

Hayashi, et al

Distinct morphologic, phenotypic, and clinical-course characteristics of indolent peripheral T-cell lymphoma

Eiko Hayashi¹, Katsuyoshi Takata¹, Yasuharu Sato¹, Yukie Tashiro², Yoshiro Tachiyama³, Seiko Sawada-Kitamura⁴, Yasushi Hiramatsu⁵, Shun Sugiguchi⁶, Soichiro Nose⁷, Mitsuyoshi Hirokawa⁸, Midori Ando¹, Lamia Abd Alkader¹, Yoshinobu Maeda⁹, Mitsune Tanimoto⁹, and Tadashi Yoshino¹

¹Department of Pathology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan; ²Department of Pathology, Imakiire General Hospital, Kagoshima, Japan; ³Division of Pathology, Hiroshima-Nishi Medical Center, Ohtake, Japan; ⁴Division of Pathology, Kanazawa University Hospital, Kanazawa, Japan; ⁵Department of Hematology and Oncology, Himeji Red-cross Hospital, Himeji, Japan; ⁶Department of Pathology, Tonami General Hospital, Tonami, Japan; ⁷Department of Pathology, Okayama Saiseikai General Hospital, Okayama, Japan; ⁸Department of Diagnostic Pathology, Kuma Hospital, Kobe, Japan; and ⁹Department of Hematology and Oncology, Okayama University Graduate School of

Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

Address correspondence to:

Katsuyoshi Takata, MD, PhD, Department of Pathology, Okayama University Graduate

School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho,

Okayama City, Okayama 700-8558, Japan

E-mail: katsuyoshi.t@h5.dion.ne.jp

Tel. +81 86 235 7150

Fax. +81 86 235 7156

Running title: Low-grade T-cell lymphoma

Key words: Indolent PTCL, CD20, Ki-67, memory T-cell, good prognosis

Funding

This work was supported in part by a Grant-in-Aid for Cancer Research (21-6-3) from the Ministry of Health, Labour and Welfare, Tokyo, Japan.

Abstract

Peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) consists of a heterogeneous group of lymphomas. Patients generally show an aggressive clinical course and very poor outcome. Although the 2008 WHO classification of PTCL-NOS includes 3 variants, low-grade lymphoma is not included. Of 277 PTCL-NOS cases recorded in our consultation files, we examined the clinicopathological characteristics of 10 patients with T-cell lymphomas composed of small-sized cells with slight nuclear atypia. Eight patients showed extranodal involvement (5 patients, spleen; 3 patients, thyroid), and 5 patients were at clinical stage I or II. Histologically, all samples presented diffuse infiltrate of small lymphoid cells, with few mitotic figures. Immunohistologically, all samples were positive for CD3, and CD20 was detected in 5 samples. All samples showed a low Ki-67 labeling index (mean, 1.05%), and 7 samples were positive for central memory T-cell markers. Clonal T-cell receptor γ chain and/or α - β chain gene rearrangements were detected in all 10 patients. Five patients received chemotherapy, whereas for 3 patients, treatment consisted only of observation following surgical resection of the spleen or thyroid. Nine patients were alive at a median follow-up time of 19.5 months, whereas 1 patient died of an unrelated disease. The present study strongly indicates that T-cell lymphoma with small-sized lymphoma cells

and a low Ki-67 labeling index is a distinct variant. Recognition of this novel lymphoma subtype, which should not be defined merely as PTCL-NOS, should be seriously considered.

Introduction

Peripheral T-cell lymphomas, not otherwise specified (PTCL-NOS) constitute a heterogeneous group of nodal and extranodal mature T-cell lymphomas that do not correspond to any of the specifically defined entities of mature T-cell lymphoma in the current WHO Classification system [1]. PTCL-NOS account for 25.9% of PTCLs according to the International Peripheral T-cell Lymphoma Project [2] and 7.21% of all lymphomas in Japan [3].

PTCL-NOS include lymphomas that can be categorized into various groups genetically and biologically; therefore, this category has been referred to as a “wastebasket” category [4]. Histologically, PTCL-NOS show paracortical or diffuse infiltrates, with effacement of the normal lymph-node architecture. In most cases, numerous medium-sized or large cells with many mitotic figures are noted. In rare cases, small lymphoid cells predominate, but the cells show atypia and have irregular nuclei. Although PTCL-NOS is a heterogeneous category, it is generally thought that PTCL-NOS are aggressive lymphomas characterized by a poor response to therapy, frequent relapses, and a low 5-year overall survival rate [5].

In the present study, we described a group of 10 patients that was morphologically and

phenotypically unique, having small, monotonous lymphoid cells characterized by slight nuclear atypia, a low Ki-67 labeling index (LI), and good prognosis compared to those of typical PTCL-NOS patients.

To the best of our knowledge, no previous clinicopathological studies have focused on these unique T-cell lymphomas, excluding a few case reports [6, 7]. The immunohistochemical and genotypic characteristics of this disease were examined in the current study, and we attempted to determine the clinical significance of these findings during the clinical follow-up.

Materials and Methods

Patient selection and clinical data

We chose 10 PTCL-NOS patients characterized by an infiltrate of monotonous, small-sized lymphoid cells with slight nuclear atypia and a low Ki-67 LI (<5%), from among 277 cases of PTCL-NOS recorded in the 2006–2011 pathology consultation files of the Department of Pathology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences. Among all 277 cases, no patients had the same histopathology but a higher Ki-67 LI. Fourteen of the 277 cases had small to medium

sized lymphoid cells with irregular nuclei, and showed high Ki-67 LI (>30%). Therefore, we excluded these cases in the present study. There was no case composed of large-sized lymphoid cells and showing a low Ki-67 LI. Because the samples of all 10 patients that we included had a Ki-67 LI of 5% or less in our study, we defined the cut-off as 5%, although it is commonly placed at 30% [8]. Clinical data were available for all patients. We collected data on age, gender, complete blood count, lactate dehydrogenase (LDH) level, B symptoms, disease at other sites, bone marrow involvement, performance status (PS), Ann Arbor stage [9], international prognostic index (IPI) [10], prognostic index for PTCL-unspecified (PIT) [11], history of prior immunosuppressive therapy or immune system disorder, type of treatment, response to therapy, date of last follow-up, and status. HIV-positive or human T-cell leukemia virus-positive patients were excluded. Informed consent for examination was obtained for the use of all samples.

