 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.

Table 1. Clinical characteristics of all patients with reported classic Hodgkin lymphoma and immunohistochemical features of all cases with reported subsequent non-Hodgkin lymphoma.

Diagnostic category		Sex		Age at primary lymphoma in years	Ann Arbor stage		Interval CHL-NHL diagnosis in months	Immunophenotype of Hodgkin(-like) cells in primary diagnosis showing number and percentage (positive/tested)				
		Male N (%)	Female N (%)	Median (range)	Stage I/II N (%)	Stage III/IV N (%)	Median (range)	CD20+ N (%)	CD79a+ N (%)	PAX-5+ N (%)	CD15+ N (%)	EBER+ N (%)
CHL with no reported NHL*	N=2,615	1,446 (55)	1,169 (45)	36 (15-75)	1,528 (59)	1,077 (41)	No NHL	Not reviewed				
CHL diagnosis not confirmed	N=29	18 (62)	11 (38)	46 (23-73)	10 (34)	19 (66)	46 (1-160)	16/29 (55)	14/27 (52)	26/29 (90)	10/27 (37)	20/28 (71)
AITL/PTCL-TFH/PTCL-NOS	N=15	7 (47)	8 (53)	49 (23-73)	3 (20)	12 (80)	36 (3-160)	7/15 (47)	6/15 (40)	15/15 (100)	7/14 (50)	12/14 (86)
DLBCL EBV+	N=8	7 (88)	1 (13)	39.5 (24-72)	5 (63)	3 (38)	36 (1-112)	6/8 (75)	5/6 (83)	8/8 (100)	1/7 (14)	8/8 (100)
CD30+ T-cell lymphoma	N=3	2 (67)	1 (33)	46 (42-73)	2 (67)	1 (33)	86 (83-92)	0/3 (0)	0/3 (0)	0/3 (0)	2/3 (67)	0/3 (0)
Other B-cell proliferations	N=3	2 (67)	1 (33)	61 (46-66)	0 (0)	3 (100)	65 (13-72)	3/3 (100)	3/3 (100)	3/3 (100)	0/3 (0)	0/3 (0)
CHL diagnosis confirmed	N=25	15 (60)	10 (40)	39.5 (24-72)	11 (46)	13 (54)	57 (6-149)	3/24 (13)	7/23 (30)	24/24 (100)	19/23 (83)	4/22 (18)
Subsequent PMCL/MGZL	N=6	4 (67)	2 (33)	53 (40-60)	2 (40)	3 (60)	38 (6-70)	2/6 (33)	2/6 (33)	6/6 (100)	4/6 (67)	1/6 (17)
Other subsequent BCL	N=19	11 (58)	8 (42)	49 (19-73)	9 (47)	10 (53)	68 (13-149)	1/18 (6)	5/17 (29)	18/18 (100)	15/17 (88)	3/16 (19)

*: no pathology review performed; **: any positive staining, details in *Online Supplementary Table S1*; AITL: angio-immunoblastic T-cell lymphoma; CHL: classic Hodgkin lymphoma; DLBCL: diffuse large B-cell lymphoma; EBER: EBV-encoded RNA *in-situ* hybridization; MGZL: mediastinal gray zone lymphoma; NHL: non-Hodgkin 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.

