Tandem autologous hematopoietic stem cell transplantation for treatment of adult T-cell lymphoblastic lymphoma: a multiple center prospective study in China

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ABSTRACT

T-cell lymphoblastic lymphoma (T-LBL) is a highly aggressive form of lymphoma with poor clinical outcomes and no standard treatment regimen. In this study, we assessed the safety and efficacy of tandem autologous hematopoietic stem cell transplantation (auto-HSCT) for adult T-LBL and evaluated prognostic factors affecting survival. A total of 181 newly-diagnosed adult T-LBL patients were enrolled: 89 patients were treated with chemotherapy alone, 46 were allocated to the single auto-HSCT group, 46 were treated with tandem auto-HSCT. Median follow-up time was 37 months; the 3-year progression/relapse rate of the tandem auto-HSCT group was significantly lower than that of the single auto-HSCT and chemotherapy groups (26.5% *vs.* 53.1% and 54.8%). The 3-year progression-free survival (PFS) and overall survival (OS) rates of the tandem auto-HSCT group (73.5% and 76.3%) were significantly higher than those of the single auto-HSCT group (46.9% and 58.3%) and the chemotherapy group (45.1% and 57.1%). In the tandem auto-HSCT group, age and disease status after the first transplant impacted OS and PFS. Multivariate analysis identified that disease status after the first transplant was the only independent prognostic factor for patients treated with tandem-HSCT. In addition, diagnostic models of the initial $CD8^+CD28^+/CD8^+CD28^-$ T-cell ratio in predicting the disease status were found to be significant. Taken together, tandem auto-HSCT can be considered an optimal strategy for adult T-LBL patients. (Study registered at: *ChiCTR-ONN-16008480*).

Introduction

Lymphoblastic lymphoma (LBL) accounts for 1-2% of all non-Hodgkin lymphomas (NHL) and is considered a highly aggressive lymphoma with poor clinical outcomes. T-cell LBL (T-LBL) accounts for approximately 90% of all LBL and occurs mostly in children and adolescents; it predominantly affects males.[1] Pathologically, the typical characteristics presented by T-LBL patients are often large anterior-mediastinal mass, hydropericardium and pleural effusion. In addition, some T-LBL patients may also present bone marrow (BM) involvement and central nervous system (CNS) infiltration.[2,3] Currently there is still no standard clinical treatment regimen, with multi-agent high-dose chemotherapy regimens (that mimic the treat-

ment of other lymphoma types) representing first-line treatment for most patients.[4] Intensive chemotherapy regimens (such as hyper-CVAD [cyclophosphamide, vincristine sulfate, adriamycin and dexamethasone] and acute lymphoblastic leukemia [ALL]-like regimens for children) have also been used in treating adult T-LBL patients. Subsequently, complete remission (CR) rates have been significantly improved to 90-95% along with a long-term disease-free survival rate of 50-55%.[5,6] However, 5-year OS does not exceed 67%, and 30-40% of patients will relapse. Thus, additional therapeutic strategies are urgently needed in the treatment of T-LBL.

Auto-hematopoietic stem cell transplantation (auto-HSCT) was one potential treatment regimen for T-LBL patients; however, the therapeutic effect of auto-HSCT treatment for these patients is still controversial. A recent retrospective study indicated that auto-HSCT could improve OS,[7] and demonstrated that auto-HSCT could reduce early relapse to some degree.[8] However, the high 5-year relapse rate was still the primary reason for restricting the application of this approach, as the cumulative relapse rate of T-LBL patients during the 5 years after receiving auto-HSCT in the CR1 stage was as high as 56%.[9] Survival of patients with relapsed disease was generally <12 months, and only 10% of these patients could survive long term.[10,11] Even after remission with second-line regimens, auto-HSCT for relapsed patients still yielded poor results and did not improve OS.[12] Compared with auto-HSCT, the overall effect of allogeneic hematopoietic stem cell transplantation (allo-HSCT) was associated with fewer relapses, but higher treatment-related mortality (TRM) offset any potential survival benefit.[13] Thus, due to the high rates of relapse following auto-HSCT, and the increased TRM in patients treated with allo-HSCT, new treatment strategies must be designed, not only to reduce the relapse rate of T-LBL but also to improve OS and PFS.

Consecutive or 'tandem' auto-HSCT might represent a novel regimen in the treatment of T-LBL. Tandem auto-HSCT function as a second round of an *in vivo* 'reset' through repeated ultra-high-dose chemotherapy, and could eliminate residual tumor cells in the body more thoroughly, thus reducing the risk of post-transplant minimal residual disease (MRD) and relapse. Tandem-HSCT had been successfully applied in treating certain brain tumors (such as neuroblastoma and glioma), and achieved good clinical outcome.[14-16] In recent years, several groups have indicated that tandem auto-HSCT could reduce the relapse rate of refractory, relapsed, aggressive lymphomas (such as diffuse large B-cell lymphoma, mantle cell lymphoma [MCL], and peripheral T-cell lymphoma) and improve long-term survival.[17,18] Tandem auto-HSCT has been shown to be superior to salvage chemotherapy for relapsed patients; however, whether tandem auto-HSCT is effective for newly diagnosed T-LBL patients is still not known.

In this scenario, a multi-center prospective clinical trial was designed to explore the therapeutic effect of tandem auto-HSCT treatment for adult T-LBL patients. Newly diagnosed adult T-LBL patients with stage III/IV disease were given 2-3 courses of induction therapy according to the Intensive Chemotherapy-Berlin-Frankfurt-Münster study group (IC-BFM) 2002 regimens. After achieving CR or a partial response (PR), the patients were divided into three groups, matched by age and gender: the chemotherapy (no-HSCT) group, the single auto-HSCT group, and the tandem auto-HSCT group. The objectives of this study were to evaluate the therapeutic effect and safety of tandem auto-HSCT, and to assess factors affecting survival.

