

Epstein–Barr virus infection patterns in nodular lymphocyte-predominant Hodgkin lymphoma

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Aims: To investigate Epstein-Barr virus (EBV) latency types in 19 cases of EBV-positive nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), as such information is currently incomplete.

Methods and results: Immunohistochemistry (IHC) for CD20, CD79a, PAX5, OCT2, CD30, CD15, CD3 and programmed cell death protein 1 was performed. For EBV detection, *in-situ* hybridisation (ISH) for EBV-encoded RNA (EBER) was employed combined with IHC for EBV-encoded latent membrane protein (LMP)-1, EBV-encoded nuclear antigen (EBNA)-2, and EBV-encoded BZLF1. In 95% of the cases, neoplastic cells with features of Hodgkin and Reed–Sternberg (HRS) cells were present, mostly showing expression of CD30. In all cases, the B-cell phenotype was largely intact, and delineation from classic Hodgkin lymphoma (CHL) was further supported by myocyte enhancer factor 2B (MEF2B) detection. All tumour cells were EBER-positive except in two cases. EBV

latency type II was most frequent (89%) and type I was rare. Cases with latency type I were CD30-negative. Five cases contained some BZLF1-positive and/or EBNA-2-positive bystander lymphocytes.

Conclusions: As HRS morphology of neoplastic cells and CD30 expression are frequent features of EBV-positive NLPHL, preservation of the B-cell transcription programme, MEF2B expression combined with NLPHL-typical architecture and background composition facilitate distinction from CHL. EBER ISH is the method of choice to identify these cases. The majority present with EBV latency type II, and only rare cases present with latency type I, which can be associated with missing CD30 expression. The presence of occasional bystander lymphocytes expressing BZLF1 and/or EBNA-2 and the partial EBV infection of neoplastic cells in some cases could indicate that EBV is either not primarily involved or is only a transient driver in the pathogenesis of EBV-positive NLPHL.

Keywords: EBV, Hodgkin lymphoma, latency type, NLPHL

Introduction

Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) represents a rare form of Hodgkin lymphoma

(HL). At least a proportion of the neoplastic cells of NLPHL can show some overlapping morphological and immunophenotypic features with the tumour cells [Hodgkin and Reed–Sternberg cells (HRS) cells] of

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classic HL (CHL), which may lead to diagnostic problems. However, several significant differences usually allow reliable discrimination between these two HL entities. In particular, besides differences in clinical presentation and prognosis, the composition of the background infiltrate, the topographic distribution of the neoplastic cells and the preservation of the B-cell transcription programme are unique to NLPHL. There are anecdotal reports from the 1990s about Epstein–Barr virus (EBV)-positive NLPHL,^{1–13} but a sizeable multi-institutional study could not detect an EBV association in NLPHL.¹⁴ As it is ultimately unclear whether most of these earlier reports may have also included cases of lymphocyte-rich CHL (LRCHL), which can show some overlap with NLPHL and thus can lead to erroneous diagnosis,¹⁴ the majority of the data have implied that EBV negativity can be another feature helping to distinguish NLPHL from CHL.¹⁴ Nevertheless, two more recent studies described somewhat larger numbers of NLPHL cases with EBV infection of the tumour cells.^{15,16} Although these studies presented unequivocal evidence for an occasional association between NLPHL and EBV, only partial information regarding the EBV latency type in these cases has been presented. In order to close this gap, we performed a study on archival material from the Lymphoma Reference Centre at the Institute of Pathology, University of Würzburg, Germany.

Materials and methods

CASE SELECTION AND EVALUATION

For this retrospective study, the archive of the Lymphoma Reference Centre at the Institute of Pathology of the University Würzburg was screened (2000–2020) for cases diagnosed as NLPHL with indications of an EBV association. Ethics approval was granted by the Ethics Committee of the University of Würzburg (no. 2022011101). This search identified 20 NLPHL cases that showed unequivocal EBV infection of the neoplastic cells. All cases were subjected to a consensus immunohistochemistry (IHC) panel (Table 1) and reviewed by a group of expert haematopathologists (A.R., I.A., E.H., and A.Z.). Cases with borderline features between NLPHL and CHL were subjected to additional immunohistochemical detection of myocyte enhancer factor 2B (MEF2B), which has been identified as a highly sensitive and specific molecule that can help in the differentiation of NLPHL from CHL, supporting a diagnosis of NLPHL in challenging

