主論文の要約

Prognostic significance of CD20 expression and Epstein-Barr virus (EBV) association in classical Hodgkin lymphoma in Japan: A clinicopathologic study

（本邦の古典的ホジキンリンパ腫における、CD20 発現および Epstein-Barr ウィルス感染の予後指標としての意義に関する臨床病理学的検討）

名古屋大学大学院医学系研究科　分子総合医学専攻
高次医用科学講座　臓器病態診断学分野
（指導：中村 栄男　教授）
Ahmed Ali Elsayed Ali

【Introduction】

Classical Hodgkin Lymphoma (CHL) is characterized by the presence of Hodgkin and Reed–Sternberg (HRS) cells in a mixed inflammatory background. The origin of HRS cells from germinal center B-lymphocytes has been recently elucidated in most cases, whereas in few cases the HRS cells show a T-cell origin. However, the B-cell origin of HRS cells is not usually detectable on immunophenotypic analysis. Markers of B lineage such as CD20, CD19 and CD79a are generally down-regulated in the HRS cells but the expression of the B cell-specific transcription factor PAX5 is usually retained. In addition, HRS cells may express T-cell, cytotoxic molecule (CM) or follicular dendritic cell (FDC) phenotype in variable rates. The expression of CD20 has been demonstrated immunohistochemically on HRS cells in approximately 5-50% of CHL patients, however, the clinicopathologic significance of its expression is still controversial. A proportion of cases of CHL are associated with EBV, where the virus is believed to play a crucial role in the pathogenesis. In this study, we investigated the clinicopathologic significance of CD20 expression and EBV-association in CHL.

【Materials and methods】

A total of 389 cases of CHL were included in the study. Approval for the study was provided by the institutional review board of Nagoya University. The diagnosis and subtyping of CHL was established by histopathologic and IHC criteria in accordance with the 2008 WHO classification. All patients were negative for human T-cell leukemia virus type 1, human immunodeficiency virus and T/CM phenotype. Tissue samples were fixed in 10% formalin and embedded in paraffin, followed by staining with hematoxylin and eosin of 5-μm-thick sections. Formalin-fixed paraffin sections were subjected to immunoperoxidase studies using the avidin-biotin peroxidase complex method. Monoclonal antibodies used were CD3, CD8, CD45RO, CD20, CD21, CD30, fascin, EMA, ALK1, CD4, CD5, CD15, TIA-1, granzyme B and Pax5. Reaction for the reagents was considered positive when more than 5% of the H-RS cells are stained, although in practice many of the positive samples showed reaction in more than 10% of cells. The presence of EBV small ribonucleic acids was determined by means of in situ hybridization using EBER oligonucleotides on formalin fixed, paraffin-embedded sections. Statistical analysis of data was performed with STATA software.

【Results】

Clinicopathologic characteristics: Patients' characteristics are summarized in **Table 1**. Patients consisted of 125 females and 262 males with median age of 48 years (range, 4-89 years). They included 74 CD20-positive ($CD20^+$) cases (19%) (**Fig. 1**) and 315 CD20-negative ($CD20^-$) cases (81%). On comparison, $CD20^+$ cases showed significantly older age at onset (P=0.018), less involvement of cervical lymph nodes (P=0.042), and more involvement of

cubital lymph nodes (P=0.045) and the pancreas (P=0.032). $CD20^-$ cases were more significantly associated with elevated white blood cell (WBC) count >15,000/mm^3 (P=0.037) and with elevated soluble interleukin (IL)-2 receptor >4000 U/mL (P=0.035).

EBV distribution in CHL: EBV-positive (EBV^+) CHL cases comprised 44% of our series (n=173) in contrast to 216 EBV-negative (EBV^-) cases (56%). **Table 2** summarizes a comparison of EBV^+ and EBV^- cases. EBV^+ CHL patients showed significantly older age at onset (P<0.0001), higher ratio of males (P<0.001), less mediastinal involvement (P<0.001), more involvement of para-aortic and retroperitoneal lymph nodes (P=0.038 for both), and less occurrence of WBC count >15,000/mm^3 (P=0.009). Patients with EBV^+ CHL were significantly associated with aggressive clinical parameters, namely performance status (PS)>1 (P=0.026), presence of B-symptoms (P=0.036), and thrombocytopenia (P=0.025). The international prognostic index (IPI) score was therefore significantly higher in the EBV^+ group (P=0.001). Histologically, mixed cellularity (MC) subtype was significantly more frequent in EBV^+ group (P<0.001) while nodular sclerosis (NS) subtype was significantly more frequent in EBV^- group (P<0.001).

Immunophenotypic characteristics: Phenotypic features are listed in **Table 3**. EBV positivity was significantly higher in $CD20^+$ than in $CD20^-$ cases (P=0.002). The expression of CD15 was significantly higher in $CD20^-$ than $CD20^+$ cases (P=0.048). The percentages of $CD20^+$ and EBV^+ CHL patients showed significant positive correlation with increasing age with the frequency peak for CD20-positivity in the 41-60 years range (P=0.035) and for EBV-positivity in patients older than 60 years (P<0.001) **(Table 4)**.