Methods

Study design and patient selection

This prospective phase II study was conducted between February 2005 and November 2013 at five centers in southwestern China. (Study registered at: *ChiCTR-ONN-16008480*.) Eligible adults (n=181) aged 18-59 years with stage III/IV, newly diagnosed T-LBL were enrolled: 87 patients from Xinqiao Hospital, 25 from the General Hospital of Kunming Military Region of PLA, 23 from the Second Affiliated Hospital of Chongqing Medical University, 23 from Yunan Provincial People's Hospital, and 23 from the Second Yunnan Provincial Peoples' Hospital. The study was performed in accordance with the Declaration of Helsinki and local laws. The protocol was approved by the ethics committee of each center, and written informed consent was obtained from all patients.

Treatment strategies

Induction therapy - all patients were given induction therapy according to the IC-BFM 2002 regimens. After CR/PR was achieved, the patients were divided into three groups: 89 patients were treated with consolidation chemotherapy according to the IC-BFM 2002 regimens, 46 were treated with single auto-HSCT, and the other 46 patients were treated with tandem auto-HSCT. During the induction therapy, all the patients were given standard intrathecal injection to prevent CNS lymphoma. Patients with CNS involvement were given triple intrathecal therapy to return cerebrospinal fluid levels to normal. Patients with large mediastinal masses were not given local radiotherapy in this study.

Conditioning regimens - stem cell mobilization and collection details can be found in the *Online Supplementary Appendix*. For all patients receiving auto-HSCT, as melphalan was not used in China, other myeloablation regimens were utilized during conditioning. The BEAC regimen (carmustine: 0.2 mg/m^2 x 1 day [d]; etoposide: 100 mg/m^2 x 4 d; cytosine arabinoside: 100 mg/m^2, q12 hours [h] x 4 d; cyclophosphamide: 1.5 g/m^2 x 4 d) was adopted as the conditioning chemotherapy regimen for the single auto-HSCT and the first transplant of the tandem auto-HSCT. The IAC regimen (idarubicin: 10 mg/m^2 x 3 d; cyclophosphamide: 1 g/m^2 x 2 d; cytosine arabinoside: 1 g/m^2, q12 h x 4 d) was adopted as the conditioning treatment for the second transplant of the tandem auto-HSCT. The interval between the two transplants was 3-6 months. Stem cells were infused 48 h after the final dose of each of the regimens. All patients received prophylaxes for bacterial infection, fungal infection, herpes simplex virus infection, and pneumocystis pneumonia, as in our previous work.[19]

Statistical analysis

Treatment responses were classified according to uniform international standards. The χ^2 test was used to analyze the intergroup difference in clinical features, and the Mann-Whitney U test was used to analyze age difference. The Kaplan-Meier estimator was used to estimate the survival curves. The log-rank test was performed for intergroup comparison. Cox regression analysis was used to analyze risk factors. As baseline of chemotherapy group and HSCT group was imbalanced, to reduce the influence of potential confounders, propensity score matching was applied. $P<0.05$ was considered statistically significant. SPSS (version 22.0) was used to carry out all the above statistical analyses.

Table 1. Patients' demographic and clinical characteristics in the chemotherapy (Chemo) and autologous hematopoietic stem cell transplantation (auto-HSCT) groups.

Characteristics	All (%)	Chemo (%)	Single HSCT (%)	Tandem HSCT (%)	P
Median age, years (range)	32 (18-53)	34 (18-53)	28 (18-43)	29 (18-50)	0.803
Gender, Male	91 (50.2)	47 (52.8)	19 (41.3)	25 (54.3)	
ECOG score					0.51
0-1	84 (46.4)	44 (49.4)	22 (47.8)	18 (39.1)	
2	97 (53.6)	45 (50.6)	24 (52.2)	28 (60.9)	
Stage					0.076
III	67 (37)	39 (43.8)	17 (37.0)	11 (23.9)	
IV	114 (63)	50 (56.2)	29 (63.0)	35 (76.1)	
B symptom	75 (41.4)	40 (44.9)	17 (37.0)	18 (39.1)	0.627
aaIPI score					0.446
2	65 (35.9)	36 (40.4)	15 (32.6)	14 (30.4)	
3	116 (64.1)	53 (59.6)	31 (67.4)	32 (69.6)	
BM involvement	64 (35.3)	32 (35.9)	13 (28.3)	19 (41.3)	0.419
Mediastinal masses	68 (37.6)	26 (29.2)	20 (43.5)	22 (47.8)	0.067
CNS involvement	7 (3.8)	4 (4.4)	2 (4.3)	1 (2.2)	0.788
Remission status					0.853
CR		47 (52.8)	22 (47.8)	24 (52.2)	
PR		42 (47.2)	24 (52.2)	22 (47.8)	
Immunophenotypic classification based on flow cytometry					
Pre-T-LBL		11	3	5	0.914
Pro-T-LBL		13	5	8	
Cortical T-LBL		2	2	1	
Medullary T-LBL		6	3	5	
Immunophenotypic classification based on immunohistochemical staining					
Early T-LBL		17	9	17	0.17
Non-early T-LBL		47	37	29	

ECOG: Eastern Cooperative Oncology Group; aaIPI: age-adjusted International Prognostic Index; BM: bone marrow; CNS: central nervous system; CR: complete remission; PR: partial response; T-LBL: T-cell lymphoblastic lymphoma.

Results

Patients' characteristics

Median age of the patients in this study was 32 years (range: 18-59 years). At disease onset, 75 patients (41.4%) exhibited symptom B, 68 patients (37.6%) had mediastinal masses, and 7 patients (3.8%) had CNS infiltration; 114 patients (63%) were stage IV (by Ann Arbor staging) at diagnosis. Sixty-four patients (35.3%) were diagnosed with BM and/or peripheral blood (PB) involvement, and nine of them were diagnosed through BM flow cytometry detection. No significant differences between the three groups (chemotherapy, single auto-HSCT, and tandem auto-HSCT) were found with respect to remission status, age, gender, Eastern Cooperative Oncology Group (ECOG) score, stage, symptom B or age-adjusted International Prognostic Index (aaIPI) score (Table 1).

