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Post-transplant persistent lymphopenia is a strong predictor of late survival in isolated intestine and multivisceral transplantation

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Conflict of interest
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Summary

Absolute lymphocyte count (ALC) has been identified as a prognostic factor in liver transplantation. We hypothesized that a lower ALC may be linked to poor outcomes in isolated intestinal/multivisceral transplantation (IIT/MVT). The aim of this study was to investigate the prognostic impact of ALC in IIT/MVT. A total 141 IIT/MVT patients were eligible for the study. Post-transplant ALCs (at 3, 6, and 12 months) were evaluated, and prognostic impact of trend of ALC during the first year was investigated. Of these 141 patients, 108 patients survived in the first year (1-year survivors). One-year survivors were categorized according to post-transplant ALC at each time point. When ALC was decreased throughout the first year (post-transplant persistent lymphopenia: <500/μl at 3, 6, and 12 months), patient survival ($P < 0.001$, hazard ratio = 5.09) and graft survival ($P < 0.001$, hazard ratio = 5.15) after the first year was significantly worse, and this remained to be an independent risk factor. Negative impact of persistent lymphopenia on patient and graft survival was significant regardless of type of intestinal graft. Infection leading to mortality occurred more frequently in the persistent lymphopenia group (43% vs. 24%). Trend of post-transplant ALC may be a strong predictive marker for long-term outcome in 1-year survivors after IIT/MVT.

Introduction

Outcomes after isolated intestinal/multivisceral transplantation (IIT/MVT) have been improving, and as of 2014, 1-year patient and graft survival was reported to be 75–80% and 70–75%, respectively [1]. However, patient and graft outcomes, especially long-term, are still significantly inferior to that of other solid organ transplantation. The survival curve for IIT/MVT does not plateau over years, as it does with other organ transplantation, probably due to high incidence of rejection, infection, and other morbidities [1–5]. Readmission rate after IIT/MVT reached almost 100%, suggesting that intestine recipients more often require extensive ongoing medical care, not only in the early post-transplant period [1]. It is important to understand prognostic factors in IIT/MVT recipients affecting not only the early post-transplant course, but also the long-term. We need to pay more attention to patients who successfully go through early post-transplant period, and detecting prognostic factors for this particular group will be helpful to provide appropriate long-term post-transplant care.

Lymphopenia is associated with patient frailty leading to poor outcome [6–9]. Recently, it was reported that

peritransplant absolute lymphocyte count (ALC) in liver transplant recipients would be a reliable prognostic factor for survival, post-transplant infectious complication, and recurrence of disease such as cytomegalovirus (CMV) infection, hepatitis C, and hepatocellular carcinoma [10–13]. Because ALC is regarded as a marker of nutritional status [14,15], the trend of post-transplant ALC following IIT/MVT may reflect function of the transplant intestine. We hypothesized that ALC might be a useful surrogate marker to predict outcomes after IIT/MVT. The aim of this study was to investigate the association between peritransplant ALC and outcomes after IIT/MVT, especially in 1-year survivors, focusing on prognostic factors after the first year.

Patients and methods

Patient selection

Between 2003 and 2012, 182 patients underwent IIT/MVT at Indiana University Hospital, which included IIT, modified MVT (stomach, intestine, and pancreas), and MVT (liver, stomach, intestine, and pancreas) with or without kidney transplant. The medical records were retrospectively reviewed. Age at transplant of 15-year old or younger was excluded from this study, because of their different normal range of ALC. Retrospective analysis of the transplant database has been approved by the Institutional Review Board at the Indiana University School of Medicine.

Surgical procedure

The organ procurement was performed by standard techniques described by our group and elsewhere [16,17]. Transplant procedures for each type of intestinal graft were also previously described by our group [18,19]. Distal intestinal continuity was re-established by performing an ileocolic anastomosis with terminal ileostomy, if the recipient had adequate length and condition of their native colon. Proximal gastrointestinal continuity was restored with an anastomosis between the donor stomach and the recipient esophagus in MVT and modified MVT, and between the donor jejunum/duodenum and the recipient jejunum in IIT. Initially, kidney transplant was performed simultaneously; however, this method has recently been replaced by delaying kidney transplant (usually 24–48 h after the visceral transplant) to stabilize the recipient's hemodynamic state prior to the kidney transplant, which was the same strategy applied to liver and kidney transplant recipients at our transplant center [20].

