Epstein-Barr Virus Genome in Non-Hodgkin’s Lymphomas Occurring in Immunocompetent Patients: Highest Prevalence in Nonlymphoblastic T-Cell Lymphoma and Correlation With a Poor Prognosis

By Francesco d’Amore, Preben Johansen, Annette Houmand, Dennis D. Weisenburger, and Leif S. Mortensen

A series of 520 cases of non-Hodgkin’s lymphoma (NHL; 374 of B-cell, 130 of T-cell, 5 of non-B/non-T-cell, and 11 of undetermined phenotype) was analyzed for the presence of Epstein-Barr virus (EBV) using RNA in situ hybridization (ISH). The aims of the study were to assess the frequency of EBV-encoded small nuclear RNAs 1 and 2 (EBER), abundant immediate early nuclear antigens (EBERs), and latent membrane protein-1 (LMP-1) in cases covering the entire histologic spectrum of NHL, and to analyze whether EBV status had prognostic relevance with regard to patient survival. EBER positivity was found in 25 of 374 (7%) B-NHL and 40 of 130 (31%) T-NHL (P < .00005) cases, but in only 16 cases with non-B/non-T-cell or undetermined phenotype. Among T-NHL cases, EBER positivity was confined to angioimmunoblastic, lymphadenopathy-like lymphoma (11 of 13 cases, 85%), Lennert’s lymphoma (five of seven cases, 71%), and pleomorphic T-NHL (24 of 67 cases, 36%). Mycosis fungoides, lymphoblastic, and CD30-positive anaplastic large T-cell NHL cases were consistently EBV-negative. Double-labeling by RISH and immunophenotyping demonstrated the presence of EBV in neoplastic T cells, but no CD21 expression was found in the EBER-positive T-NHL cases. LMP-1 was expressed in 12 of 40 (30%) EBER-positive T-NHL and 5 of 25 (20%) EBER-positive B-NHL cases. For both T- and B-NHL, no correlation was found for EBV positivity and age, sex, clinical stage, or serum level of lactate dehydrogenase (LDH) at diagnosis. However, in T-NHL but not B-NHL, EBER positivity correlated with the presence of constitutional symptoms and a poor performance score (PS >1; scale, 0 to 4). EBER status did not have any prognostic significance in B-NHL, but it had a negative prognostic impact in high-grade T-NHL. Ten-year survival of EBER-negative vs EBER-positive cases: 33% vs 14%; P = .01. A multivariate analysis including all B- and T-NHL of intermediate/high-grade histology showed that EBER positivity in T-NHL was one of the three most significant factors recognized by the final prognostic model, only surpassed by PS greater than 1 and age greater than 67 years, and more powerful than B symptoms, an elevated LDH, or disseminated disease (clinical stage greater than II). We conclude that patients with EBV-positive T-NHL have a very poor clinical outcome, that EBV status should be considered as additional useful information in the classification of T-NHL, and that EBV-positive T-NHL should be treated as a separate entity in the future.

© 1996 by The American Society of Hematology.
presence of EBV, as evidenced by EBER RISH, has prognostic relevance with regard to patient survival in specific NHL subsets.

MATERIALS AND METHODS

Patient Population and Clinicopathologic Assessment

For the present study, 520 patients were randomly selected from the database of the Danish Lymphoma Study Group, the LYFO Registry. This is a population-based registry of all new cases of NHL diagnosed in western Denmark (Jutland and Funen; 2.8 million inhabitants) that was activated on January 1, 1983, and is ongoing. The organization of the registry, including clinical and histologic assessment criteria, has been previously described.13 For the purpose of the study, eligible cases were randomly selected from each NHL histologic subtype to cover the entire spectrum of NHL. Eligibility criteria were (1) morphologically and immunophenotypically confirmed NHL diagnosis, (2) availability of pretreatment clinical parameters and treatment and follow-up data, and (3) access to formalin-fixed, paraffin-embedded samples containing a sufficient amount of representative tumor tissue obtained before primary treatment. Furthermore, cases selected for the study were reviewed morphologically and immunophenotypically by one of the investigators (P.J.), who is a member of the pathology panel of the Danish Lymphoma Study Group. For T-NHL subtypes, the terminology of the updated Kiel classification12 was used, and the cases were subdivided into low-grade and high-grade categories. Although originally placed in the low-grade category by the updated Kiel classification12 was used, and the cases were subdivided into low-grade and high-grade categories. Although originally placed in the low-grade category by the updated Kiel classification, the AILD subtype was included within the high-grade category in accordance with previous reports on the aggressive clinical behavior of this subtype.14 With regard to Lennert’s lymphoma, survival data of the LYFO Registry cases were consistent with inclusion in the low-grade category (see Results). However, because an aggressive clinical behavior has also been reported for Lennert’s lymphoma,13 the survival analysis of high-grade T-NHL was performed both with and without the inclusion of Lennert’s lymphoma. Pleomorphic and immunoblastic T-NHL cases were considered as one group (PTCL). The cases of T cell-rich B-NHL (TCRB) were identified according to the criteria proposed by Jaffe et al15 and, for the purpose of survival analysis, were grouped into the high-grade B-NHL category. All patients included in this study were white patients who had no evidence of overt congenital or acquired immunodeficiency.

Tissue Specimens

Neutral buffered formalin is the routine tissue fixative related to the highest degree of nucleic acid preservation.16 Therefore, for the purpose of this study, only formalin-fixed, paraffin-embedded tissue specimens were used. The large majority of tissue samples had a fixation time of 12 to 48 hours. For IH and RISH, 5- to 6-μm thick tissue sections were cut and mounted on glass slides coated with 3-amino-propyltriethoxysilane (Sigma, St Louis, MO). All slides were stained with hematoxylin and eosin, and Giemsa stains.