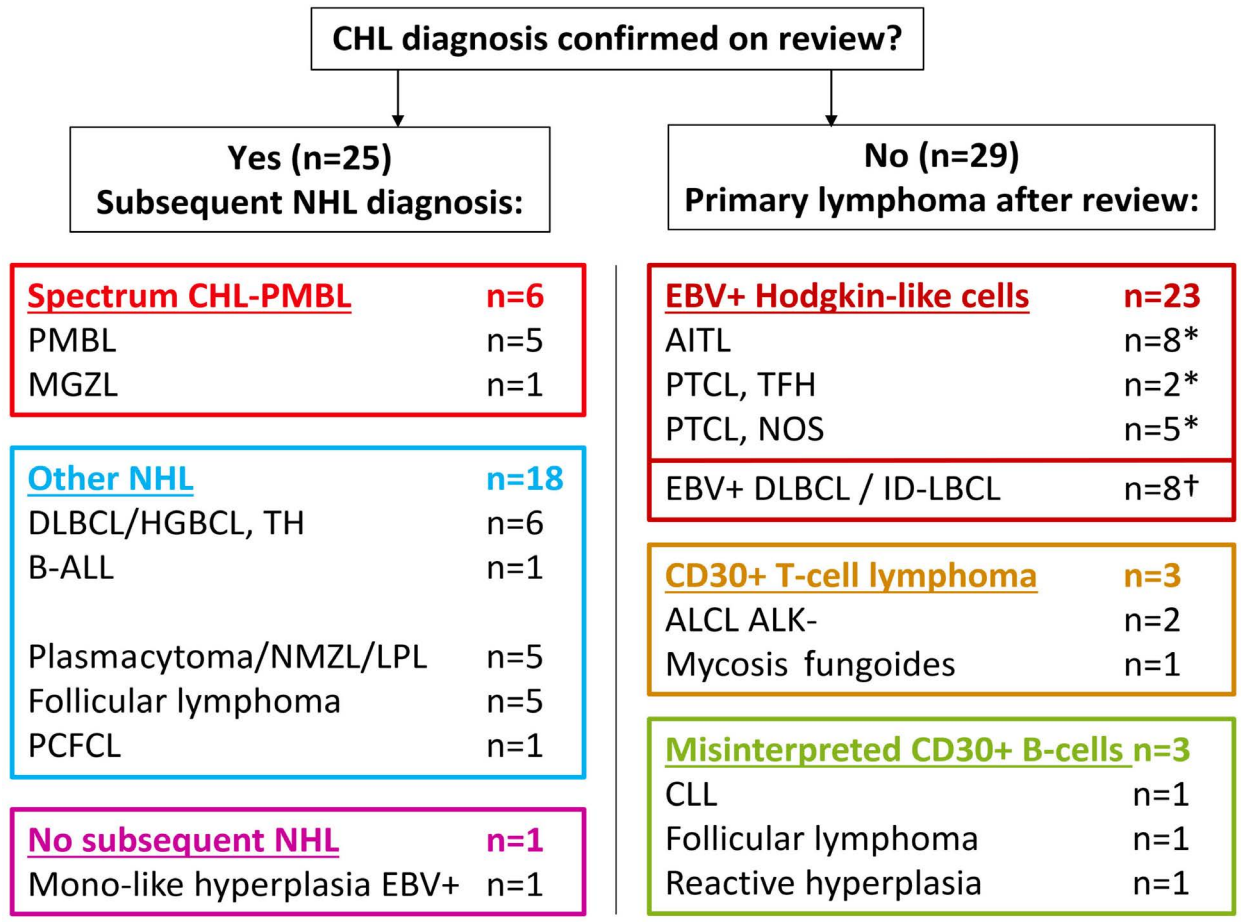

Figure 3. Overview of subsequent diagnoses in patients with a confirmed diagnosis of classic Hodgkin lymphoma on review and of alternative diagnoses in patients in whom the diagnosis of classic Hodgkin lymphoma was not confirmed. *: one case was highly likely an alternative diagnosis although lacking unequivocal indicators such as marker loss in the absence of sufficient DNA for molecular testing; †: two cases were highly likely Epstein-Barr virus-positive diffuse large B-cell lymphoma, although no unequivocal indicators could be found in the absence of sufficient material for additional diagnostic testing; AITL: angioimmunoblastic 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; HGBCL, TH: high-grade large B-cell lymphoma, triple hit; ID-LBCL: immunodeficiency-related large B-cell lymphoma; LPL: lymphoplasmacytic lymphoma; MGZL: mediastinal gray 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.

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, poor specimen quality or unavailability of biopsy material precluded further immunohistochemistry or molecular studies for a definite diagnosis and these cases were termed equivocal between CHL and PTCL with a preference for PTCL.

Epstein-Barr virus-positive diffuse large B-cell lymphoma mimicking classic Hodgkin lymphoma

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] The 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 (human immunodeficiency virus [HIV] infection, 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$^-$ indolent B-cell lymphoma (differential diagnosis nodal marginal zone lymphoma/lymphoplasmacytic lymphoma).

Equivocal review diagnosis of Epstein-Barr virus diffuse large B-cell lymphoma

In two cases, the primary CHL diagnosis was highly suspicious for EBV$^+$ DLBCL. Patient #22 presented with isolated cerebral localization with EBER$^+$ Hodgkin-like cells 9 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 there was no information on CD79a, while subsequent diagnosis of EBV$^+$ DLBCL could be made unequivocally.