Post-transplant management

The immunosuppression protocol utilized has been described in previous reports as well [18,21,22]. The induction immunosuppression regimen consisted of rabbit antithymocyte globulin (RATG; 2 mg/kg starting on the day of transplant, 3–5 doses every 48 h) with rituximab [150 mg/m^2 on postoperative day (POD) 1] and high-dose steroids which are rapidly tapered to maintenance doses. Tacrolimus and prednisone, with monthly basiliximab, were administered as maintenance immunosuppression. Mycophenolate mofetil was not used in our series because of its potential risk of drug-induced enteritis. Enteral feeding usually started on POD 2 or 3 through a jejunostomy tube and was advanced to a goal rate as tolerated. Total parenteral nutrition started immediately after surgery and was withdrawn as enteral feeding advanced. An intestine biopsy was performed through the ileostomy for routine surveillance, as well as for clinical findings including increased output from the ileostomy, gastrointestinal symptoms, and fever of unknown etiology to rule out acute rejection and/or viral infection [23]. Post-transplant CMV prophylaxis regimen was decided according to donor (D) and recipient (R) serostatus of CMV immunoglobulin G antibody [(+) positive; (−) negative]. Ganciclovir 5 mg/kg is initiated in the operation room and is given twice a day for the first 2 weeks. Then, valganciclovir 900 mg is given orally once a day for 1 year post-transplant. For moderate or high-risk serostatus group (R+ or D+/R−), CMV immunoglobulin is given (1–10 doses over 4 months post-transplant). All results of CMV polymerase chain reaction and/or antigenemia were reviewed, and all positive results were considered to be CMV viremia. Tissue invasive CMV enteritis was diagnosed on biopsy with hematoxylin and eosin stain with or without immunohistochemistry. Acute rejection was diagnosed based on biopsy results, and moderate or severe acute rejection was considered significant. Rejection treatment regimen consisted of RATG in addition to a steroid pulse with rapid tapering, varying slightly based on severity of rejection and response to treatment. The ileostomy was usually taken down 3–6 months after the transplant for patients who had distal intestinal continuity.

Analysis of prognostic factors for patient and graft survival

Analysis of prognostic factors for patient and graft survival consisted of two parts. First, pretransplant recipient factors, donor factors, and surgical factors were assessed to evaluate prognostic factors for overall patient and graft survival in the entire group. Pretransplant ALC was included in this analysis. Second, patient and graft survival was assessed in the patients who survived in the first year (1-year survivors), so that we could determine the potential impact of trend of ALC in the first year and post-transplant lymphopenia on survival after the first year. Patients who died within the first year were excluded from the second

analysis. This analysis included peritransplant factors, post-transplant complications such as acute rejection, and viral infection, as well as patient characteristics. Post-transplant ALCs at 3, 6, and 12 months were evaluated in this second analysis to investigate its impact on outcomes after the first year in 1-year survivors.

Peritransplant ALCs were routinely included in laboratory tests, and the results of ALCs were collected with retrospective chart review. Pre- and post-transplant ALCs were evaluated as potential prognostic factors in continuous manner and with categorization. The cutoff levels of ALC were decided by receiver operating characteristics (ROC) curve analysis and according to the previous literature [10,11]. Trend of post-transplant ALC was analyzed separately, and persistent post-transplant lymphopenia was defined as ALC <500/μl all three points post-transplant (at 3, 6, and 12 months) [10]. These were included in the prognostic factor analysis for patient and graft survival after the first year.

Statistical analysis

The data were summarized using mean with standard deviation or median with interquartile range (IQR)/range for continuous variables and percentage for discrete variables. The Mann–Whitney *U*-test was used for two group comparisons. Analysis of risk factors for survival was performed using Cox's proportional hazards regression model. Patient and graft survival rates were estimated using the Kaplan–Meier method, and differences in the curves were analyzed using a log-rank test. Association with post-transplant persistent lymphopenia was evaluated using logistic regression model. On the multivariate Cox regression analysis for patient survival after the first year, clinically relevant variables, which were likely associated with long-term patient survival and post-transplant ALCs, were predefined and included. Forward selection was used in the multivariate analysis, considering the low number of events. SPSS version 19.0 (IBM, New York, NY, USA) was used for statistical analysis, and the level of significance was set at 0.05.

Results

Of the 182 patients who underwent IIT/MVT from 2003 to 2012, 141 (male = 69, female = 72) met the inclusion criteria for this study and were analyzed. The median age was 51 years (range 16–67 years). The median follow-up time was 2.5 years (IQR 1.1–4.6 years). Thirty-five patients (25%) underwent IIT, 25 (18%) underwent modified MVT, and 81 (57%) underwent MVT. Kidney transplant was performed in four patients combined with IIT, one with modified MVT, and 19 with MVT. One-year patient and graft survival rates were 87% and 84% for IIT, 96% and 88% for modified MVT, 76% and 74% for MVT, and 46% and 46% for IIT/MVT combined with kidney transplant ($P = 0.001$ and $P = 0.02$, respectively).

Analysis of pretransplant patient, donor, and operative characteristics as possible prognostic factors for patient and graft survival

Pretransplant factors, including pretransplant recipient ALC, donor, and operative characteristics, were evaluated to determine prognostic factors for patient and graft survival after IIT/MVT. On univariate analysis, pretransplant ALC showed tendency to worsen patient survival in continuous manner ($P = 0.07$, hazard ratio = 1.33 per 100/μl down). With categorization based on threshold of ALCs at 250, 500, and 1000/μl, ALC of 500–1000/μl showed significantly worse survival in the group of ALC of 500–1000/μl compared with ALC >1000/μl ($P = 0.03$, hazard ratio = 1.84), but ALC of 250–499/μl or <250/μl did not show association with worse survival ($P = 0.87$ and $P = 0.29$, respectively).

On multivariate analysis of patient survival, African American recipient race ($P = 0.001$, hazard ratio = 4.6), and inclusion of kidney graft ($P = 0.001$, hazard ratio = 2.95) remained as independent prognostic factors. RATG induction showed significant association with better outcome in continuous manner ($P < 0.001$, hazard ratio = 0.81 per 1 mg/kg increase). Pretransplant ALC was not considered an independent risk factor either as a continuous or categorized value.

Trend of post-transplant ALC as a prognostic factor in 1-year survivors

ALC significantly decreased after transplant and recovered over the first year. Median (IQR) ALC was 960/μl (570–1690/μl) before transplant, but decreased to 230/μl (120–410/μl) at 3 months, 470/μl (270–710/μl) at 6 months, and 900/μl (400–1420/μl) at 12 months. Of 141 patients, 108 (77%) survived the first year and intestinal graft was viable in 104 (74%). Patient survival rates after the first year of these 108 patients were 87% at 2 year, 77% at 3 year, and 69% at 5 year.

Trend of post-transplant ALC in the first year was evaluated as one of the possible prognostic factors for survival after the first year in patients who were alive at 1 year (1-year survivors), along with other recipient and operative factors (Table 1). Analysis of patient and graft survival included 108 patients who were alive at 12 months and 104 patients whose intestinal grafts were viable at 12 months. As potential prognostic markers for survival after the first year, the cutoff levels for ALC at pretransplant, 3, 6, and 12 months were decided by ROC curve analysis. Area

Table 1. Analysis of possible prognostic factors for patient survival after the first year (1-year survivors, $n = 108$; mortality after the first year $n = 30$).

		Univariate analysis		Multivariate analysis	
Variable	Observation (%)	*P* value	HR (95% CI)	*P* value	HR (95% CI)
Recipient age (per year)	108	0.005	1.05 (1.02–1.08)	0.24	–
Recipient, female	57 (53)	0.45	0.76 (0.37–1.56)		
Recipient, African American (Ref. Caucasian)	4 (4)	0.28	2.23 (0.52–9.46)		
Primary diagnosis					
Short gut syndrome	56 (52)				
Malignant tumor	15 (14)	0.57	0.7 (0.2–2.41)		
Diffuse portomesentric vein thrombosis	32 (30)	0.74	1.14 (0.51–2.55)		
Pseudo-obstruction	5 (5)	0.9	1.1 (0.25–4.85)		
Type of intestine graft					
Isolated intestine (Ref)	29 (27)				
Modified MVT	23 (21)	0.23	0.53 (0.19–1.49)		
MVT	56 (52)	0.07	0.48 (0.22–1.06)		
Inclusion of kidney graft	11 (10)	0.45	1.51 (0.53–4.35)		
Inclusion of liver graft	56 (52)	0.17	0.6 (0.29–1.25)		
Native splenectomy	79 (73)	0.06	0.5 (0.24–1.02)	0.67	–
Retransplantation	6 (6)	0.31	0.04 (0.0–18.38)		
Pretransplant ALC					
>1000/μl (Ref)	58 (54)				
500–1000/μl	30 (28)	0.59	1.24 (0.56–2.74)		
250–499/μl	12 (11)	0.14	0.22 (0.03–1.63)		
<250 μl	7 (7)	0.93	1.07 (0.25–4.68)		
Continuous manner (per 100/μl down)		0.82	1.0 (0.97–1.04)	0.65	–
Post-transplant ALC at 3 months					
>500/μl (Ref)	21 (20)				
250–500/μl	32 (30)	0.47	1.56 (0.47–5.22)		
125–249/μl	26 (24)	0.78	1.21 (0.32–4.54)		
<125/μl	28 (26)	0.02	3.79 (1.22–11.79)		
Continuous manner (per 100/μl down)		0.06	1.19 (0.99–1.43)		
Post-transplant ALC at 6 months (per 100/μl down)					
>1000/μl (Ref)	15 (14)				
500–1000/μl	36 (35)	0.76	1.28 (0.27–6.18)		
250–499/μl	29 (27)	0.4	1.97 (0.41–9.51)		
<250 μl	26 (25)	0.02	6.18 (1.4–27.35)		
Continuous manner (per 100/μl down)		0.004	1.24 (1.07–1.43)		
Post-transplant ALC at 12 months (per 100/μl down)	104	0.001			
>1000/μl (Ref)	45 (43)				
500–1000/μl	27 (26)	0.002	6.41 (2.03–20.29)		
250–499/μl	18 (17)	0.008	5.61 (1.57–20.01)		
<250 μl	14 (13)	<0.001	14.01 (4.09–48.03)		
Continuous manner (per 100/μl down)		0.001	1.15 (1.06–1.24)		
Post-transplant persistent lymphopenia	29 (28)	<0.001	3.94 (1.87–8.31)	<0.001	5.09 (2.28–11.34)
Acute rejection within the first year*	27 (25)	0.4	1.39 (0.65–2.97)	0.51	–
CMV infection within the first year	8 (7)	0.55	1.44 (0.44–4.77)	0.39	–
RATG induction (per 1 mg/kg increase)	–	0.009	0.82 (0.71–0.95)	0.002	0.78 (0.67–0.91)
Rituximab induction	103 (95)	0.02	0.25 (0.08–0.83)	0.43	–
Maintenance IL-2 receptor antagonist (per dose)	87‡	0.87	1.0 (0.94–1.1)		