Immunohistochemistry

Immunohistochemical analysis was performed on paraffin or, when available, frozen sections using the following antibodies (Abs): for B cells, CD20 (L26; DAKO, Glostrup, Denmark); two Abs for T-cells, CD3 (polyclonal CD3; DAKO) and CD45RO (UCHL-1; DAKO); anti-CD30 Ab (Ber-H2; DAKO) for LMP-1 receptor/EBV receptor CD21 Ab (1F8, DAKO, on paraffin sections, or B2, Biogenex, Mainz, Germany, on frozen sections); anti–low-affinity IgE receptor CD23 Ab (MHH6; DAKO) only applicable on frozen tissue sections; and anti–LMP-1 Ab, consisting of a pool of four monoclonal Abs recognizing three different epitopes of the LMP-1 molecule (CS 1-4; DAKO). Staining for LMP-1 was performed on paraffin sections and, when available, frozen tissue sections. For all Ab stains, positive controls (tissue sections with known positivity for the relevant antigen) and negative controls (identical staining procedure but without primary antibody) were included. For LMP-1 stains, paraffin-embedded tissue sections from Hodgkin’s and NHL cases of known LMP-1 positivity were used as controls. Before immunohistochemical staining, antigen demasking was performed by either proteolytic digestion or microwave pretreatment. Microwave pretreatment (15 minutes in 10 mmol/L citrate buffer, pH 6.0) was the optimal antigen retrieval procedure for LMP-1 staining of paraffin-embedded tissue sections. Ab binding was detected by a standard three-step immunoperoxidase technique using the labeled streptavidin-biotin method (LSAB; DAKO); peroxidase activity was revealed using 3-amino-9-ethylcarbazole (AEC; Sigma), and the slides were counterstained with Mayer’s hematoxylin.

RISH

Probes and controls. For EBER detection, a mixture of five fluorescein-conjugated oligodeoxyribonucleotides (30-mers; EBER, DAKO) complementary to the two EBV-encoded small nuclear RNAs17 was used. EBERs are actively transcribed in latently infected cells. For BHLF detection, a mixture of three fluorescein-conjugated oligodeoxyribonucleotides (30-mers; BHLF, DAKO) was used. They were complementary to two abundant immediate-early mRNAs encoding proteins belonging to the EB-A-D subgroup of EBV-encoded antigens.18 BHLF is expressed in permissively infected cells. Omission of the labeled probe, preincubation with ribonuclease A (Boehringer Mannheim, Mannheim, Germany), or substitution of the probe with a 3'-fluorescein–conjugated 30-base randomor oligonucleotide (provided by J.J. Hyldig, DAKO) all resulted in negative stains. Incubation of tissue sections with deoxyribonucleoside (Boehringer Mannheim) did not affect RISH signals. For evaluation of total RNA integrity, a fluorescein-conjugated, single-stranded, antisense DNA probe (β-actin; DAKO) was used. This probe hybridizes to all of exon 3 and part of exon 4 of the mRNA transcript of the human β-actin gene. β-Actin is widely expressed in a variety of cell and tissue types, including lymphoid tissue, and can, therefore, serve as an appropriate control for mRNA preservation.19 Hybridization with the sense strand oligonucleotide of the human β-actin gene (DAKO) yielded a negative stain.

RISH protocol. Sections were dewaxed and rehydrated, digested with proteinase K, and postfixed for 20 minutes in 4% paraformaldehyde. Subsequently, the sections were allowed to prehybridize for 60 minutes at 45°C in a solution consisting of 50% formamide, 20% dextran sulfate, 0.2% polyvinyl pyrrolidone, 0.2% ficoll, 0.1% sodium pyrophosphate, 5 mmol/L NaEDTA, and 50 mmol/L Tris/HCl, pH 7.6. Hybridization with the relevant probe was performed overnight at 45°C. After hybridization, sections were washed for 2 minutes in 2x standard saline citrate (SSC) at room temperature, followed by two washes in preheated (45°C) 2x SSC containing 50% formamide and two final washes of 5 minutes each in 2x SSC at room temperature. For visualization of hybridization signals, the alkaline phosphatase antialkaline phosphatase (APAAP) method20 was used in association with Fast Red TR salt (4-chloro-2-methylbenzeneazidionium; Sigma). For double-labeling purposes (RISH/IH), nitroblue tetrazolium (NBT)/brom-chlorindoxyl phosphate (BCIP) was used for visualization of the hybridization signals. Mayer’s hematoxylin was used for nuclear counterstaining.

Definition and patterns of positivity. The number of EBER-positive cells was visually estimated by light microscopy. The scoring system was analogous to that reported by Weiss et al.21 Briefly, scores were reported as the mean of 10 (or less if limited by section
size) medium power fields (10× ocular lens and 20× objective) and were subdivided into four groups: no positive cells (negative), less than 10 positive cells (+), 10 to 40 positive cells (++), and greater than 40 positive cells (+++). Examples of +, ++, and +++ patterns of positivity are shown in Fig 1A, B, and C, respectively.

Double Labeling

After performance of the RISH protocol, including the APAAP detection step, endogenous peroxidase activity was inactivated by 0.3% H₂O₂. Membrane antigens were then detected by the peroxidase-based LSAB technique (DAKO).

Treatment Regimens and Follow-Up

The clinical and histologic assessment criteria, treatment regimens, and follow-up procedures applied to patients from the LYFO Registry have been described in detail previously. All patients were treated according to common general guidelines resulting in a rather homogeneous therapeutic background. Cases of intermediate-/high-grade histology were treated with polychemotherapy, ie, cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone (CHOP) or CHOP-like regimen.

Statistical Analysis

Age distributions were compared by the non-parametric Mann-Whitney test. Differences between crosstabulated values in frequency tables were evaluated statistically by the Pearson χ² test. Duration of survival was calculated from the date of conclusive histologic diagnosis to the date when the patient was last known to be alive or to the date of death. Survival curves were calculated by the method of Kaplan and Meier. As an initial step, the prognostic significance of a variety of features, including EBV status, was estimated in a univariate fashion by the Tarone-Ware test. Among those with univariate significance, independent factors of prognostic value for survival were subsequently identified by a Cox regression analysis. Only cases without missing values among the variables selected for the Cox analysis after the univariate step were included at the multivariate level. The BMDP statistical program package (Statistical Software, Los Angeles, CA) was used. Programs 1D and 4F were used for data description and the frequency tables, and programs 1L and 2L were used for univariate and multivariate survival analysis.

