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bjh research paper

Clinical significance of early T-cell precursor acute lymphoblastic leukaemia: results of the Tokyo Children's Cancer Study Group Study L99-15

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Summary

Early T-cell precursor acute lymphoblastic leukaemia (ETP-ALL) is a recently identified subtype of T-ALL with distinctive gene expression and cell marker profiles, poor response to chemotherapy and a very high risk of relapse. We determined the reliability of restricted panel of cell markers to identify EPT-ALL using a previously classified cohort. Then, we applied the cell marker profile that best discriminated ETP-ALL to a cohort of 91 patients with T-ALL enrolled in the Tokyo Children's Cancer Study Group L99-15 study, which included allogeneic stem cell transplantation (allo-SCT) for patients with poor prednisone response. Five of the 91 patients (5·5%) met the ETP-ALL criteria. There were no significant differences in presenting clinical features between these and the remaining 86 patients. Response to early remission induction therapy was inferior in ETP-ALL as compared with T-ALL. The ETP-ALL subgroup showed a significantly poorer event-free survival (4-year rate; 40%) than the T-ALL subgroup (70%, $P = 0·014$). Of note, three of four relapsed ETP-ALL patients survived after allo-SCT, indicating that allo-SCT can be effective for this drug-resistant subtype of T-ALL.

Keywords: acute lymphoblastic leukaemia, childhood, Early T-cell precursor, cell marker profile, allogeneic stem cell transplantation.

In approximately 15% of patients with childhood acute lymphoblastic leukaemia (ALL) leukaemic lymphoblasts have an immunophenotype corresponding to immature T cells (Pullen *et al*, 1999). Although the prognosis of childhood T-cell acute lymphoblastic leukaemia (T-ALL) has dramatically improved (Goldberg *et al*, 2003; Pui *et al*, 2009), patients with

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T-ALL continue to have an increased risk of relapse compared to those with B-precursor ALL (Pullen *et al*, 1999; Pui & Evans, 2006; Pui *et al*, 2008). In childhood B-precursor ALL patients, clinical presenting features (age and leucocyte count at diagnosis) and chromosomal translocations predict therapeutic outcome, and have been used for risk-specific adjustments in therapeutic intensity (Pui & Evans, 2006; Pui *et al*, 2008). Much effort has been put into identifying prognostically relevant clinical and biological features for childhood T-ALL (Schneider *et al*, 2000; Weng *et al*, 2004; Gottardo *et al*, 2007; Winter *et al*, 2007; Dalmazzo *et al*, 2009; Karrman *et al*, 2009; Attarbaschi *et al*, 2010; Cleaver *et al*, 2010; Zuurbier *et al*, 2010), but none is sufficiently discriminatory to be used for treatment stratification in contemporary protocols.

A recent study identified a distinct biological subtype of T-ALL, early T-cell precursor ALL (ETP-ALL) (Coustan-Smith *et al*, 2009), characterized by a gene expression profile recapitulating that of normal ETP cells, a subpopulation of thymocytes that retain multi-lineage differentiation potential (Bell & Bhandoola, 2008). ETP-ALL can be recognized by a distinctive cell surface antigen profile: lack of CD1a and CD8, weak CD5, and expression of one or more myeloid- or stem cell-related antigens. Notably, ETP-ALL was associated with an inferior clearance of leukaemia cells after the first phase of remission induction therapy and extremely poor event-free and overall survival in patients treated on intensified chemotherapeutic protocols both at the St Jude Children's Research Hospital and the Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP) (Coustan-Smith *et al*, 2009).

To verify the impact of prognostic significance of ETP-ALL, we studied patients with T-ALL in the Tokyo Children's Cancer Study Group (TCCSG) L99-15 study (Manabe *et al*, 2008). Because the cell marker panel used in this multicentre protocol did not include all of the markers required for diagnosis of ETP-ALL, we first establish a scoring system based on a more limited panel which could effectively differentiate ETP-ALL from T-ALL in a previously reported cohort (Coustan-Smith *et al*, 2009). Using this scoring system, we retrospectively identified patients with ETP-ALL in the TCCSG L99-15 study, and determined their presenting features, response to chemotherapy, and rates of relapse.

Materials and methods

Patients

Seven hundred and seventy patients (1–18 years of age) diagnosed with ALL were consecutively enrolled in the TCCSG L99-15 study from February 1999 to July 2003 (Manabe *et al*, 2008). The diagnosis of ALL was based on morphological, biochemical, and flow cytometric features of leukaemia cells. Flow cytometric analysis was performed in institutional or commercial laboratories, and the results were reviewed by members of the TCCSG diagnostic committee. Among 754 eligible ALL patients, 91 patients were diagnosed as T-ALL based on the expression of CD7 and at least one other T-cell marker on leukaemia cells with negative myeloperoxidase reaction (<3%); 90 patients were initially enrolled as T-ALL (Manabe *et al*, 2008) and one patient whose initial diagnosis was unclassified leukaemia was retrospectively diagnosed as T-ALL. The study was approved by the institutional review boards of the participating institutions or the equivalent organization with written informed consent from the parents or guardians of the patients.

For testing the usefulness of the scoring system, previously reported data of flow cytometric analyses of T-ALL patients from the St Jude Children's Research Hospital (St Jude cohort) were studied (Coustan-Smith *et al*, 2009). In the St Jude cohort, based on the findings of the flow cytometric analysis and gene expression profile, 17 patients were diagnosed as having ETP-ALL and 122 patients as having typical T-ALL (Coustan-Smith *et al*, 2009).