MVT, multivisceral transplantation; ALC, absolute lymphocyte count; CMV, cytomegalovirus; RATG, rabbit antithymocyte globulin; HR, hazard ratio; CI, confidence interval.

*Moderate or severe acute cellular rejection.

‡Insufficient data in 21 patients.

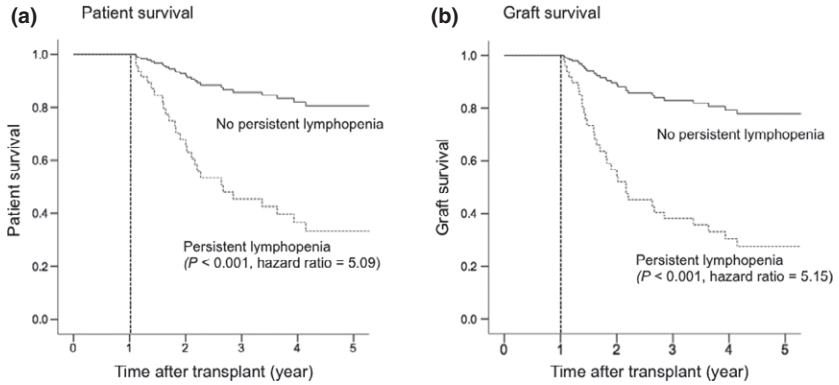

Figure 1 Adjusted patient and graft survival curves in 1-year survivors based on status of post-transplant persistent lymphopenia. (a) Patient survival. Post-transplant persistent lymphopenia group (<500/μl at 3, 6, and 12 months after IIT/MVT) showed significantly worse survival in comparison with no persistent lymphopenia group ($P < 0.001$, hazard ratio = 5.09). (b) Graft survival. Post-transplant persistent lymphopenia group showed significantly worse survival in comparison with no persistent lymphopenia group ($P < 0.001$, hazard ratio = 5.15).

under curve values for pretransplant, at 3, 6, and 12 months were 0.51, 0.62, 0.7, and 0.73, respectively, and *P* values were 0.92 for pretransplant, 0.055 for 3 months, 0.002 for 6 months, and <0.001 for 12 months. While no appropriate cutoff level was determined for pretrasplant ALC, the following best cutoff level was determined based on the maximum sum of sensitivity and specificity; 125/μl at 3 months (sensitivity 0.41 and specificity 0.82), 270/μl at 6 months (sensitivity 0.48 and specificity 0.84), and 1020/μl at 12 months (sensitivity 0.86 and specificity 0.52). Patients were categorized into four groups using cutoff levels of 250, 500, and 1000/μl for ALC at 6 and 12 months and 125, 250, and 500/μl for ALC at 3 months, which included the best cutoff level at each point (each value was rounded 25/μl, and 125/μl at 3 months, 250/μl at 6 months, and 1000/μl at 12 months were considered to be the best cutoff levels).