RESULTS

General Patient Characteristics

The general patient characteristics at diagnosis regardless of EBV status are reported in Table 1, wherein a comparison of the distribution of major clinicopathologic features among
the B- and T-NHL cases is also shown. B- and T-NHL cases were comparable as to age, clinical stage, performance score (PS), and serum level of lactate dehydrogenase (s-LDH) at diagnosis. Although not significantly different, patients within the T-NHL group were younger, as evidenced by lower mean and median ages (6 and 4 years lower, respectively). Compared with the B-NHL group, there was a modest excess of males and a more marked excess of patients presenting with constitutional symptoms in T-NHL. The B-NHL group had significantly more low-grade cases than the T-NHL group (41% vs 15%, \( P < .0005 \)). The B- to T-cell phenotype ratio in the present series was 2.9:1. The relatively high fraction of T-NHL cases reflects a positive selection effort performed to obtain a sufficiently high number of T-NHL cases to improve statistical confidence in the analysis of survival data.

**RISH**

Of the 374 cases with a B-cell phenotype, 25 (7%) were EBER-positive as compared with 40 of 130 (31%) T-NHL cases (\( P < .00005 \)). Of the five cases with a non-B/non-T phenotype, all of which were CD30-positive, anaplastic large cell lymphomas (ALCL), one was EBER-positive. None of the 11 NHL cases with an undetermined phenotype was EBER-positive. In Table 2, the RISH findings for EBER are summarized in relation to phenotype and histology. Among the 25 EBER-positive B-NHL cases, only two cases, a Burkitt’s lymphoma and a diffuse large cell lymphoma, showed extensive EBER positivity (+ + +). Of the remaining 23 cases, four (one diffuse large, two diffuse mixed, and one follicular mixed NHL) had intermediate positivity (+ + ), and 19 (ie, the large majority) contained only a few scattered EBER-positive cells (+). The latter group was heterogeneous, including a variety of histologic subtypes within the low-grade and intermediate/high-grade categories. Interestingly, two of the three TCRB cases contained a small number of EBER-positive cells that were morphologically heterogeneous and did not seem to belong consistently to the neoplastic large B-cell population.

The distribution pattern of EBER positivity among the T-NHL cases was markedly different from that found in B-NHL. Low-grade cutaneous and high-grade lymphoblastic T-NHL were consistently EBER-negative, as were the nine cases of CD30-positive ALCL of T-cell phenotype. EBER positivity was confined to three T-NHL subtypes: AILD, with 11 of 13 (85%) cases positive; Lennert’s lymphoma, with five of seven cases (71%) positive; and PTCL. PTCL was the T-NHL subtype with the largest number of screened patients (\( N = 67 \)), and more than a third (36%) of all cases had some degree of EBER positivity. One third of the EBER-positive PTCL cases had extensive EBER positivity (+ + +), and the EBER-positive cells from a morphologic and phenotypic point of view clearly coincided with the tumor cell population. PTCL accounted for 73% (8 of 11) of all +++ positivity cases. For AILD and Lennert’s lymphoma, extensively positive (+ + +) cases accounted for only 1 of 11 (9%) cases and none of five cases, respectively. BHLF positivity was rare and could only be detected in 3 of 520 screened cases (0.6%). All three cases were extensively EBER-positive (+ + +); two were PTCL, and one was AILD.

**Immunohistochemistry**

**Phenotype of EBER-positive cells.** Phenotypic lineage analysis of EBER-positive cells was performed by combined RISH and IH on all T-NHL cases and all strongly positive (+ + + and ++ ) B-NHL cases. The findings of the phenotypic analysis are shown in Table 3, and the cases can be categorized into four major groups. In the first group, the majority of EBER-positive cells also expressed membrane-associated CD3 and/or CD45RO (Fig 2). All PTCL cases with strong (+ + and +++ ) EBER positivity could be assigned to this group. In the second group, the majority of EBER-positive cells expressed membrane-associated CD20. In the two cases showing strong (++++ ) positivity (Burkitt’s lymphoma and diffuse large cell lymphoma), the EBER-positive cells coincided morphologically with the tumor cell population. In the third group, EBER-positive cells were a mixed population, in which some of the cells were CD3- and/or CD45RO-positive and others were CD20-positive.
POOR PROGNOSIS IN EBV-POSITIVE T-CELL LYMPHOMAS

The majority of AILD cases, including the one with extensive EBER positivity, belonged to this group. In the last group, the majority of EBER-positive cells did not react with the tested B- and T-cell markers. However, some cells that did not react with CD3, CD45RO, or CD20 was a common finding in all cases.

**LMP-1.** The results of immunohistochemical detection of LMP-1 are also shown in Table 3. In the strongly EBER-positive (+++ and +++) groups, LMP-1 expression was found in only 55% of the cases, whereas the weakly EBER-positive (+) group had only 11% positive cases. Overall, LMP-1 expression was found in 12 of 40 (30%) EBER-positive T-NHL and five of 25 (20%) EBER-positive B-NHL cases.

**CD21 and CD23.** A total of 23 EBER-positive T-NHL cases were screened for CD21 expression in both paraffin and frozen sections. A marked difference in CD21 expression between T-NHL subtypes was found. None of the 13 EBER-positive PTCL cases expressed CD21. Conversely, all of the AILD (N = 8) and Lennert’s lymphoma (N = 2) cases had a strong reactivity for CD21 with a dendritic staining pattern related to proliferating follicular dendritic cells within the tumor areas. A morphologic evaluation on adjacent tissue sections revealed that EBER-positive lymphoid cells did not express CD21. The expression of CD23 expression in T-NHL closely followed that of CD21 in terms of histologic subtypes and reactivity pattern.