Immunohistochemistry and hybridization studies

Tissue samples were fixed in 10% formalin and embedded in paraffin. Staining was performed using paraffin sections with an automated Bond-max stainer (Leica

Biosystems, Melbourne, Vic., Australia). The primary antibodies and probes used are summarized in Table 1. For CD20, CD3, CD5, CD7, CD4, CD8, CD79a, CD45RO, CCR7, CD62L, PD1, CD56, CD57, CXCL13, granzyme B, TIA-1, TCR γ , TCR δ , and TCR β antigens, positivity was identified when at least 30% of the lymphoma cells were positive for their respective antibodies. The Ki-67 LI was determined by counting at least 1,000 tumor cells in each sample, and the index was calculated as a percentage.

For flow cytometry (FCM), the following antibodies were used: CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD22, and CD23, along with Ig κ and Ig λ light chains.

For the indirect double immunofluorescence study, paraffin sections were stained with CD3 and CD20. The following primary antibodies (clone, dilutions) were used: CD20 (L26, 1:200; Novocastra, Newcastle-upon-Tyne, UK) and CD3 (rabbit polyclonal, 1:100; Abcam, Tokyo, Japan). The indirect double immunofluorescence study was performed according to standard procedures, as described previously [12].

Molecular genetic analysis

Molecular genetic analysis was performed using polymerase chain reaction (PCR) or

Southern blot hybridization. PCR was used in the analysis of gene rearrangements of both TCR and the immunoglobulin heavy chain (IgH). PCR was performed according to standard procedures, as described previously [13-16]. For Southern blot hybridization, high-molecular-weight genomic DNA was extracted from fresh tissue taken from the patients and used for the detection of TCR γ gene rearrangements or IgH gene rearrangements, according to a method described previously [17].

Results

Patient summary

The clinical features of the 10 patients are summarized in Table 2. The age of the patients (5 men and 5 women) ranged from 41 to 82 years, with a median age of 61.5 years. Eight patients showed extranodal site involvement, with the extranodal site being the spleen in most cases (5 patients), followed by the thyroid gland (3 patients).

Additionally, in 2 of 5 patients who had an affected spleen, the disease was localized to that organ. Those patients with thyroid gland involvement also demonstrated involvement of the regional lymph nodes (2 patients) or systemic lymph nodes (1 patient). Three of 10 patients had systemic lymph node involvement. Bone marrow

involvement was observed in 3 of 9 patients. One patient increased lymphocyte counts, and in another patient, 2% of cells in the peripheral blood had chromosomal abnormalities. We confirmed there was no azurophilic granule or prominent nucleoli in the tumor cell, or atypical lymphocytes in any of the patients' smears. Results for clinical stage were as follows: stage I, 2 patients; stage II, 3 patients; stage III, 1 patient; and stage IV, 4 patients. Nine patients had an IPI of low or low-intermediate risk. In the PIT, 2 patients were group 1, and 6 patients were group 2. Patient no. 5 had B symptoms and hypergammaglobulinemia, whereas patient no. 10 had autoimmune hemolytic anemia and elevated serum LDH. None of the patients demonstrated bulky disease (size of lymph node ≥ 10 cm) with a history of prior immunosuppressive therapy or immune system disorder.

Compared to the study of PTCL-NOS by the international PTCL-NOS project [5], our patient series had low IPI scores ($p < 0.001$), low PIT group scores ($p = 0.002$), and a normal LDH level ($p = 0.035$; Table 3). Furthermore, compared with another report that described clinical information in more detail [18], our patient series had low clinical stages ($p = 0.025$), low IPI scores ($p = 0.015$), and a normal LDH level ($p = 0.025$; Table 3).

Morphological and immunophenotypic features

Regardless of whether the lesion was nodal or extranodal, all samples presented diffuse infiltrate of small lymphoid cells (Figure 1 a–f). These cells had oval or slightly irregular nuclei and pale cytoplasm. In cases with thyroid lesions, tumor cells infiltrated the follicular epithelium, resembling a lymphoepithelial lesion involved in mucosa-associated lymphoid tissue (MALT) lymphoma (Figure 1 d). Samples from all patients were positive for CD3, TCR β , and surface antigen of central memory T-cells (CD45RO and CCR7). Seven samples were positive for another antigen of central memory T-cells (CD62L). Three of the 10 samples demonstrated either complete loss or decreased staining intensity for CD5 or CD7. Half of the samples showed the helper T-cell phenotype (CD4+, CD8-), whereas the other half showed the cytotoxic T-cell phenotype (CD4-, CD8+). Only the clinical stage tended to be high in the cytotoxic phenotype samples ($P=0.07$), however we found no significant differences in clinicopathological data between samples with the cytotoxic T-cell phenotype and those with the helper T-cell phenotype. All CD8-positive samples were positive for granzyme B or TIA-1. Both T follicular helper cell markers (PD1 and CXCL13) were positive in 3 samples, and these samples were also positive for CD4. Although CD57 was positive in patient no. 3, this patient did not show any LGL, and the patient's lymphocyte counts

did not increase. All samples in the study were negative for CD79a, CD56, TCR δ , TCR γ , and EBER ISH. An overview of marker expression is presented in Table 4, and Figure 1 g-x shows the immunohistochemical staining of patient no. 9. Mitosis was very rare, and Ki-67 LI was low (range, 0.28–3.04%; mean, 1.05%; Table 4).

Five samples had CD20 expression. Figure 2 a–d shows CD20 expression, as measured using flow cytometry, and the double immunofluorescent study of CD3 (green) and CD20 (red) in patient no. 7. We found that CD20 was coexpressed in CD3-positive tumor cells.

Molecular genetic findings

TCR γ chain and/or TCR α - β chain gene rearrangements were detected in all 10 patients. In 7 of these patients, these rearrangements were detected using PCR analysis; in the remaining 3 patients, the rearrangements were detected using Southern blot hybridization (Figure 3 a–b). However, we could not detect any immunoglobulin heavy-chain gene rearrangements in any of the 10 patients, using either method.