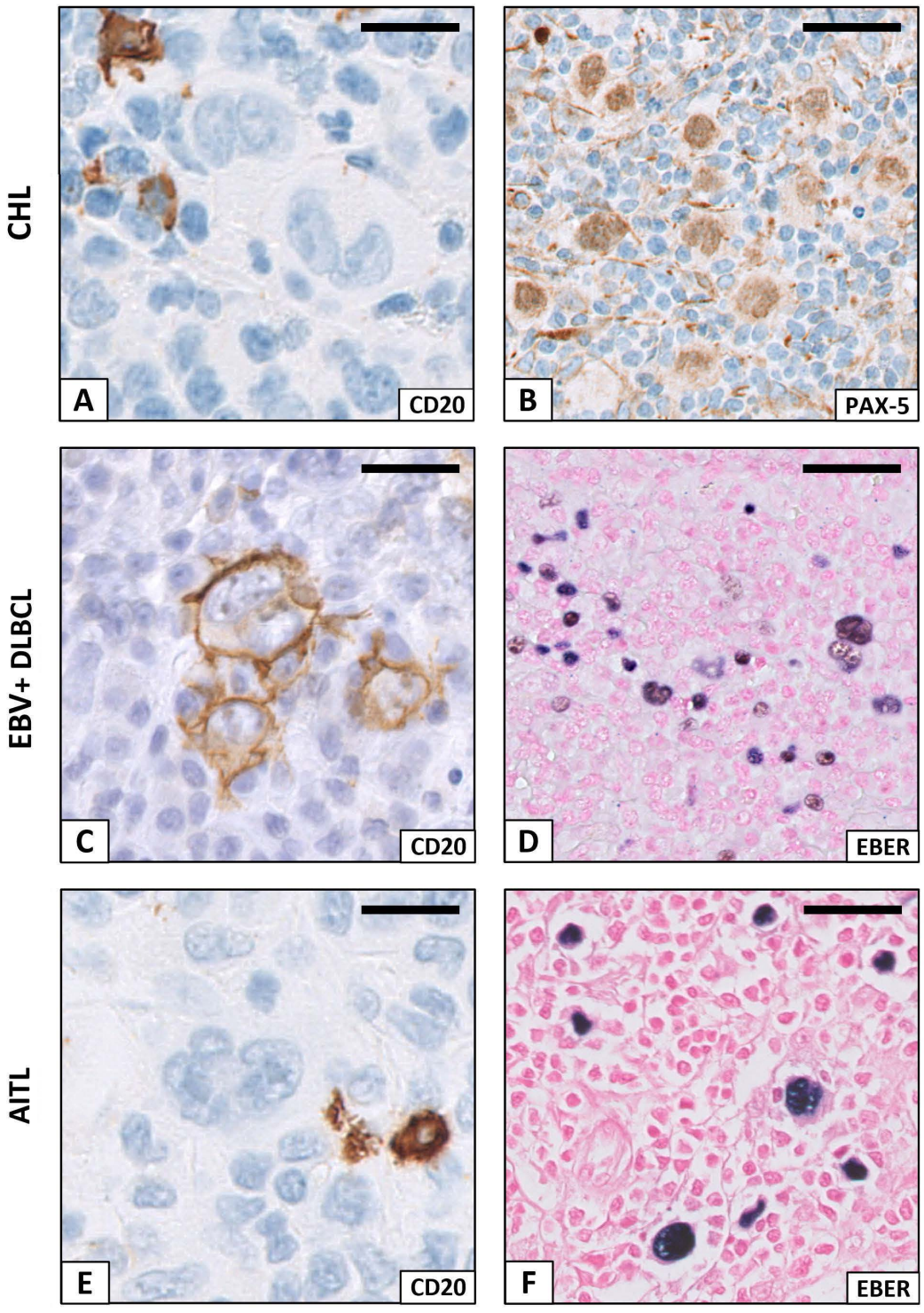

Figure 4. Morphological and immunohistochemical features of classic Hodgkin lymphoma and angio-immunoblastic T-cell lymphoma and Epstein-Barr virus-positive diffuse large B-cell lymphoma mimicking classic Hodgkin lymphoma. (A, B) Case #52. A case of confirmed classical Hodgkin lymphoma from a cervical lymph node with CD20$^-$, EBER$^+$ (not shown) Hodgkin-type cells all of similar size (B, illustrated by PAX-5); (C, D) Case #17 is a case of Epstein-Barr virus-positive diffuse large B-cell lymphoma from an axillary lymph node in a patient positive for human immunodeficiency virus with CD20$^+$/EBER$^+$ Hodgkin-like cells. EBER demonstrates a variation in size and morphology of the tumor cells. (E, F) Case #5. A case of angio-immunoblastic T-cell lymphoma from an axillary lymph node with CD20$^-$/EBER$^+$ Hodgkin-like cells, also showing variable size and morphology of tumor cells. Further details regarding histology are noted in *Online Supplementary Table S1*. AITL: angio-immunoblastic T-cell lymphoma; CHL: classical Hodgkin lymphoma; DLBCL: diffuse large B-cell lymphoma; EBER: Epstein Barr virus-encoded RNA *in-situ* hybridization. Scale bars: A, C, E: 20 μm; B, D, F: 40 μm.