**Correlation of EBER Positivity With Clinicopathologic Presentation Features**

For both T- and B-NHL, no correlation was seen between EBER positivity and age, sex, clinical stage, or s-LDH at diagnosis. EBER positivity correlated with the presence of constitutional symptoms and PS greater than 1 in T-NHL, but not in B-NHL. Of the eight PTCL cases with strong (++) EBER positivity, with a median survival of 6 months and mean age of 53 years (range, 29 to 82 years), four (50%) presented with localized disease, a normal s-LDH, and a good PS (World Health Organization [WHO] grade 0-1) at diagnosis. The same discrepancy between EBER positivity

---

**Table 2. Correlation Between Histology, Phenotype, and EBER Status**

<table>
<thead>
<tr>
<th>Histologic Subtype</th>
<th>Total No. of Cases</th>
<th>EBER Status (no. of cases)</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>+</td>
</tr>
<tr>
<td>Low grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-cell phenotype</td>
<td>154</td>
<td>145</td>
<td>8</td>
</tr>
<tr>
<td>Lymphocytic</td>
<td>29</td>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>Lymphoctic, plasmacytoid</td>
<td>43</td>
<td>43</td>
<td>0</td>
</tr>
<tr>
<td>Follicular small cleaved and mixed cell</td>
<td>49</td>
<td>45</td>
<td>3</td>
</tr>
<tr>
<td>MALT B-NHL</td>
<td>28</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>Unclassifiable</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>T-cell phenotype</td>
<td>20</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>Low-grade cutaneous*</td>
<td>13</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Lennert’s lymphoma</td>
<td>7</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Phenotype undetermined</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

**Intermediate/high grade**

| B-cell phenotype                  | 232               | 216      | 11 | 3  | 2  | 16                  | (6.9)     |
| Follicular large cell             | 4                 | 4        | 0  | 0  | 0  | 0                   | (0.0)     |
| Diffuse small cleaved cell        | 50                | 48       | 2  | 0  | 0  | 2                   | (4.0)     |
| Diffuse mixed cell                | 35                | 30       | 3  | 2  | 0  | 5                   | (14.3)    |
| Lymphoplasmacytoid, polymorphic   | 11                | 11       | 0  | 0  | 0  | 0                   | (0.0)     |
| CD30-positive ALCL                | 7                 | 7        | 0  | 0  | 0  | 0                   | (0.0)     |
| Diffuse large cell                | 74                | 71       | 1  | 1  | 1  | 3                   | (4.1)     |
| T-cell-rich                       | 3                 | 1        | 2  | 0  | 0  | 2                   | (66.7)    |
| Immunoblastic                     | 21                | 20       | 1  | 0  | 0  | 1                   | (4.8)     |
| Small noncleaved, Burkitt’s       | 12                | 9        | 2  | 0  | 1  | 3                   | (25.0)    |
| Small noncleaved, non-Burkitt’s   | 12                | 12       | 0  | 0  | 0  | 0                   | (0.0)     |
| Unclassifiable                    | 3                 | 3        | 0  | 0  | 0  | 0                   | (0.0)     |
| T-cell phenotype                  | 110               | 75       | 20 | 6  | 9  | 35                  | (31.8)    |
| PTCL                              | 67                | 43       | 13 | 3  | 8  | 24                  | (35.8)    |
| AILD                              | 13                | 2        | 7  | 3  | 1  | 11                  | (84.6)    |
| CD30-positive ALCL                | 9                 | 9        | 0  | 0  | 0  | 0                   | (0.0)     |
| Lymphoblastic                     | 20                | 20       | 0  | 0  | 0  | 0                   | (0.0)     |
| Unclassifiable                    | 1                 | 1        | 0  | 0  | 0  | 0                   | (0.0)     |
| Non-B/non-T cell phenotype         | 5                 | 4        | 1  | 0  | 0  | 1                   | (20.0)    |
| CD30-positive ALCL                | 5                 | 4        | 1  | 0  | 0  | 1                   | (20.0)    |
| Phenotype undetermined            | 8                 | 8        | 0  | 0  | 0  | 0                   | (0.0)     |
| Total                            | 520               | 454      | 44 | 11 | 11 | 66                  | (12.7)    |

Abbreviation: MALT, mucosa-associated lymphoid tissue.

* Mycosis fungoides, Sézary syndrome.
Table 3. Correlation Between EBER Positivity, Histology, and Immunohistochemical Findings

<table>
<thead>
<tr>
<th>EBER Status/Lineage/Histology</th>
<th>No. of Cases</th>
<th>T B</th>
<th>B/T</th>
<th>Null</th>
<th>LMP-1 Positivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>++ pattern</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-cell</td>
<td>11</td>
<td>6/11 (55)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse large cell</td>
<td>1</td>
<td>1/1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small noncleaved, Burkitt's</td>
<td>1</td>
<td>1/1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T cell</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTCL</td>
<td>8</td>
<td>8/8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AILD</td>
<td>1</td>
<td></td>
<td>1/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ pattern</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-cell</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse mixed cell</td>
<td>1</td>
<td>1/1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse large cell</td>
<td>2</td>
<td>2/2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T cell</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTCL</td>
<td>3</td>
<td>3/3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AILD</td>
<td>3</td>
<td></td>
<td>3/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lennert’s lymphoma</td>
<td>1</td>
<td></td>
<td>1/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ pattern</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-cell</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytic</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular small cleaved and mixed cell</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MALT</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse small cleaved cell</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse mixed cell</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse large cell</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-cell-rich</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunoblastic</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small noncleaved, Burkitt’s</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T cell</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AILD</td>
<td>7</td>
<td>1/7</td>
<td>4/7</td>
<td>2/7</td>
<td>0/7</td>
</tr>
<tr>
<td>Lennert’s lymphoma</td>
<td>4</td>
<td></td>
<td>3/4</td>
<td>1/4</td>
<td>1/4</td>
</tr>
<tr>
<td>Non-B/non-T</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD30-positive ALCL</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: ND, not determined.