On univariate analysis, post-transplant ALCs at 6 and 12 months were significantly associated with outcome in continuous manner (6 months, $P = 0.004$, hazard ratio = 1.24 per 100/μl down; 12 months, $P = 0.001$, hazard ratio = 1.15 per 100/μl down). ALC <125/μl at 3 months [$P = 0.02$, hazard ratio = 3.79 (Ref. > 500/μl)] and ALC <250/μl at 6 months [$P = 0.02$, hazard ratio = 6.18 (Ref. > 1000/μl)] showed significantly higher risk of mortality. The risk was clearly stratified by ALC at 12 months [$P = 0.002$, hazard ratio = 6.41 for ALC 500–1000/μl, $P = 0.002$, hazard ratio = 5.61 for ALC 250–499/μl, $P = 0.002$, hazard ratio = 14.01 for ALC <250/μl (Ref. > 1000/μl)]. Of 108 patients, 29 showed post-transplant persistent lymphopenia (ALC < 500/μl at 3, 6, and 12 months). When ALC decreased throughout the first year, patient survival after the first year was significantly worse ($P < 0.001$, hazard ratio = 3.94). On multivariate analysis, post-transplant persistent lymphopenia was identified to be an independent prognostic factor for patient survival ($P < 0.001$, hazard ratio = 5.09; Fig. 1a). RATG induction showed significant association with better outcome in continuous manner ($P = 0.002$, hazard ratio = 0.78 per 1 mg/kg increase), which was considered to be an independent factor improving patient survival in 1-year survivors.

Risk factor analysis for graft loss after the first year was conducted (Table 2). After adjusting the risk with the confounding factors, post-transplant persistent lymphopenia remained an independent prognostic factor for graft survival after the first year in 104 patients who had viable intestinal graft at 1 year ($P < 0.001$, hazard ratio = 5.15; Fig. 1b). RATG induction also remained to be an independent factor improving graft survival ($P = 0.002$, hazard ratio = 0.79 per 1 mg/kg increase).

Table 2. Analysis of possible prognostic factors for graft survival after the first year (patients with viable intestine graft at 1 year, $n = 104$; graft loss after the first year $n = 34$).

Variable	Observation (%)	Multivariate analysis	
		P value	HR (95% CI)
Post-transplant persistent lymphopenia	29 (29)	<0.001	5.15 (2.44–10.89)
Native splenectomy	28 (27)	0.47	–
RATG induction (per 1 mg/kg increase)	–	0.002	0.79 (0.68–0.92)
Rituximab induction	98 (94)	0.14	–
Acute rejection within the first year*	24 (23)	0.19	–
CMV infection within the first year	8 (8)	0.055	2.9 (0.98–8.6)
Recipient age	–	0.62	–
Pretransplant ALC	–	0.49	–

ALC, absolute lymphocyte count; RATG, rabbit antithymocyte globulin; HR, hazard ratio; CI, confidence interval.
*Moderate or severe acute cellular rejection.