CD30$^+$ T-cell lymphoma mimicking classic Hodgkin lymphoma

In three patients, the primary CHL diagnosis was recognized as CD30$^+$ T-cell lymphoma on review, since there was a lack of defining B-cell lineage markers while expression of T-cell markers was confirmed. Two cases were classified as anaplastic large cell lymphoma, ALK$^-$, and one case could be recognized as regional lymph node involvement of mycosis fungoides, 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 (#27) and follicular lymphoma (#28), relapsing as such. Case #29 presented with reactive plasma cell hyperplasia and subsequently as indolent B-cell lymphoma (differential diagnosis nodal marginal zone lymphoma/lymphoplasmacytic lymphoma) 13 months later.

Immunophenotype of Hodgkin(-like) cells

Compared to Hodgkin-type cells in patients with 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*<0.001) and significantly less often positive for CD15 (10/27 *vs*. 19/23; *P*=0.002). Differences in CD79a expression were not statistically 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 *Online Supplementary Table S1*.

Clinical-pathological correlations

Patients recognized as having 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

having 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 a 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 misdiagnoses. 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 the primary CHL diagnosis (2006-2013). No significant differences were noted in the interval between the primary and secondary lymphoma episodes for confirmed CHL and mimickers.

Risk of secondary non-Hodgkin lymphoma after classic Hodgkin lymphoma

Based on the present selection of cases and original pathology diagnoses, risk calculations show a standardized incidence ratio of developing NHL after CHL of 7.79 (95% CI: 5.78-10.3). Based on diagnoses after pathology review, the standardized incidence ratio was significantly lower at 4.39 (95% CI: 2.92-6.35; *P*=0.015) when still including the equivocal cases (highly likely misdiagnoses) as CHL, and 3.61 (95% CI: 2.29-5.42; *P*=0.002) when 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 2,615 CHL patients without a 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 standardized incidence ratios.[24]

Discussion

The WHO Classification of lymphoma is dynamic and continuously incorporates novel insights into lymphoma biology, which in turn affects 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 standardized incidence ratio of 3.61-4.39. Although general expert pathology review is reported to show reclassification of 6.7% of cases 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%.[20,24] In light of the relatively low *a priori* incidence of NHL, the absolute risk of secondary NHL is therefore very low. This revised view sheds a quite different light on CHL risk assessments and underscores the importance of pathology review in epidemiological studies.[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 diagnostic pitfalls. The Hodgkin-like B cells display varying phenotypes with a spectrum ranging from CHL to DLBCL immunophenotype and are most often EBER$^+$ (Figure 4). Thus, subsequent "overgrowth" of this population at relapse, resulting in EBV$^+$ DLBCL, may not be unexpected and was observed in four of 15 AITL/PTCL cases in our series. This aspect also contributes to difficulties in differentiating between these entities.[25,26]

Likewise, CHL-like B-cell proliferations are part of the spectrum of immunodeficiency-related B-lymphoproliferative disorders. In settings of overt immunodeficiency, such as HIV infection or after solid organ transplantation, this may not pose a major differential diagnostic problem. In elderly patients with presumed immune senescence, this may be more controversial.[15]

In addition to recalculating 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 immunodeficiency-related B-lymphoproliferative disorders, as was apparent in our series. In such cases, cor-

relation with clinical information, including clinical staging, disease distribution (lack of mediastinal involvement, exclusively infradiaphragmatic lymphadenopathy) and potential immunodeficiency states (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.[9,10,27-32] Therefore, PMBL and MGZL after CHL may be considered a form of relapse rather than a second malignancy.[18,32] This may be different for other types of subsequent indolent and aggressive B-cell lymphoma classes that in our study included DLBCL (EBV$^-$, n=5), triple-hit high-grade B-cell lymphoma (n=1), B-cell acute lymphoblastic leukemia (n=1) and various types of indolent B-cell lymphoma (n=11). While rare cases are reported in which there is a common clonal origin of synchronous and metachronous CHL and NHL, it is currently unknown whether this is a universal phenomenon or rather the exception.[33,34]

At the level of individual patients, an adequate diagnosis is obviously required to determine appropriate treatment strategies and guide communication on the expected outcome.[35,36] The problem of misdiagnosis also has an impact on the interpretation of clinical trials in CHL patients, especially for those with high stage and relapsed/refractory disease, as was recently shown.[20] 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. We have, therefore, refrained from subjective conclusions in cases in which 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 patients with recurring lymphoma. 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 pathologists and hemato-oncologists in establishing a diagnosis of CHL, exploring its differential diagnosis, and parameters that may serve to avoid pitfalls.

Disclosures

No conflicts of interest to disclose.

Contributions

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

Acknowledgments

We dedicate this study to the memory of Professor Ton Hagenbeek who passed away in May 2021. Ton's critical attitude and keen interest in innovations in the field of hematology and hematopathology will remain an inspiration.

Funding

This study was financially supported by the van Vlissingen Lymphoma Foundation.

Data-sharing statement

Anonymized study data are available from the corresponding author.

References

1. Driessen J, Visser O, Zijlstra JM, et al. Primary therapy and relative survival in classical Hodgkin lymphoma: a nationwide population-based study in the Netherlands, 1989-2017. Leukemia. 2021;35(2):494-505.
2. Schaapveld M, Aleman BM, van Eggermond AM, et al. Second cancer risk up to 40 years after treatment for Hodgkin's lymphoma. N Engl J Med. 2015;373(26):2499-2511.
3. Dores GM, Metayer C, Curtis RE, et al. Second malignant neoplasms among long-term survivors of Hodgkin's disease: a population-based evaluation over 25 years. J Clin Oncol. 2002;20(16):3484-3494.
4. Swerdlow AJ, Higgins CD, Smith P, et al. Second cancer risk after chemotherapy for Hodgkin's lymphoma: a collaborative British cohort study. J Clin Oncol. 2011;29(31):4096-4104.