- Phenotypes are defined as follows: T, CD3- and/or CD45RO-positive; B, CD20-positive; B/T, mixed population containing CD3/CD45RO-positive cells and CD20-positive cells; Null; majority of EBER-positive cells not reactive with T- and B-cell markers.

and other prognostic factors was seen in one of three (33%) PTCL cases with strong (++) EBER positivity, and 4 of 13 (31%) PTCL cases with weak (+) EBER positivity. Of the two B-NHL cases with strong (++) EBER positivity, both had advanced-stage disease and an elevated s-LDH.

Correlation between EBER status and anatomic site. Of the 130 T-NHL cases included in the study, 54 (42%) were extranodal. Of the 40 EBER-positive T-NHL cases, 29 (73%) were nodal and 11 (27%) were extranodal. Of the 11 EBER-positive extranodal T-NHL cases, five (46%) were primarily localized to the oro-/nasopharyngeal region and represented the totality of the oro-/nasopharyngeal T-NHL cases screened. The remaining six EBER-positive extranodal T-NHL cases were localized to the skin (2 cases of 25 screened), lung (one of eight screened), small intestine (1 case of 10 screened), and connective tissue (two of six screened). Of the eight PTCL cases with strong (++) EBER positivity, six were extranodal, and four of these were localized to the oro-/nasopharyngeal region. Similar to their T-NHL counterparts, EBER-positive B-NHL cases were also predominantly nodal (19 of 25 cases, 76%). The six EBER-positive extranodal B-NHL cases were localized to the gastrointestinal tract (three cases), salivary glands (two cases), and uterus (one case). The only EBER-positive case of non-B/non-T phenotype was nodal.

Survival Analysis

Univariate analysis. For survival analysis at the univariate level, the following parameters were considered: age, sex, clinical stage, PS, B symptoms, s-LDH, phenotype, histologic grade, EBER status at diagnosis, and LMP-1 expression. EBER status did not have any prognostic influence on B-NHL in general (7-year survival of EBER-negative v EBER-positive cases, 51% v 36%; median survival, 4.4 v 2.9 years) or on intermediate-/high-grade B-NHL in particular (7-year survival of EBER-negative v EBER-positive cases, 37% v 38%; median survival, 2.8 v 2.7 years; Fig 3A and B). Conversely, EBER positivity had a significant
negative prognostic impact on T-NHL in general (7-year survival of EBER-negative vs EBER-positive cases, 31% vs 20%; median survival, 1.9 years vs 8 months) and on high-grade T-NHL (Lennert’s lymphoma not included) in particular (7-year survival of EBER-negative vs EBER-positive cases, 33% vs 14%; median survival, 1.5 years vs 6 months; Fig 3C and D). A significant adverse effect of EBER positivity on high-grade T-NHL survival was still present, although less marked, if the Lennert’s lymphoma cases were included in that group (P = .03). The three T-NHL subtypes accounting for the totality of EBER-positive T-NHL cases differed significantly in their 7-year-survival values regardless of EBER status, with Lennert’s lymphoma as the subtype with the most favorable prognosis (PTCL, 14%; AILD, 31%; and Lennert’s lymphoma, 57%; P = .027). Interestingly, survival of EBER-positive high-grade T-NHL cases (Lennert’s lymphoma cases not included) was equally poor regardless of the degree of EBER positivity (7-year survival of +++, ++, and + cases: 10%, 33%, and 11%; median survival: 7 months, 20 months, and 6 months; P = .50). Among EBER-positive T-NHL, no survival difference was found between LMP-1-negative and LMP-1-positive cases.

Multivariate analysis. A multivariate Cox regression analysis was performed on cases of intermediate-/high-grade histology (Table 4). EBER positivity in T-NHL was one of the three most important prognostic features for intermediate-/high-grade NHL, only surpassed by PS greater than 1 and age greater than 67 years. As a prognostic factor, EBER positivity in T-NHL was more powerful than B symptoms, elevated s-LDH, or disseminated disease (clinical stage greater than II).

DISCUSSION

The results of this study indicate that the distribution of EBV-positive cases within the histologic spectrum of NHL is nonrandom. The likelihood that the EBV distribution pattern within our case material reflects the true biologic picture, at least within a white NHL population, is enhanced by the population-based origin of the case material that was randomly selected for this study. EBER positivity was found in about one third of all T-NHL cases, an overall frequency fourfold to fivefold higher than that seen in B-NHL. Furthermore, EBER positivity was confined to a subset of T-NHL subtypes (ie, AILD, Lennert’s lymphoma, and PTCL), whereas low-grade cutaneous, lymphoblastic, and CD30-positive ALCL cases were consistently EBV-negative. In both B- and T-NHL, EBV occurred in nodal as well as extranodal cases. In agreement with previous reports, a preferential localization of EBV-positive extranodal T-NHL cases to the oro-/nasopharyngeal region was observed.