G-banding karyotype analysis was performed in 3 patients. In patient no. 9, this analysis showed abnormal karyotypes (Table 4). In patient no. 6, there was only a single

cell with an apparent chromosomal abnormality.

Response to treatment and survival

The median follow-up period was 19.5 months. Only patient no. 10 died, and this death resulted from autoimmune hemolytic anemia. The other 9 patients are alive, with or without disease. Treatment and outcome data for all patients are shown in Table 2. Six patients were treated with chemotherapy or allogeneic bone marrow transplantation, and 4 achieved complete response (CR). Three patients received only observation after splenectomy or thyroidectomy, and did not experience recurrence.

Discussion

In this study, we presented a new, distinct subgroup of T-cell lymphomas with good prognosis; this subgroup appears to be a subset of PTCL-NOS. The 10 patients with this condition showed similar histological findings, and the disease was characterized by infiltration of small lymphoid cells with slight nuclear atypia and low Ki-67 LI. Half of the patient samples showed the helper T-cell phenotype (CD4+), and the other half showed the cytotoxic phenotype (CD8+). Cytotoxic molecule positive PTCL-NOS was

associated with several clinical factors that indicate poor prognosis [18, 19]. In this study, however, we could find no significant differences in clinicopathological data between samples with the cytotoxic phenotype and those with the helper T-cell phenotype. In addition, patient no. 8, who had the cytotoxic phenotype, is alive and without disease after splenectomy alone. This clinical behavior is unusual in general for PTCL-NOS. Actually, the cytotoxic markers become prognostic factors in PTCL-NOS, but in our present series, the cytotoxic markers might have displayed a different behavior.

It may be necessary to distinguish the disease observed in our patient series from T-cell large granular lymphocytic leukemia (T-LGL), T-cell prolymphocytic leukemia (T-PLL), and chronic lymphoproliferative disorders of NK cells (CLPD-NK) due to the fact that small lymphoid cells proliferate. T-LGL patients were distinguished from our patient series by their large granules and the displayed phenotype. T-PLL usually had elevated lymphocyte counts ($>100 \times 10^3 \mu\text{l}$) and prominent nucleoli in lymphoid cells (1). CLPD-NK are characterized by an increase in peripheral blood NK cells ($>2 \times 10^3 \mu\text{l}$) and are positive for CD16 and CD56 (1). On the other hand, it may be necessary to distinguish our patient series from T-LGL, T-PLL, and chronic adult T-cell leukemia/lymphoma (ATLL) due to the fact that the clinical course was indolent. Our

patient series can be distinguished by the T-LGL and T-PLL in their smear and the phenotype described above. ATLL showed monoclonal integration of HTLV-1. All patients in our series were negative for HTLV-1. Therefore, we excluded chronic ATLL.

While some patients were treated with chemotherapy and 1 patient received bone marrow transplantation, all patients, excluding patient no. 3, are alive, with or without disease, and 3 patients did not relapse after surgical resection. We thought that these clinical behaviors differentiated our series from the general PTCL-NOS population.

CD20, a selective marker for B-cells, was expressed in half of our patient series. The presence of CD20 is generally considered specific for the B-cell lineage on both benign and neoplastic lymphocytes. T-cell lymphoma with expression of CD20 is a rare, but well-recognized phenomenon, and CD20 expression has been reported in some patients [6, 7, 20-22]. Although the clinicopathological spectrum of CD20-positive T-cell lymphomas is still not well understood, this condition is known to occur frequently in elderly men and to show an aggressive clinical course in many patients [7]

Another interesting result of the immunohistochemical staining was that all patient samples were found to be positive for CD45RO and CCR7, whereas 7 were positive for CD62L. These antibodies are expressed in central memory T-cells (T_{CM}). Naive T-cells

differentiate into lymphocytes of various type such as T_{CM} , effector memory T-cells, regulatory T-cells, and follicular helper T-cells after antigen stimulation[23]. In a study of helper T-cell differentiation of PTCL-NOS, it was reported that only 2 of 10 patients expressed both CCR7 and CD62L [24]. In contrast, 7 of the patients in our series showed samples that were positive for both of these antigens. This result suggests that this type of indolent T-cell lymphoma derives from organ-specific T_{CM} . Furthermore, this characteristic suggests that this type of indolent T-cell lymphoma is localized to extranodal sites that are somewhat similar to MALT lymphoma [1, 25, 26].

Although PTCL-NOSs are predominantly nodal lymphomas [27], there was extensive extranodal site involvement in our patient series. PTCL-NOS at the extranodal sites, especially the spleen and the thyroid, might be associated with a high frequency of this type of indolent T-cell lymphoma. Physicians may need to pay careful attention to PTCL-NOS in these sites because of the possibilities of indolent T-cell lymphoma.

Xiao et al. reported a case of T-cell lymphoma with indolent clinical behavior that was positive for CD20 [6]. Rahemtullah et al. described a 9-patient series of CD20-positive T-cell lymphomas, in which 1 patient presented with the same morphological and immunophenotypical characters as the patients in our present series [7]. These other 2 reports may concern the same subset of patients as does our series. Perry et al. reported

7 cases of indolent T-cell lymphoproliferative disease of the gastrointestinal tract [28], and these patient samples consisted of small lymphoid cells with slightly irregular nuclei, clear cytoplasm, and low Ki-67 LI (5-10%). This lymphoproliferative disease might be similar to the one occurring in our patient series. To the best of our knowledge, ours is the first report to describe a clinicopathological analysis of this new, distinct subgroup of T-cell lymphomas.

In conclusion, indolent T-cell lymphoma has distinct morphological, immunophenotypical, and clinical outcomes. Therefore, it may need to be classified as an independent disease. More cases and long-term prognoses must be evaluated to clarify the pathophysiology of this subgroup.

Disclosure/Conflict of Interest

The authors declare no competing financial interests.

Acknowledgements

The authors would like to specially thank Dr. K Mizobuchi, Dr. T Morito, Dr. S Takata, Dr. M Tamura, Ms. H Nakamura, Ms. M Okabe, and Mr. T Igawa. The authors would also like to thank the Central Research Laboratory, Okayama University Medical School, Okayama, Japan.