Survival: There was no significant difference in overall survival (OS) and progression-free survival (PFS) between $CD20^+$ and $CD20^-$ CHL cases (P=0.87 and P=0.38, respectively) **(Table 1)**. Patients with EBV^+ CHL showed a tendency to poor prognosis in term of OS compared with EBV^- patients, but without statistical significance (P=0.09). PFS showed no significant difference between EBV^+ and EBV^- cases (P=0.3) **(Table 2)**.

Prognostic factors: Analyses of prognostic factors are shown in **Tables 5 and 6**. Univariate analysis identified 11 prognostic factors for OS in CHL patients: age (P=0.049), involvement of more than one extranodal site (P=0.001), advanced clinical stage (III/IV; P<0.001), presence of B-symptoms (P=0.002), performance status >1 (P<0.001), hemoglobin level <10.5 g/dL (P=0.003), thrombocytopenia (P=0.044), serum albumin level <3.5 g/dL (P<0.001), elevated serum lactate dehydrogenase (LDH) level (P=0.015), elevated soluble IL-2 receptor >4000 U/mL (P=0.015) and IPI score (High-intermediate and high risk; P<0.001). Multivariate analysis found the presence of B-symptoms (P=0.031), presence of performance status >1 (P=0.023), thrombocytopenia (P=0.018), elevated serum LDH level (P=0.026), and EBV infection (P=0.042) but not CD20-positivity were identified as significant prognostic factors for OS. Univariate analysis identified five prognostic factors for PFS in CHL patients: involvement of more than one extranodal site (P=0.003), advanced

clinical stage (III/IV; P=0.001), performance status >1 (P=0.047), hemoglobin level <10.5 g/dL (P=0.024), and serum albumin <3.5 g/dL (P=0.002). On multivariate analysis, only advanced clinical stage (III/IV; P=0.016) was proven to be of independent prognostic impact on PFS.

New prognostic model for CHL: The IPI could stratify the prognosis of CHL in this analysis; however, other factors, including EBV positivity (but not CD20 positivity), presence of B-symptoms, and thrombocytopenia in addition to elevated serum LDH level and performance status >1 were identified as prognostic factors for OS on multivariate analysis. We thus attempted to construct a new prognostic model with these five prognostic factors. We classified patients into three risk groups with the use of the following terms: low risk, 0 or 1 adverse factor; intermediate risk, 2 or 3 factors; high risk, 4 or 5 factors. This novel prognostic model for CHL could stratify the prognosis of patients with CHL (P<0.0001). For 144 patients (58%) classified into the low-risk group, 5-year OS was 91%. For 92 patients (37%) in the intermediate group, 5-year OS was 66%; for 11 patients (5%) in the high-risk group, 5-year OS was 36% (P<0.0001; **Fig. 2**).

【Discussion】

In this study, EBV positivity (but not CD20 positivity), the presence of B-symptoms, presence of performance status >1, thrombocytopenia, and elevated serum LDH level were independent poor prognostic factors for OS in CHL patients. Our new prognostic model including these five adverse factors could stratify the prognosis of CHL patients with very high statistical significance (P<0.0001). EBV is an important factor involved in the pathogenesis of Hodgkin lymphoma and therefore worthy of its inclusion in a prognostic model for CHL. This study was done on 389 consecutive cases of CHL. Seventy-four cases (19%) were $CD20^+$ which was within the reported range 5 - 50% of CHL patients. We found that the $CD20^+$ cases were different from $CD20^-$ cases regarding the age at onset and the frequency of EBV association with no significant difference in OS or PFS between the two groups. In this study, EBV^+ CHL constituted 44% of patients. The percent of EBV^+ CHL cases varies between developed and developing countries. In North America and European countries, the reported proportion of EBV^+ CHL cases varies from 26-50%, while in developing countries the proportion of EBV^+ CHL is higher. EBV^+ patients were more likely to have MC subtype than EBV^- patients who had more frequently NS subtype. In our series, we found a tendency to poor prognosis in term of OS in EBV^+ CHL patients compared with EBV^- patients, but without statistical significance (P=0.09).

【Conclusions】

IPI was successful in stratification of the OS of CHL cases in this analysis. Only two of five prognostic factors in our analysis, namely elevated serum LDH level and the presence of

performance status >1, were adopted in IPI; thus, the attempt to construct a new prognostic model with newly identified factors might be reasonable. Our novel prognostic model included EBV infection, which is an important factor involved in the pathogenesis of Hodgkin lymphoma and thus, it is quite reasonable to be included. EBV^+ CHL is significantly associated with older age at onset; thus, it may be more adequate to substitute the age > 60 factor of IPI with the EBV status. Because EBV infection prevails in developing and East Asian countries, our new prognostic model may be more applicable for patients of these countries. We recommend examination of EBV association in CHL patients in routine pathologic practice especially in East Asian and developing countries.