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Lymphopenia at diagnosis predicts survival of patients with immunodeficiency-associated lymphoproliferative disorders

Mizuki Watanabe[1], Junya Kanda[1], Masakatsu Hishizawa[1], Momoko Nishikori[1], Tadakazu Kondo[1], Kouhei Yamashita[1], Akifumi Takaori-Kondo[1]

[1]Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

†Correspondence: Junya Kanda, M.D.
Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, 54 Kawaharacho, Shogoin, Sakyo-ku, Kyoto, Japan, 606-8507.
Tel: +81-75-751-3152; Fax: +81-75-751-3153; e-mail: jkanda16@kuhp.kyoto-u.ac.jp

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Running head: Lymphopenia predicts outcomes of PTLD and OIIA-LPD

Article Summary: Little is known about the impacts of immunocompromised status on clinical outcomes in patients with immunodeficiency-associated LPD. We found that lymphopenia at the diagnosis of LPDs could be a novel predictive factor for inferior OS and PFS in these patients.

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Abstract

Introduction: The number of patients who are administered immunosuppressive agents has been increasing. Accordingly, more patients face higher risks for developing immunodeficiency-associated lymphoproliferative disorders (LPD). Although immunodeficiency-associated LPD are distinct from other lymphoid neoplasms in terms of their immunocompromised backgrounds, little is known about the impact of lymphopenia at diagnosis on survival in patients with these LPD.

Patients and Methods: Seventy-one immunodeficiency-associated LPD in Kyoto University Hospital (post-transplant LPD (PTLD), n=26; other iatrogenic immunodeficiency-associated LPD, n=45) were reviewed and analyzed.

Results: The median age at diagnosis was 63 y (range, 3-83). Diffuse large B-cell lymphoma was the most common subtype (n=33), followed by Hodgkin lymphoma (n=12), B-cell monomorphic LPD not specified (n=11) and polymorphic LPD or early-phase diseases (n=15). The median follow-up period for survivors was 2.5 years and overall survival (OS) and progression-free survival (PFS) at 2.5 years were 75% and 67%, respectively. Multivariate analysis showed that lymphopenia (≤ 800/μl) at diagnosis predicted inferior OS (HR, 3.72; P=0.043) and PFS (HR, 3.82; P=0.012). Serum albumin values also strongly affected OS (>3.18 g/dL vs. ≤3.18 g/dL; HR, 0.21; P=0.010) and PFS (HR, 0.26; P=0.013).

Conclusion: Lymphopenia at diagnosis is suggested to predict inferior OS and PFS in patients with immunodeficiency-associated LPDs. Immunocompromised status might affect disease progression in these distinct lymphoid neoplasms growing under immunocompromised backgrounds.

Keywords: *immunodeficiency-associated lymphoproliferative disorders, PTLD, immune-suppressive patients, Lymphopenia*

Introduction

As a result of recent advances in medical care, the number of patients who receive various immunosuppressive agents has been increasing. These patients are known to be at risk for the development of immunodeficiency-associated lymphoproliferative disorders (LPD) under an iatrogenic immunocompromised status.[1–3] Although aberrant infection of Epstein-Barr virus (EBV) in immunosuppressed lymphocytes has been suggested to play a key role in the pathogenesis of these LPD,[4–7] the overall context of this unique disease entity is not yet completely understood.

This insufficient understanding of iatrogenic immunodeficiency-associated LPD is partly attributed to their clinical, histopathological and genetic heterogeneity.[1,8–10] They include post-transplant lymphoproliferative disorders (PTLD) that arise in patients after solid organ transplantations or hematopoietic stem cell transplantation (HSCT), and other iatrogenic immunodeficiency-associated LPD that arise in patients treated with various immunosuppressive agents for any reason. They include various pathological subtypes including non-destructive hyperplasia of lymphocytes, polymorphic LPDs and several aggressive types of malignant lymphomas. Their genetic landscapes have not yet been completely revealed. In recent studies, PTLD has been considered to consist of genetically distinct populations: EBV-related or others[6,11–13] and germinal center B-cell-like (GCB) or non-GCB subtype.[14] There was also a hypothesis that some subtypes of PTLD might actually be a coincidental occurrence of lymphoid neoplasms among post-HSCT patients,[11,15] although this view has not yet reached a consensus. The genetic backgrounds of other iatrogenic immunodeficiency-associated LPD such as MTX-associated LPD have been scarcely examined.

Regardless of this heterogeneity, LPD growing with an immunocompromised background are known to present worse clinical outcomes than those without such a background.[1,16,17] In the analysis of PTLD, the International Prognostic Index (IPI) score, hypoalbuminemia and the treatment response for rituximab have been suggested as prognostic factors for survival.[1,16,18,19] However, these prognostic factors have been less discussed in other iatrogenic immunodeficiency-associated LPDs.[20,21] Moreover, although all these iatrogenic immunodeficiency-associated LPDs share immunocompromised backgrounds[22] with some pathological features derived from aberrant viral infection,[7,23,24] the impact of the immunosuppressive status in each patient on clinical outcomes has rarely been assessed.[25] This should be more carefully examined, since it reflects not only the patient's morbidity but also the anti-viral or anti-tumor effects of lymphocytes.

In this study, we analyzed the impact of lymphopenia on survival in patients with iatrogenic immunodeficiency-associated LPDs. We chose total lymphocyte count as a clinical factor to evaluate patients' immunosuppressive status since it is easy to obtain and is always examined as an index for immune reconstitution in routine practice.