cases.¹⁷ After a second review of all cases, one case had to be omitted because it showed immunophenotypic and morphological features favouring a diagnosis of CHL, leading to 19 genuine EBV-positive NLPHLs out of 749 cases with representative tissue material available (2.5%). One of those (case 17) has been previously published as a case report.¹⁸ The cases were further categorized according to the architectural and immunohistological patterns described by Fan *et al.*¹⁹ All cases were sent to the Würzburg Lymphoma Reference Centre for establishment of a primary diagnosis before therapy. Clinical data were not available.

HISTOLOGICAL AND IMMUNOHISTOLOGICAL METHODS

Histological sections (2 µm) were cut and stained with haematoxylin and eosin, Giemsa and periodic acid Schiff for routine histological evaluation. Immunohistochemical staining was performed on formalin-fixed paraffin-embedded tissue slides according to the manufacturers' instructions and standard protocols (Table 1).

IN-SITU HYBRIDISATION (ISH)

For detection of EBV-encoded RNAs (EBER-1 and EBER-2), ISH was performed on tissue sections by use of the Ventana ready-to-use kit, according to the appropriate protocols, within an automated immunostainer (Benchmark XT; Ventana/Roche, Tucson, AZ, USA).

Results

The patient characteristics and the histomorphological and immunohistochemical findings are summarized in Table 2.

PATIENT AND SAMPLE CHARACTERISTICS

Patient age ranged from 9 years to 85 years (median, 39 years). Five of six paediatric patients were males, and among the adult patients the majority were also males (8/13). According to the information provided by the submitting pathologists, most biopsied lymph nodes were cervical (10), followed by axillary (two), inguinal (two), abdominal (two), and mediastinal (one). For two cases, we did not receive any information about the localisation. As stated in Materials and

Table 1. Immunohistochemical antibody panel

Antibody	Supplier	Clone	Dilution	Expression pattern
CD30	Santa Cruz (Santa Cruz, CA, USA)	BerH2	1:200	Membranous/Golgi zone
CD20	Agilent/Dako (Waldbronn, Germany)	L26	1:500	Membranous
CD79a	Agilent/Dako (Waldbronn, Germany)	JCB117	1:400	Membranous
PAX5	BD Biosciences (Heidelberg, Germany)	24	1:100	Nuclear
OCT2	Santa Cruz (Santa Cruz, CA, USA)	Polyclonal	1:3000	Nuclear
CD15	Agilent/Dako (Waldbronn, Germany)	CARB-3	1:800	Membranous
CD3	Agilent/Dako (Waldbronn, Germany)	F7.2.38	1:800	Membranous
CD5	Leica Biosystems, (Nussloch Germany)	4C7	1:500	Membranous
PD-1	Laboratory G. Roncador, Centro National de Investigaciones Oncologicas/CNIO, (Madrid, Spain)	NAT-105 CE3	1:200	Membranous
CD21	Agilent/Dako (Waldbronn, Germany)	1F8	1:200	Membranous
MEF2B	Atlas antibodies (Bromma, Sweden)	polyclonal	1:400	Nuclear
LMP-1	Agilent/Dako (Waldbronn, Germany)	Cocktail of monoclonal antibodies CS1–4	1:800	Membranous
EBNA-2	Abcam (Cambridge, UK)	PE2	1:100	Nuclear
EBV BZLF1	Santa Cruz (Santa Cruz, CA, USA)	BZ1	1:20	Nuclear+cytoplasmic

EBNA, Epstein–Barr virus-encoded nuclear antigen; EBV, Epstein–Barr virus; LMP, latent membrane protein; MEF2B, myocyte enhancer factor 2B; PD-1, programmed cell death protein 1.

methods, no information regarding EBV loads, serological studies, disease stage, follow-up or treatment was available.