A comparative analysis between the chemotherapy group and the HSCT group showed that there was a statistically significant difference in the presence of mediastinal mass between the two groups when propensity score matching analysis was applied. Seventy-four pairs of patients were eligible for survival analysis. Patients' characteristics are summarized in *Online Supplementary Table S1*.

Progression/relapse rate

Because CR/PR of all the patients in this study was achieved through induction therapy prior to grouping, the progression/relapse rates of different consolidation treatments were analyzed. In the auto-HSCT groups (including both single auto-HSCT and tandem auto-HSCT group), 1-year and 3-year progression/relapse rates were 19.9% and 39.6%, respectively, both of which were significantly lower than those of the chemotherapy group (36.1% and 54.8%, respectively; P=0.04) (Figure 1A). With respect to the auto-HSCT groups, no statistical differences were found in 1-year progression/relapse rates between the tandem auto-HSCT group and the single auto-HSCT group (12.1% *vs.* 18.6%), but the 3-year progression/relapse rate of the tandem auto-HSCT group was significantly lower than that of the single auto-HSCT group (26.5% *vs.* 53.1%; P=0.003), indicating the advantage of tandem auto-HSCT treatment in long-term disease control (Figure 1B).

Survival analysis

Median follow-up time was 37 months (range: 5-81 months). The 3-year PFS and OS rates of the auto-HSCT groups were 60.4% and 66.3%, respectively, which were significantly higher than those of the chemotherapy group (45.1% and 57.1%, respectively; P<0.05) (Figure 2A and B). The 3-year PFS and OS rates among the 46 patients treated with single auto-HSCT were 46.9% and 58.3%, respectively. The 3-year PFS and OS rates among the patients treated with tandem auto-HSCT were 73.5% and 76.3%, respectively, which were significantly higher than

those of the chemotherapy group and the single auto-HSCT group (*P*=0.01 and *P*=0.02, respectively) (Figure 2C and D).

Moreover, prognostic value of immunophenotyping on T-LBL patients was also evaluated when patients were divided into pre-T-LBL, pro-T-LBL, cortical T-LBL and medullary T-LBL groups according to the European Group for Immunological Characterization of Leukemias (EGIL) classification. Sixty-four patients were eligible for subgroup analysis based on flow cytometric results; survival analysis of patients showed that there was no difference in PFS between the four subgroups of T-LBL (*Online Supplementary Figure S1A*). When classification was divided into early T-cell subtype and non-early T-cell subtype according to immunohistochemical surface marker, 25 cases could not be classified due to the fact that some required markers were incomplete. PFS of the early T-cell group was much poorer compared to that of non-early T-cell group, but this difference was not significant (*P*=0.06) (*Online Supplementary Figure S1B*). In addition, when all the patients were divided into the chemotherapy group and the auto-HSCT group, in the chemotherapy group, patients with early T-cell subtype had a significantly poor survival compared with the non-early T-cell group, but a comparison with the auto-HSCT group showed no significant difference, suggesting that auto-HSCT might overcome the deleterious effect of the early T-cell subgroup on prognosis (*Online Supplementary Figure S1C and D*).

Factors influencing toxicity related to tandem auto-hematopoietic stem cell transplantation and patient prognosis

Because two high-dose pretreatments were carried out on patients in the tandem auto-HSCT group, treatment-related toxicity was one of the major concerns in this study. The median times for leukocyte and platelet reconstitution after the first transplant were 12 d (range: 9-21 d) and 14 d (range: 11-17 d) respectively, and the median times for leukocyte and platelet reconstitution after the second transplant were 14 d (range: 10-17 d) and 14 d (range: 8-40 d), respectively. There was no significant difference in the time for post-transplant hematopoietic reconstitution between the two transplants.

Among the major transplant-related complications,

only the occurrence rate of gastrointestinal symptoms (66.7%) was significantly higher during the first transplant than during the second transplant (33.3%), while there were no significant differences between the two transplants in the occurrence rates of other complications (e.g., hemorrhage and fever). Two patients died from transplant-related causes due to intracranial hemorrhage and infectious shock. Major transplant-related complications during the two transplants are shown in *Online Supplementary Table S2*.

Univariate and multivariate analysis

Information about the 46 patients in the tandem auto-HSCT group was collected and analyzed to determine independent prognostic factors. The results showed that aaIPI score, disease stage, B symptom, mediastinal mass, CNS and BM involvement, and disease status before first transplant had no influence on PFS or OS (*P*>0.05) (Figure 3A-J). Univariate analysis showed that age and disease status after the first transplant had influences on OS and PFS. Multivariate analysis showed that only disease status after the first transplant had an influence on OS and PFS (Tables 2 and 3). For patients <32 years of age, the 3-year OS rate and 3-year PFS rate were 94.4% and 88.1%, respectively; these rates were significantly higher than those among patients >32 years of age (58.5% and 50.4%, respectively; *P*<0.05) (Figure 4A and B). The 3-year OS and PFS rates among patients who were considered to be in CR after the first transplant were 86.8% and 74.9%, respectively. These rates were significantly higher than those patients who achieved PR after the first transplant (28.1% and 28.1%, respectively; *P*<0.05) (Figure 4C and D).