With regard to T-NHL, the results of the present and of those other studies, that assessed EBV frequency by EBER RISH, were fairly comparable. In T-NHL, the EBV frequency varied between 31%19 and 47%;16 for PTCL, was in the order of 71% (present study) to 75%19 for Lennert’s lymphoma, and was consistently over 80% for AILD19,21 (present study). The infrequent association of EBV with CD30-positive ALCL was also in keeping with several, but not all, previous findings. Anagnostopoulos et al65 reported EBV positivity assessed by Southern blot analysis of EBV DNA in only 1 of 22 CD30-positive ALCL cases. The positive case of that series was of non-B/non-T-cell phenotype. More recently, Lopategui et al46 analyzed 20 American and 14 Asian cases of CD30-positive ALCL. Immunophenotypically, 15 cases were of B- and 15 of T-cell phenotype, three were non-B/non-T, and one expressed both B- and T-cell markers. EBER RISH revealed the presence of EBV in 3 of 14 (21%) Asian cases (one B- and two T-cell) and 1 (T-cell) of 20 (5%) American cases. However, using a highly sensitive nested polymerase chain reaction (PCR; total of 80 cycles), Ross et al67 were able to amplify EBV DNA in eight of 20 (100%) cases of CD30-positive ALCL. A single round of PCR (40 cycles) revealed EBV DNA in only four of the eight cases, while both isotopic and nonisotopic in situ hybridization for EBV DNA were negative in all tested specimens. These results emphasize the difficulty of comparing data from different studies due to technique-dependent variability and indicate the need for interpretative caution when using extremely sensitive detection methods. Another point that emerged from our study, and which seems to apply to all Asian and white series screened for EBV so far, is the consistent negativity of lymphoblastic T-NHL cases, possibly reflecting an in vivo refractoriness to EBV infection of prethymic and early thymic T cells. In B-NHL, there seems to be a lower concordance of EBV frequency among different series. Hamilton-Dutoit and Pallesen15 identified EBV-encoded proteins in 4 of 112 (4%) B-NHL cases of various subtypes. However, isotopic DNA in situ hybridization could only confirm the presence of EBV DNA in two of these cases, yielding a final EBV frequency in B-NHL of 2%. This low figure probably reflects limitations in method sensitivity, because the same analysis revealed EBV frequencies (confirmed by genomic analysis) for AILD and PTCL of 25% and 12%, respectively. Ohshima et al48 reported on 56 B-NHL cases in which the EBV frequency was assessed using Southern blot, isotopic in situ hybridization, and PCR analyses. Both Southern blot and isotopic in situ hybridization analysis identified 5% of the cases as being EBV-positive, whereas 7% were found to be positive by PCR. A recent study by Hummel et al49 used EBV DNA PCR and EBER RISH to evaluate EBV frequency in a large series (208 cases) of European B-NHL cases. Twenty-six percent of the cases were found to be positive for EBV (PCR = RISH), a result threefold to fourfold higher than that found in our study. When comparing the two studies, some histologic subtypes show marked differences in the number of EBV-positive cases; eg, immunoblastic (3 of 10 cases reported by Hummel et al49 vs none of 43 in the present series), immunoblastic B-NHL (9 of 28 vs 1 of 21), and CD30-positive ALCL of B-cell phenotype (6 of 16 vs none of 7). With regard to CD30-positive ALCL of B-cell phenotype, the frequency found in our study is much closer to that reported by Lopategui et al,46 who reported no EBER positivity among the 10 American cases screened. However, the results of Hummel et al49 and the present data also show some similarities: ie, EBV frequency in small lymphocytic B-NHL (8% vs 7%, respectively) and Burkitt’s lymphoma (30% vs 25%, respectively). As the two studies are comparable as to methodology and...
Fig 3. Survival curves for B- and T-cell lymphomas according to EBER status (EBER-negative, solid line; EBER-positive, broken line). (A) EBER-negative (n = 349) versus EBER-positive (n = 25) B-NHL cases; (B) EBER-negative (n = 204) versus EBER-positive (n = 16) intermediate-/high-grade B-NHL cases; (C) EBER-negative (n = 90) versus EBER-positive (n = 40) T-NHL cases; and (D) EBER-negative (n = 68) versus EBER-positive (n = 35) high-grade T-NHL cases.

ethnic patient background, the observed incidence differences in some histologic subtypes are difficult to explain. A theoretic possibility could be different patient selection criteria resulting in an over- or underselection of EBV-positive cases. However, neither series included immunocompromised patients, and, on the basis of the available data, other potential differences in patient selection criteria cannot be assessed. Immunophenotypic analysis of EBER-positive T-NHL cases by RISH/IDH double-labeling demonstrated the presence of EBV in neoplastic T cells. However, the proportion of EBV-positive neoplastic T cells varied markedly among the T-NHL cases. This finding supports the hypothesis that EBV infection is merely a secondary event, i.e., occurring within malignant T cells after neoplastic transformation. However, previous studies applying EBV terminal analysis on EBV-positive PTCL13,50 have clearly demonstrated the clonal nature of EBV infection in those cases. In view of these data, it appears possible that EBV-positive T-NHL represents an oligoclonal disorder in which EBV-negative and EBV-positive clones coexist. The EBV-infected neoplastic T cells would then, on the basis of EBV-induced molecular events as described for B cells, such as impairment of tumor suppressor gene function51 and protection from apoptosis,52 outgrow the EBV-negative component of the tumor cell population. To further investigate this hypothesis, it will be crucial to elucidate the mechanism of EBV entry into normal and malignant T cells and to observe the dynamics of EBV infection in T-NHL, focusing on those cases where sequential biopsies are available.

<table>
<thead>
<tr>
<th>Variable</th>
<th>P</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS (WHO 0-4) &gt;1</td>
<td>&lt;.00005</td>
<td>2.71</td>
</tr>
<tr>
<td>Age &gt;67 years</td>
<td>&lt;.00005</td>
<td>1.91</td>
</tr>
<tr>
<td>EBER-positive T-NHL</td>
<td>.028</td>
<td>1.61</td>
</tr>
<tr>
<td>Presence of B symptoms</td>
<td>.051</td>
<td>1.37</td>
</tr>
<tr>
<td>Elevated s-LDH at diagnosis</td>
<td>.054</td>
<td>1.36</td>
</tr>
<tr>
<td>Clinical stage &gt;II</td>
<td>.080</td>
<td>1.33</td>
</tr>
</tbody>
</table>

Abbreviation: RR, relative risk.
In line with previous reports, we did not find any expression of CD21 on tumor cells of EBER-positive T-NHL cases. While well-characterized for B cells, the mechanism of EBV adsorption and cell penetration is still unclear for T cells. A number of studies have reported the finding of EBV-receptor (CD21 or CD21-like molecules) on subsets of normal, immature (CD1a-positive, CD3-dull or -negative) human thymocytes and human cell lines of T-cell origin. It was further observed that the monoclonal antibodies used, such as those derived from clones HB5, B2, or OKB7, had a differential reactivity with thymocytes, with HB5 yielding the best staining in most of the studies. This differential Ab reactivity can explain the CD21 negativity found in the aforementioned studies of EBV-positive T-NHL, in which anti-CD21 from clones IF8 and/or B2 were used, but may also suggest that the EBV-binding molecule on thymocytes is similar but not identical with, the CD21 molecule cloned and characterized for B lymphocytes. In fact, based on studies performed on the human leukemic CD21-negative T cell line HSB-2, Hedrick et al have described a 70-kD protein that binds to EBV gp 350/220, the viral ligand of CD21. The 70-kD protein binds gp 350/220 at epitopes related to, but not identical with, those bound by CD21.