HISTOPATHOLOGICAL FINDINGS

All cases showed at least a partial nodular architectural pattern. Classic patterns A and B (according to Fan *et al.*¹⁹) were the only patterns observed in three cases, and another seven cases showed predominantly a classic A pattern. Seven cases showed the variant patterns C, D, and F, whereas two cases showed focal progression to T-cell/histiocyte-rich large B-cell-lymphoma-like areas (pattern E). Five cases showed pure patterns; the remaining 14 showed a mixed pattern. The nodular areas contained a mixture of lymphoid cells with small nuclei, a variable number of histiocytes, and large atypical neoplastic cells. In all cases, the majority of the neoplastic cells showed morphological features consistent with LP cells. However, in almost all cases (18/19), in addition to the typical LP cell

morphology, a varying number of neoplastic cells showed some cytomorphological features of HRS cells, with pleomorphic nuclei and prominent nucleoli (Figure 1A). In seven cases, a large number of HRS cells could be easily identified at $\times 10$ objective magnification. In nine additional cases, the number of the HRS cells was low, but they were present in all areas of the infiltrate, whereas, in two further cases, only few such cells could be identified after careful screening at higher magnification ($\times 20$). Epithelioid histiocytes were present in all cases; they formed aggregates in eight cases and granulomas in one case. Most cases (14/19) contained eosinophils in the background infiltrate. Although, mainly, these were rare and could be identified only after a thorough search at high magnification, seven cases contained areas with up to one eosinophil per high-power field (HPF), and one case showed up to five eosinophils/HPF. Two cases showed focal necrosis. Residual germinal centres could be identified in five cases. Focal capsular fibrosis was present in six cases, and focal

Table 2. Morphological features, immunophenotype of neoplastic cells and Epstein-Barr virus (EBV) infection patterns in EBV-positive nodular lymphocyte-predominant Hodgkin lymphoma

Case	Age (years)/sex	Anatomical site (LN)	Architectural pattern ¹⁹	HRS-like tumour cells	EBER	LMP-1	EBNA-2	BZLF1	CD20	CD79a	PAX5	OCT2	CD30	CD15	MEF2B
1	46/M	NA	A + B	Many	+	-	-	vf byst	+w	+w	+w	+s	-	-	ND
2	24/M	Cervical	C + D	Many	+	+	-	-	+s	+s	+v	+s	+s	-	+
3	56/M	Cervical	A + B	Many	+	+	vf byst	-	+s	+w	+w	+s	+v	vf	+
4	11/F	Cervical	C + A	Few	+	+	+	+	+s	-	+w	+s	-/+w	-	+
5	38/M	Axillary	A	Few	+	+	vf byst	-	+v	-/+v	+w	NA	-/+w	-	ND
6	69/F	Axillary	A + C	Many	+	+	+	+	+s	+w	+v	+s	+w/-	-	ND
7	85/F	Cervical	A + D	Some	+	+	+	vf byst	+s	+v	+v	+s	+v	vf	ND
8	47/F	Cervical	A + D	Some	+	+	-	-	+s	-	+w	+s	+w	-	ND
9	48/M	Mediastinal	C + E	Some	+	+	+	Several byst	+s	+s	+v	+s	+s	vf	ND
10	65/F	Cervical	D + C	Some	+	+	-	-	+s	+s	+s	+s	+v	vf	ND
11	25/M	Inguinal	A + C	Many	+	+	-	-	+s	-/+w	+w	+s	+w	-	ND
12	9/M	Abdominal	C	Some	+	+	-	-	+s	+v	+w	+s	+v	-	ND
13	26/M	Abdominal	A	Some	+	+	-	-	+s	+s	+v	+s	+v	-	ND
14	15/M	Inguinal	A + C	Many	+	+	+	NA	+s	+v	+v	+s	+s	-	ND
15	76/M	Cervical	C	Some	+/-	+	-	-	+s	+/-v	+w	+s	+v	-	ND
16	11/M	NA	F	No	+	+	-	-	+s	+w/-	+v	+s	+v	-	ND
17*	60/F	Cervical	A + C	Many	+/-	-	-	-	+s	+s	+v	+s	-	-	ND
18	13/M	Cervical	A + C	Some	+	+	+	vf byst	+s	+w	+w	+s	+v/-	-	ND
19	12/M	Cervical	C + E	Some	+	+	+	vf byst	+s	+w	+w	+s	-/+w	-	ND