CD8+CD28+/CD8+CD28⁻ equilibrium could predict the disease status of T-cell lymphoblastic lymphoma patients

Recently, CD8+CD28⁻ T cells have attracted interest because of their critical role in immunomodulation. They are recognized as a unique subpopulation of regulatory T cells that have an extensive and direct effect, such as by inhibiting T-cell activation and proliferation, and decreasing the secretion of proinflammatory cytokines by activated T cells. Here, flow cytometry showed that a lower
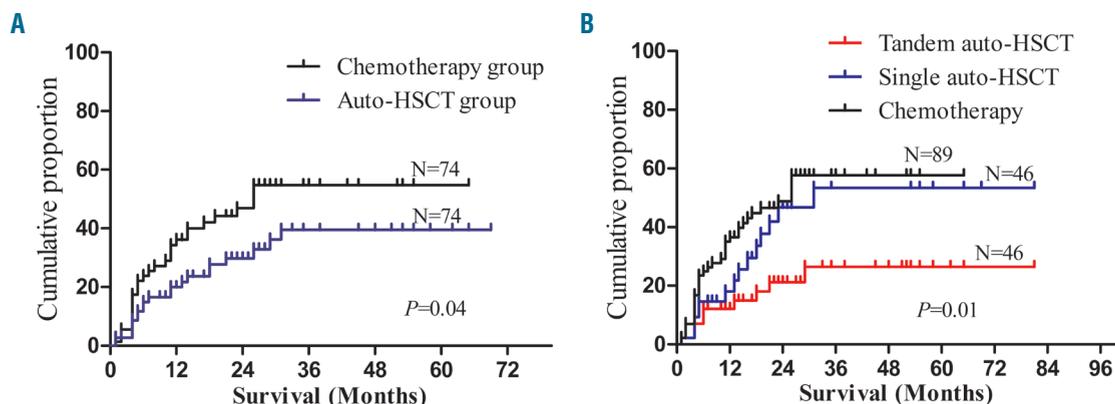

Figure 1. Cumulative relapse rate of the entire patients' cohort stratified by treatment strategies. (A) Cumulative relapse rate between chemotherapy group and autologous hematopoietic stem cell transplantation (auto-HSCT) group, (B) cumulative relapse rate between chemotherapy group, single auto-HSCT group and tandem auto-HSCT. N: number.

number of CD8$^+$CD28$^-$ T cells (5.84±2.06%) were seen in the patients with newly diagnosed T-LBL, compared with the patients who achieved PR (11.19±2.13%) and CR (18.91±3.38%) (Figure 5A-C). During follow-up, when patients relapsed, the number of CD8$^+$CD28$^-$ T cells (7.59±1.53%) decreased compared to that of patients with CR or PR. Meanwhile, the CD8$^+$CD28$^+$/CD8$^+$CD28$^-$ ratio of the patients in CR or with PR was significantly lower than that of newly diagnosed patients; when patients in CR or with PR relapsed, the ratio would increase, suggesting that the number of CD8$^+$CD28$^-$ T cells and the CD8$^+$CD28$^+$/CD8$^+$CD28$^-$ ratio might reflect the disease

status of T-LBL patients. Interestingly, the number of CD8$^+$CD28$^+$ and CD8$^+$CD28$^-$ T cells and the CD8$^+$CD28$^+$/CD8$^+$CD28$^-$ ratio were found to be statistically significant in predicting disease status. The AUC and the 95% confidence intervals are shown in Figure 5D. ROC curves showed that the CD8$^+$CD28$^+$/CD8$^+$CD28$^-$ ratio had the largest AUC (0.91) together with the best sensitivity (93%) and specificity (85%), suggesting that the CD8$^+$CD28$^+$/CD8$^+$CD28$^-$ balance had the best prediction efficiency (Figure 5E). In addition, purified CD8$^+$CD28$^+$ and CD8$^+$CD28$^-$ T cells were isolated to test their direct suppressive activity, results from two donors
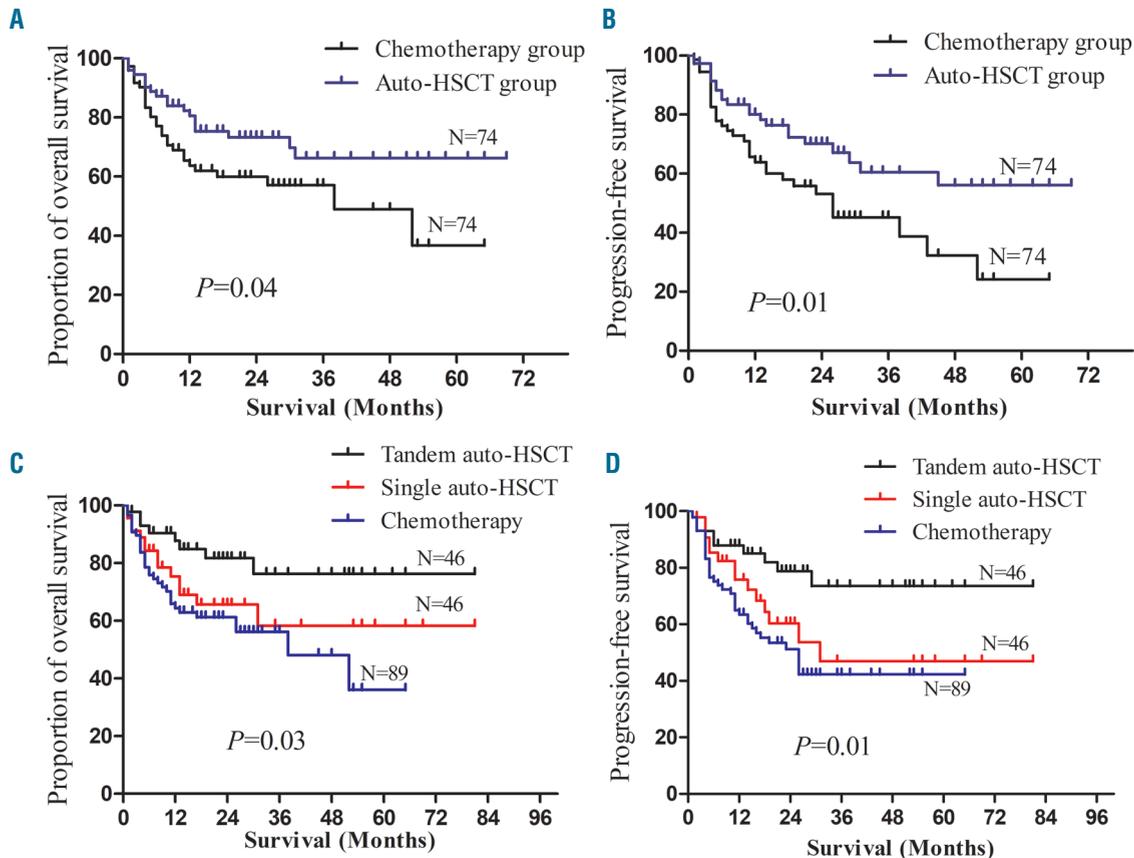

Figure 2. Kaplan-Meier estimated overall survival (OS) and progression-free survival (PFS) of different treatment strategies. Kaplan-Meier estimated OS (A) and PFS (C) of the entire patient cohort stratified by chemotherapy group and autologous hematopoietic stem cell transplantation (auto-HSCT). Kaplan-Meier estimated OS (B) and PFS (D) of the entire patient cohort stratified by chemotherapy group, single auto-HSCT group and tandem auto-HSCT. N: number.

Table 2. Univariate and multivariate analyses of the overall survival of patients with tandem autologous hematopoietic stem cell transplantation (HSCT).

Prognostic variables	Univariate analysis HR (95%CI)	P	Multivariate analysis HR (95%CI)	P
aaIPI scores	0.59 (0.293-1.19)	0.141	1.676 (0.204-13.773)	0.631
Clinical stage	0.94 (0.19-4.664)	0.94	0.34 (0.033-3.491)	0.364
B symptoms	3.487 (0.829-14.664)	0.088	1.676 (0.204-13.773)	0.631
BM involvement	2.877 (0.685-12.07)	0.149	9.539 (0.636-13.02)	0.103
Mediastinum involvement	0.211 (0.045-1.078)	0.062	0.247 (0.019-3.246)	0.287
Age	0.11 (0.14-0.903)	0.043	0.079 (0.003-2.181)	0.134
Disease status before HSCT	0.409 (0.097-1.718)	0.222	1.017 (0.119-8.695)	0.988
Disease status after 1st HSCT	0.181 (0.045-0.727)	0.002	0.009 (0.00-0.364)	0.013

HR: hazard ratio; CI: confidence interval; aaIPI: age-adjusted International Prognostic Index; BM: bone marrow.

(Patient 1: newly diagnosed; Patient 2: in CR) demonstrated that both CD8⁺CD28⁺ and CD8⁺CD28⁻ T cells were unable to mediate suppressive activity (Figure 5F and H) after incubation with autologous monocytes, IL2 and granulocyte-macrophage colony-stimulating factor (GM-CSF) for one week; only CD8⁺CD28⁻ T cells could show suppressive activity (Figure 5G and I). Cytotoxic assay demonstrated that stimulated CD8⁺CD28⁻ T cells could
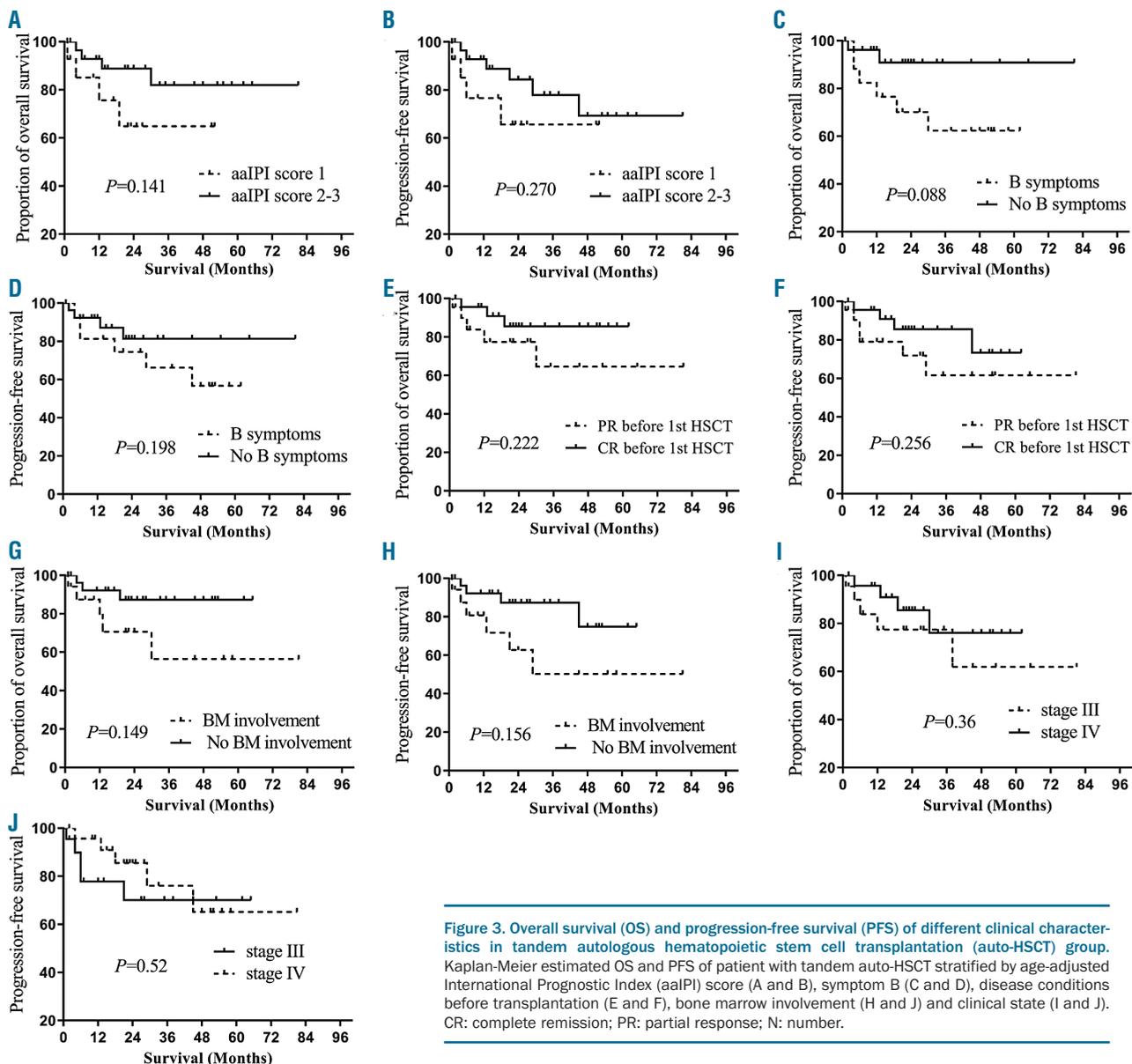