Treatment protocol

Details of the treatment regimen have been previously reported (Manabe *et al*, 2008). After 1 week oral administration of prednisolone (60 mg/m^2), patients were stratified into three treatment subgroups: those with $<0{\cdot}001 \times 10^9$ blasts/l in peripheral blood were categorized as intermediate risk (IR), those with $0{\cdot}001$–$0{\cdot}999 \times 10^9$ blasts/l as high risk (HR), and those with $\geq 1{\cdot}0 \times 10^9$ blasts/l were categorized as HR-SCT, and regarded as candidates for allogeneic stem cell transplantation (allo-SCT) in first remission. The determination of blasts in peripheral blood and bone marrow was done by microscopic evaluation at each institution. Patients in all three of the treatment subgroups underwent identical induction therapy composed of prednisolone, vincristine, cyclophosphamide, daunorubicin and asparaginase with triple intrathecal injection therapy. IR patients were randomized to receive high-dose cytarabine (2 g/m^2) or cytarabine (75 g/m^2) plus cyclophosphamide and 6-mercaptopurine in the post-remission induction intensification phase. HR and HR-SCT patients were treated with high-dose cytarabine in the post-remission induction intensification phase. Patients with an initial white blood cell (WBC) count $\geq 100 \times 10^9$ blasts/l in the IR and HR subgroups received prophylactic cranial irradiation (12 Gy for patients aged 1–6 years, and 18 Gy for patients aged 7 years and older). HR-SCT patients underwent allo-SCT in first remission; the recommended timing for SCT was after four or five courses of intensification therapy (corresponding to 7–8 months after diagnosis).

Statistical analysis

Clinical features of patients were compared using the chi-square test, and blast counts of bone marrow and peripheral blood were compared using the Mann–Whitney test. To compare cell surface antigen expression levels between patients with ETP-ALL and patients with typical T-ALL in

the St Jude cohort, we performed either the student's *t*-test or the *t*-test for unequal variances based on the *F* value for sample variances. The duration of event-free survival was defined as the time from the initiation of therapy to either treatment failure (relapse, death, or diagnosis of secondary cancer) or to the last day when the patient was confirmed to be in remission. The probabilities of event-free survival and overall survival were estimated by the Kaplan–Meier analysis, and were tested for significance using log-rank test. For univariate and multivariate analysis, the Cox proportional hazards model was employed to assess risk factors on even-free survival. *P* values <0·05 were considered statistically significant.

Results

Development of an ETP-ALL scoring system

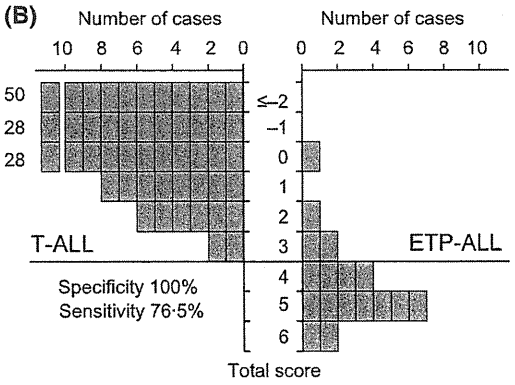
ETP-ALL shows a distinctive immature immunophenotype characterized by lack of CD1a and CD8 expression, weak CD5 expression with <75% positive blasts, and expression of one or more of the following myeloid or stem cell antigens on at least 25% of lymphoblasts: CD117, CD34, HLA-DR, CD13, CD33, CD11b and/or CD65 (Coustan-Smith *et al*, 2009). Among the T-ALL patients enrolled in the TCCSGL99-15 study, data for some of the markers were only available in a limited group of patients (e.g. CD1a and CD11b were available for 67% and 7%

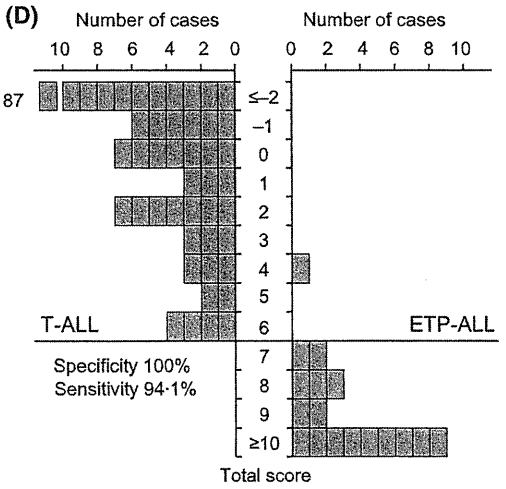
(A)

Scoring system based on 6 marker expression		
Score	–1	+1
CD5	≥5%	<75%
CD8	≥5%	<5%
CD13		≥25%
CD33		≥25%
CD34		≥25%
HLA-DR		≥25%

(C)

Scoring system based on 11 marker expression				
Score	–2	–1	+1	+2
CD5	≥75%			<75%
CD8	≥5%			<5%
CD13			≥25%	≥75%
CD33			≥25%	≥75%
CD34			≥25%	≥75%
HLA-DR			≥25%	≥75%
CD2		≥75%	<20%	
CD3		≥75%	<20%	
CD4		≥75%	<20%	
CD10		≥75%	<20%	
CD56			≥20%	





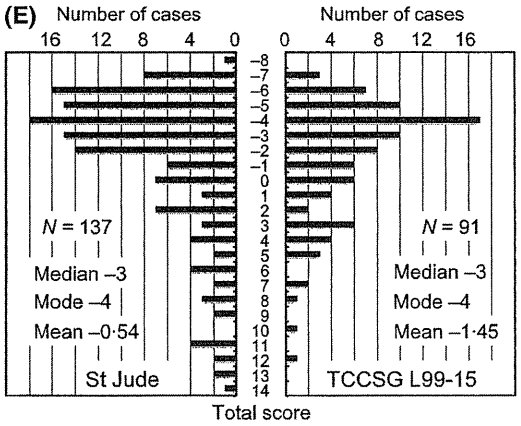


Fig 1. Establishment of a scoring system for immunophenotypical diagnosis of early T-cell precursor acute lymphoblastic leukaemia (ETP-ALL). (A) Scoring system based on the expression of six cell surface markers. (B) Distribution of total score of 6-marker expression in 17 ETP-ALL cases (right) and 122 T-ALL cases (left) of the St Jude cohort. (C) Scoring system based on the expression of 11 markers. (D) Distribution of total score of 11-marker expression in ETP-ALL patients (right) and T-ALL patients (left) of the St Jude cohort. (E) Distribution of total score of 11-marker expression in 139 T-ALL cases of the St Jude cohort (left) and 91 T-ALL cases of the TCCSG L99-15 study (right).

Table I. Profiling of cell surface marker expression of ETP-ALL patients.

Score	CD1a	CD2	CD3	CD4	CD5	CD7	CD8	CD10	CD13	CD33	CD34	CD56	HLA-DR	Others
12	NT	3·4	12·7	1·3	1·9	68·0	1·2	1·0	67·7	61·9	61·9	0·7	52·1	cyCD3$^+$
10	0·4	2·5	1·6	0·5	26·2	99·0	2·2	1·4	92·4	3·4	0·8	0·3	4·4	
8	0·5	93·8	1·7	NT	4·1	97·6	NT	0·5	93·5	6·2	99·2	7·4	37·2	CD11b$^+$
7	0·8	11·7	2·7	18·1	71·5	96·7	1·9	66·2	17·5	0·3	16·8	NT	1·3	
7	0·2	78·8	0·3	0·6	1·1	95·7	2·3	72·0	81·9	12·6	2·4	NT	3·2	

NT, not tested.

of patients respectively) or not available at all (CD65 and CD117). Thus, we devised a scoring system based on the expression of six markers; CD5, CD8, CD13, CD33, CD34 and HLA-DR (Fig 1A) and applied it to the St Jude cohort, which included 17 ETP-ALL and 122 non-ETP T-ALL patients (Coustan-Smith *et al*, 2009). As shown in Fig 1B, the total score for all of the 122 typical T-ALL cases was a maximum of three, while it was four or more in 13 of the 17 ETP-ALL cases. Thus, the specificity was 100%, and the sensitivity 77%.

We next focused on the expression levels of the other antigens that were originally not included in the definition of ETP-ALL. In the St Jude cohort (Coustan-Smith *et al*, 2009), the expression levels of CD2 ($P < 0·01$, *t*-test), sCD3 ($P < 0·01$), CD4 ($P < 0·01$), and CD10 ($P = 0·035$) were significantly lower in ETP-ALL than in typical T-ALL, whereas the expression level of CD56 was significantly higher ($P = 0·018$) in ETP-ALL than in typical T-ALL. Thus, we established a second scoring system by the combination of these five additional markers (CD2, sCD3, CD4, CD10 and CD56) with the six used in the first analysis (CD5, CD8, CD13, CD33, CD34 and HLA-DR) (Fig 1C). When we applied this scoring system to the St Jude cohort, the total score in typical T-ALL patients was always six and lower, while it was seven or more in 16 of the 17 ETP-ALL patients (Fig 1D); specificity and sensitivity were 100% and 94%, respectively.

Application of scoring system to TCCSG L99-15 study

We applied the scoring system that included the 11 markers to the TCCSG L99-15 cohort (Fig 1E). In the TCCSG L99-15 study, median and mode of total score were -3 and -4 respectively, identical to those in the St Jude cohort. Among 91 T-ALL cases of the TCCSG L99-15 study, 5 (5·5%) had a score ≥7. The cell surface antigen expression profile of these five patients is summarized in Table I. Four patients showed typical ETP-ALL immunophenotype with negative CD1a expression; the remaining patient had no CD1a expression data but their cells had an immunophenotype with the highest total score of 12. The 86 patients (94·5%) whose total score were 6 and lower (Fig 1E) were considered as having non-ETP T-ALL. Among these 86 patients, however, there were 13 whose immunophenotype showed marginal patterns with a score of 3–6 (Table SI).

Clinical features and early treatment response of ETP-ALL

Table II summarizes the patient characteristics of ETP-ALL ($n = 5$) and T-ALL ($n = 86$). Distributions of gender, higher initial WBC count, age, National Cancer Institute (NCI) risk group, mediastinal mass, French-American-British

Table II. Demographic characteristics of the patients.

		T-ALL	ETP-ALL	χ^2-test
		$N = 86$	$N = 5$	P
Sex	Male	67	2	0·089
	Female	19	3	
WBC	≥100 × 10^9/l	42 (48·8%)	1 (20%)	0·36
Age	≥10 Years old	39 (45·3%)	3 (60%)	0·66
NCI risk group	Standard	14	1	1·0
	High	72	4	
Mediastinal mass	Yes	51 (59·3%)	3 (60%)	1·0
FAB classification	L1	59	2	0·32
	L2	25	3	
CNS involvement	Yes	3 (3·5%)	0 (0%)	1·0
Treatment subgroup	IR	22	1	
	HR	33	0	
	HR-SCT	31	4	
	HR-SCT%	36·0%	80%	0·070
Remission failure	Yes	4/85 (4·7%)	0/5 (0%)	1·0
Relapse	BM	15	4	
	CNS	2	0	
	Thymus	1	0	
	BM + thymus	2	0	
	Unknown	2	0	
SCT	Yes	42/85 (49·4%)	5/5 (100%)	0·057
Status at SCT	CR1	28	2	
	CR2	3	2	
	CR3	1	0	
	Failure	1	0	
	Rel1	3	1	
	Rel2	1	0	
	Unknown	5	0	

WBC, white blood cell count; NCI, National Cancer Institute; FAB, French-American-British; CNS, central nervous system; IR, intermediate risk; HR, high risk; SCT, allogeneic stem-cell transplantation; BM, bone marrow; CR1, first remission; CR2, second remission; CR3, third remission; Rel1, first relapse; Rel2, second relapse.

classification, and central nervous system involvement were not significantly different between the two groups. Clinical features at diagnosis of 13 borderline patients were similar to those of remaining 73 T-ALL patients (data not shown). Karyotypic analysis showed that two patients with ETP-ALL had +4 abnormality, which was not observed in the remaining patients (data not shown). Blast counts in peripheral blood (Fig 2A) and bone marrow (Fig 2B) at diagnosis were not significantly different between the two groups.

Although peripheral blast counts after 1 week monotherapy with prednisolone (Fig 2C) were similar between patients with ETP-ALL and T-ALL, bone marrow blast counts on day 14 remission induction therapy (Fig 2D) and blast counts in peripheral blood on day 15 (Fig 2E) were higher in patients with EPT-ALL ($P = 0{\cdot}057$ and $P = 0{\cdot}004$ by Mann–Whitney test respectively). Interestingly, among the 13 phenotypically borderline cases, blast counts in peripheral blood on day 8 and those in bone marrow on day 14 were significantly higher than those in remaining T-ALL patients (Fig S1).

Treatment outcome of ETP-ALL

Induction failures were observed in four of the patients with T-ALL but in none of those with ETP-ALL (Table II). Relapse occurred in 22 of the 82 (26·8%) patients with T-ALL who achieve remission and in four of the five patients with ETP-ALL. Due to HR-SCT classification and/or relapse, allo-SCT was performed in 41 patients (49·4%) with T-ALL and all five patients with ETP-ALL. With a median follow-up of 5·3 years, the estimated 4-year rate of event-free survival (Fig 3A) was 70·9% [95% confidence interval (CI), 61·1–80·7] for patients with T-ALL as compared to 40·0% (95% CI, 0–82·9) for those with ETP-ALL ($P = 0{\cdot}014$ by log-rank test). In a univariate analysis, ETP-ALL was a significant adverse risk factor for relapse ($P = 0{\cdot}048$). In a multivariate analysis including EPT-ALL, responses to prednisolone, NCI risk group, therapeutic subgroup, and gender as category terms, ETP-ALL was significant risk factor for relapse ($P = 0{\cdot}014$). Among the five patients with ETP-ALL (Table III), three patients who relapsed

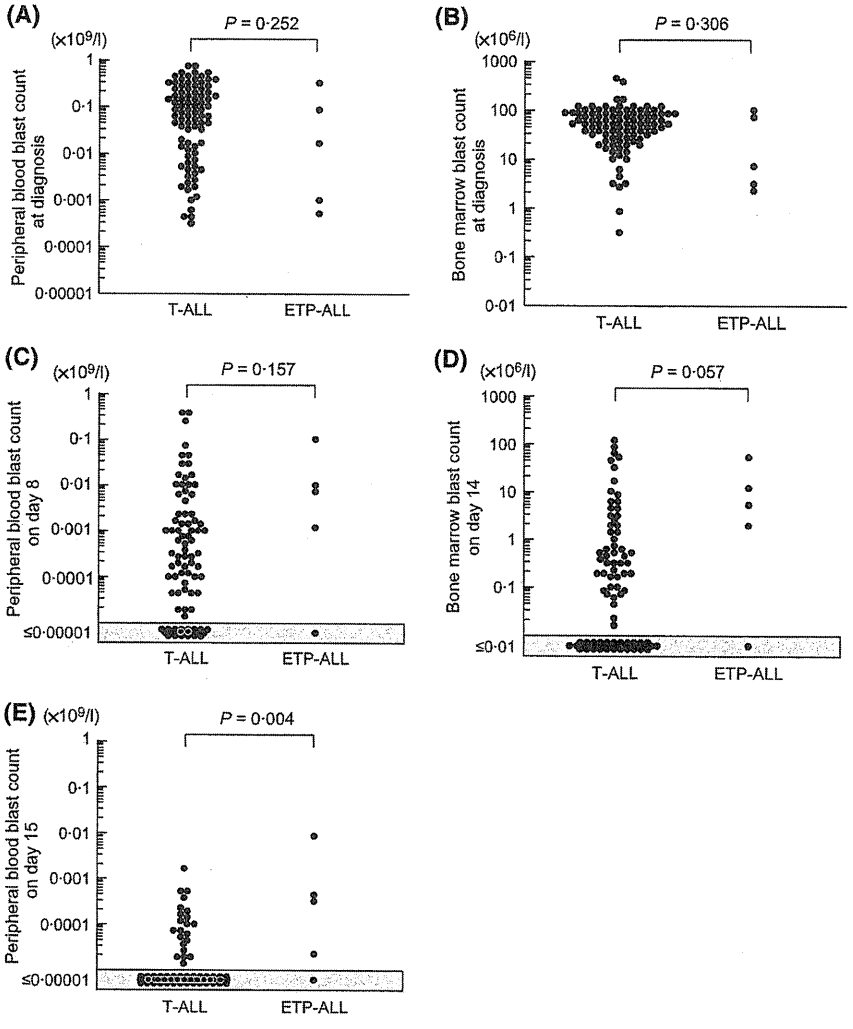


Fig 2. Comparison of blast counts in the patients having ETP-ALL with those in the patients having T-ALL. Blast counts in (A) peripheral blood at diagnosis, (B) bone marrow at diagnosis, (C) peripheral blood on day 8, (D) bone marrow on day 14, and (E) peripheral blood on day 15 were compared by Mann–Whitney analysis, and each *P* value is indicated at the top of figures.

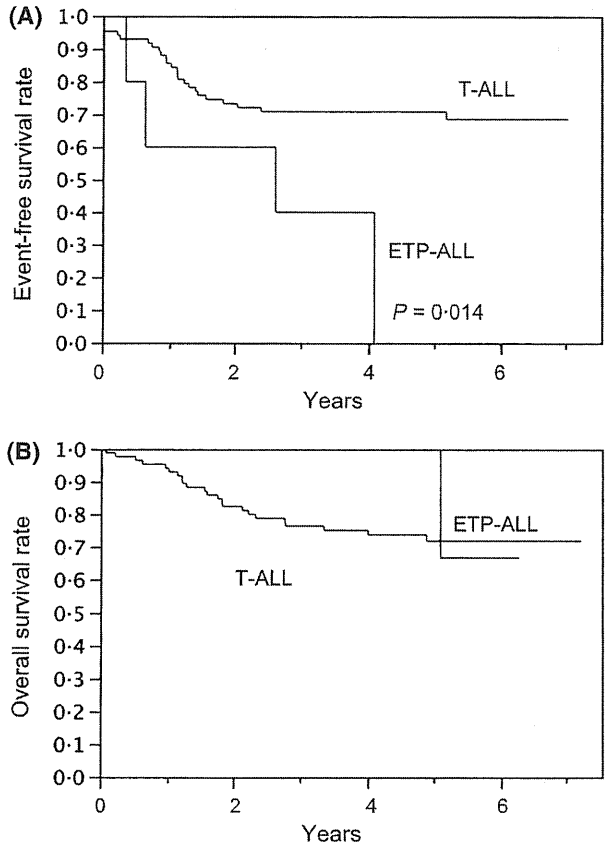


Fig 3. Kaplan–Meier plots of (A) event-free survival and (B) overall survival of the patients with ETP-ALL and T-ALL patients.

3, 7 and 48 months from diagnosis remained in second remission for 64, 67 and 8 months after allo-SCT. Thus, four of the five patients with ETP-ALL are alive in first (one patient) or second remission (three patients) (Fig 3B). Due to poor prednisolone response, 8 of the 13 borderline patients (62%) underwent allo-SCT in first remission at 6–9 months (median 7·5 months) after diagnosis, and all eight patients are alive in first remission (Table SII). As a result, the event-free survival of 13 borderline patients was similar (76·9%; 95% CI, 54·0–99·8) to that of the remaining T-ALL patients and significantly better than that of bona fide ETP-ALL patients ($P = 0·031$, log-rank test).

Discussion

As cure rates for children with ALL approach 90%, it has become ever more important to identify small subgroup of patients who are resistant to modern intensive chemotherapy. Among patients with T-ALL, reliable prognostic indicators have been lacking (Pui & Evans, 2006; Pui *et al*, 2008). Reportedly, however, patients with ETP-ALL have a particularly poor response to chemotherapy (Coustan-Smith *et al*, 2009) suggesting that alternative treatment approaches are needed for this leukaemia subtype. In the present study, we sought to determine the prevalence of ETP-ALL among patients with T-ALL enrolled in our TCCSG L99-15 study and assess their treatment outcome. Because of the limited panel of markers tested at diagnosis, we first devised a scoring system that allowed the identification of ETP-ALL among a previously reported cohort with 100% specificity and 94% sensitivity. The five patients with ETP-ALL identified among those enrolled in the TCCSG L99-15 study had a significantly poorer response to initial therapy as indicated by higher blast counts of peripheral blood at day 15, consistent with the previous findings of higher minimal residual disease levels observed among patients with ETP-ALL in both St Jude and AIEOP cohorts (Coustan-Smith *et al*, 2009). The event-free survival of ETP-ALL patients was significantly inferior in comparison with that of T-ALL patients. Four of the five patients with ETP-ALL enrolled in our study relapsed, confirming the dismal response to therapy of this T-ALL subtype. However, three of these four patients are alive in second remission after receiving allo-SCT, suggesting that allo-SCT should be considered as a frontline therapy for patients with ETP-ALL in first remission.

The prevalence of ETP-ALL in our study (5·5%) was lower than that determined in the St Jude's (12·2%) and AIEOP (13·0%) cohorts (Coustan-Smith *et al*, 2009). One possibility is that some cases with ETP-ALL may have been misclassified as typical T-ALL owing to the limited panel of markers used. Thus, we examined the borderline cases where patient immunophenotype showed marginal patterns, and found that

Table III. Clinical features of stem-cell transplantation in patients with ETP-ALL.

	SCT				Relapse		Final outcome		
Score	Status	Donor	Source	Time from diagnosis (months)	Site	Time from diagnosis (months)	Status	Survival	Time from diagnosis (months)
12	CR2	Unrelated	CB	6	BM	3	CR2	Alive	70
10	CR1	Sibling	BM	8	BM	31	Rel3	Dead	58
8	CR1	Unrelated	BM	8	No		CR1	Alive	31
7	Rel1	Sibling	PBSC	8	BM	7	CR2	Alive	75
7	CR2	Unrelated	BM	53	BM	48	CR2	Alive	61

SCT, allogeneic stem cell transplantation; CB, cord blood cell; BM, bone marrow; PBSC, peripheral blood stem cell; CR1, first remission; CR2, second remission; Rel1, first relapse; Rel3, third relapse.

almost two thirds of these borderline patients underwent allo-SCT early in first remission because of poor responses to prednisolone and early phase of induction therapy. Of note, all of these patients are alive in first remission, and the resultant event-free survival of the borderline patients was significantly better than that of bona fide ETP-ALL patients. These observations suggest that allo-SCT improves final outcome of the borderline subgroup even if some ETP-ALL patients are included. This seems to be consistent with the previous findings by the Berlin-Frankfürt-Münster group, which reported that allo-SCT was superior to chemotherapy alone in high-risk childhood T-ALL (Schrauder *et al*, 2006). Another possible explanation is that some cases may have been classified as acute myeloid leukaemia because of the expression of multiple myeloid markers and therefore were not enrolled in TCCSG L99-15. The possibility of differences in prevalence due to the different ancestry of the various cohorts should also be considered.

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Authorship

T.I., N.K. and D.C. analysed data and wrote the paper. E.C., A.K., M.K. and H.T. analysed data. K.K., A.M., M.K., K.I., Y.H., M.T. and K.S. designed the research study. A.O. designed the research study, analysed data, and wrote the paper.

Conflict of Interest

These authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig S1. Comparison of blast counts between borderline patients and remaining T-ALL patients.

Table SI. Profiling of cell surface marker expression of borderline patients.

Table SII. Clinical features of stem-cell transplantation in borderline patients.

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The Utility of Performing the Initial Lumbar Puncture on Day 8 in Remission Induction Therapy for Childhood Acute Lymphoblastic Leukemia: TCCSG L99-15 Study

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Background. Traumatic lumbar puncture with leukemic blasts (TLP+), which has been reported to occur 5–10%, in the previous studies, adversely affects the outcome of children with acute lymphoblastic leukemia (ALL). Based on the results from our previous study, we deferred the initial lumbar puncture until day 8 in remission induction therapy in order to reduce the frequency of cases with TLP+. ***Procedure.*** The study was conducted as a prospective cohort study within the Tokyo Children's Cancer Study Group (TCCSG) L99-15 study. Between April 1999 and June 2003, 754 children with newly diagnosed ALL enrolled. The patients received the initial intrathecal chemotherapy after 7 days of prednisolone treatment. The incidence of central nervous system (CNS)-positive (the presence of leukemic blasts in cerebrospinal fluid or cranial nerve palsy) including TLP+ cases and cumulative incidence of CNS relapse were examined. ***Results.*** The incidence of CNS-positive and TLP+ was 2.9% (n = 22) and 0.8% (n = 6), respectively. These incidences were much lower than those in the representative study groups employing the initial IT on day 1. Of 22 patients with CNS-positive, only one patient relapsed in CNS, whereas 22 of the remaining CNS-negative 723 patients suffered from CNS relapse. Overall, event-free survival at 4 year was 78.2 ± 1.6%. Four-year cumulative incidence of any CNS relapse was 3.3 ± 0.7%, which improved from our previous study in spite of limiting the use of cranial irradiation. ***Conclusions.*** Our strategy reduced the frequency of CNS-positive patients who required reinforcement of CNS-directed therapy without compromising overall outcome. Pediatr Blood Cancer 2012; 58:23–30. © 2011 Wiley Periodicals, Inc.

Key words: acute lymphoblastic leukemia (ALL); central nervous system (CNS) relapse; chemotherapy; chemotherapy neurotoxicities

INTRODUCTION

The long-term cure rate in childhood acute lymphoblastic leukemia (ALL) is as high as 80% [1,2], and this is largely attributable to sufficient central nervous system (CNS)-directed therapy, such as prophylactic cranial irradiation (pCRT) and intrathecal chemotherapy (IT) [3]. However, administering CNS-directed therapy always results in a dilemma between sufficient intensity and treatment-related toxicity. Especially, the use of pCRT should be avoided because of its substantial risk for late complications such as second malignancies, endocrinopathies, neurocognitive dysfunctions, and neurotoxic effects [4–6]. On the other hand, CNS relapse still occurs in 2–5% of children with ALL and remains to be a treatment obstacle [7]. Therefore, proper assessment of risk factors for CNS relapse is required.

Risk factors for CNS relapse include a T-cell immunophenotype, hyperleukocytosis, specific genetic alterations such as *t*(9;22) and *t*(4;11), and the presence of CNS involvement [7]. The prognostic significance of the presence of leukemic blasts in the cerebrospinal fluid (CSF) without pleocytosis, referred to as CNS-2, has varied in clinical trials and is heavily dependent on the effectiveness of systemic and CNS-directed therapy [8–11]. By contrast, traumatic lumbar puncture with leukemic blasts (TLP+) adversely affects the outcome of patients with ALL as well as overt CNS leukemia [2,9–11]. Though controversy over whether TLP+ is an iatrogenic event or intrinsically inevitable status still exists, clinicians should pay attention to keep the rate of TLP+ to a minimum.

To overcome the problem concerning TLP+, the Tokyo Children's Cancer Study Group (TCCSG) adopted a strategy to postpone the initial lumbar puncture (LP) and IT until 7 days after prednisolone (PSL) monotherapy in the L89-12 study in order to decrease circulating leukemic blasts substantially before the initial LP/IT [12]. This strategy yielded a significant reduction of TLP+ [12], but the net effect on the outcome was not clarified because over 80% of the patients received cranial irradiation in the L89-12 study [12,13]. We hypothesized that deferring the initial LP/IT until day 8 in remission induction therapy could reduce the frequency of cases with TLP+ without compromising outcome, even if the indication for pCRT is restricted. Here, we report the results of the L99-15 study adopting the day 8 LP/IT strategy and restricting the indication for pCRT.

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METHODS

Study Design

The study was conducted as a prospective cohort study within the TCCSG L99-15 randomized clinical trial compared with the historical controls.

Patients

From April 1999 to June 2003, 770 children aged between 1 and 18 years who were newly diagnosed with ALL were consecutively enrolled on the TCCSG L99-15 study. Sixteen patients were excluded because of the following reasons; fatal complication before start of treatment (n = 8; intracranial hemorrhage 6, respiratory failure 1, renal failure 1), and lack of data (n = 8). Finally, 754 patients were evaluable for analysis. The trial was approved by the institutional review boards of all participating institutions. Written informed consent was obtained from parents or guardians, and from the patients when appropriate from their age and understanding.

Diagnosis

The diagnosis of ALL was based on morphological, biochemical, and immunophenotypical features of leukemic blasts, including leukemic blast morphology on May- or Wright- Giemsa-stained bone marrow (BM) smears, negative staining for myeloperoxidase, and reactivity with monoclonal antibodies to B- or T-lineage-associated lymphoid differentiation antigens, as described previously [14]. Remission was defined as the presence of fewer than 5% leukemic blasts determined by morphology.

CNS status was evaluated by reviewing morphology of CSF cytospin preparation at each institution. If more than or equal to 5 leukocytes/μL were counted and leukemic blasts were identified in the CSF, CNS-3 was assigned. If leukemic blasts were detected with fewer CSF leukocytes than 5/μL, the CNS status was defined as CNS-2. If CSF was not traumatic and leukemic blasts were not identified in the CSF specimen, CNS-1 was assigned. If more than 10 erythrocytes/μL were present in the CSF, the CNS status was defined as traumatic lumbar puncture (TLP), which was further classified into 2 groups based on the presence of leukemic blasts in the CSF, TLP+, and TLP−. If symptoms due to intracranial infiltrates, such as cranial nerve palsy, were present in spite of normal CSF findings, the CNS status was defined as CNS-1s. In this analysis, we regrouped patients with CNS-1s, CNS-2, CNS-3, and TLP+ into a CNS-positive group, whereas patients with CNS-1 or TLP− were regrouped into a CNS-negative group.

Risk Classification

The patients were stratified into 3 provisional risk groups at the time of diagnosis according to initial leukocyte counts and age at diagnosis (Initial SR: less than 20×10^9 leukocytes/L and age between 1 and 6 years. Initial IR: neither SR nor HR criteria were met. Initial HR: 50×10^9 leukocytes/L or more and age 10 years or older, or 100×10^9 leukocytes/L regardless of age). Thereafter, the patients were reclassified based on immunophenotype, cytogenetics, and treatment response. Sensitivity to PSL was determined after 7-day monotherapy of PSL. The presence of 1,000 leukemic blasts/μL or more in peripheral blood (PB) on day 8 was defined as PSL poor-responder (PPR), fewer than 1,000 leukemic blasts/μL as PSL good-responder (PGR) [12]. Of those who were determined as PGR, we further defined patients with no detectable leukemic blasts in PB on day 8 as Day8NoBlast [15]. Finally, the definition of SR was non-T cell immunophenotype, initial SR, no PPR, and no HR criteria. Those in SR who harbored *t*(1;19) (E2A-PBX1) were moved up to IR. Those under either of the following were included in IR: initial SR and PPR; initial IR and no PPR; initial HR and Day8NoBlast; and T-cell immunophenotype and Day8NoBlast. Similarly, HR included the following: initial IR and PPR; initial HR and 1–999 leukemic blasts/μL in PB; T-cell immunophenotype and 1–999 leukemic blasts/μL in PB; and CNS leukemia. Patients who did not achieve remission between the 43rd and 50th day after the initiation of remission induction therapy, those with the Philadelphia chromosome (BCR-ABL) or 11q23 (MLL) rearrangements, and PPR and initial HR or T-cell immunophenotype were allocated to the HR and underwent allogeneic stem cell transplantation (n = 57).

Treatment

The outline of the treatment regimen in each risk group is shown in Supplemental Figure 1 and the details were previously described [15]. The initial LP for CNS evaluation was performed on day 8 of remission induction treatment after 7-day monotherapy of PSL concurrently with IT chemotherapy; double IT consisting of methotrexate (MTX) and hydrocortisone (HDC) in SR, triple IT consisting of MTX, HDC, and cytarabine in IR and HR. The use of anesthesia and the criteria for platelet transfusion were determined by each treating physician. According to the CNS status, CNS-directed therapy was modified as follows: patients with CNS-2 received additional doses of triple IT; patients with CNS-1s or CNS-3 received additional doses of triple IT and therapeutic CRT with 18 Gy. Patients with TLP regardless of the presence of leukemic blasts in CSF did not receive any reinforcement of CNS directed therapy. The number of IT chemotherapy was 11 for SR patients, ranged from 9 to 10 for IR patients, and ranged from 17 to 19 for HR patients. Patients whose initial white blood cell (WBC) count exceeded 100×10^9/L were allocated to undergo pCRT. In total, 65 patients (8.6%) received pCRT. The dose of pCRT was 12 Gy for patients aged 1–6, and 18 Gy for patients aged 7 or older. The median follow-up period of the patients was 5.1 years.

Statistical Analysis

We performed a cohort study. Prior data indicated that the incidence of CNS-positive and TLP+ among the historical controls was 0.10–0.20 and 0.05–0.07, respectively. If the true failure rates for experimental subjects was 0.05 and 0.01 according to the previous L89-12 study, we would need to study 581 and 222 cohort subjects to be able to reject the null hypothesis that the failure rates for experimental and control subjects were equal with probability (power) 0.9. The Type I error probability associated with this test of this null hypothesis was 0.05. We used an uncorrected chi-squared statistic to evaluate this null hypothesis. The number of patients who enrolled onto the TCCSG 99-15 study was enough to evaluate our hypothesis. The duration of event-free survival (EFS) was defined as the time from the initiation of therapy to either treatment failure (relapse, death from any cause, or diagnosis of secondary cancer) or to the day of last follow-up when the patient was confirmed to be in remission.

Patients who did not attain a complete remission (CR) after the first induction phase or who died before the confirmation of remission were considered to have failed at day 0. Overall survival (OS) was defined as the time from initiation of therapy to death from any cause or the time of the last follow-up. The probability of EFS and OS was estimated by the Kaplan–Meier method, and was tested for significance using the log-rank test. The Cox proportional hazards model was employed to assess independent effects of risk factors on EFS. An isolated CNS relapse was defined as one without simultaneous relapse at another site, while a combined CNS relapse was one accompanied by relapse in the BM or any other extramedullary sites. Cumulative incidence of isolated and combined CNS relapse was estimated by the Kaplan–Meier method for patients who achieved CR. Differences in the distribution of individual parameters among patient subsets were analyzed using the chi-square test for categorized variables and the Mann–Whitney U test for continuous variables. All calculations were performed by PC-SAS (SAS Institute Inc., PC-SAS, version 8, 2000, Cary, NC).