byst, bystander lymphocytes; EBER, Epstein-Barr virus-encoded RNA; EBNA, Epstein-Barr virus-encoded nuclear antigen; F, female; HRS, Hodgkin and Reed-Sternberg; LMP, latent membrane protein; LN, lymph node; M, male; MEF2B, myocyte enhancer factor 2B; NA, not available; ND, not done; s, strong expression intensity; v, variable intensity; vf, very few; w, weak expression intensity; +, positive, -, negative, +/-, predominance of positively labeled cells; -/+ , predominance of negative cells. Cases with EBV latency type I are in bold; the rest showed latency type II.

Fan *et al.* architectural patterns: A, 'classic' B-cell-rich nodular; B, serpiginous/interconnected nodular; C, nodular with prominent extranodular neoplastic cells; D, nodular with a T-cell-rich background; E, diffuse, T-cell-rich B-cell lymphoma-like; F, diffuse 'moth-eaten' with a B-cell-rich background.

*Previously published as a case report.

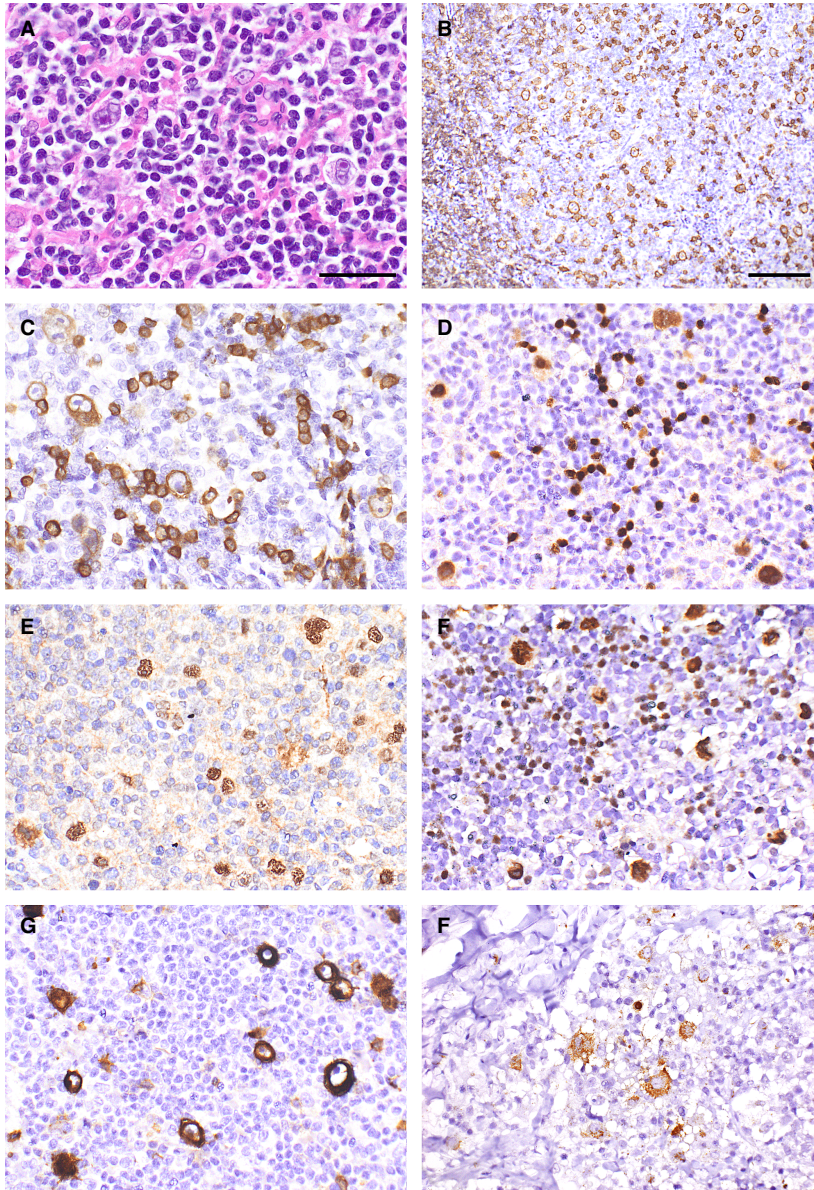


Figure 1. Immunohistochemical features of an Epstein–Barr virus-positive nodular lymphocyte-predominant Hodgkin lymphoma (case 2). **A**, Several of the neoplastic cells show features of Hodgkin and Reed–Sternberg cells. Haematoxylin and eosin. scale bar: 50 μ m. **B**, Immunohistochemistry reveals the expression of B-cell-characteristic antigens: The neoplastic cells express CD20 with similar intensity as the bystander B cells. (scale bar: 100 μ m). **C**, CD79a is also expressed by all neoplastic cells. **D**, PAX5 expression is of variable intensity. **E**, The neoplastic cells selectively express myocyte enhancer factor 2B. **F**, All neoplastic cells express OCT2 with higher intensity than the bystander B cells. **G**, Most neoplastic cells show strong expression of CD30 in this case. **H**, Only in a few cases do sparse neoplastic cells express CD15 (case 3).

intersecting fibrotic bands were found in seven cases and were usually associated with capsular fibrosis (5/7).

IMMUNOHISTOCHEMICAL ANALYSIS

The neoplastic cells expressed the B-cell-characteristic/specific antigens CD20 (19/19), CD79a

(17/19), PAX5 (19/19), and OCT2 (18/18). The vast majority showed strong and diffuse expression of CD20 (Figure 1B). CD79a expression was diffuse (Figure 1C), but with variable intensity in 13 of 17 cases. PAX5 expression was diffuse and either weak (10/19) or variable (8/19) (Figure 1D); only one case showed diffuse and strong PAX5 expression. OCT2 was robustly (diffuse and strong) expressed in all