Figure 3. Overall survival (OS) and progression-free survival (PFS) of different clinical characteristics in tandem autologous hematopoietic stem cell transplantation (auto-HSCT) group. Kaplan-Meier estimated OS and PFS of patient with tandem auto-HSCT stratified by age-adjusted International Prognostic Index (aaIPI) score (A and B), symptom B (C and D), disease conditions before transplantation (E and F), bone marrow involvement (H and J) and clinical state (I and J). CR: complete remission; PR: partial response; N: number.

Table 3. Univariate and multivariate analyses of the progression-free survival of patients with tandem autologous hematopoietic stem cell transplantation (HSCT).

Prognostic variables	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P*	HR (95%CI)	*P*
aaIPI scores	0.704 (0.372-1.335)	0.282	0.57 (0.203-1.6)	0.286
Clinical stage	1.294 (0.275-6.099)	0.744	1.181 (0.170-8.815)	0.86 6
B symptoms	2.274 (0.651-7.942)	0.198	1.345 (0.231-7.837)	0.742
BM involvement	2.506 (0.704-8.919)	0.156	3.177 (0.592-17.035)	0.177
Mediastinum involvement	0.257 (0.065-1.02)	0.053	0.32 (0.053-1.914)	0.212
Age	0.204 (0.043-0.971)	0.046	0.194 (0.026-1.442)	0.109
Disease status before HSCT	0.445 (0.125-1.582)	0.211	0.582 (0.119-2.854)	0.505
Disease status after 1st HSCT	0.256 (0.071-0.918)	0.009	0.116 (0.017-0.804)	0.029

HR: hazard ratio; CI: confidence interval; aaIPI: age-adjusted International Prognostic Index; BM: bone marrow.

decrease the cytotoxic T lymphocyte (CTL)-mediated cytotoxic ability.

Discussion

ALL-like treatment regimens for children are currently recommended as induction treatment for all T-LBL patients.[20] Accordingly, all patients in this study were given induction therapy according to the ALL-like treatment regimens for children. Overall, 93 (51.4%) of the 181 patients achieved CR after 2-3 treatment courses. CR rates are lower than those reported by other centers,[5] which might be due to the advanced disease stage of the patients selected in this study (all stage III/IV). Following induction therapy, all patients were randomly assigned to three groups: chemotherapy (no HSCT), auto-HSCT or tandem auto-HSCT. The chemotherapy group included 89 patients, and the single and tandem auto-HSCT groups included 92 patients in total. Overall, both 1-year and 3-year progression/relapse rates of the auto-HSCT groups were lower than those of the chemotherapy group, and the lower rates associated with better OS: the 3-year OS and 3-year PFS rates of the auto-HSCT groups were significantly higher than those of the chemotherapy group. Results from this study indicate that combining auto-HSCT and increasing intensity of chemotherapy for adult T-LBL patients can reduce the relapse rate and enhance T-LBL patient survival compared with routine chemotherapy.

Tremendous progress has been made in targeting the lymphoma surface molecule, especially in B-cell NHL, such as mAb directed against CD20, CD40, CD52 and CCR4, but little advance has been achieved in T-cell NHL.[21] Tandem auto-HSCT had been shown to reduce the risk of post-transplant MRD as well as relapse ratio. For this reason, this approach had been tested in clinical trials in the treatment of myeloma.[22,23] Recently, in the context of lymphoma, Le-Gouill *et al*.[13] conducted tandem auto-HSCT for 15 refractory recurrent high-risk NHL patients with a median follow-up time of 20 months (range: 0-55 months) and achieved an OS rate of 67%. A study by Espigado *et al.* also indicated that the 12-year OS rate among malignant lymphoma patients treated with tandem auto-HSCT was as high as 71%, significantly higher than patients who did not undergo transplant.[11] Furthermore, the disease status achieved before the second HSCT could impact patient prognosis; the 12-year OS rate among patients who achieved CR before the second transplant was significantly higher than that of patients who did not achieve CR. These studies demonstrated that tandem auto-HSCT treatment was effective in treating relapse / refractory lymphoma and myeloma; however, no large-scale study of the use of tandem auto-HSCT for treatment of T-LBL has thus far been reported.