References

1. Swerdlow S, Campo E, Harris N, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: International Agency for Research on Cancer, 2008.
2. Vose J, Armitage J, Weisenburger D, Project IT-CL. International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. *J Clin Oncol* 2008; 26: 4124-4130.
3. Lymphoma Study Group of Japanese Pathologists. The world health organization classification of malignant lymphomas in japan: incidence of recently recognized entities. *Pathol Int* 2000; 50: 696-702
4. Jaffe ES. The 2008 WHO classification of lymphomas: implications for clinical practice and translational research. *Hematology Am Soc Hematol Educ Program* 2009; 1: 523-531.
5. Weisenburger DD, Savage KJ, Harris NL, Gascoyne RD, Jaffe ES, MacLennan KA, Rüdiger T, Pileri S, Nakamura S, Nathwani B, Campo E, Berger F, Coiffier B, Kim WS, Holte H, Federico M, Au WY, Tobinai K, Armitage JO, Vose JM, Project IPT-cL. Peripheral T-cell lymphoma, not otherwise specified: a report of 340 cases from the International Peripheral T-cell Lymphoma Project. *Blood* 2011; 117: 3402-3408.
6. Xiao WB, Wang ZM, Wang LJ. CD20-positive T-cell lymphoma with indolent clinical behaviour. *J Int Med Res* 2010; 38: 1170-1174.
7. Rahemtullah A, Longtine JA, Harris NL, Dorn M, Zembowicz A, Quintanilla-Fend L, Preffer FI, Ferry JA. CD20+ T-cell lymphoma: clinicopathologic analysis of 9 cases and a review of the literature. *Am J Surg Pathol* 2008; 32: 1593-1607.
8. Louissaint A, Ackerman AM, Dias-Santagata D, Ferry JA, Hochberg EP, Huang MS, Iafrate AJ, Lara DO, Pinkus GS, Salaverria I, Siddiquee Z, Siebert R, Weinstein HJ, Zukerberg LR, Harris NL, Hasserjian RP. Pediatric-type nodal follicular lymphoma: an indolent clonal proliferation in children and adults with high proliferation index and no BCL2 rearrangement. *Blood* 2012; 120: 2395-2404.
9. Carbone PP, Kaplan HS, Musshoff K, Smithers DW, Tubiana M. Report of the Committee on Hodgkin's Disease Staging Classification. *Cancer Res* 1971; 31: 1860-1861.
10. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. A predictive model for aggressive non-Hodgkin's lymphoma. *N Engl J Med* 1993; 329: 987-994.
11. Gallamini A, Stelitano C, Calvi R, Bellei M, Mattei D, Vitolo U, Morabito F, Martelli M, Brusamolino E, Iannitto E, Zaja F, Cortelazzo S, Rigacci L, Devizzi L, Todeschini G, Santini G, Brugiattelli M, Federico M, Linfomi II. Peripheral T-cell lymphoma unspecified (PTCL-U): a new prognostic model from a retrospective multicentric clinical study. *Blood*

2004; 103: 2474-2479.

12. Sato Y, Takata K, Ichimura K, Tanaka T, Morito T, Tamura M, Yoshino T. IgG4-producing marginal zone B-cell lymphoma. *Int J Hematol* 2008; 88: 428-433.

13. Mannami T, Yoshino T, Oshima K, Takase S, Kondo E, Ohara N, Nakagawa H, Ohtsuki H, Harada M, Akagi T. Clinical, histopathological, and immunogenetic analysis of ocular adnexal lymphoproliferative disorders: characterization of malt lymphoma and reactive lymphoid hyperplasia. *Mod Pathol* 2001; 14: 641-649.

14. Sato Y, Nakamura N, Nakamura S, Sakugawa S, Ichimura K, Tanaka T, Ohara N, Oka T, Kondo E, Yoshino T. Deviated VH4 immunoglobulin gene usage is found among thyroid mucosa-associated lymphoid tissue lymphomas, similar to the usage at other sites, but is not found in thyroid diffuse large B-cell lymphomas. *Mod Pathol* 2006; 19: 1578-1584.

15. Sato Y, Ichimura K, Tanaka T, Takata K, Morito T, Sato H, Kondo E, Yanai H, Ohara N, Oka T, Yoshino T. Duodenal follicular lymphomas share common characteristics with mucosa-associated lymphoid tissue lymphomas. *J Clin Pathol* 2008; 61: 377-381.

16. van Dongen JJ, Langerak AW, Brüggemann M, Evans PA, Hummel M, Lavender FL, Delabesse E, Davi F, Schuurin E, García-Sanz R, van Krieken JH, Droese J, González D, Bastard C, White HE, Spaargaren M, González M, Parreira A, Smith JL, Morgan GJ, Kneba M, Macintyre EA. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia* 2003; 17: 2257-2317.

17. Cossman J, Zehnbauser B, Garrett CT, Smith LJ, Williams M, Jaffe ES, Hanson LO, Love J. Gene rearrangements in the diagnosis of lymphoma/leukemia. Guidelines for use based on a multiinstitutional study. *Am J Clin Pathol* 1991; 95: 347-354.

18. Went P, Agostinelli C, Gallamini A, Piccaluga PP, Ascani S, Sabattini E, Bacci F, Falini B, Motta T, Paulli M, Artusi T, Piccioli M, Zinzani PL, Pileri SA. Marker expression in peripheral T-cell lymphoma: a proposed clinical-pathologic prognostic score. *J Clin Oncol* 2006; 24: 2472-2479.

19. Asano N, Suzuki R, Kagami Y, Ishida F, Kitamura K, Fukutani H, Morishima Y, Takeuchi K, Nakamura S. Clinicopathologic and prognostic significance of cytotoxic molecule expression in nodal peripheral T-cell lymphoma, unspecified. *Am J Surg Pathol* 2005; 29: 1284-1293.

20. Yao X, Teruya-Feldstein J, Raffeld M, Sorbara L, Jaffe ES. Peripheral T-cell lymphoma with aberrant expression of CD79a and CD20: a diagnostic pitfall. *Mod Pathol* 2001; 14: 105-110.