cases (Figure 1F). The majority of cases (17/19) showed CD30 expression in the neoplastic cells (Figure 1G). In most of these (12/17), we observed CD30 expression in all neoplastic cells, whereas, in five cases, only partial/focal expression could be identified. The expression intensity was mostly variable (eight cases) or weak (six cases), whereas all neoplastic cells showed strong expression in three cases. Only a very few neoplastic cells expressed CD15 in four of 19 cases (Figure 1H). In two cases that contained large numbers of neoplastic cells with features of HRS cells that were also CD30-positive, and in one case showing no expression of CD79a, diffuse and strong expression of MEF2B was detectable (Figure 1E). In all cases, we observed T-cell rosettes (CD3/CD5) surrounding the neoplastic cells, and these rosettes expressed PD-1.

EBV EXPRESSION PATTERNS

By definition, all 19 cases harboured EBV-positive neoplastic cells. Whereas, in most cases (17/19), all neoplastic cells were positively labelled (Figure 2A), two cases also contained a low number of EBV-negative neoplastic cells (Figure 2B). Furthermore, in seven cases, a low number of lymphoid bystander cells were positively labelled (Figure 2C). Regarding

the EBV latency types, most cases were of latency type II (EBER-positive and LMP-1-positive, 17/19) (Figure 2D), whereas the remaining two cases showed latency type I (EBER-positive and LMP-1-negative). No EBNA-2-expressing neoplastic cells could be identified in this series, whereas single EBNA-2-positive bystander cells were present in five cases (Figure 3A). Three of these cases also showed single bystander cells expressing BZLF1 (Figure 3B), and one case contained several BZLF1-expressing bystander lymphocytes (Figure 3C).

Discussion

This study shows that EBV infection of the tumour cells in NLPHL, although a rare phenomenon, may occur in quite appreciable numbers in lymphoma reference centres, which receive diagnostically challenging cases for consultation. A search of our archives led to the identification of 19 such cases, which is a larger number than those in the two largest published studies on the subject. However, the frequency of EBV positivity in NLPHL in our patient population (2.5%) is somewhat lower than previously published.^{15,16} The goal of this study was to analyse in more detail the types of EBV latency, as previous studies mostly performed only EBER ISH, and only a