To evaluate the safety and efficacy of tandem auto-HSCT treatment for adult T-LBL patients, the clinical outcome of tandem auto-HSCT and single auto-HSCT treatment were further compared. As revealed by this study, there was no statistical difference in 1-year progression/relapse rates between tandem auto-HSCT treatment and single auto-HSCT treatment, but the 3-
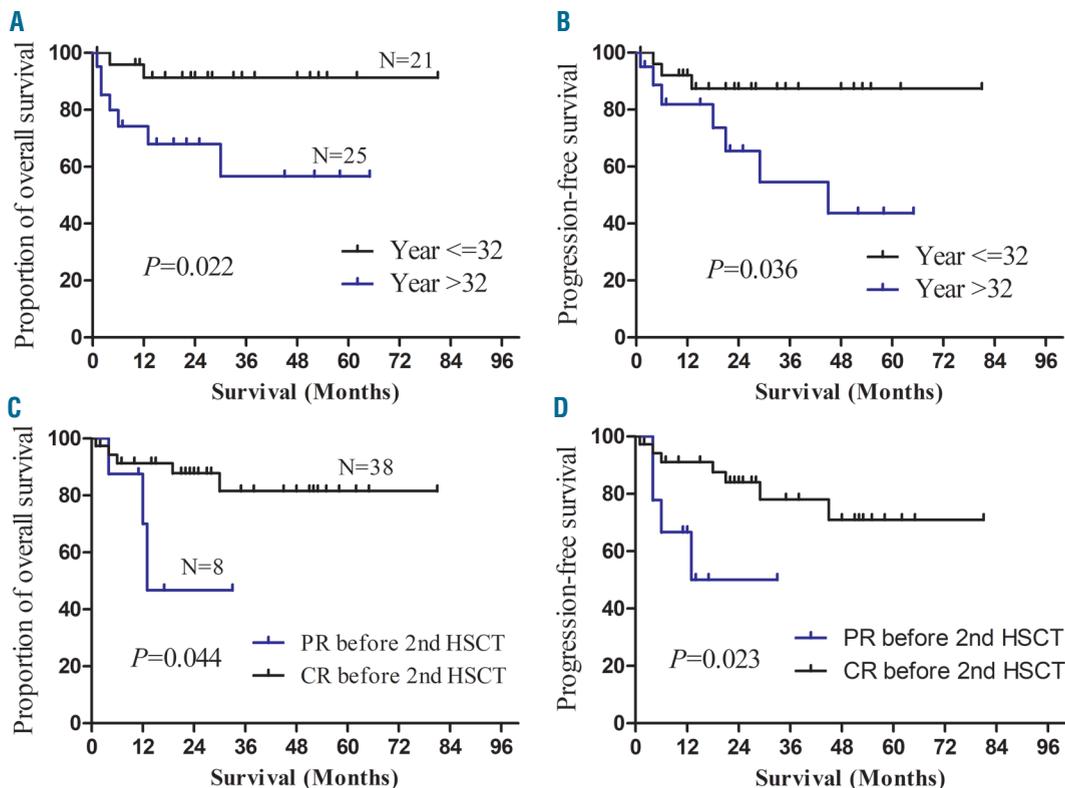

Figure 4. Overall survival (OS) and progression-free survival (PFS) of age and disease status after first hematopoietic stem cell transplantation (HSCT) in tandem autologous (auto)-HSCT group. Kaplan-Meier estimated OS and PFS of patients with tandem auto-HSCT stratified by year (A and B) and disease status after first auto-HSCT group (C and D). CR: complete remission; PR: partial response; N: number.

year progression/relapse rate of the tandem auto-HSCT group was significantly lower than that of the single auto-HSCT group. We attributed this to the fact that tandem auto-HSCT was much more effective, removing residual tumor cells in the body, thereby deepening remission status. Compared to the single auto-HSCT group and the chemotherapy group, the 3-year progression/relapse rate was significantly reduced in the tandem auto-HSCT group. The increase in survival rates might also be related to the comparable safety profiles of the single auto-HSCT and tandem auto-HSCT treatment regimens. As indicated by this study, there was no significant difference in the rates of the common complications between the two transplants, and the occurrence rate of gastrointestinal symptoms during the second transplant was significantly lower than that during the first. This might be due to the fact that the intensity of conditioning regimens during the second transplant was lower than that during the first. Two patients (2.1%) in the tandem auto-HSCT group died of transplant-related causes (intracranial hemorrhage and infectious shock, respectively), but the death rate was not higher than the rates of transplant-related death in treating lymphomas with auto-HSCT reported by other centers. Our results suggest, therefore, that the safety

level of tandem auto-HSCT treatment for adult T-LBL patients is acceptable.