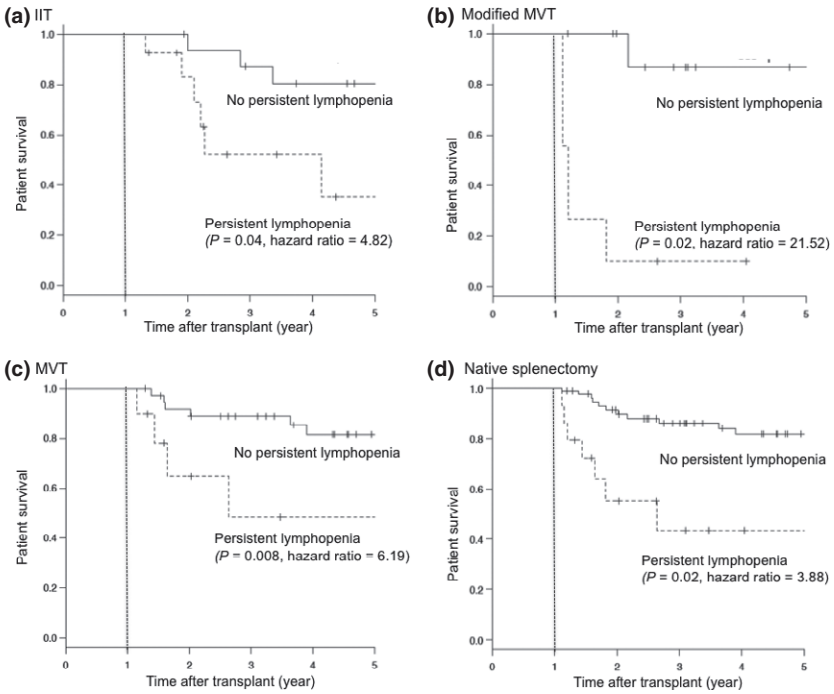

Figure 2 Adjusted patient survival curves in 1-year survivors according to graft type. Post-transplant persistent lymphopenia group showed significantly worse survival in comparison with no persistent lymphopenia group regardless of intestinal graft type. (a) isolated intestinal graft ($P = 0.04$, hazard ratio = 4.82), (b) modified multivisceral graft ($P = 0.02$, hazard ratio = 21.52), (c) multivisceral graft ($P = 0.008$, hazard ratio = 6.19), and (d) native splenectomy group (modified MVT and MVT; $P = 0.02$, hazard ratio = 3.88).

Subgroup analysis was conducted to address the difference of intestinal graft type. With adjusting the risk by Cox regression models, post-transplant persistent lymphopenia was considered to be an independent prognostic factors in all types of intestinal graft (IIT, modified MVT, and MVT; Fig. 2a–c). The status of native spleen did not change the significant prognostic impact of post-transplant persistent lymphopenia (Fig. 2d).

Associated factors with post-transplant lymphopenia

Potential factors, which might lead to post-transplant persistent lymphopenia, were assessed (Table 3). Recipient age at transplant showed significant association with post-transplant persistent lymphopenia on univariate analysis. Post-transplant ALC at 6 and 12 months was significantly higher in the native splenectomy group (modified MVT and MVT group). Trend of median (IQR) ALC in the native splenectomy and preserved native spleen groups were 250/μl (118–470/μl) and 230/μl (160–405/μl) at 3 months ($P = 0.78$), 530/μl (315–840/μl) and 330/μl (140–477/μl) at 6 months ($P = 0.001$), and 980/μl (560–1600/μl) and 530/μl (238–998/μl) at 12 months ($P = 0.004$). The presence of native spleen (preserved native spleen) was significantly associated with post-transplant persistent lymphopenia on univariate analysis. All eight patients who had CMV infection in the first year received ganciclovir followed by valganciclovir treatment, and all 27 patients who had acute rejection in the first year were treated with RATG. These complications and treatments, which could induce lymphopenia, were not associated with persistent post-transplant lymphopenia ($P = 0.56$ and $P = 0.63$). On multivariate analysis, recipient age ($P = 0.01$, HR = 1.05 per year) and persevered native spleen (isolated intestine graft; $P = 0.005$, HR = 4.24) remained independent factors predicting post-transplant persistent lymphopenia.

Post-transplant complications and cause of death associated with persistent lymphopenia

Associations between persistent lymphopenia and complications, including CMV infection, acute rejection, and malnutrition, were assessed. Five of 29 patients who showed persistent lymphopenia had CMV infection in the second year, whereas 2 of 73 without persistent lymphopenia had CMV infection. According to Kaplan–Meier curve analysis, cumulative incidence of CMV infection was 20% in the persistent lymphopenia group and 4% in the nonpersistent lymphopenia group in the second year ($P = 0.08$, log-rank test). Sensitivity, specificity, and positive and negative predictive values of persistent lymphopenia for

Table 3. Risk factors associated with post-transplant persistent lymphopenia.

	Post-transplant persistent lymphopenia*		Univariate		Multivariate	
	Yes (n = 29)	No (n = 73)	P value	Odds ratio (95% CI)	P value†	Odds ratio (95% CI)
Recipient age (per year)	–	–	0.004	1.06 (1.02–1.1)	0.01	1.05 (1.01–1.1)
Primary diagnosis						
Portal hypertension	9	22	0.93	1.04 (0.41–2.65)	0.81	–
Inclusion of liver graft	10	43	0.03	0.37 (0.15–0.9)	0.32	–
Inclusion of kidney graft	2	8	0.54	0.6 (0.12–3.02)	0.46	–
Preserved native spleen	14	14	0.004	3.93 (1.55–10.0)	0.005	4.24 (1.56–11.54)
Pretransplant ALC						
<1000/μl	15	31	0.32	1.56 (0.65–3.75)	0.1	–
Continuous manner (per 100/μl down)	–	–	0.21	0.97 (0.92–1.02)		
Donor age (year)	–	–	0.67	0.99 (0.94–1.04)	0.79	–
RATG induction therapy						
≥10 mg/kg	25	64	0.71	0.78 (0.22–2.83)	0.87	–
Continuous manner (per 1 mg/kg increase)	–	–	0.94	0.99 (0.79–1.24)		
Rituximab induction	27	69	0.78	0.78 (0.14–4.53)	0.81	–
CMV infection in the first year	3	5	0.56	1.57 (0.35–7.04)	0.44	–
Rejection in the first year	9	15	0.26	1.74 (0.66–4.59)	0.33	–

ALC, absolute lymphocyte count; RATG, rabbit antithymocyte globulin; CMV, cytomegalovirus.
*ALC < 500/μl at 3, 6, and 12 months.
†Forward selection is used.