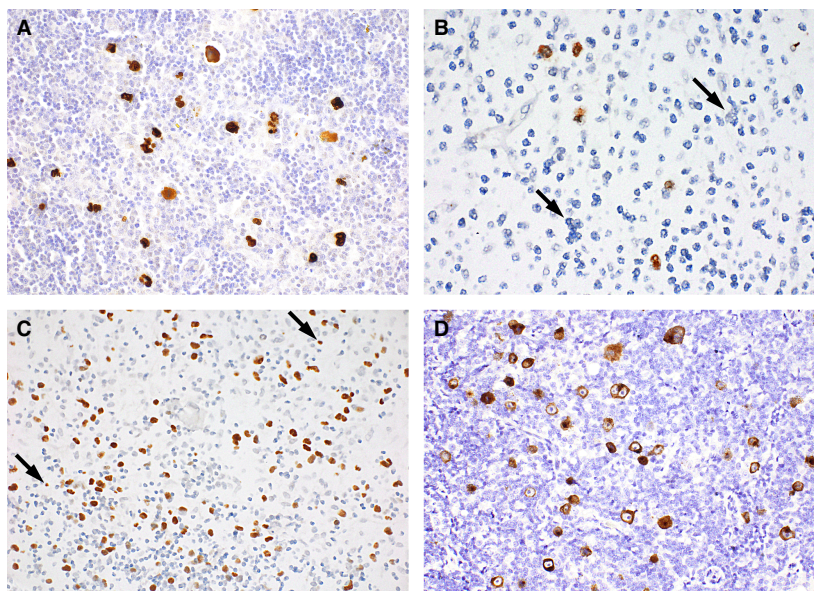


Figure 2. Epstein–Barr virus (EBV) infection patterns in different EBV-positive nodular lymphocyte-predominant Hodgkin lymphomas. A, In most cases, all neoplastic cells are labelled by EBV-encoded RNA (EBER) *in-situ* hybridisation (case 18). B, In two cases, EBER *in-situ* hybridisation highlights the fact that some neoplastic cells (arrows) are not EBV-infected. C, Besides EBV-positive neoplastic cells, several cases contain EBV-positive bystander lymphocytes (case 9; bystander cells highlighted by arrows). D, Except for two cases, all cases showed expression of EBV-encoded latent membrane protein-1 by all EBV-infected neoplastic cells (case 2).

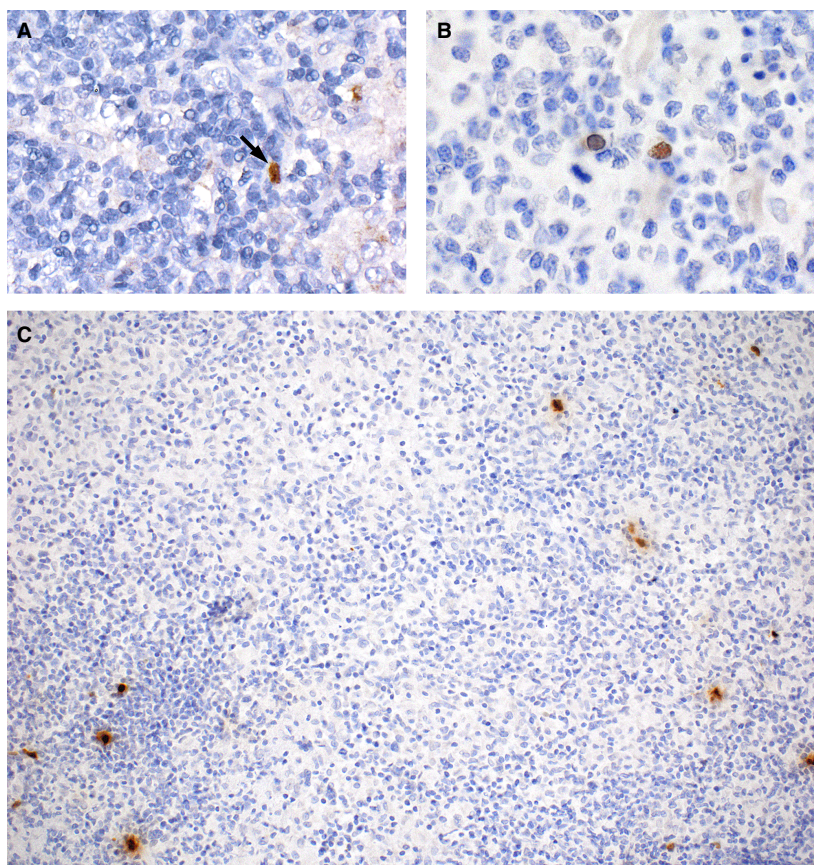


Figure 3. Expression of Epstein–Barr virus (EBV)-encoded nuclear antigen-2 (EBNA-2) and/or BZLF1 by bystander cells in EBV-positive nodular lymphocyte-predominant Hodgkin lymphoma. **A**, Bystander lymphocytes expressing EBNA-2 (case 7). **B**, The same case also contains lymphocytes expressing BZLF1. **C**, One case (case 9) showed numerous BZLF1-positive lymphocytes in the background infiltrate.

fraction of positive cases were additionally screened for possible LMP-1 expression. The presented results have implications for diagnostic routine and for the biology of NLPHL, as discussed in more detail below.

As in previous reports, we have observed that EBV-positive NLPHL can pose diagnostic problems, especially in cases harbouring considerable numbers of neoplastic cells with cytomorphological features of HRS cells, as seen in 16 of 19 (84%) of our cases. In addition, eight cases contained eosinophilic granulocytes in noteworthy numbers in the background infiltrate, which may also be present in NLPHL but add to the diagnostic dilemma of distinguishing it from CHL. This overlap between NLPHL and CHL was further enhanced by the fact that most of our cases (17/19) showed CD30 expression by the neoplastic cells, which was quite diffuse in 12 cases, albeit of variable intensity. Four cases also showed CD15 expression in very few neoplastic cells. Reliable differentiation of EBV-positive NLPHL from CHL (especially LRCHL) can be achieved by analysing both the immunophenotype

of the neoplastic cells and their background infiltrate. Regarding the neoplastic cells, all cases of the present series showed a preserved B-cell transcription programme. The neoplastic cells showed strong expression of OCT2 and CD20. In contrast, although PAX5 expression was present in the neoplastic cells of all cases, we mostly observed weak or variable intensity. The expression pattern of CD79a was more variable, in terms of both the number of positive cells and intensity, ranging from strong and diffuse to variable/weak or partial expression, and two cases were negative. This variability in the expression patterns of PAX5 and CD79a might be caused by the EBV infection, which can alter the immunophenotype of infected B cells.^{20,21} In three cases that posed diagnostic difficulties, because of either strong expression of CD30, absence of CD79a expression, or weak expression of both PAX5 and CD79a, detection of robust expression of MEF2B by the neoplastic cells supported the diagnosis of NLPHL.¹⁷ Regarding the background infiltrate, all cases showed rosettes of PD-

1-positive T cells, a feature constantly seen in NLPHL. In addition, all cases showed at least partially a nodular architecture pattern, mostly the classic pattern A according to Fan *et al.*¹⁹

Investigation of the EBV-positive NLPHL cases for the EBV latency patterns revealed that none of the cases showed latency pattern III. Most EBV-positive NLPHLs (17/19) showed latency pattern II. Moreover, in line with the observation of Huppman *et al.*,¹⁶ we encountered two cases with EBV latency type I. This heterogeneity in the latency pattern adds to the differences between NLPHL and CHL, in which latency pattern II is always present. We did not observe differences in morphology and growth pattern between latency type I and latency type II cases; however, according to our observations, cases with type I EBV latency unexpectedly did not show CD30 expression by the neoplastic cells. Similar findings have been reported by Huppman *et al.* (case 6).¹⁶ These data demonstrate that such cases can be missed when only LMP-1 IHC is employed to search for a possible EBV association in NLPHL, and if only CD30-expressing NLPHL cases are examined in this regard.

Another interesting difference between EBV-positive CHL and NLPHL is the finding in two of our cases that not all tumour cells showed EBER signals, as has also been described previously by Wang *et al.*¹⁵ There are at least two possible scenarios to explain this phenomenon. One possibility is that EBV might have initially infected all neoplastic cells, and a proportion later abandoned viral episomes. This 'hit-and-run' scenario of EBV coincides with the question of whether EBV may be only a transient driver of lymphomagenesis. This scenario proposes that the transforming events initially provided by EBV are later functionally replaced by stable genetic changes in the host cell. In this case, the viral episome, whose replication is inherently imperfect after each cell cycle, is gradually lost from the neoplastic clone.²² This loss of EBV episomes has been observed not only in the EBV-positive Burkitt lymphoma cell line Akata²³ but also in a small series of samples from Burkitt lymphoma patients that showed sporadic loss of EBV DNA after tumour initiation.²⁴ Data from a more recent study using high-sensitivity methods provided additional evidence that this 'hit-and-run' scenario might be more frequent than currently acknowledged.²⁵

Another possibility is that partial infection of the neoplastic cells could indicate a secondary infection. We have proposed this scenario in previous articles,^{26,27} and it has also gained some support from others.^{15,28} In these cases, the patients are thought to have some degree of immunosuppression, allowing

EBV reactivation. This scenario leads to the question of whether immune dysregulation or immunodeficiency causes EBV infection of NLPHL. There are single reports of NLPHL in the context of congenital immune dysregulation syndromes^{29,30}; however, these cases were not associated with EBV. Nevertheless, there is some indirect and direct evidence supporting this scenario. Indirect evidence is the presence of additional EBER-positive bystander lymphocytes, as found in seven of our cases and also in the study of Wang *et al.*¹⁵ In addition, EBV latency types II (as observed in most of our cases) and III tend to occur in patients with increasing degrees of immunosuppression. A more direct indication of EBV reactivation is the finding of some bystander cells in four cases of our series that expressed BZLF1. This transcription factor, also named ZEBRA, Zta, Z, or EB-1 when expressed in latently infected cells, can switch EBV from latency to the lytic cycle. BZLF1 can also reactivate transcriptionally silent host genes and can thus affect key cellular pathways implicated in angiogenesis, cell cycle control, proliferation, and apoptosis.³¹ Further direct evidence supporting this concept is the observation in five of our cases of single bystander lymphocytes expressing EBNA-2. EBNA-2 is the earliest expressed latent protein³²; it can drive the cells through the first G₁ phase and activates the promoters necessary to produce all latent proteins expressed in the EBV growth programme.³³ Thus, these EBNA-2-expressing bystander lymphocytes could represent early infected lymphocytes due to recent EBV reactivation.

In summary, EBV-positive NLPHL cases might pose a diagnostic problem because of the atypical morphological features of the neoplastic cells combined with the frequent expression of CD30. As noted in previous studies, the preservation of the B-cell-transcription programme, MEF2B expression, and an NLPHL-typical microenvironment and architecture of the infiltrate, allow reliable distinction from CHL. EBER ISH is the method of choice to identify all of these cases. The majority of cases present with EBV latency type II and, more rarely, with latency type I, which can be associated with missing CD30 expression. The presence of occasional bystander cells expressing BZLF1 and/or EBNA-2 and the partial infection of neoplastic cells in some cases are possible indicators that EBV infection in NLPHL might be caused by some degree of immune dysregulation or immunodeficiency that has led to EBV reactivation. Nevertheless, a 'hit-and-run' scenario for EBV cannot be excluded. Further molecular studies, e.g. of the mutational load of EBV-positive and EBV-negative NLPHL cases, as

already performed in CHL,³⁴ may further elucidate the role of EBV in the pathogenesis of NLPHL.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

All authors contributed to the conception and design of the study. E. Gerhard-Hartmann, A. Zamó, A. Rosenwald, I. Anagnostopoulos and M. Rosenfeldt provided study material and specimens, and histopathological diagnoses. E. Gerhard-Hartmann, L.-M. Schinagl, I. Anagnostopoulos and M. Rosenfeldt collected and assembled data. E. Gerhard-Hartmann, K. Jöhrens, A. Zamó, I. Anagnostopoulos and M. Rosenfeldt performed data analysis. I. Anagnostopoulos supervised the project. I. Anagnostopoulos and E. Gerhard-Hartmann wrote the first draft of the manuscript, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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