21. Mohrmann RL, Arber DA. CD20-Positive peripheral T-cell lymphoma: report of a

- case after nodular sclerosis Hodgkin's disease and review of the literature. *Mod Pathol* 2000; 13: 1244-1252.
22. Yokose N, Ogata K, Sugisaki Y, Mori S, Yamada T, An E, Dan K. CD20-positive T cell leukemia/lymphoma: case report and review of the literature. *Ann Hematol* 2001; 80: 372-375.
23. Sallusto F, Lenig D, Förster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 1999; 401: 708-712.
24. Matsumoto Y, Horiike S, Ohshiro M, Yamamoto M, Sasaki N, Tsutsumi Y, Kobayashi T, Shimizu D, Uchiyama H, Kuroda J, Nomura K, Shimazaki C, Taniwaki M. Expression of master regulators of helper T-cell differentiation in peripheral T-cell lymphoma, not otherwise specified, by immunohistochemical analysis. *Am J Clin Pathol* 2010; 133: 281-290.
25. Du M, Diss TC, Xu C, Peng H, Isaacson PG, Pan L. Ongoing mutation in MALT lymphoma immunoglobulin gene suggests that antigen stimulation plays a role in the clonal expansion. *Leukemia* 1996; 10: 1190-1197.
26. Qin Y, Greiner A, Trunk MJ, Schmausser B, Ott MM, Müller-Hermelink HK. Somatic hypermutation in low-grade mucosa-associated lymphoid tissue-type B-cell lymphoma. *Blood* 1995; 86: 3528-3534.
27. Rizvi MA, Evens AM, Tallman MS, Nelson BP, Rosen ST. T-cell non-Hodgkin lymphoma. *Blood* 2006; 107: 1255-1264.
28. Perry A, Warnke R, Hu Q, Rosenwald A, Gaulard P, Copie-Bergman C, Geissinger E, Wang HY, Weisenburger D, Jaffe ES. Indolent T-cell lymphoproliferative disease of the gastrointestinal tract: report of seven cases. *J Hematopathol* 2012; 5: 224.

Table 1. Antibodies used for immunohistochemical analysis			
Antibody or Probe	Source	Clone	Dilution
CD20	Novocastra, Newcastle-upon-Tyne, UK	L26	1:200
CD3	Novocastra	LN10	1:200
CD5	Novocastra	4C7	1:100
CD7	Novocastra	CD7-580	1:50
CD8	Nichirei, Tokyo, Japan	C8/144B	1:100
CD4	Nichirei	1F6	1:40
CD79a	Dako, Glostrup, Denmark	JCB117	1:40
CD45RO	Dako	UCHL1	1:50
CD62L	Abcam, Tokyo, Japan	rabbit polyclonal	1:100
CD56	Leica Biosystems, Melbourne, Vic., Australia	1B6	1:30
CD57	Leica Biosystems	NK-1	1:50
granzyme B	Nichirei	GrB-7	ready to use
TIA-1	Beckman Coulter, Brea, CA, USA	2G9	1:500
PD1	Abcam, Tokyo, Japan	NAT	1:200
CCR7	Epitomics, CA, USA	Y59	1:5000
CXCL13	R&D Systems, Minneapolis, MN, USA	goat polyclonal	1:50
Ki-67	Novocastra	MIB-1	1:5000
Human Pan TCR $\gamma\delta$	Thermo scientific, Waltham, MA, USA	5A6.E9	1:20
TCR1151	Thermo scientific	8A3	1:50
TCR1153	Thermo scientific	γ 3.20	1:80
EBV-encoded small RNA	INFORM EBER, Leica Biosystems	-	-

Table 2. Patient summary

Patient No.	Age	Gender	Clinical manifestation	Sites involvement	PB		Stage	PS	IPI score	PTT	Treatment	Response	Follow-up period (months)	Follow-up status
					WBC(/ μ l)	Ly(%)								
1	60	F	Thyromegaly	Thyroid and cervical LN	5770	33.8	II	0	0	Unknown	Unknown	CR	62	NEDA
2	67	F	Thyromegaly	Thyroid and cervical LN	15600	59.2	II	0	1	2	Thyroidectomy and observation	CR	36	NEDA
3	52	M	Right lower quadrant of abdomen mass	Abdominal pelvic and cervical LN	7260	16.7	III	0	1	1	CHOP	CR	25	NEDA
4	82	F	Cervical LN swelling	Cervical and axilla LN	6100	48.6	II	0	1	2	CHOP	CR	21	NEDA
5	63	M	Splenomegaly	Spleen	1510	17.2	Is	0	1	2	Splenectomy and observation	CR	15	NEDA
6	41	M	Pancytopenia	Cervical LN, BM, spleen, liver	9000	35	IV	0	2	2	EPOCH, allo-BMT	CR	29	NEDA
7	59	M	Thyromegaly	Thyroid, systemic LN, BM	3500	38	IV	0	2	2	R-CHOP	CR	18	NEDA
8	69	F	Splenomegaly	Spleen	900	71	Is	0	1	2	Splenectomy and observation	CR	17	NEDA
9	45	M	Splenomegaly	Spleen, PB	2090	63	IV	0	1	1	Splenectomy and CHOP	SD	12	AWD
10	80	F	Anemia, splenomegaly	Spleen, BM, liver, mediastinum and retroperitoneal LN	2800	33	IV	1	4	4	Steroid monotherapy	-	2	Dead

LN, Lymph node; PB, peripheral blood; BM, Bone marrow; WBC, white blood cell; Ly, lymphocyte; PS, performance status; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone; R-CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone with rituximab; EPOCH, Etoposide, cyclophosphamide, doxorubicin, vincristine, prednisolone; allo-BMT, allogeneic bone marrow transplantation; SD, Stable disease; CR, Complete response; AWD, Alive with disease; NEDA, No evidence of disease and alive