Methods

Data collection

Clinical data of patients who were pathologically and clinically diagnosed with PTLD or other iatrogenic immunodeficiency-associated LPD over the past 20 years were collected from electronic medical records in Kyoto University Hospital. Diagnosis was based on the WHO classification at the time and also reviewed

according to the WHO classification of 2017 (revised 4th edition) when analyzed. Details of lymphomas and the results of blood examinations such as total lymphocyte count and serum albumin value at diagnosis were also collected from the records. Those associated with primary immune disorders or human immunodeficiency virus (HIV) infections were excluded. All patients gave their informed consent prior to their inclusion in the study. The Institutional Review Board of Kyoto University Hospital, where this study was organized, approved this study.

Statistics

The primary endpoint of this study was overall survival (OS) and the secondary endpoint was progression-free survival (PFS). OS was examined by calculating deaths from any cause; survivors at the last follow-up were censored. PFS was examined by calculating progression/relapse of LPD/lymphomas or death from any cause. Descriptive statistics were used to summarize variables related to patient characteristics. OS and PFS were evaluated by Kaplan-Meier methods and the Cox regression hazards model was used in univariate and multivariate analyses to assess the prognostic significance of the total lymphocyte count at diagnosis. Multivariate analysis was performed using covariates that were selected by preceding stepwise selection in the Cox model with a P-value threshold of under 0.2. Covariates assessed were recipients' sex, clinical background (PTLD, other iatrogenic immunodeficiency-associated LPDs), histological characteristics (monomorphic, polymorphic, or early-phase diseases), International Prognostic Index (IPI) value, primary treatment (rituximab-containing chemotherapies, other chemotherapies or focal radiation, no treatments or reduction in immunosuppressive agents), EBER positivity, serum albumin value at diagnosis and year at diagnosis (1998–2013, 2013–2017).

Results

Patient characteristics (Table 1)

A total of 71 patients (PTLD, n=26; Other iatrogenic immunodeficiency-associated LPD, n=45) were included, 65 of whom had data of total lymphocyte counts at the diagnosis of LPD (52-87412/μL). The median age at transplantation was 63 y (range, 3-83) and the median follow-up period for survivors was 2.5 years. Diffuse large B-cell lymphoma was diagnosed in 33 patients (PTLD, n=12; Others, n=21), Hodgkin lymphoma in 12 (PTLD, n=3; Others, n=9), monomorphic B-cell LPD not specified in 11 (PTLD, n=3; Others, n=7) and polymorphic LPD or early-phase diseases in 15 (PTLD, n=7; Others, n=8). As for the initial treatment, immunosuppressive agents were reduced in 34 patients (PTLD, n=4; Others, n=30), rituximab-containing chemotherapies were given in 23 patients (PTLD, n=14, Others, n=9), other chemotherapies were given in 7 (PTLD, n=4; Others, n=3), and radiation or nothing was given in 5 (PTLD, n=4: Others, n=1). The median value of serum albumin at diagnosis was 3.18 g/dl. We set a threshold value of 800/μL (10^9/L) absolute lymphocyte counts as the lymphopenia definition by calculating the optimal threshold value using a receiver-operating characteristic (ROC) curve. A total of 26 patients (PTLD, n=14; Others, n=12) were diagnosed with lymphopenia (≤800/μl) and 39 patients were not (PTLD, n=11; Others, n=28).

Impact of total lymphocyte count at diagnosis on OS

The impact of total lymphocyte count on OS was illustrated with reference to a Lymphopenia group (total lymphocyte count ≤800 /μl at diagnosis) and a No-Lymphopenia group (total lymphocyte count >800 /μl at diagnosis) (Figure 1a). Overall, the 2.5-year OS was 74.8% (Lymphopenia group, 38.8%; No-

Lymphopenia group, 93.1%).

In the multivariate analysis, lymphopenia at diagnosis was associated with inferior OS (HR, 3.72; P= 0.043; Table 2). Serum albumin values (>3.18 g/dL vs. ≤3.18 g/dL; HR, 0.21; P=0.010) and high IPI (high vs. low to high-intermediate; HR, 4.37; P=0.003) also affected the OS. A subgroup multivariate analysis to assess the impact of lymphopenia according to the clinical background showed a similar trend (Figure 1b, 1c), although statistical significance was observed only in patients with other iatrogenic immunodeficiency-associated LPD (HR, 26.67; P=0.012). Progression of lymphoma or LPD was the most common cause of death in the lymphopenia group (PTLD, n=3; Others, n=6), followed by transplant-related mortality (PTLD, n=5) (Table 3).

Impact of lymphopenia at diagnosis on PFS

The impact of the total lymphocyte count on PFS was illustrated with reference to a Lymphopenia group and a No-Lymphopenia group (Figure 2a). Overall, the 2.5-year PFS was 67.1% (Lymphopenia group, 36.7%; No-Lymphopenia group, 86.6%).

In a multivariate analysis, lymphopenia was independently associated with inferior PFS (HR, 3.82; P=0.012; Table 4). Serum albumin values also showed a strong impact (>3.18 g/dL vs. ≤3.18 g/dL; HR, 0.26; P=0.013). Trends of inferior PFS in patients with high IPI (high vs. low to high-intermediate; HR, 3.04; P=0.067) and superior PFS in those who received rituximab-containing chemotherapy as primary treatment (rituximab-containing chemotherapies vs. other chemotherapies; HR, 0.16; P=0.081) were suggested. Although the non-lymphopenia group showed a trend of superior PFS (Figure 2b, 2c), its statistical

impact was apparent only in patients with other iatrogenic immunodeficiency-associated LPDs (HR, 9.66; P=0.010), but not in patients with PTLD (HR, 0.66; P=0.703) (Table 5).

Discussion

The results of our study demonstrated that lymphopenia at diagnosis may predict inferior survival in patients with immunodeficiency-associated LPD, despite its histological heterogeneity. Contrary to the expectation that this high mortality among patients with lymphopenia reflects their fragility with respect to various infections or intensive chemotherapy,[21] the major cause of death was disease progression. Whereas the possibility of rituximab as a primary treatment might have had some impacts on disease suppression, the impact of lymphopenia was independently associated with a higher risk of mortality.

These results suggest that lymphopenia itself could influence disease progression among immunocompromised patients. Several biological expectations could support this hypothesis. First, tumor pathogenesis of these LPD depends partially on the underlying infection of oncoviruses such as EBV. Immunocompromised status in lymphopenia patients could progress aberrant expansion of these oncoviruses. Second, anti-tumor effects of lymphocytes are thought to be less efficient in patients with fewer lymphocytes. Studies on graft-versus-lymphoma (GVL) effects[26] or on Programmed cell death 1 (PD1)-Programmed cell Death 1-Ligand 1 (PDL1) inhibition[27] have revealed that the anti-tumor effects of lymphocytes play important roles in suppressing tumor cells. As suggested in several malignant diseases,[28] the total number of lymphocytes might reflect their tumor-suppressive efficacy against lymphoma cells as well,

with a clear impact especially among immunocompromised patients. These considerations that follow our results could explain why some immunodeficiency-associated LPD shrink after the cessation or reduction of immunosuppressive agents.[24,29,30] They also support a previous suggestion that earlier recovery of lymphocytes after the cessation or reduction of immunosuppressive agents can predict a lower frequency of disease progression.[31]

Possibility of rituximab application, higher IPI value and hypoalbuminemia were reconfirmed as strong prognostic factors for overall survival in our analysis. However, similar to the results of the phase 2 PTLD-1 trial, [32] rituximab-containing chemotherapies such as R-CHOP did not dramatically improve overall survival. The investigation of risk-dependent strategies and the results of other regimens examined in ongoing clinical trials are awaited.[16,33] Based on our hypothesis, promotion of the anti-tumor effects of lymphocytes might be another potent strategy to improve clinical outcomes of iatrogenic immunodeficiency-associated LPD. Since frequent somatic alterations in genes encoding PD-L1/PD-L2 were suggested to contribute to the tumor pathogenesis of lymphomas associated with prior EBV infection, [34]PD1-PDL1 inhibitors could be considered as a therapeutic option in EBV-related immunodeficiency-associated LPD.[35,36] Although more detailed investigation is warranted, modulation of tumor microenvironment should be a potent target in the treatment strategy of immunodeficiency-associated LPD including PTLD.[37] Nevertheless, it is often a big issue to improve and balance immunoreactivities of lymphocytes among patients in post-transplant status or with autoimmune diseases.[38,39] EBV targeted cell therapies using virus-specific T-cells derived from patients' own lymphocytes or from third party T-cells have been suggested to be an emerging option with favorable outcomes in patients with EBV-related PTLD.[40–44] Since similar efficacy could be expected for EBV-

related immunodeficiency-associated LPD other than PTLD, there is a call for clinical trials for refractory/relapsed cases. Off-the-shelf products are awaited to broaden the application of these novel agents.

Our study has several limitations. First, this was a retrospective analysis in a small, heterogeneous population and some factors could not be collected from clinical records. As shown via the indefinite impact of lymphopenia in the subgroup analysis of patients with PTLD, the heterogeneity and small number of cases might have obscured the results of multivariate analysis. A prospective study using a larger cohort is mandatory to confirm the reproducibility of our findings.

Second, although clinically suggestive, our proposed explanation of our results has not yet been proved biologically and a more detailed biological approach is necessary. Third, lymphocyte subsets were not evaluated in this study. To discuss the anti-tumor effects of lymphocytes more in detail, evaluation of T-lymphocyte subsets might be of importance. Third,

Conclusion

Lymphopenia at diagnosis may potentially predict inferior OS and PFS in patients with immunodeficiency-associated LPDs. It might reflect the characteristics of the mechanism of disease progression for these distinct lymphoid neoplasms growing under immunocompromised backgrounds. A more detailed analysis in a larger cohort is needed to clarify the tumor pathology of these LPD and to investigate better risk-stratified treatment strategies against them.

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Compliance with Ethical Standards

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References

1. Dierickx D, Tousseyn T, Gheysens O. How i treat posttransplant lymphoproliferative disorders. Blood 2015;126(20):2274–2283.
2. Mariette X, Cazals-Hatem D, Warszawki J, Liote F, Balandraud N, Sibilia J. Lymphomas in rheumatoid arthritis patients treated with methotrexate: A 3-year prospective study in France. Blood 2002;99(11):3909–3915.
3. Hasserjian RP, Chen S, Perkins SL, et al. Immunomodulator agent-related lymphoproliferative disorders. Mod Pathol 2009;22(12):1532–1540.
4. Chetty R, Biddolph SC, Kaklamanis L, et al. EBV latent membrane protein (LMP-1) and bcl-2 protein expression in Reed-Sternberg-like cells in post-transplant lymphoproliferative disorders. Histopathology 1996;28(3):257–260.
5. Stevens SJC, Verschuuren EAM, Pronk I, et al. Frequent monitoring of Epstein-Barr virus DNA load in unfractionated whole blood is essential for early detection of posttransplant lymphoproliferative disease in high-risk patients. Blood 2001;97(5):1165–1171.
6. Timms JM, Bell A, Flavell JR, et al. Target cells of Epstein-Barr-virus

(EBV)-positive post-transplant lymphoproliferative disease: Similarities to EBV-positive Hodgkin's lymphoma. Lancet 2003;361(9353):217–223.

7. Miyazaki T, Fujimaki K, Shirasugi Y, et al. Remission of lymphoma after withdrawal of methotrexate in rheumatoid arthritis: Relationship with type of latent Epstein-Barr virus infection. Am J Hematol 2007;82(12):1106–1109.

8. Al-Mansour Z, Nelson BP, Evens AM. Post-transplant lymphoproliferative disease (PTLD): Risk factors, diagnosis, and current treatment strategies. Curr Hematol Malig Rep 2013;8(3):173–183.

9. Evens AM, Choquet S, Kroll-Desrosiers AR, et al. Primary CNS posttransplant lymphoproliferative disease (PTLD): An international report of 84 cases in the modern era. Am J Transplant 2013;13(6):1512–1522.

10. Rosenberg AS, Klein AK, Ruthazer R, Evens AM. Hodgkin lymphoma post-transplant lymphoproliferative disorder: A comparative analysis of clinical characteristics, prognosis, and survival. Am J Hematol 2016;91(6):560–565.

11. Morscio J, Dierickx D, Ferreiro JF, et al. Gene expression profiling reveals clear differences between EBV-positive and EBV-negative posttransplant lymphoproliferative disorders. Am J Transplant 2013;13(5):1305–1316.

12. Menter T, Dickenmann M, Juskevicius D, Steiger J, Dirnhofer S, Tzankov A. Comprehensive phenotypic characterization of PTLD reveals potential reliance on EBV or NF-κB signalling instead of B-cell receptor signalling. Hematol Oncol 2017;35(2):187–197.

13. Menter T, Juskevicius D, Alikian M, et al. Mutational landscape of B-cell post-transplant lymphoproliferative disorders. Br J Haematol 2017;178(1):48–56.

14. Vakiani E, Basso K, Klein U, et al. Genetic and phenotypic analysis of B-

cell post-transplant lymphoproliferative disorders provides insights into disease biology. Hematol Oncol 2008;26(4):199–211.

15. Margolskee E, Jobanputra V, Jain P, et al. Genetic landscape of T- and NK-cell post-transplant lymphoproliferative disorders. Oncotarget 2016;7(25):37636–37648.
16. DeStefano CB, Desai SH, Shenoy AG, Catlett JP. Management of post-transplant lymphoproliferative disorders. Br J Haematol 2018;182(3):330–343.
17. Hoshida Y, Xu JX, Fujita S, et al. Lymphoproliferative disorders in rheumatoid arthritis: Clinicopathological analysis of 76 cases in relation to methotrexate medication. J Rheumatol 2007;34(2):322–331.
18. Xu L-P, Zhang C-L, Mo X-D, et al. Epstein-Barr Virus–Related Post-Transplantation Lymphoproliferative Disorder after Unmanipulated Human Leukocyte Antigen Haploidentical Hematopoietic Stem Cell Transplantation: Incidence, Risk Factors, Treatment, and Clinical Outcomes. Biol Blood Marrow Transplant 2015;21(12):2185–2191.
19. Montanari F, Radeski D, Seshan V, Alobeid B, Bhagat G, O'Connor OA. Recursive partitioning analysis of prognostic factors in post-transplant lymphoproliferative disorders (PTLD): a 120 case single institution series. Br J Haematol 2015;171(4):491–500.
20. Lam GY. Lymphoproliferative disorders in inflammatory bowel disease patients on immunosuppression: Lessons from other inflammatory disorders. World J Gastrointest Pathophysiol 2015;6(4):181.
21. Trusson R, Serre JE, Szwarc I, et al. Treatment Response and Outcomes in Post-transplantation Lymphoproliferative Disease vs Lymphoma in Immunocompetent Patients. Transplant Proc 2016;48(6):1927–1933.
22. Balandraud N, Meynard JB, Auger I, et al. Epstein-Barr virus load in the

peripheral blood of patients with rheumatoid arthritis: Accurate quantification using real-time polymerase chain reaction. Arthritis Rheum 2003;48(5):1223–1228.

23. Baecklund E, Backlin C, Iliadou A, et al. Characteristics of diffuse large B cell lymphomas in rheumatoid arthritis. Arthritis Rheum 2006;54(12):3774–3781.
24. Niitsu N, Okamoto M, Nakamine H, Hirano M. Clinicopathologic correlations of diffuse large B-cell lymphoma in rheumatoid arthritis patients treated with methotrexate. Cancer Sci 2010;101(5):1309–1313.
25. Zimmermann H, Babel N, Dierickx D, et al. Immunosuppression Is Associated With Clinical Features and Relapse Risk of B Cell Posttransplant Lymphoproliferative Disorder. Transplantation 2018;102(11):1914–1923.
26. Grigg A, Ritchie D. Graft-versus-lymphoma effects: Clinical review, policy proposals, and immunobiology. Biol Blood Marrow Transplant 2004;10(9):579–590.
27. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. Int Immunol 2007;19(7):813–824.
28. Ménétrier-Caux C, Ray-Coquard I, Blay J-Y, Caux C. Lymphopenia in Cancer Patients and its Effects on Response to Immunotherapy: an opportunity for combination with Cytokines? J Immunother Cancer 2019;7(1):85.
29. Baird RD, Van Zyl-Smit RN, Dilke T, Scott SE, Rassam SMB. Spontaneous remission of low-grade B-cell non-Hodgkin's lymphoma following withdrawal of methotrexate in a patient with rheumatoid arthritis: Case report and review of the literature. Br J Haematol 2002;118(2):567–568.

30. Salloum E, Cooper DL, Howe G, et al. Spontaneous regression of lymphoproliferative disorders in patients treated with methotrexate for rheumatoid arthritis and other rheumatic diseases. J Clin Oncol 1996;14(6):1943–1949.
31. Inui Y, Matsuoka H, Yakushijin K, et al. Methotrexate-associated lymphoproliferative disorders: management by watchful waiting and observation of early lymphocyte recovery after methotrexate withdrawal. Leuk Lymphoma 2015;56(11):3045–3051.
32. Trappe R, Oertel S, Leblond V, et al. Sequential treatment with rituximab followed by CHOP chemotherapy in adult B-cell post-transplant lymphoproliferative disorder (PTLD): The prospective international multicentre phase 2 PTLD-1 trial. Lancet Oncol 2012;13(2):196–206.
33. DeStefano CB, Malkovska V, Rafei H, et al. DA-EPOCH-R for post-transplant lymphoproliferative disorders. Eur J Haematol 2017;99(3):283–285.
34. Kataoka K, Miyoshi H, Sakata S, et al. Frequent structural variations involving programmed death ligands in Epstein-Barr virus-associated lymphomas. Leukemia 2019;33(7):1687–1699.
35. Kinch A, Sundström C, Baecklund E, Backlin C, Molin D, Enblad G. Expression of PD-1, PD-L1, and PD-L2 in posttransplant lymphoproliferative disorder after solid organ transplantation. Leuk Lymphoma 2019;60(2):376–384.
36. Miyoshi H, Kiyasu J, Kato T, et al. PD-L1 expression on neoplastic or stromal cells is respectively a poor or good prognostic factor for adult T-cell leukemia/lymphoma. Blood 2016;128(10):1374–1381.
37. Marcelis L, Tousseyn T. The Tumor Microenvironment in Post-Transplant Lymphoproliferative Disorders. Cancer Microenviron 2019;12(1):3–16.

38. Ashrafi F, Shahidi S, Ebrahimi Z, Mortazavi M. Outcome of rapamycin therapy for post-transplant-lymphoproliferative disorder after kidney transplantation: case series. Int J Hematol stem cell Res 2015;9(1):26–32.
39. Crane GM, Powell H, Kostadinov R, et al. Primary CNS lymphoproliferative disease, mycophenolate and calcineurin inhibitor usage. Oncotarget 2015;6(32):33849–33866.
40. Burns DM, Crawford DH. Epstein-Barr virus-specific cytotoxic T-lymphocytes for adoptive immunotherapy of post-transplant lymphoproliferative disease. Blood Rev 2004;18(3):193–209.
41. Ricciardelli I, Blundell MP, Brewin J, Thrasher A, Pule M, Amrolia PJ. Towards gene therapy for EBV-associated posttransplant lymphoma with genetically modi fied EBV-specific cytotoxic T cells. Blood 2014;124(16):2514–2523.
42. Barker JN, Doubrovina E, Sauter C, et al. Successful treatment of EBV-associated posttransplantation lymphoma after cord blood transplantation using third-party EBV-specific cytotoxic T lymphocytes. Blood 2010;116(23):5045–5049.
43. Bollard CM, Savoldo B, Rooney CM, Heslop HE. Adoptive T-Cell Therapy for EBV-Associated Post-Transplant Lymphoproliferative Disease. Acta Haematol 2003;110(2–3):139–148.
44. Bollard CM, Rooney CM, Heslop HE. T-cell therapy in the treatment of post-transplant lymphoproliferative disease. Nat Rev Clin Oncol 2012;9(9):510–519.

Figure legends

Figure 1. Overall survival

Probability of overall survival for total patients (a), post-transplant lymphoproliferative disorders (PTLD) (b), and other iatrogenic lymphoproliferative disorders (c) with reference to a Lymphopenia group (total lymphocyte count <=800/μl at diagnosis) and a No-Lymphopenia group (total lymphocyte count >800/μl at diagnosis).

Figure 2. Progression-free survival

Probability of progression-free survival for total patients (a), post-transplant lymphoproliferative disorders (PTLD) (b), and other iatrogenic lymphoproliferative disorders (c) with reference to a Lymphopenia group (total lymphocyte count <=800/μl at diagnosis) and a No-Lymphopenia group (total lymphocyte count >800/μl at diagnosis).

Tables

Lymphopenia predicts outcomes of PTLD and OIIA-LPD

Table 1 **Patient characteristics**

Group by clinical background (n=71)		Total	PTLD value		Other iatrogenic LPD value		Variance
		n*1	n*1	%*2	n*1	%*2	P=value
Age*3 median(range)		63 (3-83)	54 (3-70)		66 (40-83)		
Gender	Male	26	15	57.5	11	24.4	0.009
	Female	45	11	42.3	34	35.6	
Year at diagnosis	1998-2013	31	10	38.5	21	46.7	0.502
	2014-2018	40	16	11.5	24	53.3	
Disease subtype	DLBCL/BL	33	12	46.2	21	46.7	0.722
	Monomorphic B-cell	11	4	15.4	7	15.6	
	Hodgkin lymphoma	12	3	11.5	9	20.0	
	Polymorphic/early	15	7	26.9	8	17.8	
IPI	Low	11	3	11.5	8	17.8	0.321
	Low-Intermediate	13	5	19.2	8	17.8	
	High-Intermediate	18	5	19.2	13	28.9	
	High	20	11	42.3	9	20.0	
	Missing	9	2	7.7	7	15.6	
EBER-ISH	positive	38	16	61.5	22	48.9	0.583
	negative	22	7	26.9	15	33.3	

	Missing	11	3	11.5	8	17.8	
Initial treatment	Reduction of ISA	34	4	15.4	30	66.7	<0.001
	non-RTX contained	7	4	15.4	3	6.7	
	RTX contained	23	14	53.8	9	20.0	
	Radiation	2	1	3.8	1	2.2	
	Nothing	3	3	11.5	0	0.0	
	Missing	2	0	0	2	4.4	
Serum albumin	< 3.18 (g/dl)	33	16	61.5	17	37.8	0.088
	≧3.18 (g/dl)	36	10	38.5	26	57.8	
	Missing	2	0	0	2	4.4	
Lymphocyte count	<= 800 (/μl)	26	14	65.4	12	37.8	0.063
	> 800 (/μl)	39	11	30.8	28	51.1	
	Missing	6	1	3.8	5	11.1	

*1n indicates the number of patients with each characteristic

*2% indicates the percentage of patients in each group

*3Age indicates patient's age at diagnosis

*4Serum albumin indicates serum albumin value at diagnosis

*5Lymphocyte count indicates the total lymphocyte count at diagnosis

Abbreviations: DLBCL, diffuse large B-cell lymphoma; PTLD, post-transplant lymphoproliferative disease; LPD, lymphoproliferative disorder; BL, Burkitt lymphoma; IPI, international prognostic index; EBER-ISH, EBV-encoded small RNA-in situ hybridization; ISA, immunosuppressive agents; RTX, rituximab

Table 2 **Multivariate analysis of OS**

Variables		Results		
		HR	95% CI	P-Value
Gender	Male	1.00		reference
	Female	0.38	(0.11-1.25)	0.110
Primary treatment strategy	other chemotherapies or focal radiation	1.00		reference
	rituximab-containing chemotherapies	0.16	(0.02-1.34)	0.091
	nothing / reduction of immunosuppressive agents	0.45	(0.08-2.70)	0.076
IPI at diagnosis	Low, Low-Int	1.00		reference
	High, High-Int	4.37	(1.12-16.96)	0.033
Serum albumin level at diagnosis	< 3.18 (g/dl)	1.00		reference
	≥ 3.18 (g/dl)	0.21	(0.06-0.68)	0.010
Total lymphocyte count at diagnosis	> 800 (/μl)	1.00		reference
	<= 800 (/μl)	3.72	(1.04-13.23)	0.043

Table 3 **Number of lymphocytes at diagnosis and cause of death**

Group by total lymphocyte count at diagnosis		<= 800 (/μl)	> 800 (/μl)	Total
Disease subtype	Cause of deaths	n*[1]		
PTLD	Lymphomas/LPD	2	1	3
	Transplant-related (GVHD/rejection/infection)	5	1	6
	Others	1	0	1
Other iatrogenic LPDs	Lymphomas/LPDs	6	2	8
	Infection	0	2	2
	Others	1	0	1

*[1]n indicates the number of patients with each characteristic

Abbreviations: PTLD, post-transplant lymphoproliferative disease; LPD, lymphoproliferative disorder; GVHD, graft-versus-host disease

Table 4 **Multivariate analysis of PFS**

Variables		Results		
		HR	95% CI	P-Value
Primary treatment strategy	other chemotherapies or focal radiation	1.00		reference
	rituximab-containing chemotherapies	0.16	(0.02-1.25)	0.081
	nothing / reduction of immunosuppressive agents	0.68	(0.12-3.63)	0.649
IPI at diagnosis	Low, Low-Int	1.00		reference
	High, High-Int	3.94	(0.93-10.00)	0.067
Serum albumin level at diagnosis	< 3.18 (g/dl)	1.00		reference
	≥ 3.18 (g/dl)	0.26	(0.09-0.76)	0.013
Total lymphocyte count at diagnosis	> 800 (/μl)	1.00		reference
	<= 800 (/μl)	3.82	(1.34-10.91)	0.012

Table 5 **Subgroup analysis (PTLD and other iatrogenic LPDs) of OS, PFS**

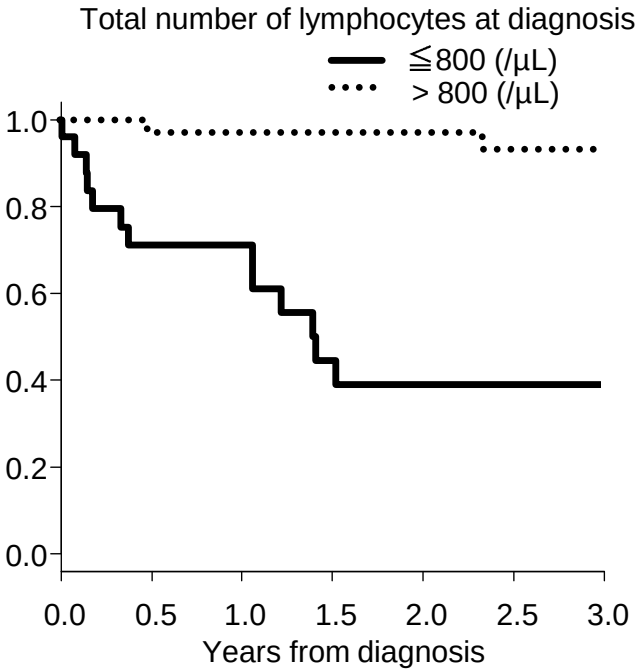
Variables		Overall Survival			Progression Free Survival		
		HR	95% CI	P-Value	HR	95% CI	P-Value
Total							
Total lymphocyte count	> 800 (/µl)	1.00		reference	1.00		reference
at diagnosis	<= 800 (/µl)	3.72	(1.04-13.23)	0.043	3.82	(1.34-10.91)	0.012
PTLD							
Total lymphocyte count	> 800 (/µl)	1.00		reference	1.00		reference
at diagnosis	<= 800 (/µl)	0.30	(0.01-6.56)	0.446	0.66	(0.08-5.72)	0.703
Other iatrogenic LPDs							
Total lymphocyte count	> 800 (/µl)	1		reference	1.00		reference
at diagnosis	<= 800 (/µl)	26.67	(2.05-346.11)	0.012	9.66	(1.71-54.55)	0.010

Abbreviations: PTLD, post-transplant lymphoproliferative disease; LPD, lymphoproliferative disorder; IPI, international prognostic index