5. Chihara D, Dores G, Flowers C, Morton LM. The bidirectional increased risk of B-cell lymphoma and T-cell lymphoma. Blood. 2021;138(9):785-789.
6. Bell D, Gaillard F. WHO classification of tumours of haematopoietic and lymphoid tissues. Revised 4th edition. Lyon: International Agency for Research on Cancer IARC; 2010.
7. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood. 1994;84(5):1361-1392.
8. Kritharis A, Pilichowska M, Evens AM. How I manage patients with grey zone lymphoma. Br J Haematol. 2016;174(3):345-350.
9. Sarkozy C, Chong L, Takata K, et al. Gene expression profiling of gray zone lymphoma. Blood Adv. 2020;4(11):2523-2535.
10. Sarkozy C, Hung SS, Chavez EA, et al. Mutational landscape of gray zone lymphoma. Blood. 2021;137(13):1765-1776.
11. Attygalle AD, Cabecadas J, Gaulard P, et al. Peripheral T-cell and NK-cell lymphomas and their mimics; taking a step forward - report on the lymphoma workshop of the XVIth meeting of the European Association for Haematopathology and the Society for Hematopathology. Histopathology. 2014;64(2):171-199.
12. Bröckelmann PJ, Goergen H, Fuchs M, et al. Impact of centralized diagnostic review on quality of initial staging in Hodgkin lymphoma: experience of the German Hodgkin Study Group. Br J Haematol. 2015;171(4):547-556.
13. Hartmann S, Goncharova O, Portyanko A, et al. CD30 expression in neoplastic T cells of follicular T cell lymphoma is a helpful diagnostic tool in the differential diagnosis of Hodgkin lymphoma. Mod Pathol. 2019;32(1):37-47.
14. Nicolae A, Pittaluga S, Venkataraman G, et al. Peripheral T-cell lymphomas of follicular T-helper cell derivation with Hodgkin/Reed-Sternberg cells of B-cell lineage: both EBV-positive and EBV-negative variants exist. Am J Surg Pathol. 2013;37(6):816-826.
15. de Jong D, Roemer MG, Chan JK, et al. B-cell and classical Hodgkin lymphomas associated with immunodeficiency: 2015 SH/EAHP Workshop Report-Part 2. Am J Clin Pathol. 2017;147(2):153-170.
16. Natkunam Y, Gratzinger D, Chadburn A, et al. Immunodeficiency-associated lymphoproliferative disorders: time for reappraisal? Blood. 2018;132(18):1871-1878.
17. Pitman SD, Huang Q, Zuppan CW, et al. Hodgkin lymphoma-like posttransplant lymphoproliferative disorder (HL-like PTLD) simulates monomorphic B-cell PTLD both clinically and pathologically. Am J Surg Pathol. 2006;30(4):470-476.
18. Wang HW, Balakrishna JP, Pittaluga S, Jaffe ES. Diagnosis of Hodgkin lymphoma in the modern era. Br J Haematol. 2019;184(1):45-59.
19. Casparie M, Tiebosch AT, Burger G, et al. Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. Cell Oncol. 2007;29(1):19-24.
20. Kersten MJ, Driessen J, Zijlstra JM, et al. Combining brentuximab vedotin with dexamethasone, high-dose cytarabine and cisplatin as salvage treatment in relapsed or refractory Hodgkin lymphoma: the phase II HOVON/LLPC Transplant BRaVE study. Haematologica. 2021;106(4):1129-1137.
21. Langerak AW, Groenen PJ, Bruggemann M, et al. EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. Leukemia. 2012;26(10):2159-2171.
22. Sie D, Snijders PJ, Meijer GA, et al. Performance of amplicon-based next generation DNA sequencing for diagnostic gene mutation profiling in oncopathology. Cell Oncol. 2014;37(5):353-361.
23. Breslow NE, Day NE. Statistical methods in cancer research. Volume II--the design and analysis of cohort studies. IARC Sci Publ. 1987;(82):1-406.
24. Laurent C, Baron M, Amara N, et al. Impact of expert pathologic review of lymphoma diagnosis: study of patients from the French Lymphopath network. J Clin Oncol. 2017;35(18):2008-2017.
25. Gratzinger D, de Jong D, Jaffe ES, et al. T- and NK-cell lymphomas and systemic lymphoproliferative disorders and the immunodeficiency setting: 2015 SH/EAHP Workshop Report-Part 4. Am J Clin Pathol. 2017;147(2):188-203.
26. Xu Y, McKenna RW, Hoang MP, Collins RH, Kroft SH. Composite angioimmunoblastic T-cell lymphoma and diffuse large B-cell lymphoma: a case report and review of the literature. Am J Clin Pathol. 2002;118(6):848-854.
27. Eberle FC, Salaverria I, Steidl C, et al. Gray zone lymphoma: chromosomal aberrations with immunophenotypic and clinical correlations. Mod Pathol. 2011;24(12):1586-1597.
28. Green MR, Monti S, Rodig SJ, et al. Integrative analysis reveals selective 9p24.1 amplification, increased PD-1 ligand expression, and further induction via JAK2 in nodular sclerosing Hodgkin lymphoma and primary mediastinal large B-cell lymphoma. Blood. 2010;116(17):3268-3277.
29. Rosenwald A, Wright G, Leroy K, et al. Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. J Exp Med. 2003;198(6):851-862.
30. Savage KJ, Monti S, Kutok JL, et al. The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin lymphoma. Blood. 2003;102(12):3871-3879.
31. Liu PP, Wang KF, Xia Y, et al. Racial patterns of patients with primary mediastinal large B-cell lymphoma. Medicine (Baltimore). 2016;95(27):1-7.
32. Traverse-Glehen A, Pittaluga S, Gaulard P, et al. Mediastinal gray zone lymphoma: the missing link between classic Hodgkin's lymphoma and mediastinal large B-cell lymphoma. Am J Surg Pathol. 2005;29(11):1411-1421.
33. Küppers R, Dührsen U, Hansmann ML. Pathogenesis, diagnosis, and treatment of composite lymphomas. Lancet Oncol. 2014;15(10):e435-e446.
34. Dobson R, Du PY, Rásó-Barnett L, et al. Early detection of T-cell lymphoma with T follicular helper phenotype by RHOA mutation analysis. Haematologica. 2022;107(2):489-499.
35. Crombie JL, LaCasce AS. Epstein Barr virus associated B-cell lymphomas and iatrogenic lymphoproliferative disorders. Front Oncol. 2019;9:109.
36. Lunning MA, Vose JM. Angioimmunoblastic T-cell lymphoma: the many-faced lymphoma. Blood. 2017;129(9):1095-1102.