Table 3. Comparison of our patient series and PTCL-NOS					
	Our patient series	PTCL-NOS [5]		PTCL/U [18]	
	n/N(%)	n/N(%)	<i>p</i>	n/N(%)	<i>p</i>
Age ; median	61.5	60	-	60	-
Male: female	1:1	1.9:1	0.7 ^a	3:2	0.714 ^a
LDH >normal,	1/10(10)	158/323(49)	0.035 ^a	42/90(47)	0.025 ^a
Performance status ≥2	0/10(0)	60/334(18)	0.293 ^a	20/93(22)	0.103 ^a
B symptoms	1/10(10)	118/340(35)	0.198 ^a	31/88(35)	0.1 ^a
bulky disease (≥ 10 cm)	0/10(0)	19/285(7)	0.85 ^a	11/85(13)	0.273 ^a
BM involvement	3/9(33)	68/322(21)	0.639 ^a	31/85(36)	0.581 ^a
Stage					
I	2/10(20)	45/334(14)	0.213 ^b	1/93(1)	0.025 ^b
II	3/10(30)	57/334(17)		19/93(20)	
III/ IV	5/10(50)	232/334(69)		73/93(79)	
IPI , Score					
0/1	7/10(70)	- (28)	< .001 ^b	24/90(27)	0.015 ^b
2	2/10(20)	- (35)		31/90(34)	
3	0/10(0)	- (22)		23/90(26)	
4/5	1/10(10)	- (15)		12/90(13)	
PIT, group					
1	2/9(22)	- (20)	0.002 ^b	17/85(20)	0.165 ^b
2	6/9(67)	- (38)		27/85(32)	
3	0/9(0)	- (29)		29/85(34)	
4	1/9(11)	- (13)		12/85(14)	
^a <i>P</i> value by Fisher's exact probability test or chi-square for independence test					
^b <i>P</i> value by Mann-Whitney nonparametric test					
[5] Weisenburger DD et al. 2011					
[18] Went P et al. 2006					

Table 4. Immunophenotypic features and molecular genetic findings of our patient series

Patient No.	CD3	CD5	CD7	CD4	CD8	CD20	CD79a	CD45RO	CCR7	CD62L	CXCL13	PD1	CD56	CD57	TIA-1	granzyme B	$\alpha\beta$ -TCR	$\gamma\delta$ -TCR	EBER	Ki-67 LI(%)	TCR rearrangement	IgH rearrangement	G-band
1	+	+	+	-	-	-	-	+	+	-	-	+	-	-	-	-	+	-	-	0.89	Clonal	Germine	^a
2	+	+	-	+	-	w+	-	+	+	+	-	w+	-	-	-	-	+	-	-	0.48	Clonal	Germine	N.D.
3	+	+	+	+	-	w+	-	+	+	+	-	+	+	-	-	-	+	-	-	0.63	Clonal	Germine	N.D.
4	+	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	-	-	1.9	Clonal	Germine	N.D.
5	+	+	+	+	-	-	-	+	+	+	+	w+	-	-	-	-	+	-	-	1.02	Clonal	Germine	N.D.
6	+	w+	w+	-	+	w+	-	+	+	+	-	-	-	-	+	+	+	-	-	1.66	Clonal	Germine	^b
7	+	+	+	-	+	-	-	+	+	+	-	+	-	-	+	+	+	-	-	1.07	Clonal	Germine	N.D.
8	+	+	+	-	+	-	-	+	+	+	-	-	-	-	+	-	+	-	-	3.04	Clonal	Germine	N.D.
9	+	+	-	-	+	w+	-	+	+	+	-	w+	-	-	+	+	+	-	-	0.28	Clonal	Germine	^c
10	+	+	+	-	+	-	-	+	+	+	-	-	-	-	+	-	+	-	-	1.07	Clonal	Germine	N.D.

w+, weakly positive; N.D., Not determined

^a undetected^bThe karyotype was interpreted according to the ISCN, as follow: 46, XY, add(7)(q11.2), der(7)add(7)(p15)add(7)(q32) [1]/46, XY[9]^c 46, XY, t(2;18)(q31;q21), add(11)(q23) [5]/46, kerm, add(10)(p11.2) [7]/46, XY[7]

