主論文の要旨

Clinicopathological Study of 30 Cases of Peripheral T-cell Lymphoma with Hodgkin and Reed-Sternberg-like B-cells from Japan

（本邦におけるホジキン・リードスタンバーグ細胞様 B 細胞を伴う末梢性 T 細胞性リンパ腫 30 例の臨床病理学的検討）

名古屋大学大学院医学系研究科　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 Elsayed Eladl Ahmed

【Introduction】

Peripheral T-cell lymphomas (PTCLs) encompass a heterogeneous group of lymphomas that vary in their clinical and pathological features. Recently, a subset of PTCL, not otherwise specified (NOS), was reported to express the follicular helper T-cell (T_{FH}) phenotype, similar to angioimmunoblastic T-cell lymphoma (AITL). The presence of a population of B-cells in the background of neoplastic T-cell proliferation was documented in AITL and PTCL-TFH and regarded as a diagnostic hallmark. These B-cells are frequently Epstein-Barr virus (EBV)-positive. In rare cases, these B-cells may closely resemble Hodgkin and Reed-Sternberg (HRS)-cells in morphology and immunohistochemistry. Here, we investigated the clinicopathological features of a series of 30 PTCL cases with scattered HRS-like cells of B-cell lineage from Japan.

【Materials and methods】

A total of 30 cases of PTCL with HRS-like B-cells (12 AITL, 11 PTCL-TFH and 7 PTCL-NOS) were included in the study. Approval for the study was provided by the institutional review board of Nagoya University. The diagnosis was established by histopathologic and IHC criteria in accordance with the 2008 WHO classification. 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, CD4, CD8, CD10, CD20, CD79a, CD15, CD30, PD-1, CXCL13, PD-L1, Bcl-6, FDC, perforin, TIA-1, LMP-1, βF1, CCR4 and Pax5. The presence of EBV small ribonucleic acids was determined by means of in situ hybridization using EBER oligonucleotides on formalin fixed, paraffin-embedded sections. DNA was extracted from formalin-fixed tissues, and PCR of the T-cell receptor γ chain (TCR-γ) gene and immunoglobulin heavy chain (IgH) gene rearrangement were performed.

【Results】

Clinicopathologic characteristics: Patients' characteristics are summarized in **table-1**. They were 18 male and 12 female, ranging from 39 to 91 years with a median age of 77 years. 80% of patients were older than 60 years. 90% of patients were diagnosed with advanced clinical Ann-Arbor staging and 40% had B-symptoms. Laboratory data at presentation revealed increased white blood cells count, elevated LDH level and elevated level of soluble interleukin-2 receptor in 30%, 37% and 39% of patients, respectively. 53% of patients were classified as High-Intermediate and High risk groups according to the International Prognostic Index (IPI) and 50% of them as groups 3 and 4 according to the Prognostic Index of T-cell lymphoma (PIT).
AITL cases (n=12): presented with the proliferation of medium-sized tumor cells with pale cytoplasm and round to angulated nuclei surrounding hyperplastic HEVs. The background contained mixed reactive inflammatory cells with scattered large HRS-like cells. Tumor cells

were $CD3^+$ (100%), $CD10^+$ (60%), $PD\text{-}1^+$ (83%) and $CXCL\text{-}13^+$ (100%). The B-cell lineage of the HRS-like cells was maintained in all cases; nine of the 12 were $CD20^+$ and the $CD20^-$ cases were $PAX\text{-}5^+$. Nine of the 12 cases were $CD30^+$. EBER was detected in HRS-like cells in 9/12 cases. **(Figure-1).**

PTCL-TFH cases (n=11): presented with tumour cells ranged from mildly atypical small-to-medium-sized cells with hyperchromatic nuclei to more pleomorphic medium-to-large-sized cells. Focal AITL-like features were detected in some cases, but the overall pattern was not that of AITL. All cases had scattered large HRS-like cells among the tumor cells. Tumor cells were $CD3^+$ (100%). A double $PD\text{-}1^+$ and $CXCL\text{-}13^+$ reaction was detected in 10 cases (91%). HRS-like cells were of B-cell lineage in all cases, with nine $CD20^+$ cases and 11 $CD30^+$. The $CD20^-$ cases were $CD79a^+$ and $PAX\text{-}5^+$. The HRS-like cells were $EBER^+$ in 8/11 cases. **(Figure-2).**

PTCL-NOS cases (n=7): four cases had a mixed pattern cell appearance containing medium-sized tumor cells, and the other three exhibited high grade morphology with pleomorphic medium-to-large-sized tumor cells. HRS-like cells were scattered throughout the lesions. Tumor cells were $CD3^+$ (100%), $CD5^+$ (71%) and cytotoxic molecule $(CM)^+$ (43%). All cases were negative for both PD-1 and CXCL-13. HRS-like cells were of B-cell lineage with a $CD20^+$ and/or $PAX\text{-}5^+$ reaction in all seven cases and a $CD30^+$ reaction in 6/7 cases. The HRS-like cells were $EBER^+$ in 3/7 cases. **(Figure 3). Table-2** summarizes the clinicopathological features of PTCL-NOS patients.

Molecular features: PCR studies for TCR-γ and IgH gene rearrangement were performed in 28 cases. Clonal TCR-γ gene rearrangement was detected in 16 cases and clonal IgH gene rearrangement in two cases. No significant difference was found regarding OS and PFS between cases with clonal TCR-γ gene rearrangement and other cases lacking clonal TCR-γ gene rearrangement (P=0.937 and P=0.689, respectively).

PD-L1 expression: All 30 cases were negative for PD-L1 expression on both tumor cells and HRS-like cells.

Therapeutic and survival data: The median follow-up period was 22 months (range, 1-65 months). The 3-year OS and PFS rates were 44% and 27%, respectively. Eleven patients experienced relapse, and 18 died from the disease or its complications. One patient with PTCL-TFH relapsed as EBV^- diffuse large B-cell lymphoma (DLBCL) approximately 30 months after his first diagnosis and died 1 year later. No clonal TCR-γ or IgH gene rearrangement was detected in this case.

Clinicopathological features of the three groups: Expression of T_{FH}-related antigens was detected in 23 cases (12 AITL and 11 PTCL cases with HRS-like cells). **Table-3** compares the AITL and PTCL-TFH cases. Both groups had overlapping clinicopathological features with no significant differences and tended to present in elderly patients, most with an advanced clinical stage. Furthermore, in a comparison of the 23 cases with T_{FH} phenotype and the 7 PTCL-NOS cases, no significant clinicopathological differences were found. Both groups tend to affect

older patients and to present with an advanced clinical stage. The OS and PFS curves demonstrated no significant differences among the three groups **(Figure 4A, B).**

EBV status and its clinical impact: HRS-like cells expressed EBER in most of the cases (67%). PTCL cases with $EBER^+$ HRS-like cells exhibited male predominance and tended to be associated with more than one extranodal site affected and B-symptoms, but without reaching significance **(Table-4).** Regarding survival data, patients with $EBER^+$ HRS-like cells had inferior OS and PFS compared to those with $EBER^-$ HRS-like cells, but with no statistical significance (P=0.235 and P=0.108, respectively; **Figure 4C, D**).

【Discussion】

In this study, we highlighted the presence of HRS-like cells of B-cell origin in a few PTCL subgroups among Japanese patients. Many of the cases expressed T_{FH} phenotype on tumor cells and were associated with EBV reactivation. EBV harboring was detected on HRS-like cells in 20 (67%) of our cases. According to our data, the EBER status of HRS-like cells did not significantly influence the clinical parameters of the PTCL cases, whereas patients with $EBER^+$ HRS-like cells tended to have more than one affected extranodal site and B-symptoms. Moreover, no significant difference in OS or PFS was found, though PTCL cases with $EBER^+$ HRS-like cells had inferior curves compared to cases with $EBER^-$ HRS-like cells.

Recently, another subgroup of PTCL-NOS with T_{FH} phenotype was recognized, which showed overlapping features with AITL and may represent a stage in the spectrum of AITL. This overlap between both groups was also demonstrated in this study. According to our data, both AITL and PTCL-TFH with HRS-like cells have an elderly onset (78 and 76 years, respectively), and they have similar clinical parameters, including staging, IPI and PIT scores, and other laboratory data. Indeed, patients of both groups showed an overlapping OS and PFS curves. These findings provide additional support for the assertion that AITL and PTCL-TFH constitute a continuous spectrum of disease, even among cases with HRS-like cells. Also, no significant differences were detected between PTCL-NOS patients and other patients who expressed the T_{FH} phenotype on their tumor cells, except for the tendency of the former group to present with more elevated serum LDH levels. In this study, PTCL-NOS cases had a similar prognosis as AITL and PTCL-TFH cases.

Monoclonal rearrangement of the IgH gene was detected in two of 28 examined cases. The remaining cases showed polyclonal rearrangement including the one case with subsequent progression to EBV^- DLBCL. In this study, the presence of HRS-like cells did not seem to reflect a higher incidence of progression to B-cell lymphoma, but the absence of follow-up biopsy in most relapsed cases prevents us from reaching a definite conclusion.

Recently, the interaction between PD-1 and its ligand PD-L1 attracted the attention for its role in suppressing T-cell immunity in cases of Hodgkin Lymphoma and other virus-associated lymphomas, providing a potential tool for immunotherapy. We investigated PD-L1 expression in all cases but none of the tumor cells and HRS-like cells were positive.

In summary, we reported 30 cases of PTCL with HRS-like cells of B-cell lineage from Japan. Many of these cases expressed the T_{FH} phenotype and were associated with EBV reactivation. They had a tendency to affect elderly patients and to be associated with advanced clinical stages and dismal prognosis, but the presence of HRS-like cells was not necessarily an indication for a subsequent development of secondary B-cell lymphoma or adverse prognosis.