Age is an important prognostic factor in T-LBL. For example, Hanne *et al.* used the combination of ALL-like high-dose chemotherapy and auto-HSCT to treat LBL patients. Median age of patients included in their study was 32 years, and the study found that the PFS and OS rates among patients <32 years of age were 54% and 69%, respectively; these rates were significantly higher than those among patients >32 years of age.[24] We also found that patient age influenced OS and PFS: patients <33 years of age had longer OS and PFS than patients >33 years of age. This might be because BM of younger patients is more resilient and can tolerate a high enough dose of chemotherapy to eliminate residual tumor cells and achieve deeper remission before transplant. This indicated that tandem auto-HSCT might represent a novel treatment method to achieve longer survival in young patients. The finding that disease status after the first transplant had an influence on OS and PFS, which was consistent with reports from other centers.[7] Multivariate Cox analysis showed that, among the factors influencing survival, only disease status after the first transplant had an influence on OS and PFS. This result was helpful in improving the induction treatment
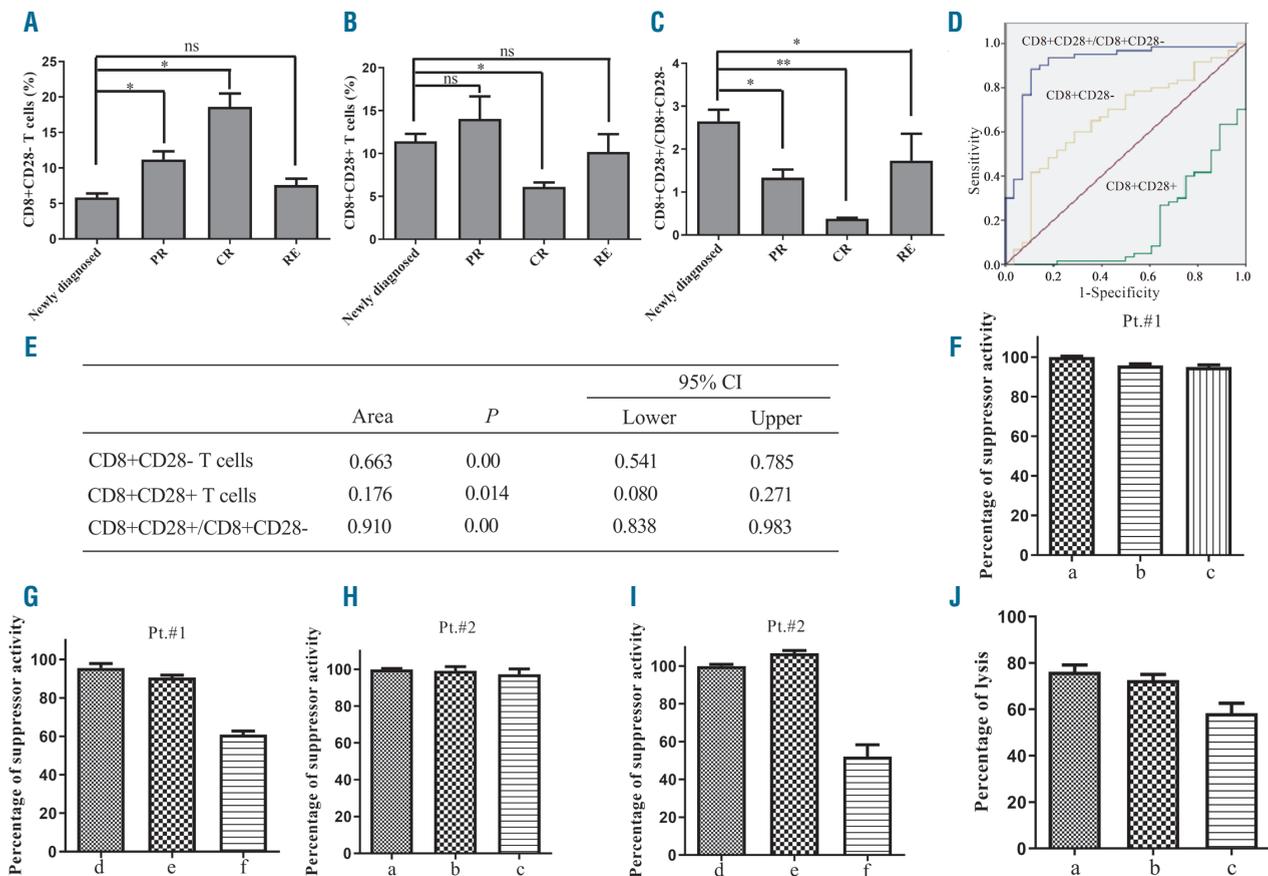

Figure 5. CD8⁺CD28⁺/CD8⁺CD28⁻ equilibrium could predict the disease status of T-cell lymphoblastic lymphoma patients. Average frequency of CD8⁺CD28⁺ T cells, CD8⁺CD28- T cells, and CD8⁺CD28⁺/CD8⁺CD28⁻ ratio in patients (A-C). Receiver operating characteristic curves of T cells and their balance in predicting the disease stage; diagonal lines in (D) are the diagnostic reference lines. (E) Area under curve (AUC) details of each subgroup. (F-I) Suppressive activities of CD8⁺CD28⁺ and CD8⁺CD28⁻ T cells were analyzed, (a and d) mononuclear cell proliferation plus anti-CD3 monoclonal antibodies in the presence of autologous irradiated monocytes, (b) as (a) plus freshly isolated CD8⁺CD28⁺ T cells, (c) as (a) plus freshly isolated CD8⁺CD28⁻ T cells, (d and e) as (b) plus IL-2 and granulocyte-macrophage colony-stimulating factor stimulation. (J) Cytotoxic function of antigen-specific cytotoxic T lymphocytes on target cells, (a) T2 cells plus p540 peptide, (b) as (a) plus stimulated CD8⁺CD28⁺ T cells, (c) as (a) plus stimulated CD8⁺CD28⁻ T cells. CR: complete remission; PR: partial response; RE: Pt: patient; CI: confidence intervals; N: number; ns: not significant.

regimen, and suggested that striving to achieve CR after the first transplant will be an important factor that will influence prognosis. OS and PFS of patients who were in PR after the first transplant was only 28.1%, which is clinically unsatisfactory. For these patients, it is recommended changing the treatment strategy, and allo-HSCT may be effective in improving their survival.

CD28 serves as co-stimulator for T-cell activation and survival, and is expressed on all naïve T cells in newborns.[25] However, as T cells experience antigen-mediated activation and differentiation, CD28 expression was gradually lost.[26] There is growing evidence that CD8+CD28+ T cells play a central role in inducing immune responses against tumor.[27] Evidence had been found that CD4+ and CD8+T cells in MCL nodes were higher, and higher peritumoral CD8+ T-cell count and CD4/CD8 ratio were correlated with longer OS;[28,29] however, there has been no research into the prognostic value of PB T-lymphocyte subset counts in T-LBL. Our research had found that peripheral CD8+CD28− T cells of newly diagnosed T-LBL was significantly lower than that of patients achieving CR or PR, but when these patients relapsed, the number of CD8+CD28− T cells would decrease again. Meanwhile, the ratio of CD8+CD28+/CD8+CD28− possess the opposite tendency for change: ROC curves confirmed that the balance of CD8+CD28+/CD8+CD28− has the best sensitivity and specificity to predict disease status. These results suggest this non-invasive testing approach could help the clinical decision-making process when selecting the most appropriate treatment.

In conclusion, compared to sequential chemotherapy, auto-HSCT could improve the OS and PFS of adult T-LBL patients. Importantly, a second auto-HSCT consolidation treatment after achieving CR through the first transplant was effective for young patients, and the safety level was comparable to patients receiving a single auto-HSCT. As far as we know, this study is the first prospective multi-center large-sample study of this treatment regimen for T-LBL. The results proved the effectiveness and safety of tandem auto-HSCT treatment for T-LBL patients, and confirmed that tandem auto-HSCT could improve OS and PFS rates in adult T-LBL.

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