Figure 1

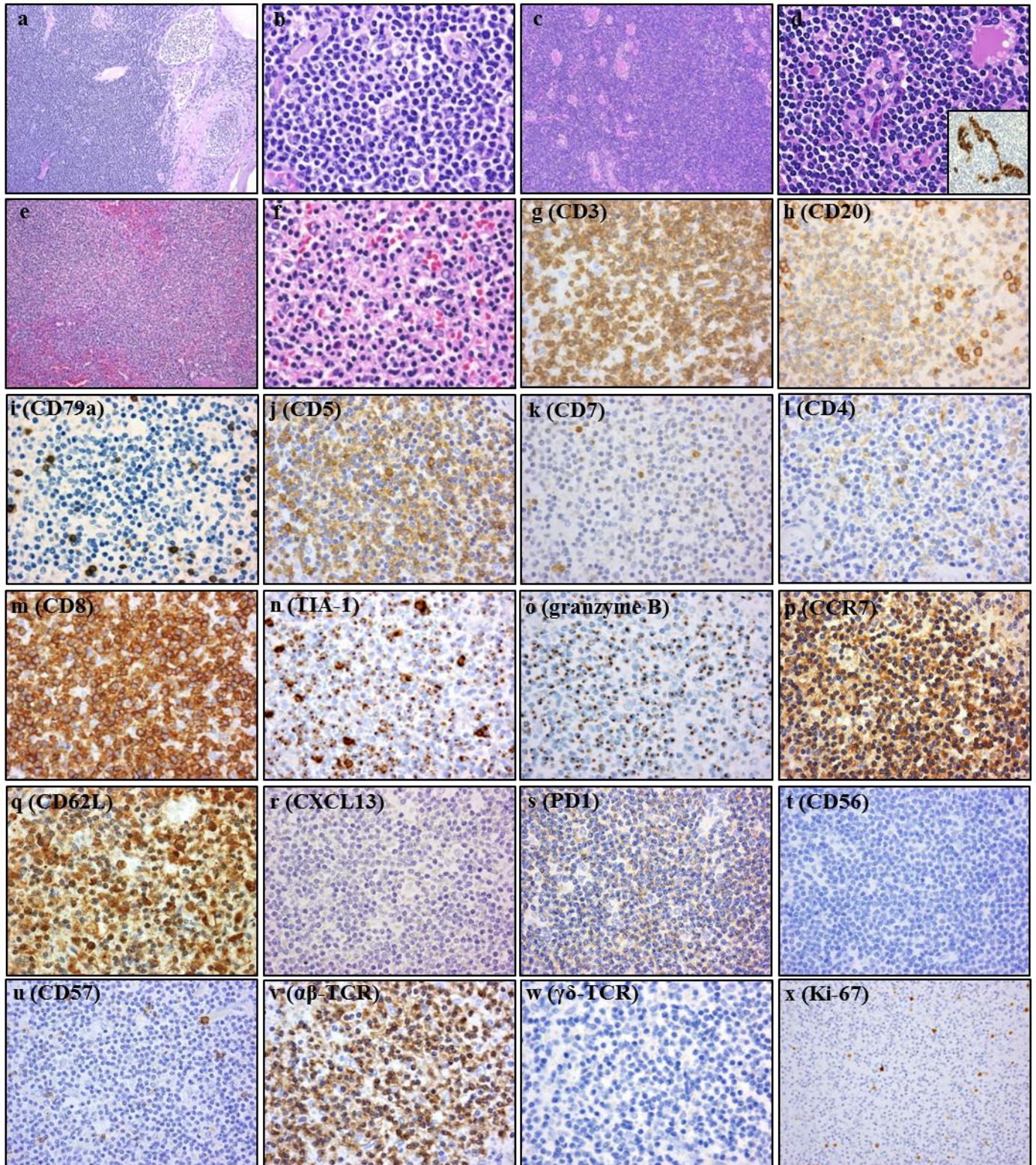


Figure 2

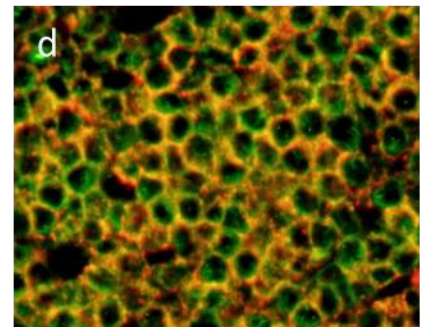
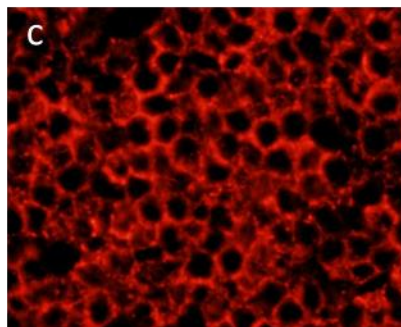
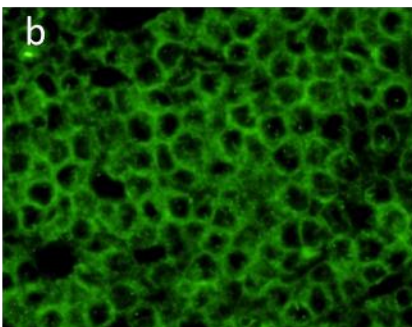
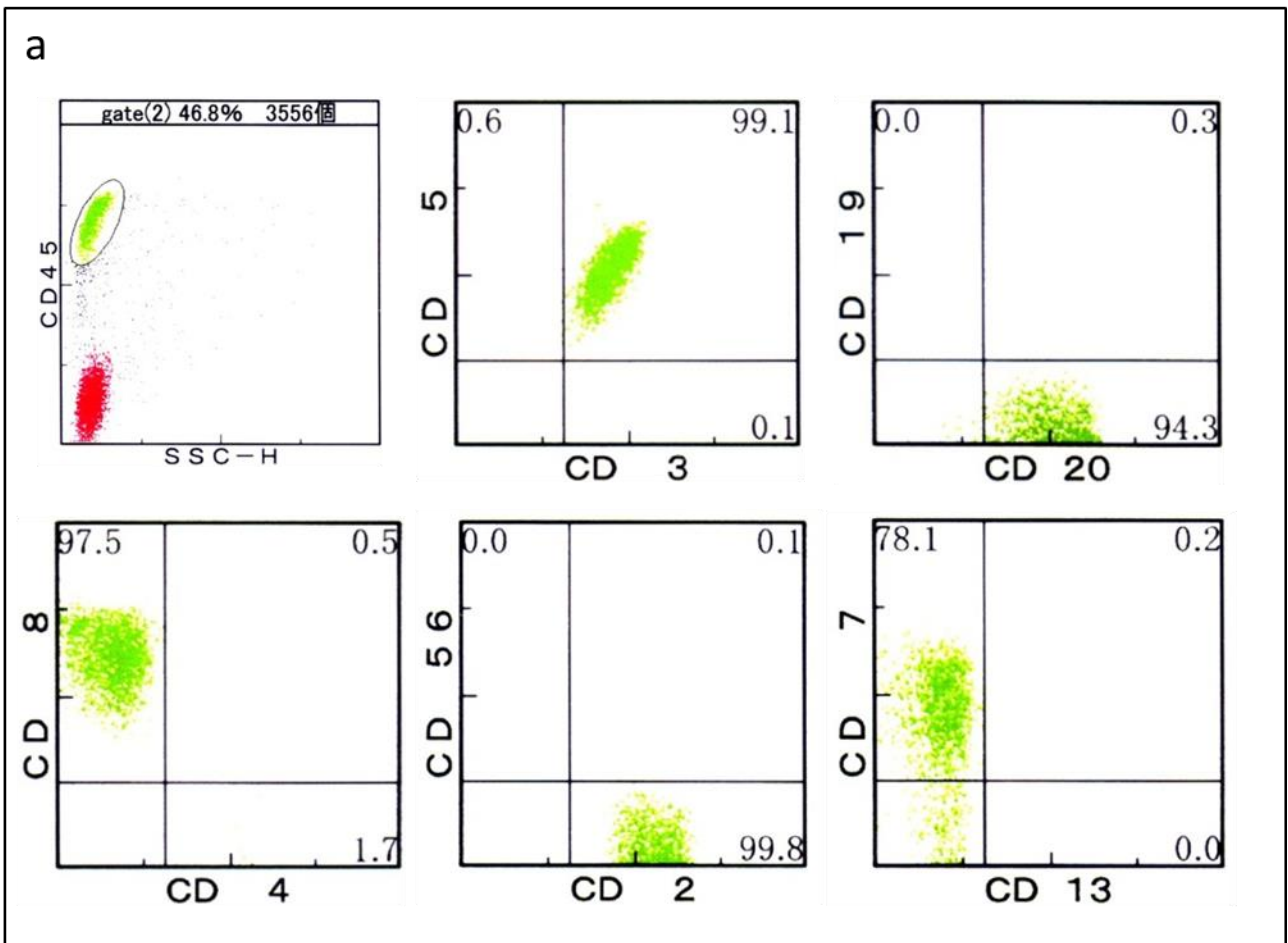