The most important finding of the present study was the identification of EBER positivity as a predictor of poor outcome in high-grade T-NHL. In fact, the combination EBER positivity and T-cell phenotype, when considered as an individual variable, was one of the strongest independent predictors of a poor outcome in intermediate/high-grade NHL, even overruling major prognostic factors such as advanced clinical stage and elevated s-LDH. Also of interest was the finding that survival seemed to be equally poor regardless of degree of EBER positivity in high-grade T-NHL. The reason for the correlation between EBV positivity and poor outcome in T-NHL is unclear. The EBV-associated T-NHL cases reported by Su et al were frequently associated with constitutional symptoms, an aggressive clinical course, and a poor prognosis—all features also found in the present case material. The same group also reported a correlation between EBV-associated PTCL and the simultaneous expression, after primary chemotherapy, of two multidrug resistance markers: ie, the multidrug resistance P-glycoprotein mdr-1 and glutathione-S-transferase π. These molecular characteristics could, if specifically related to EBV-positive T-NHL, explain the poor outcome in these cases.

A study by de Bruin et al performed on 46 cases of nodal T-NHL (26 PTCL, 10 CD30-positive ALC, 5 lymphoblastic, 4 Lennert’s, and 1 AILD) reported the prognostic value of LMP-1 expression in T-NHL. In that study, EBV positivity was found by EBV DNA PCR in 21 cases and confirmed by EBER RISH in 14 cases with satisfactorily preserved mRNA. One additional case with inadequate DNA for PCR analysis also proved to be EBV-positive by RISH. No survival difference was found between PCR-assessed EBV-negative and EBV-positive cases. The groups compared consisted of 18 EBV-positive (14 PTCL, 3 Lennert’s, and 1 AILD) and 12 EBV-negative cases (11 PTCL, 1 Lennert’s). The same conclusion applied to PTCL cases if analyzed separately. Conversely, a significant correlation between poor outcome and expression of LMP-1 was found. This finding could not be confirmed by the present study, where no survival difference was observed between those EBER-positive T-NHL cases that expressed LMP-1 and those that did not. Furthermore, a recent study on nasopharyngeal carcinoma showed that LMP-1-expressing cases had a more favorable clinical outcome than LMP-1-negative ones. In the study reported by de Bruin et al, the composition of the patient populations on the basis of LMP-1 status is not fully clarified. Eight LMP-1-positive cases were compared with 23 LMP-1-negative cases, the composition of which is not reported. The sum of these figures (N = 31) exceeds the group size expected if the survival comparison had been made within the EBV-positive group (N total = 22) as assessed by PCR and/or EBER RISH. Therefore, it seems likely that the LMP-1-negative group of 23 patients also included some, but not all, EBV-negative cases. However, this study is in agreement with ours and other reports that showed that immunohistochemically assessed LMP-1 positivity is usually only found in a fraction of EBV-associated T-NHL cases and does not represent an ideal parameter for clinical use. An insufficient sensitivity of the immunohistologic techniques applied to formalin-fixed, paraffin-embedded sections rather than frozen sections is one possible explanation for the inconsistent correlation between EBV-associated T-NHL and LMP-1 expression. However, it is unlikely to be the sole reason, as proper control tissues in the different studies yielded satisfactory staining. In a recent study reported by Suzzushima et al, LMP-1 expression was studied by molecular (reverse transcriptase PCR) and immunohistochemical methods in three cases of EBV-positive T-NHL and was found to be consistently negative. Additionally, recent molecular data have demonstrated the presence of a frequently occurring 30-base pair deletion and of single-base mutations at the carboxy-terminal part of the EBV gene (BNLF-1) encoding for LMP-1 in both white and Asian PTCL cases. Whether and how these genetic changes can affect the expression of the LMP-1 gene product and represent a prognostic tool in EBV-associated T-NHL is yet to be established.

In conclusion, we found that EBV-positive T-NHL is associated with characteristic pathologic features and a poor clinical outcome, that EBV status should be considered as additional useful information in the classification of T-NHL, and, if the adverse prognostic impact of EBV on T-NHL is confirmed by others, that a randomized approach should be used to evaluate new treatment strategies for EBV-associated T-NHL.

ACKNOWLEDGMENT

We thank Birgitte Dyhr Thomasen, Lone Christiansen, and Ole Nielsen for skillful technical assistance and Dr W.C. Chan for review and comments. We are also indebted to DAKO A/S, Denmark, and DAKO Corporation, Carpinteria, CA, for kind technical assistance and Jens Jørgen Hylidg, DAKO Denmark, for providing the negative control 30-base random oligonucleotide.

REFERENCES


POOR PROGNOSIS IN EBV-POSITIVE T-CELL LYMPHOMAS

with pleomorphic small cell lymphoma, lymphoepithelioid cell (Len- 
ner's) lymphoma and T-zone lymphoma. The Kiel Lymphoma 

1055

35. Jaffe ES, Gonzalez CL, Madeiros LJ, Raffeld M: T-cell rich 

36. Weiss LM, Chen YY: Effects of different fixatives on detection 
of nucleic acids from paraffin-embedded tissues by in situ hy-
bridization using oligonucleotide probes. J Histochem Cytochem 
39:1237, 1991

37. Glickman JD, Howe JG, Steitz JA: Structural analyses of 
EBER1 and EBER2 ribonucleoprotein particles present in Epstein-

38. Gilligan K, Rajadurai P, Resnick L, Raab-Traub N: Epstein-
Barr virus small nuclear RNAs are not expressed in permissively 
infected cells in AIDS associated leucoplaikia. Proc Natl Acad Sci 
USA 87:8790, 1990