CMV infection in the second year were 71%, 81%, 28%, and 96%, respectively (excluding patients who had CMV infection in the first year or were censored in the second year). In terms of incidence of acute rejection, there was no difference between these two groups (P = 0.74). Albumin and pre-albumin levels at 12 months were compared with assess an association between nutritional status and persistent lymphopenia. Albumin level was significantly lower in the persistent lymphopenia group [3.1 (2.5–3.7) g/dl vs. 3.6 (3.2–3.9) g/dl in the nonpersistent lymphopenia group, P = 0.02]. There was no difference in pre-albumin levels in the persistent lymphopenia group [25 (12–32) mg/dl] vs. the nonpersistent lymphopenia group [23 (18–27) mg/dl, P = 0.4].

Of 108 1-year survivors, the cause of death in 31 patients who died after the first year was evaluated according to the trend of post-transplant ALC. Infection, including bacterial, fungal, and viral infections, was the leading cause both in the persistent lymphopenia and nonpersistent lymphopenia groups. Mortality rate due to infection was slightly higher in the persistent lymphopenia group [6/14 (43%) vs. 4/17 (24%)], but the difference was not statistically significant (P = 0.44). Other leading causes of death, including post-transplant lymphoproliferative disorder associated with Epstein–Barr virus [2/14 (14%) vs. 2/17 (12%)] and cardiovascular disease [3/14 (21%) vs. 4/17 (24%)], were evenly across in the persistent lymphopenia and nonpersistent lymphopenia groups (P = 0.69). Sensitivity, specificity, and positive and negative predictive values of persistent lymphopenia for mortality in the second year were 57%, 78%, 32%, and 91%, respectively (excluding patients who were censored in the second year).

Discussion

This present study reviewed our 10-year experience of IIT/MVT and assessed the association between pre and post-transplant ALC and their patient and graft outcomes. In particular, trend of ALC in the first year was evaluated for the purpose of determining the association between post-transplant lymphopenia and outcome after the first year. The results suggest that post-transplant persistent lymphopenia may be a strong prognostic factor for long-term in 1-year survivors of IIT/MVT. While each point of ALC at 3, 6, and 12 months had different cutoff levels to predict mortality after the first year, ALC at 12 months showed the most remarkable association with prognosis. One of the most important unresolved issues in intestinal transplant is inferior long-term outcome. Because the post-transplant course in IIT/MVT tends to be quite complicated and variable, it has been difficult to determine any definitive associations between pretransplant patient conditions and long-term outcome. The importance of this study is to specifically investigate prognostic factors in 1-year survivors

after IIT/MVT. This study successfully determined the association between post-transplant persistent lymphopenia and poor patient and graft survival after the first year. ALC at 12 months may reliably stratify the risk for mortality. Considering the relatively high specificity (low probability of false positive) of post-transplant persistent lymphopenia for patient mortality and CMV infection, trend of post-transplant ALC would be useful to detect patients at high risk of negative outcomes after surviving their first year, potentially allowing close assessment and early detection of their complications, ideally leading to improved long-term outcomes in this patient population.

Pretransplant ALC was reported as one of the prognostic factors in liver transplant recipients [10,13], but we did not find obvious association between outcome and pretransplant lymphopenia. Post-transplant course in IIT/MVT is probably more multifactorial; therefore, it would be difficult to determine the impact of pretransplant lymphopenia. Because it has been recognized that patient frailty and malnutrition impact post-transplant outcome in liver transplant patients, aggressive pretransplant interventions may be warranted to improve outcome even in IIT/MVT patients such as adequate nutritional support, exercise, and early decision on candidacy for transplant before deterioration of patient condition [24]. Pretransplant ALC would reflect patient frailty and nutritional status, which would help determine latent risk in IIT/MVT patients.

The analysis of prognostic factors after the first year among 1-year survivors showed that persistent post-transplant lymphopenia significantly correlated with lower patient and graft survival. This adverse impact was remarkable regardless of type of intestinal graft. While the underlying etiology remains to be elucidated, it was speculated that post-transplant ALC might be not only a simple prognostic marker to detect high-risk patients, but also a biological parameter associated with patient immunity, frailty, malnutrition, and malfunction of the transplant intestine. Our results demonstrated that CMV infection, malnourishment, and mortality due to infection more frequently occurred in the persistent lymphopenia group, which could account for the part of the underlying biological etiologies of the negative prognostic impact. It would be interesting to investigate whether any post-transplant interventions maintaining post-transplant ALC would improve outcome after IIT/MVT.