Figure 3

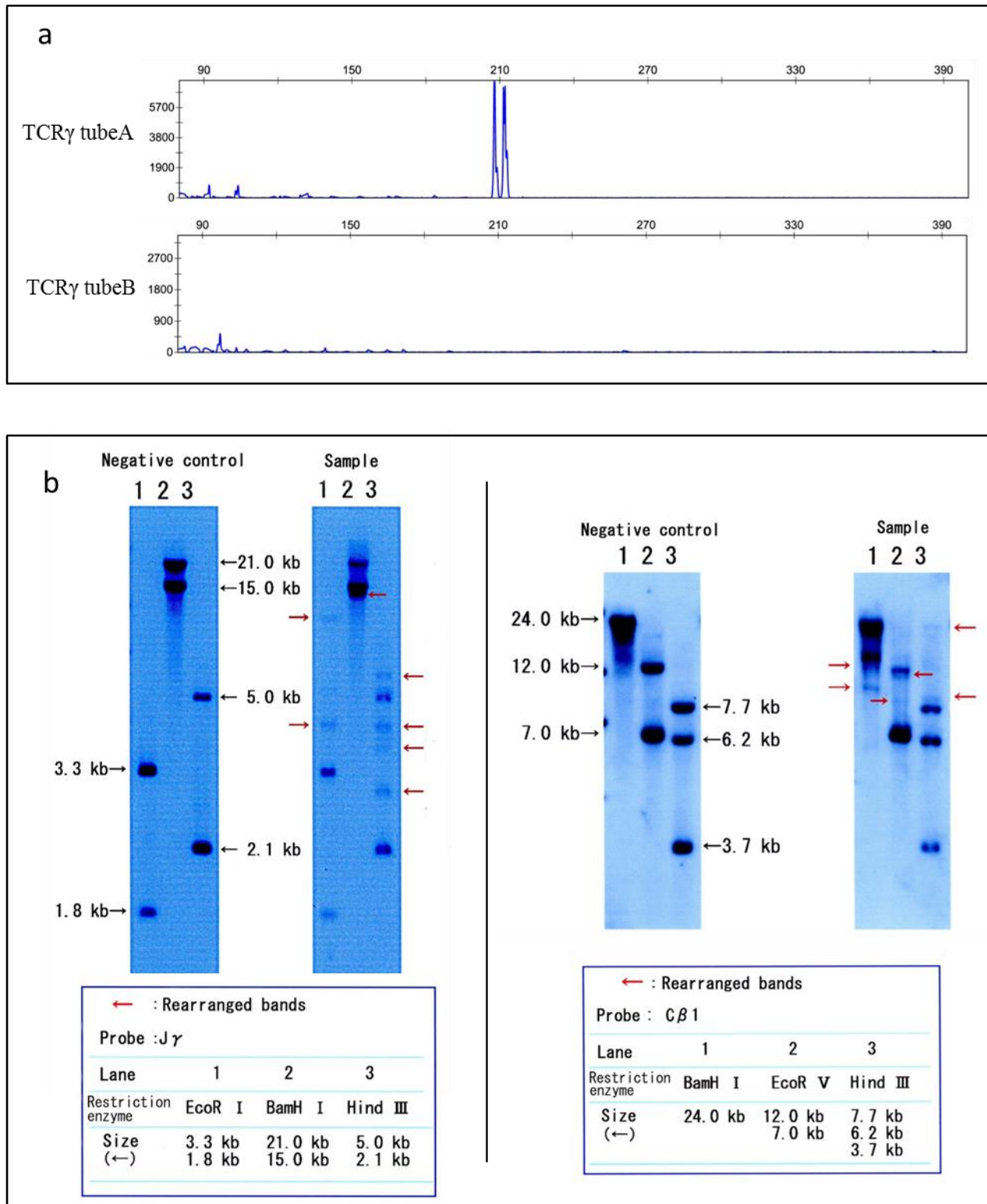


Figure legends

Figure 1. Histopathology of various lesions and immunostaining of samples.

(a, c, and e: original magnification $\times 10$ objective; x: $\times 20$; b, d, and f-w: $\times 40$)

(a, b) Lymph nodes of patient no. 6 displayed diffuse infiltration of small lymphoid cells.

These lymphoid cells showed oval or slightly irregular nuclei and pale cytoplasm on hematoxylin and eosin staining.

(c, d) Thyroid gland of patient no. 2: the thyroid follicle was present between the tumor cells, and a part of the residual follicle presented lymphoepithelial lesions. This was observed more clearly on CK AE1/AE3 staining (inset).

(e, f) Spleen of patient no. 9: the tumor cells diffusely infiltrated the red pulp.

(g-w) Immunohistochemical results of patient no. 9: The tumor cells were positive for CD3 (g), weakly positive for CD20 (h), negative for CD79a (i), positive for CD5 (j), negative for CD7 (k), negative for CD4 (l), positive for CD8 (m), positive for TIA-1 (n), positive for granzyme B (o), positive for CCR7 (p), positive for CD62L (q), negative for CXCL13 (r), weakly positive for PD1 (s), negative for CD56 (t), negative for CD57 (u), positive for $\alpha\beta$ -TCR (v), and negative for $\gamma\delta$ -TCR (w).

(x) The Ki-67 labeling index was very low at 0.28%.

Figure 2. Analysis of aberrant expression of CD20 from patient no. 7.

(a) The 2-color flow cytometry of the lymph node. The tumor cell group was positive for CD3, CD5, CD20, CD8, CD2, and CD7.

(b-d) On double immunofluorescence of CD3 (green, b) and CD20 (red, c), almost all CD3-positive cells were positive for CD20 in a merge image (yellow, d).

Figure 3. Molecular genetic analysis

(a) Oligoclonal bands around 210-bp were detected using polymerase chain reaction analysis (patient no. 7).

(b) Southern blot analysis of DNA from patient no. 3 with the J γ probe (left) and the C β 1 probe (right). Germline bands are indicated by black arrows and rearranged bands by red arrows. Clonal bands were seen in the lane 2 of the J γ probe and lane 1 and 2 of the C β 1 probe.