39. Knutson G, Spaulding D: β-actin as a positive control for 
detection of mRNA by in situ hybridization (ISH). Modern Pathol 
8:166A, 1995 (abstr)

40. Cordell JL, Falini 13, Erber WN, Ghosh A, Abdulaziz Z, 
Mac Donald S, Pulford K, Stein H, Mason DY: Immunoenzymatic 
labeling of monoclonal antibodies using immune complexes of alka-
line phosphatase and monoclonal anti-alkaline phosphatase (APAAP 

41. d’Amore F, Christensen BE, Brincker H, Pedersen NT, Thor-
ing K, Hastrup J, Pedersen M, Krogh Jensen M, Johansen P, Ander-
sen E, Bach B, Soerensen E: Clinicopathologic features and prognos-
tic factors in extranodal non-Hodgkin’s lymphomas. Eur J Cancer 
27:1201, 1991

42. d’Amore F, Brincker H, Christensen BE, Thorning K, Ped-
ersen M, Larng Nielsen J, Sandberg E, Pedersen NT, Soerensen E: 
Non-Hodgkin’s lymphoma in the elderly. A study of 602 patients 
aged 70 or older from a Danish population-based registry. Ann Oncol 
3:379, 1992

43. Tarone RE, Ware J: On distribution-free tests for equality of 
survival distributions. Biometrika 64:156, 1977

34:187, 1972

45. Anagnostopoulos I, Herbst H, Niedobitek G, Stein H: Demon-
stration of monoclonal EBV genomes in Hodgkin’s disease and Ki-
l-positive anaplastic large cell lymphoma by combined Southern 

46. Lopategui JR, Gaffey MJ, Chan JKC, Frierson HF, Sun LH, 
Bellafore FJ, Chang KL, WeissLM: Infrequent association of Ep-
stein-Barr virus with CD 30-positive anaplastic large cell lymphomas 

47. Ross CW, Schlegelmilch JA, Grogan TM, Weiss LM, Schnitzer B, 
Hanson CA: Detection of Epstein-Barr virus genome in Ki-1 (CD30)-positive, large-cell anaplastic lymphomas using the polymerase chain reaction. Am J Pathol 141:457, 1992

48. Ohshima K, Kikuchi M, Eguchi F, Masuda Y, Sumiyoshi Y, 
Mohtai H, Takeshita M, Kimura N: Analysis of Epstein-Barr viral 
genomes in lymphoid malignancy using Southern blottig, polymer-
ase chain reaction and in situ hybridization. Virchows Arch B Cell 
Pathol 59:383, 1990

49. Hummel M, Anagnostopoulos I, Korbfjnhn P, Stein H: Ep-
stein-Barr virus in B-cell non-Hodgkin’s lymphomas: Unexpected 
infector patterns and different infection incidence in low- and high-
grade types. J Pathol 175:263, 1995

50. Chen CL, Sadler RH, Walling DM, Su H, Hsieh HC, Raab-
Traub N: Epstein-Barr virus (EBV) gene expression in EBV-positive 

K: EBNA-5, an Epstein-Barr virus-encoded nuclear antigen, binds 
to the retinoblastoma and p53 proteins. Proc Natl Acad Sci USA 
90:5455, 1993

52. Gregory CD, Dive C, Henderson S, Smith CA, Williams GT, 
Gordon J, Rickinson AB: Activation of Epstein-Barr virus latent 
genes protects human B-cells from death by apoptosis. Nature 
349:612, 1991

53. Tsoukas CD, Lambris JD: Expression of CR2/EBV receptors 
on human thyocytes detected by monoclonal antibodies. Eur J 
Immunol 18:1299, 1988

54. Watry D, Hedrick JA, Siervo S, Rhodes G, Lamberti JJ, 
Lambris JD, Tsoukas CD: Infection of human thyocytes by Epstein 

55. Kaufman Paterson RL, Kelleher CA, Streib JE, Amankonah 
TD, Wu Xu J, Jones JF, Gefand EW: Activation of human thy-

56. Kaufman Paterson RL, Kelleher CA, Amankonah TD, Streib 
JE, Wu Xu Ju J, Jones JF, Gefand EW: Model of Epstein-Barr virus 
infection of human thyocytes: Expression of viral genome and 
impact on cellular receptor expression in T-lymphoblastic cell line, 
HPB-ALL. Blood 85:456, 1995

JD, Tsoukas CD: Characterization of a 70 kDa, EBV gp350/220-

58. Cheng AL, Su UJ, Chen YC, Lee TC, Wang Ch: Expression of 
p-glycoprotein and glutathione-S-transferase in recurrent lympho-
mas: The possible role of Epstein-Barr virus, immunophenotypes, 

59. Hu LF, Chen F, Zhen QF, Zhang YW, Luo Y, Zheng X, 
Winberg G, Ernberg I, Klein G: Differences in the growth pattern 
and clinical course of EBV-LMP1 expressing and nonexpressing 

60. Suzushima H, Asou N, Fujimoto T, Nishimura S, Okabo T, 
Yamasaki H, Osato M, Matsuoka M, Tsukamoto A, Takai K, Ka-
wano F, Takatsuki K: Lack of expression of EBNA-2 and LMP-1 
in T-cell neoplasms possessing Epstein-Barr virus. Blood 85:480, 
1995

61. Sandveij K, Peh SC, Andresen BS, Pallesen G: Identification of 
potential hot spots in the carboxy-terminal part of the Epstein-
Barr virus (EBV) BNLF-1 gene in both malignant and benign EBV-
associated diseases: High frequency of a 30-bp deletion in Malaysian 
and Danish peripheral T-cell lymphomas. Blood 84:4053, 1994
Epstein-Barr virus genome in non-Hodgkin's lymphomas occurring in immunocompetent patients: highest prevalence in nonlymphoblastic T-cell lymphoma and correlation with a poor prognosis. Danish Lymphoma Study Group, LYFO

F d'Amore, P Johansen, A Houmand, DD Weisenburger and LS Mortensen

Updated information and services can be found at:
http://www.bloodjournal.org/content/87/3/1045.full.html

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml