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Post-transplant lymphoproliferative disorder in kidney transplant recipients: A single-center experience in Japan

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Abstract

Post-transplant lymphoproliferative disorder is a serious complication of solid organ transplantation; however, few large studies have been performed in Asian institutions. We review our single-center experience with post-transplant lymphoproliferative disorder patients in Japan. We retrospectively evaluated patients with post-transplant lymphoproliferative disorder following kidney transplantation between January 1985 and December 2013. The patients were divided into early-onset post-transplant lymphoproliferative disorder (< 1 year) and late-onset post-transplant lymphoproliferative disorder (≥ 1 year) groups. Thirteen patients had the disorder, an incidence rate of 0.75% (13/1730). Early-onset post-transplant lymphoproliferative disorder (n=3) had not occurred for the last 2 decades. In the late-onset group (n = 10), the median time of onset was 108.7 months. The Kaplan-Meier 10-year overall survival rates were 76.9% and 95.4% in patients with and without the disorder, respectively (p=0.0001). Post-transplant lymphoproliferative disorder significantly affected transplant recipients' mortality. Late-onset occurred even > 10 years after transplantation; therefore, long-term monitoring of patients is needed.

Keywords: Early-onset, Japan, kidney transplantation, late-onset, PTLD

Introduction

Post-transplant lymphoproliferative disorder (PTLD) occurs in approximately 0.4% to 2.0% of kidney transplant recipients, and potentially influences their mortality (1-4). Previously reported survival rates have been poor, with mortality rates exceeding 50% in most studies (5-7). Although several recent studies have reported that administration of rituximab (RTX) and/or chemotherapy can effectively prevent disease progression (8-10), PTLD has remained one of the most morbid post-transplant complications. Some cases of PTLD occur within the early period after transplantation (early-onset PTLD), whereas others develop more than 1 year after transplantation (late-onset PTLD) (4, 11-13). Several differences between early- and late-onset PTLD, including risk factors, prognostic outcomes, and clinical and histopathological characteristics, have been previously described (4, 11, 12, 14).

In the present study, we retrospectively reviewed patients who developed PTLD after kidney transplantation in our department over approximately 30 years. Clinical and immunohistological characteristics were analyzed, and patient and graft outcomes were also evaluated with reference to recent studies.

Patients and methods

After institutional review board approval (ID: 3440), the Tokyo Women's Medical University medical record archive between January 1985 and December 2013 were retrospectively reviewed. This study was performed in accordance with the principles outlines in the Declaration of Helsinki. Written informed consent was obtained from all patients. Clinical and laboratory data were extracted from an electronic database and patient medical records. PTLD was pathologically diagnosed by at least two experienced pathologists after performing needle or open tissue biopsy according to the 2008 world health classification system, and divided into the following groups: early lesions, polymorphic PTLD, monomorphic PTLD, or classical Hodgkin lymphoma-type PTLD. Clinical stage was determined according to the Ann Arbor Staging System. Epstein-Barr virus (EBV) association was analyzed according to the protocol at our center using LMP-1 and/or EBNA-2 immunohistochemistry or EBER *in situ* hybridization. EBV viral load was determined using deoxyribonucleic acid (DNA)-polymerase chain reaction, and sample positivity was defined as more than 2.0×10 copies. Late-onset PTLD was defined as development more than 1 year after transplantation, whereas early-onset PTLD developed within 1 year.

Immunosuppressive protocol

In general, before 1990, cyclosporine (CYA), methylprednisolone (MP), and azathioprine (AZ) or mizoribine (MZ) were used in ABO-compatible and minor-mismatch transplants. Additionally, antilymphocyte globulin (ALG) and deoxyspergualine (DSG) were administered for ABO-incompatible transplantation. Starting in 1990, tacrolimus (FK) was used as an alternative to CYA. Since 1998, mycophenolate mofetil (MMF) has been used as an alternative to AZ or MZ. Since 2000, immunosuppressive regimens consisting of FK, MMF and MP have been generally used. Since 2004,

basiliximub (BSX) has been administered at the time of transplantation in all cases. Moreover, single-dose RTX (200mg) has been administered in ABO-minor-mismatch, -incompatible, or hyper-sensitized transplantation cases since 2005. Splenectomy was performed simultaneously to transplantation in ABO-incompatible recipients until 2005.

PTLD evaluation

As the immunosuppressive background was different between the pre-2000 and post-2000 eras, all recipients with and without PTLD were divided into four groups (one for each era, one with PTLD, and one without PTLD), and these groups were compared to each other. Moreover, PTLD patients were divided into early- and late-onset PTLD subgroups, which were individually compared and evaluated for their clinical and histopathological characteristics, and outcome of PTLD.

Statistical analysis

Recipients' demographic characteristics were compared between the two eras (pre-2000 and post-2000) using the Mann-Whitney U test or χ^2-test. Overall survival and graft survival were estimated using the Kaplan-Meier method and compared using the log-rank test. A difference was considered significant at $p < 0.05$. All analyses were performed using the JMP® 11 software package (SAS Institute Inc., Cary, NC, USA).

Results

Demographic characteristics of all recipients with and without PTLD between the pre-2000 and post-2000 eras

All recipients were divided into four subgroups as follows: patients without PTLD before 2000, those with PTLD before 2000, patients without PTLD since 2000, and those with PTLD since 2000. According to aforementioned subgroups, 540, 7, 1177, and 6 patients were categorized, respectively. Mean age at the time of transplantation was 36.3 and 44.3 years in the pre-2000 and post-2000 eras, respectively; the number of elder recipients significantly increased in the post-2000 compared to that in the pre-2000 era (p<0.0001). The rate of men was not significantly different between the pre-2000 and post-2000 eras (65.8% vs. 62.4%, respectively; p=0.168). The rate of living donor and ABO-incompatible transplantation significantly increased in the post-2000 compared to that in the pre-2000 era (p=0.0373 and p<0.0001, respectively). Notably, the uniformity of the immunosuppressive regimen, which consisted of FK, MMF, and MP, was confirmed in the post-2000 era; FK, MMF, and MP were used in 97.4%, 92.3%, and 100.0% of patients compared with 25.6%, 10.1%, and 100.0% of patients in the pre-2000 era (all, p<0.0001). The rate of EBV seropositivity seemed to be significantly different between the two eras (p<0.0001); however, this result had a lower reliability due to the high rate of unknown data, especially in the pre-2000 era (74.0%). The incident rate of overall PTLD-onset was lower in the post-2000 era than in the pre-2000 era (0.51 % vs. 1.28 %;

statistical analysis was not performed because of the small number of patients), and early-onset PTLD has not occurred since 2000.

Individual clinical and histopathological characteristics of PTLD between the early- and late-onset PTLD groups

Overall 13 patients had PTLD, accounting for an incidence rate of 0.75% (13/1730). The median age in the early- and late-onset group was 32.7 years (range 26-45) and 53.9 years (range 34-68), respectively. Only 1 PTLD patient was a woman. The reasons for end-stage kidney disease were chronic glomerulonephritis and IgA nephropathy in 7 and 3 patients, respectively, and interstitial nephritis, polycystic kidney disease, and reflux nephropathy each in 1 patient. The median duration of dialysis therapy prior to transplantation was 62.3 months (range 4-180). Five patients underwent deceased donor transplantation, and 8 patients underwent living donor transplantation. Only 1 patient, no. 10, underwent a second kidney transplantation. No patients underwent other organ transplantations. ABO was compatible, minor-mismatch, and incompatible in 8, 3, and 2 patients, respectively. Twelve patients received an initial immunosuppressive regimen consisting of calcineurin inhibitor (CYA, FK), antimetabolite (AZ, MZ, MMF), and corticosteroid (MP). Only 1 patient, no. 12, received an mTOR-inhibitor (everolimus) as part of a clinical trial. Splenectomy was performed in patient nos. 1 and 2. BSX was inducted in all 4 patients since 2004. EBV serostatus was positive in 8 patients, and negative

in 3 patients (data from 2 patients were unavailable). All patients in the early-onset group received anti-rejection treatments prior to PTLD: all received steroid pulse therapy and muromonab-CD3, and patients no. 1 and 2 additionally were administered DSG. Meanwhile, in the late-onset group, only patient no. 11 received treatments including steroid pulse therapy and DSG before PTLD. The median time from transplantation to PTLD onset was 3.67 months (range 1-8) and 108.7 months (range 14-250) in the early- and late-onset groups, respectively. All patients in the early-onset group were diagnosed as stage IV according to Ann Arbor staging, whereas 7 patients in the late-onset group were diagnosed as stage III or IV. Histopathologically, diffuse large B-cell lymphoma (DLBCL) was diagnosed in 6 patients. Non B-cell lymphoma was diagnosed in 2 patients: Hodgkin's lymphoma with mixed cellularity in patient no. 7 and monomorphic T-cell lymphoma in patient no. 10. EBV-associated PTLD occurred in 1 patient in the early-onset group (data in 2 patients unknown), and 6 patients in the late-onset group (data in 1 patient unknown). In the late-onset group, the viral load of EBV in the serum was elevated in 4 patients. The viral load in the cerebrospinal fluid (CSF) was also elevated in patient nos. 4 and 10, who had central nervous system (CNS)-PTLD despite EBV negativity in the serum. At PTLD onset, all patients in the late-onset group received an immunosuppressive regimen consisting of FK, MMF and MP because CYA was converted to FK, and AZ or MZ was converted MMF in 2000 in our department (Table 2).

Individual treatments, and patient and graft outcomes of PTLD between the early- and late-onset PTLD groups

Immunosuppression reduction (IR) was performed in all patients; antimetabolic drugs were withdrawn in all patients, and calcineurin inhibitors were withdrawn in all early-onset patients and in 4 late-onset patients. Surgery was performed in 3 patients: patient no. 3 underwent graftectomy for graft PTLD, patient no. 11 underwent surgery for an invasive tumor in the small intestine, and patient no. 13 underwent intracranial decompression for cerebral hernia due to invasive CNS tumor. Radiation therapy and chemotherapy were performed in 4 and 5 patients, respectively, and rituximab-associated chemotherapy was administered in 3 of 5 patients. The median time of follow-up was 61.7 months (range 1-183) and 46.1 months (range 2-124) in the early- and late-onset group, respectively. Two of 3 patients in the early-onset group died of PTLD 1 month after onset. In the late-onset group, 8 patients were alive; 7 and 1 patients were diagnosed as complete response and stable disease, respectively; and 2 patients died of PTLD and *Pneumocystis jiroveci* pneumonia due to bone-marrow suppression associated with chemotherapy, respectively. Two and 4 patients had graft loss in the early- and late-onset group, respectively. Among graft-loss patients, except for patient no. 5, graft function was rapidly eliminated within several months (Table 3).

Overall survival and graft survival in patients with PTLD, late-onset PTLD and without PTLD

Kaplan-Meier curves predicted worse overall survival rates for patients with overall PTLD and late-onset PTLD compared to those without PTLD (Fig. 1). While overall survival rates were significantly worse in patients with overall PTLD than those without PTLD (76.9% vs. 97.4% at 5 years, 76.9% vs. 95.4% at 10 years, 65.93% vs. 91.0% at 20 years after transplantation, p=0.0001), there was no significant difference in overall survival between patients with late-onset PTLD and patients without PTLD (p=0.078). Neither PTLD nor late-onset PTLD were significantly associated with graft survival, compared to patients without PTLD (p=0.1612, p=0.7179, respectively) (Fig. 2).

Overall survival and graft survival in patients with late-onset PTLD from onset

The 5- and 7-year overall survival rates after late-onset PTLD were 90.0% and 60.0%, respectively (Fig. 3). The 1- and 5-year graft survival rates after late-onset PTLD were 80.0% and 40.0%, respectively (Fig. 4).

Discussion

In recent years, large-scale studies for PTLD have been performed and revealed several differences between early- and late-onset PTLD, not only in risk and prognostic factors but also patient and graft outcome. However, the number of such reports has been limited in Asian institutions: to the best of

our knowledge, only 2 retrospective studies of single-center experience in China (15) and Japan (16), have been reported.

A large retrospective study reported that the risk factors of early-onset PTLD were considered to be younger age and EBV or cytomegalovirus seronegativity, whereas older age is associated with late-onset PTLD (4). In the present study, patients in the late-onset group were older than those in the early-onset group, consistent with this previous investigation. Furthermore, all patients in the early-onset group were administered antirejection drugs such as muromonab-CD3, which are regarded as a risk factor of early-onset PTLD (7, 14). Several studies reported that the pathogenesis of PTLD differs between early- and late-onset PTLD, with most early-onset PTLD cases triggered by primary EBV infection via donor lymphocytes, frequently located within the allograft, resulting from proliferating latently infected B-cells in the absence of a normal cytotoxic T-cell response under the immunosuppression. Indeed, more cases with B-cell lymphoma associated with EBV (14), or presentation in the allograft (11, 17) have been described to develop early-onset PTLD. Meanwhile, late-onset PTLD sometimes develops in an extra-nodal or CNS lesion (1), and likely presented a reduced rate of B-cell lymphoma associated with EBV and slightly more cases originating from T-cells (4). In the patients with late-onset PTLD in the present study, the rates of both B-cell lymphoma and EBV-association were apparently more frequent than have been reported previously. Additionally, extra-nodal presentation and CNS involvement were observed in 7 and 2 patients, respectively, while

no graft-PTLD developed. In patient nos. 4 and 13, we did not detect elevated EBV load in the serum but rather in the CSF, indicating EBV-associated CNS-PTLD. In patients with CNS lesions, detection of EBV load in the serum can result in negative results (18); therefore, examination of CSF samples is recommended (14). Furthermore, in patient no. 10, who had primary cutaneous PTLD, elevated EBV load was not apparent in the serum, but histopathological findings from tissue indicated a diagnosis of EBV-associated monomorphic T-cell lymphoma. These results suggest that the EBV load in the serum should not be the only method of EBV diagnosis, especially in cases involving localized lesions such as CNS or cutaneous PTLD.

Our analysis demonstrated that PTLD significantly increased the morbidity of kidney transplant recipients compared with patients without PTLD ($p<0.0001$). Early-onset PTLD patients in particular had worse progression. Patients with late-onset PTLD also demonstrated a trend toward worse outcome, although the difference was not statistically significant ($p=0.078$). High staging, CNS, and bone marrow involvement are regarded as independent risk factors for poor progression (10, 12, 14). In the present study, the patients in the early-onset group were initially diagnosed at advanced stage. Patient nos. 1 and 2, had CNS lesion and bone marrow involvement, respectively, and died immediately. Patient no. 3 had graft involvement with multiple surrounding lymph node metastasis, and graftectomy and lymph node dissection were performed. Postoperatively, the tumors did not recur or metastasize for 183 months. Graft-PTLD, which commonly develops in the early post-transplant

period, has been reported to show better outcomes (6, 12). Caillard et al. explained that graft-PTLD has a tendency to be detected earlier than other forms of PTLD due to intense monitoring of transplanted organs during the early post-transplant period and easier accessibility for surgical resection (12). Conversely, the patients in the late-onset group tended to demonstrate slower progression in the present study. We speculate that this difference is due to their better response to IR as an initial treatment. Nonetheless, patient no. 9 died 65 months after PTLD diagnosis despite chemotherapy including sequential treatment with RTX followed by cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP). Multiple and/or extranodal lesions, including the orbit, lung, testis and abdominal lymph nodes; high staging; and absence of EBV association were identified as risk factors according to previous reports (12, 14). Patient no. 10 died of *Pneumocystis jiroveci* pneumonia during chemotherapy only 2 months after PTLD diagnosis, which was caused by myelosuppression as an adverse effect of chemotherapy.

Sequential treatment with RTX followed by CHOP chemotherapy is regarded as an effective and safe treatment for PTLD patients, especially for B-cell PTLD (8). Other studies also demonstrated the effectiveness of RTX therapy; Michonneau et al. (9) reported that 5-year overall survival and disease-free survival were 69% and 80%, respectively in patients who received RTX with or without chemotherapy treatment, whereas Evens et al. (10) reported 3-year progression-free survival and overall survival were 70% and 73 %, respectively. We used RTX-associated chemotherapy in 3

patients. No patients in the early-onset group were administered RTX because the chemotherapy regimen did not include RTX at that time. Patients no. 4 and 10 were administered chemotherapy without RTX for severe kidney dysfunction. Patient no. 13 was already in a state of brain-death after the surgery; therefore overly stressful chemotherapy was not performed. Meanwhile, graft survival following late-onset PTLD appeared to be poorer than that in previous studies (19). IR is commonly performed as an initial treatment for PTLD because impairment of the immunological system is a major trigger of PTLD, although the rejection caused by reduction of immunosuppressive conditions remains an unresolved problem. In fact, some patients who received IR suffered from graft loss in the present study. Rabot et al. reported that calcineurin inhibitor withdrawal could adversely influence graft and patient survival (19). Meanwhile, RTX and CHOP chemotherapy can be effective for maintaining graft function (20).

In the present study, several interesting trends were observed. First, the rate of early-onset PTLD is apparently lower than that of late-onset PTLD, and notably, it had not occurred for the last 2 decades. Second, late-onset PTLD was more likely to occur much later in the post-transplant period; the median time to onset from transplantation was over 100 months, and the longest time was up to 250 months. There are several possible reasons for this late onset. Regarding the first reason, RTX, which is used in cases of ABO minor-mismatch, incompatibility, or hyper-sensitized transplantation, may be effective for preventing early-onset PTLD. Our previous study demonstrated that single-dose RTX

has the effect of B-cell depletion for approximately 2 years (21). The rate of overall PTLD-onset in the recent era was also lower than that in the previous era (0.51 % vs 1.28 %) despite a higher rate of negative EBV serostatus (5.95 % vs 2.59 %), as shown in Table 1. This may be associated with RTX administration. However, this hypothesis must be tested; the effectiveness of RTX has been established as a treatment of PTLD but not as PTLD prophylaxis. Therefore, more detailed research is needed regarding whether RTX can be effective as a prophylaxis therapy for early-onset PTLD. Regarding the second reason, late-onset PTLD may have been caused by the fact that the population in the post-2000 era was significantly older than that in the pre-2000 era; the age was regarded as an independent factor according to the aforementioned study (4). Third, in the recent decade between 2001 and 2010, PTLD-onset seems to have occurred rather earlier than in the previous era, and was diagnosed at advanced stage (more than stage 4 in all cases). This outcome may be caused by using MMF, which is a powerful immunosuppressive agent. Several reports have previously indicated that MMF administration may affect the high incidence of PTLD-onset (22, 23). Furthermore, compared to patients in the post-2000 era, PTLD in patient nos. 7 and 8 occurred relatively later in the transplant period despite receiving the same immunosuppressive drugs such as FK, MMF, and MP. This discrepancy may have been caused by BSX administration as an initial immunosuppressive agent, which has been used since the recent decade. MMF and a currently powerful immunosuppressive regimen, including FK, MMF, MP and BSX, may cause outbreak in earlier after transplantation or

during the more aggressive progression of PTLD.

Moreover, an epidemiological difference in EBV prevalence has been recognized between Asia and Western countries; Asian children and young adults infected with EBV at an earlier age, and more than 95% of adults are seropositive. By contrast, the rate of seropositive adolescents and young adults ranges from 50% to 70% in the West (15), which therefore may increase the rate of early-onset PTLD. Chan et al. also reported PTLD characteristics similar to our results (15). In their institution, EBV-positive PTLD represented the majority of the late-onset group, and the time from transplantation to onset was substantially longer than reported in Western countries. They speculated that the reason for this result was the higher rate of EBV positivity in Asian countries, reflecting a much longer time required for EBV lymphomagenesis as a result of reactivation of latent EBV infection (15). In our study, 7/10 late-onset PTLD cases were associated with EBV, indicating similar tendencies in these 2 Asian studies. It may be necessary to establish specific guidelines for PTLD in Asian populations, including risk factors, prognostic indicators, diagnostic criteria and outcome.

Conclusion

We reviewed PTLD patients with regard to clinical and histopathological characteristics, as well as patient and graft outcomes. The present study demonstrated that PTLD significantly impacted kidney transplant recipients' mortality, and also observed several interesting characteristics. First, the rate of

early-onset PTLD is apparently lower than that of late-onset PTLD, and notably has not occurred in the modern era. Second, late-onset PTLD was more likely to occur much later in the post-transplant period. Long-term and regular observation is needed to detect late-onset PTLD, and larger investigations re-evaluating PTLD characteristics in an Asian multi-center study should be performed, if possible.

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Conflicts of interest

The authors have no conflict of interests to declare.

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Figure legends

Figure 1: Overall survival rate after kidney transplantation for patients with any PTLD (*n*=13) vs. late-onset PTLD (*n*=10) vs. without PTLD (*n*=1730).

Kaplan-Meier curves indicated significantly worse OS rates in patients with PTLD compared patients without PTLD (76.9% vs. 97.4% at 5 years, 76.9% vs. 95.4% at 10 years, 65.9% vs. 91.0% at 20 years after transplantation, p=0.0001). OS rates was also worse in patients with late-onset PTLD than in patients without PTLD, although the difference was not significant (p=0.078).

Figure 2: Graft survival rate after kidney transplantation for patients with overall PTLD (*n*=13) vs. with late-onset PTLD (*n*=10) vs. without PTLD (*n*=1730).

Kaplan-Meier curves indicated that graft survival did not significantly differ between patients with and without PTLD (p=0.1612). Furthermore, graft survival did not significantly differ between patients with late-onset and patients without PTLD (p=0.7179).

Figure 3: Overall survival rate after outbreak of late-onset PTLD

The 5- and 7- year overall survival rates after late-onset PTLD were 90.0% and 60.0%, respectively.

Figure 4: Graft survival rate after outbreak of late-onset PTLD

The 1- and 5-year graft survival rates after late-onset PTLD were 80.0% and 40.0%, respectively.

Table 1: Demographic characteristics of recipients with and without PTLD between the pre-2000 and post-2000 eras

	Without PTLD (pre-2000)	With PTLD (pre-2000)	Without PTLD (post- 2000)	With PTLD (post-2000)	Total (pre- 2000)	Total (post- 2000)	*p* value
n	540	7	1177	6	547	1183	
Age	36.2 (20-66)	44.7 (26-62)	44.3 (20-75)	54 .0 (34-68)	36.3 (20-66)	44.3 (20-75)	<0.0001
Sex							
male/famale	353/ 187 (65.4%)	7/0	733/ 444 (62.3%)	5/1	360/ 187 (65.8%)	738/ 445 (62.4%)	0.168
ABO compatibility							<0.0001
ABO- compatible	386 (71.5%)	4	675 (57.3%)	4	390 (71.3%)	679 (57.4%)	
- minor mismatch	87 (16.1%)	1	198 (16.8%)	2	88 (16.1%)	200 (16.9%)	
- incompatible	67 (12.4%)	2	304 (25.8%)	0	69 (12.6%)	304 (25.7%)	
Donor source							0.0373
living donor transplantation	482 (89.3%)	4	1087 (92.4%)	4	486 (88.8%)	1091 (92.2%)	
deceased donor transplantation	49 (9.07%)	3	70 (5.95%)	2	52 (9.51%)	72 (6.09%)	
unknown	9 (1.67%)	0	20 (1.70%)	0	9 (1.65%)	20 (1.70%)	
Immunosuppressive drugs at KTx							
MP	540 (100.0%)	7	1177 (100.0%)	6	547 (100.0%)	1183 (100.0%)	-
CYA	404 (74.8%)	3	26 (2.21%)	1	407 (74.4%)	27 (2.28%)	<0.0001
FK	136 (25.2%)	4	1147 (97.5%)	5	140 (25.6%)	1152 (97.4%)	<0.0001
AZ	246 (45.6%)	5	17 (1.44%)	0	251 (45.9%)	17 (1.44%)	<0.0001
MZ	232 (43.0%)	1	70 (5.95%)	0	233 (42.6%)	70 (5.92%)	<0.0001
MMF	54 (10.0%)	1	1087 (92.4%)	5	55 (10.1%)	1092 (92.3%)	<0.0001
EVL	0 (0.00%)	0	1 (0.085%)	1	0 (0.00%)	2 (0.17%)	0.336
ALG	67 (12.4%)	2	0 (0.00%)	0	69 (12.6%)	0 (0.00%)	<0.0001
DSG	67 (12.4%)	2	0 (0.00%)	0	69 (12.6%)	0 (0.00%)	<0.0001
BSX	0 (0.00%)	0	896 (76.1%)	4	0 (0.00%)	900 (76.1%)	<0.0001
RTX	0 (0.00%)	0	550 (46.7%)	0	0 (0.00%)	550 (46.5%)	<0.0001
Splenectomy at the time of KTx							0.0010
yes	67 (12.4%)	2	85 (7.22%)	0	69 (12.6%)	85 (7.19%)	
no	469 (86.9%)	0	1085 (92.2%)	6	469 (85.7%)	1091 (92.2%)	
unknown	4 (0.74%)	0	7 (0.59%)	0	4 (0.73%)	7 (0.59%)	
EBV serostatus							<0.0001
positive	123 (22.8%)	4	989 (84.0%)	4	127 (23.2%)	993 (83.9%)	
negative	14 (2.59%)	1	70 (5.95%)	2	15 (2.74%)	72 (6.09%)	
unknown	403 (74.6%)	2	118 (10.0%)	0	405 (74.0%)	118 (9.97%)	
Incidence rate of PTLD-onset		7 (1.28%)		6 (0.51 %)			
early-onset PTLD		3 (0.55%)		0 (0.00 %)			
late-onset PTLD		4 (0.73%)		6 (0.51 %)			
Immunosuppressive drugs at diagnosis of PTLD							
MP		7		6			
CYA		1		0			
FK		6		6			
AZ		2		0			
MZ		1		0			
MMF		4		6			
Treatments for PTLD							
IR		7		6			
chemotherapy		1		4			
with RTX		0		3			
without RTX		1		1			
radiation		2		2			
surgery		1		2			

PTLD, post-transplant lymphoproliferative disorder; KTx, kidney transplantation; MP, methylprednisolone; CYA, cyclosporine; FK, tacrolimus; AZ, azathioprine; MZ, mizoribine; MMF, mycophenolate mofetil; EVL, everolimus; ALG, antilymphocyte globlin; DSG, deoxyspergualine; BSX, basiliximab; RTX, rituximab; EBV, Epstein-Barr virus; IR, immunosuppression reduction;

Table 2: Individual clinical and histopathological characteristics of PTLD between the early- and late-onset PTLD groups

Patient number	Age/sex	KTx (year)	Immunosuppressive drugs at KTx	EBV-serostatus	Previous anti-rejection therapy	Duration from KTx to onset (months)	Histopathology	EBV association	Presentation	Ann Arbor staging	Immunosuppressive drugs at onset
Early-onset (*n*=3)											
1	27/M	1989	CYA, AZ, MP, ALG DSG	Positive	mPSL, OKT3, DSG	8	ML	Unknown	CNS, hilar LN	4B	CYA, AZ, MP
2	26/M	1996	FK, AZ, MP, ALG DSG	Negative	mPSL, OKT3, DSG	3	DLBCL, diffuse mixed	Unknown	Neck LNs, axillary LN inguinal LNs bone marrow	4B	FK, MZ, MP
3	45/M	1997	FK, AZ, MP	Positive	mPSL, OKT3	1	B-cell lymphoma	Yes	Graft, axillary LNs inguinal LNs	4A	FK, AZ, MP
Late-onset (*n*=10)											
4	53/M	1990	CYA, MZ, MP	Unknown		250	Polymorphic B-cell lymphoma	Yes	CNS, abdominal LN	4B	FK, MMF, MP
5	47/M	1992	FK, AZ, MP	Positive		147	DLBCL	No	Cervical LN	1A	FK, MMF, MP
6	62/M	1995	CYA, AZ, MP	Unknown		193	PEL, B-cell lymphoma	Unknown	Pleura	PEL	FK, MMF, MP
7	53/M	1998	FK, MMF, MP	Positive		177	Mixed cellularity Hodgkin lymphoma	Yes	Abdominal LNs	2B	FK, MMF, MP
8	64/M	2000	FK, MMF, MP	Positive		131	DLBCL	Yes	Lung, abdominal LNs retroperitoneal LNs	3A	FK, MMF, MP
9	68/M	2001	FK, MMF, MP	Positive		68	DLBCL	No	Orbit, lung, testis abdominal LNs	4A	FK, MMF, MP
10	52/M	2004	FK, MMF, MP, BSX	Positive		23	NHL, T-cell, monomorphic	Yes	Lung, skin	4A	FK, MMF, MP
11	39/W	2006	FK, MMF, MP, BSX	Negative	mPSL, DSG	14	DLBCL	Yes	Abdominal LNs small intestine	4A	FK, MMF, MP
12	34/M	2009	CYA, EVL, MP, BSX	Negative		45	Burkitt B-cell lymphoma	Yes	Liver, abdominal LNs bone marrow	4B	FK, MMF, MP
13	67/M	2010	FK, MMF, MP, BSX	Positive		39	DLBCL	Yes	CNS	4B	FK, MMF, MP

PTLD, post-transplant lymphoproliferative disorder; KTx, kidney transplantation; EBV, Epstein-Barr viral; CGN, chronic glomerulonephritis; IgAN, IgA nephropatchy; PKD, polycystic kidney disease; CYA, cyclosporine; AZ, azathioprine; MP, methylprednisolone; ALG, antilymphocyte globulin; DSG, deoxyspergualine; MZ, mizoribine; FK, tacrolimus; MMF, mycophenolate mofetil; BSX, basiliximab; EVL, everolimus; OKT3, muromonab-CD3; mPSL, steroid pulse therapy; ; ML, malignant lymphoma; DLBCL, diffuse large B-cell lymphoma; PEL, primary effusion lymphoma; NHL; non-Hodgkin lymphoma; CNS, central nervous system; LN, lymph node

Table 3: Individual outcomes of PTLD patients between the early-and late-onset PTLD groups

Patient number	Treatments	Patient outcome	Duration from onset to death (months)	Graft outcome	Duration from onset to graft loss (months)	Follow-up period (months)
Early-onset (*n*=3)						
1	IR, radiation	Dead for PTLD	1			1
2	IR, radiation	Dead for PTLD	1	Graft loss for rejection suspected	1	1
3	IR, graftectomy	CR		Graft loss for graftectomy	1	183
Late-onset (*n*=10)						
4	IR, CYVE	CR		Graft loss for rejection	2	38
5	IR	CR		Graft loss for rejection	53	124
6	IR	CR		Graft loss for rejection suspected	0	46
7	IR	CR				26
8	IR, R-CHOP, HD-MTX	CR				42
9	IR, R-CHOP, R-MCOP, MCOP, R-ICE	Dead for PTLD	65			65
10	IR, radiation, LVD	Dead for pneumonia	2	Graft loss for rejection	1	2
11	IR, surgery	CR				88
12	IR, R-CODOX-M/ R-IVAC	CR				22
13	IR, surgery, radiation	SD				8

PTLD, post-transplant lymphoproliferative disorder; IR, immunosuppression reduction; CYVE, cytarabine-etoposide; R-CHOP, rituximab- cyclophosphamide-doxorubicin-vincristine-prednisolone; HD-MTX; high-dose-methotrexate; R-MCOP, rituximab-cyclophosphamide-vincristine-mitoxantrone-predonisolone; R-ICE, rituximab-ifosfamide-carboplatin-etoposide; LVD, 1- asparaginase-vincristine-dexamethasone ; R-CODOX-M/ R-IVAC, rituximab-cyclophosphamide-vincristine-doxorubicin-methotrexate/ ifosfamide-etoposide-cytarabine; CR, complete response; SD, stable disease

Figure 1

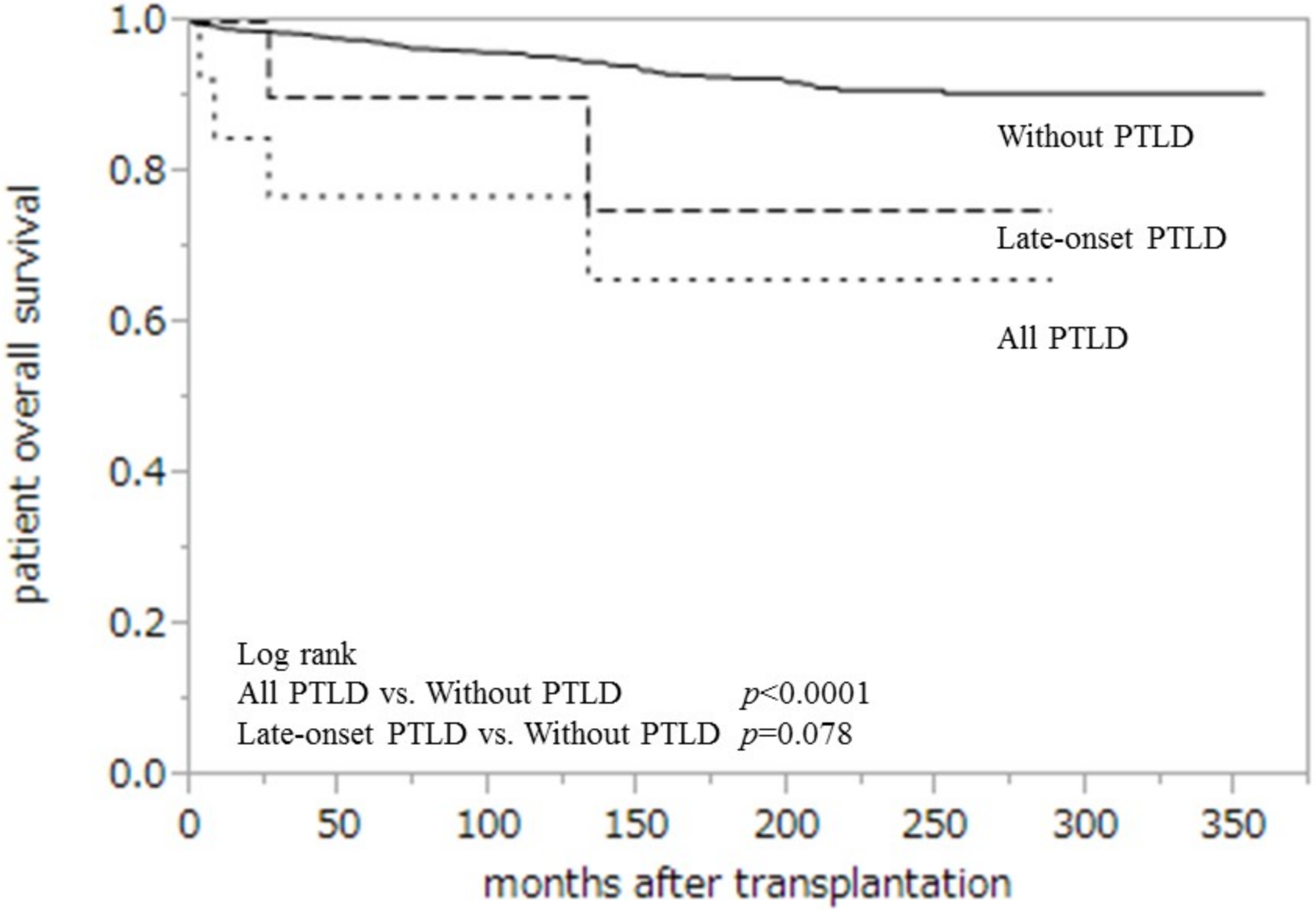

1.0
0.8
0.6
0.4
0.2
0.0
patient overall survival
Without PTLD
Late-onset PTLD
All PTLD
Log rank
All PTLD vs. Without PTLD p<0.0001
Late-onset PTLD vs. Without PTLD p=0.078
0
50
100
150
200
250
300
350
months after transplantation

Figure 2

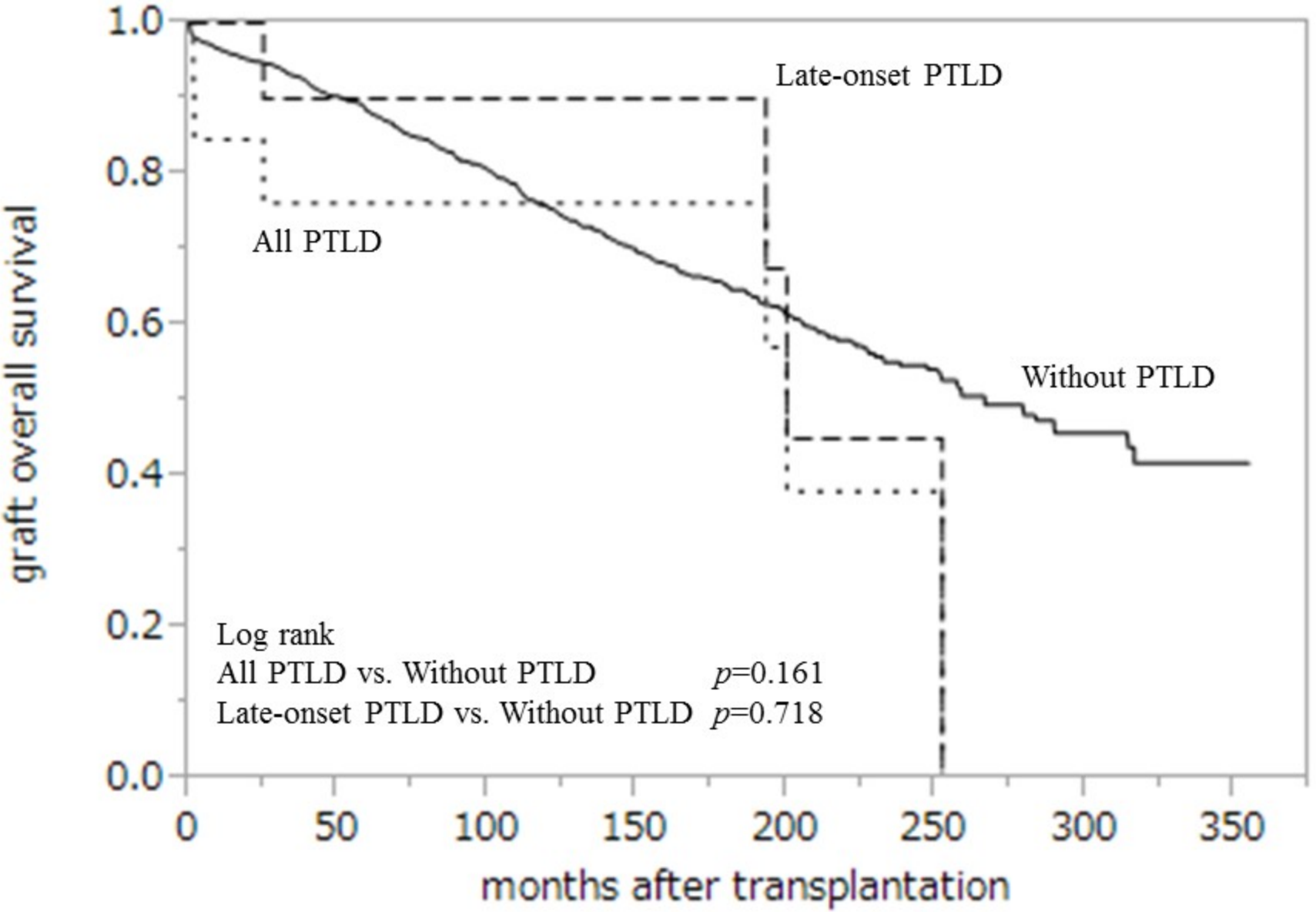

1.0
0.8
0.6
0.4
0.2
0.0
graft overall survival
Late-onset PTLD
All PTLD
Without PTLD
Log rank
All PTLD vs. Without PTLD p=0.161
Late-onset PTLD vs. Without PTLD p=0.718
0
50
100
150
200
250
300
350
months after transplantation

Figure 3

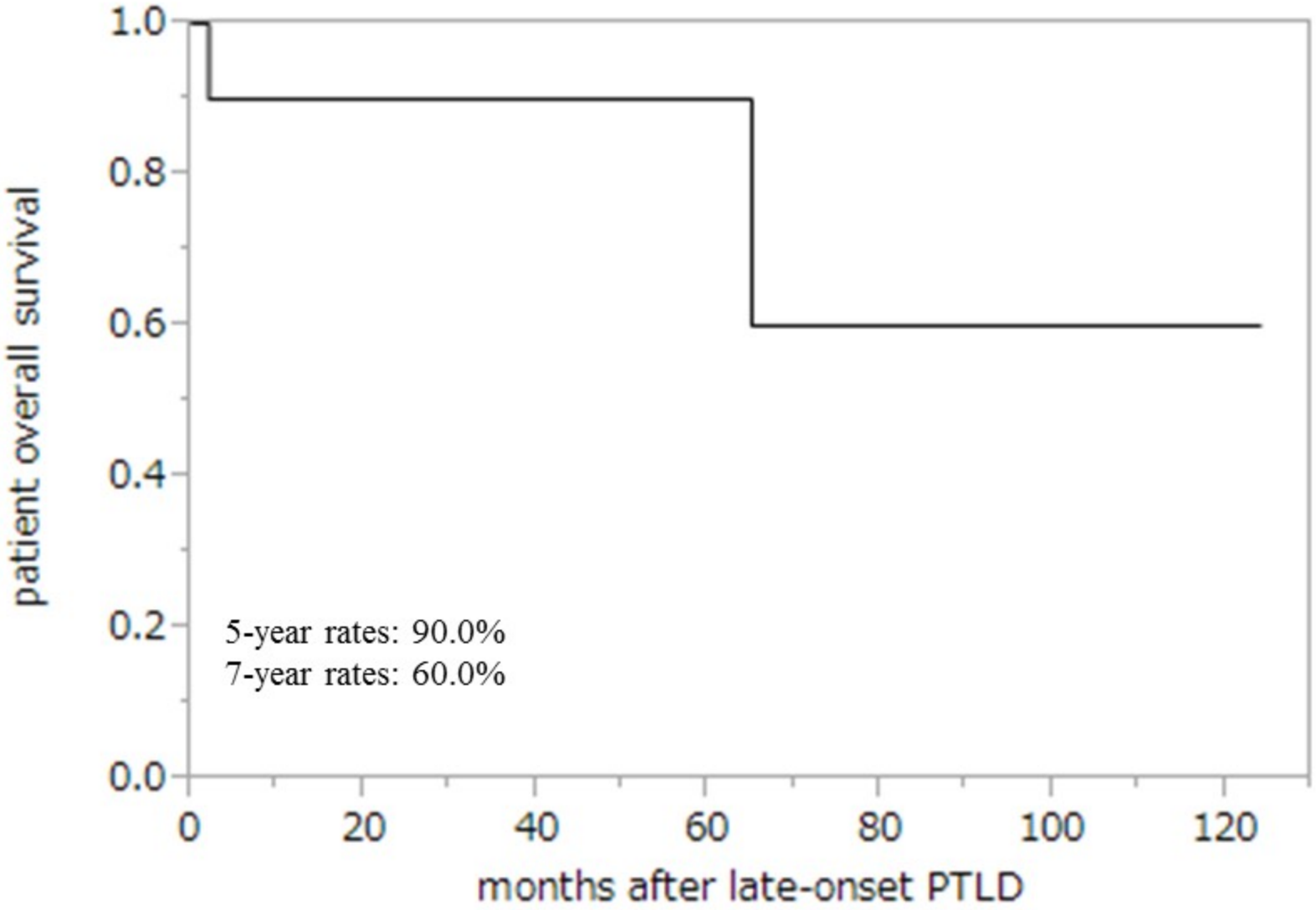
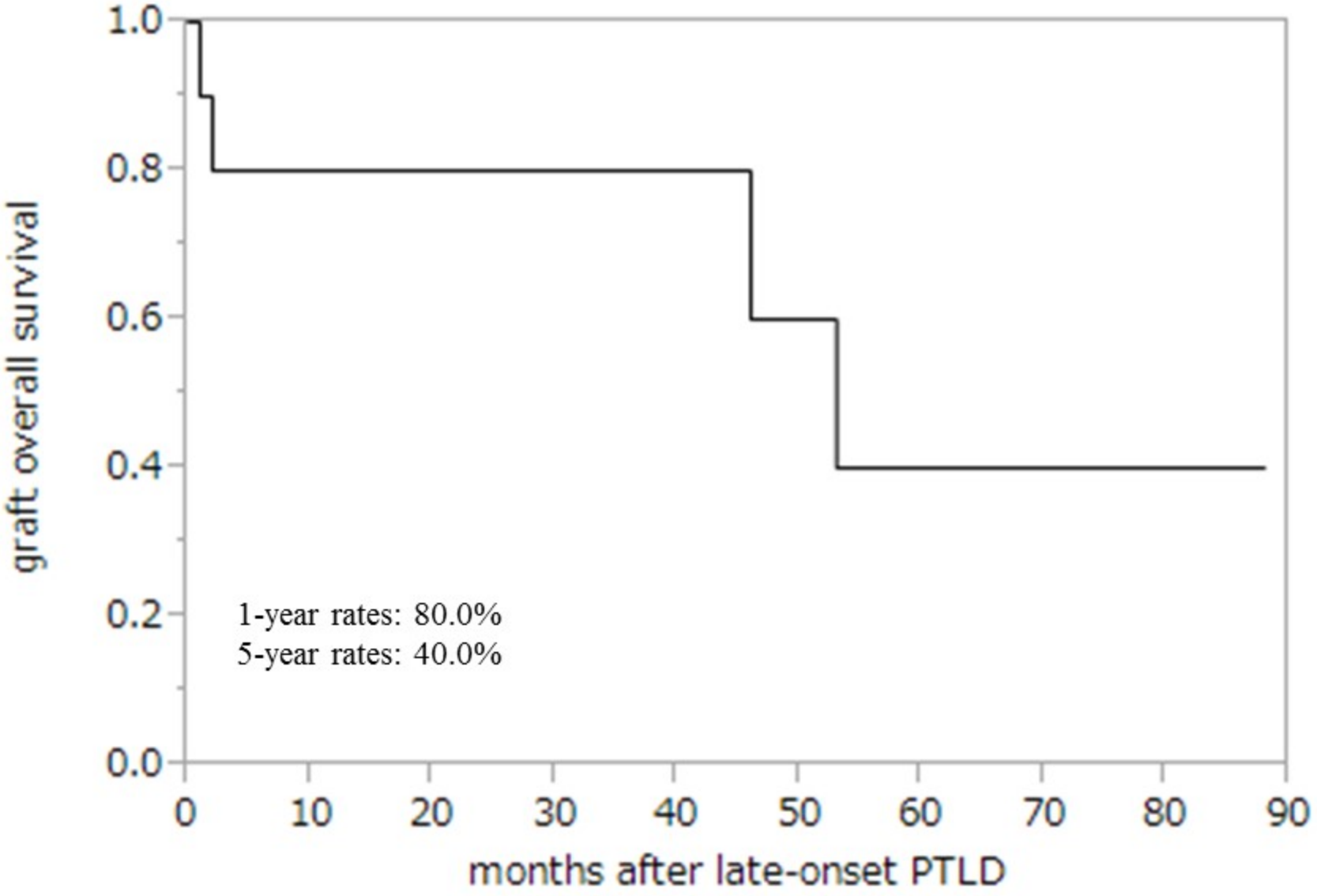

1.0
0.8
0.6
0.4
0.2
0.0
patient overall survival
5-year rates: 90.0%
7-year rates: 60.0%
0
20
40
60
80
100
120
months after late-onset PTLD

Figure 4


1.0
0.8
0.6
0.4
0.2
0.0
graft overall survival
1-year rates: 80.0%
5-year rates: 40.0%
0
10
20
30
40
50
60
70
80
90
months after late-onset PTLD