Absolute lymphocyte count decreased dramatically after IIT/MVT and recovered over 1 year after transplant. Our patient population received RATG as induction immunosuppression, which contributes to a significant depletion of lymphocytes. The recovery of ALC after administration of RATG depends on the dosage received, but it has been reported to take between 1 and 2 months. Because IIT/MVT patients were exposed to extensive surgical stress, received relatively high dose of RATG as induction immunosuppression (6–10 mg/kg in total), and 1-year course of post-transplant universal prophylaxis for CMV infection with ganciclovir followed by valganciclovir, it was not surprising to find that their ALC took longer to recover. Prolonged depletion of ALC was probably associated with significantly impaired lymphocyte function, which may make patients more susceptible to complications, especially infections. To improve outcome, it is probably important to investigate how to recover ALC after transplant. Overimmunosuppression, which may further deplete ALC, needs to be avoided to maintain post-transplant ALC and potentially to preserve lymphocyte function. Therefore, an optimal immunosuppression protocol still needs to be elucidated. Thus, multidisciplinary strategies should address the issue of post-transplant recovery of ALC, using multiple modalities.

Interestingly, induction immunosuppression with higher doses of RATG showed a significant association with better outcome in 1-year survivors. Although RATG is a potent immunosuppressant depleting lymphocytes, the dose of RATG induction therapy was not associated with post-transplant persistent lymphopenia. Adequate dose of induction probably prevented subclinical acute rejection and stabilized the graft condition such as amelioration of ischemia–reperfusion injury [25]. These potential positive effects may overweigh the risk of its lymphocyte depleting effect [10].

It should be noted that it is still unclear whether persistent post-transplant lymphopenia was causative for poor outcome or just the sequel of a suboptimal patient

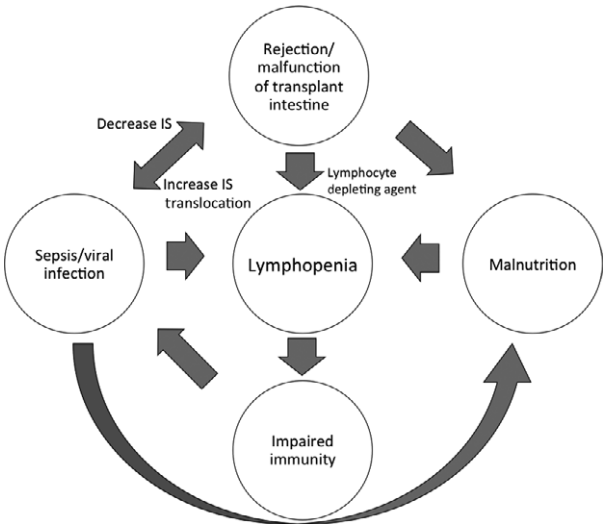

Figure 3 Complication spiral associated with lymphopenia. Lymphopenia is considered to be both cause and result and lie in the center of unfavorable situations, such as infection, malnutrition, impaired immunity, and malfunction of transplant intestine.

condition leading to poor outcome. Our results demonstrated that older recipients, who are more prone to frailty [26,27], showed significantly higher rate of post-transplant persistent lymphopenia. It is speculated that lymphopenia is considered to be both cause and result and lies in the center of unfavorable situation (Fig. 3). According to the previous studies regarding the impact of ALC on liver transplant patient outcomes, lymphopenia might be associated with impaired antiviral and antitumor immunity [10,11]. In addition, pretransplant lymphopenia was associated with higher infectious complication rates in liver transplant patients [12]. As described above, the present study showed higher incidence of CMV infection and mortality associated with infectious complications in patients with post-transplant persistent lymphopenia. Further investigations could demonstrate more clearly the clinical implication of lymphopenia in IIT/MVT patients.

In conclusion, trend of post-transplant ALC may be a strong predictive marker for long-term outcome in 1-year survivors after IIT/MVT. Further investigation of risk factors for persistent post-transplant lymphopenia is warranted to improve long-term outcome in IIT/MVT patients.

Authorship

SN: designed and performed the study, collected and analyzed data, and wrote the manuscript. RSM: performed the study, analyzed data, and wrote the manuscript. EA, BE, and TB: collected and analyzed the data and wrote the manuscript. CAK: collected and analyzed the data and performed the critical revision. JAF: designed the study, analyzed the data, and performed the critical revision. AJT: performed study, analyzed data, and did the critical revision.

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