--; could not be determined due to a lack of events

Figure 1

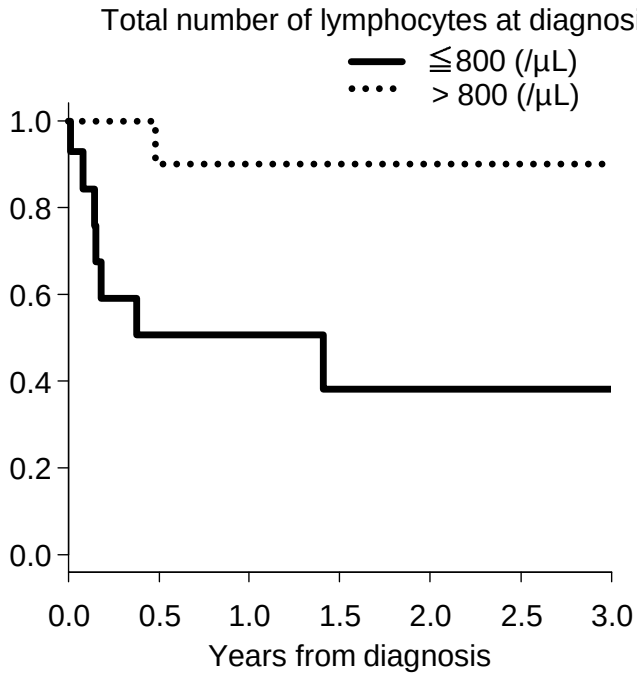
a Overall survival, total



Number at risk

≦800/μL	26	15	14	8	7	6	5
> 800 /μL	39	34	30	29	26	20	20

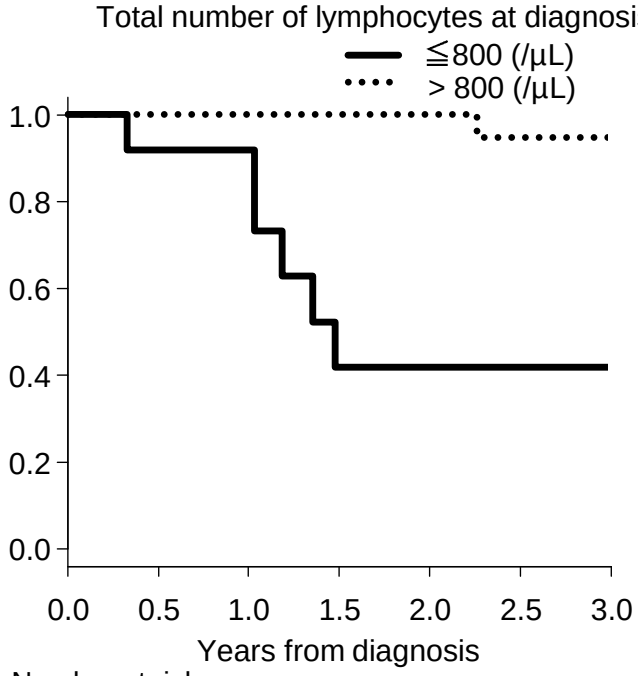
b Overall survival, PTLD



Number at risk

≦800/μL	14	5	4	3	3	2	2
> 800 /μL	11	9	8	8	6	4	4

C Overall survival, Other iatrogenic LPD

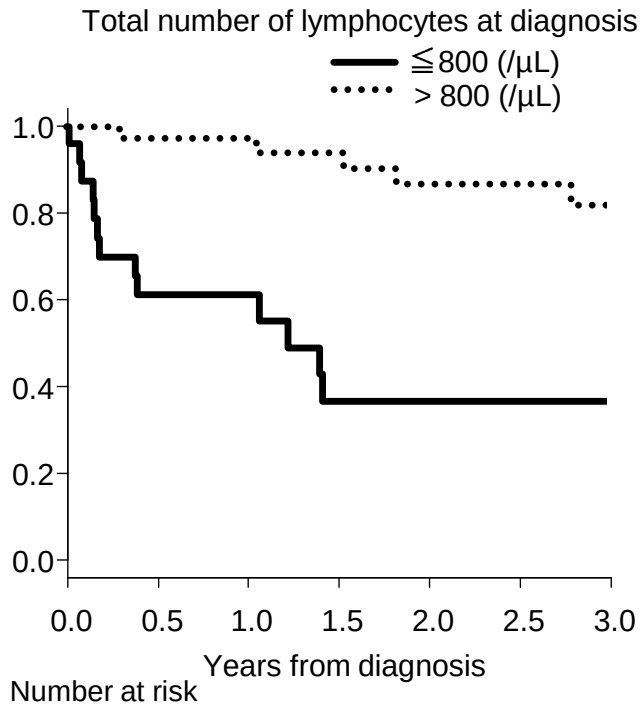


Number at risk

≦800/μL	12	10	10	5	4	4	3
> 800 /μL	28	25	22	21	20	16	16

Figure 2

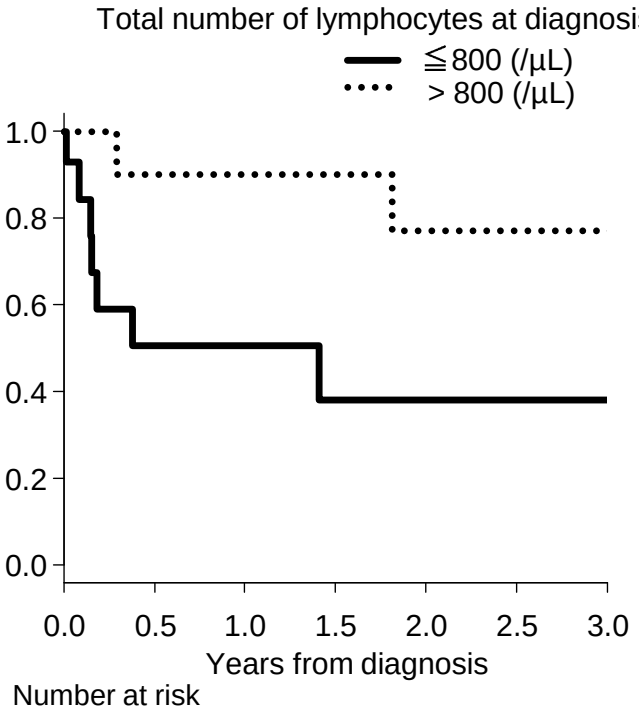
a Progression-free survival, total



Number at risk

≦800μ/L	26	11	10	6	6	5	4
> 800μ/L	39	33	29	26	23	18	17

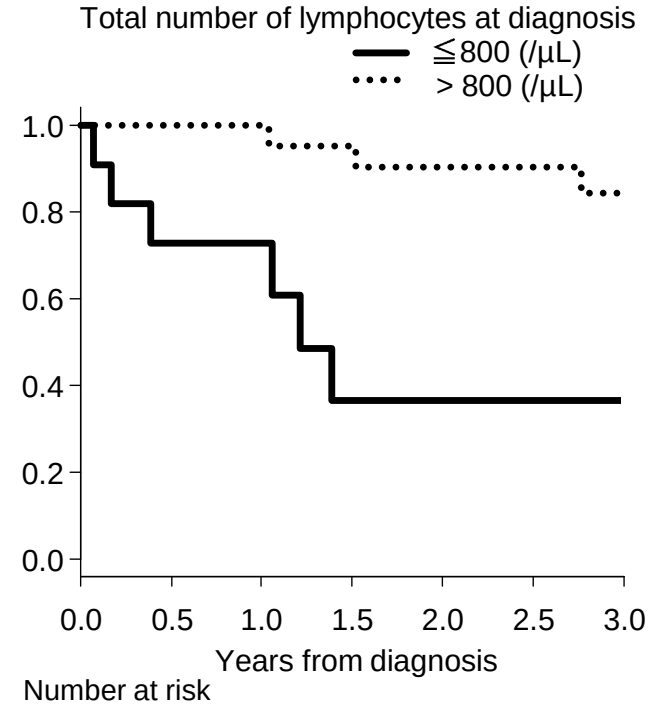
b Progression-free survival, PTLD



Number at risk

≦800μ/L	14	5	4	3	3	2	2
> 800μ/L	11	9	8	7	5	3	3

C Progression-free survival, Other iatrogenic LPD



Number at risk

≦800μ/L	12	6	6	3	3	3	2
> 800μ/L	28	24	21	19	18	15	14