RESULTS

Patient Characteristics

Table I shows the demographic characteristics of the 754 patients. Most of these characteristics (gender, initial WBC count, immunophenotype) were similar to those in our previous studies and in other study groups [1,2,16]. However, the proportion of PPR, 15.0% in the L99-15, was higher than 8.6% in the recent trial from the Berlin-Frankfurt-Munster (BFM) group [1]. The proportion of patients with each CNS status was as follows; CNS-1 88.7%, CNS-1s 0.5%, CNS-2 1.1%, CNS-3 0.5%, TLP+ 0.8%, and TLP− 7.2%. CNS status was not determined because of lack of data in 9 patients (1.2%). The CNS-positive group, which accounted for 2.9% of all the patients, bore significantly more unfavorable features such as older age, higher WBC count, higher frequency of poor risk cytogenetics (BCR-ABL and MLL rearrangement), lower frequency of good risk cytogenetics (high hyperdiploid and TEL-AML1), and higher proportion of PPR (Table II). The CNS-positive group tended to have high frequency of E2A-PBX1; however, this difference did not reach statistical significance. The distributions of gender and frequency of T-ALL were equal between the CNS-positive and CNS-negative group.

Treatment Outcome

Of the 754 patients, 737 patients (97.7%) entered complete remission. Four-year EFS and OS were 78.2 ± 1.6% and 87.6 ± 1.2%, and the cumulative incidence of any CNS relapse was 3.3 ± 0.7% (Fig. 1). Of the 22 patients in the CNS-positive group, only one patient (4.5%) with CNS-2 had CNS relapse (BM-

TABLE I. Patient Characteristics According to CNS Status Groups in the TCCSG L99-15 Study

	Total n (%)	CNS-1, n (%)	CNS-1s, n (%)	CNS-2, n (%)	CNS-3, n (%)	TLP+, n (%)	TLP−, n (%)	ND, n (%)
Overall	754	669 (88.7)	4 (0.5)	8 (1.1)	4 (0.5)	6 (0.8)	54 (7.2)	9 (1.2)
Immunophenotype								
B lineage	664 (88.1)	587 (88.4)	4 (0.6)	7 (1.1)	3 (0.5)	5 (0.8)	50 (7.5)	8 (1.2)
T	90 (11.9)	82 (91.1)	0 (0.0)	1 (1.1)	1 (1.1)	1 (1.1)	4 (4.4)	1 (1.1)
Gender								
Boy	428 (56.8)	382 (89.3)	3 (0.7)	6 (1.4)	1 (0.2)	3 (0.7)	29 (6.8)	4 (0.9)
Girl	326 (43.2)	287 (88.0)	1 (0.3)	2 (0.6)	3 (0.9)	3 (0.9)	25 (7.7)	5 (1.5)
Age (years)								
1–10	615 (81.6)	545 (88.6)	2 (0.3)	5 (0.8)	3 (0.5)	4 (0.7)	47 (7.6)	9 (1.5)
10 or older	139 (18.4)	124 (89.2)	2 (1.4)	3 (2.2)	1 (0.7)	2 (1.4)	7 (5.0)	0 (0.0)
WBC (/L)								
$<100 \times 10^9$	660 (87.5)	589 (89.2)	4 (0.6)	6 (0.9)	1 (0.2)	5 (0.8)	47 (7.1)	8 (1.2)
100×10^9 or more	94 (12.5)	80 (85.1)	0 (0.0)	2 (2.1)	3 (3.2)	1 (1.1)	7 (7.4)	1 (1.1)
Cytogenetic								
BCR-ABL	17 (2.3)	13 (76.5)	0 (0.0)	2 (11.8)	1 (5.9)	1 (5.9)	0 (0.0)	0 (0.0)
MLL rearrangement	17 (2.3)	13 (76.5)	0 (0.0)	1 (5.9)	1 (5.9)	0 (0.0)	2 (11.8)	0 (0.0)
E2A-PBX1	38 (5.0)	33 (86.8)	0 (0.0)	1 (2.6)	0 (0.0)	2 (5.3)	2 (5.3)	0 (0.0)
TEL-AML1	78 (10.3)	70 (89.7)	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	6 (7.7)	1 (1.3)
High hyperdiploid	182 (24.1)	166 (91.2)	0 (0.0)	2 (1.1)	0 (0.0)	0 (0.0)	13 (7.1)	1 (0.5)
Pseudodiploid	75 (9.9)	66 (88.0)	0 (0.0)	1 (1.3)	0 (0.0)	1 (1.3)	6 (8.0)	1 (1.3)
High hypodiploid	30 (4.0)	23 (76.7)	0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)	6 (20.0)	0 (0.0)
Normal	247 (32.8)	224 (90.7)	3 (1.2)	0 (0.0)	2 (0.8)	1 (0.4)	12 (4.9)	5 (2.0)
Day 8 blast (/μL)								
Zero	249 (33.0)	225 (90.4)	3 (1.2)	0 (0.0)	1 (0.4)	1 (0.4)	15 (6.0)	4 (1.6)
1–999	392 (52.0)	347 (88.5)	0 (0.0)	5 (1.3)	2 (0.5)	2 (0.5)	33 (8.4)	3 (0.8)
PGR (0–999)	641 (85.0)	572 (89.2)	3 (0.5)	5 (0.8)	3 (0.5)	3 (0.5)	48 (7.5)	7 (1.1)
PPR (1,000 or more)	113 (15.0)	97 (85.8)	1 (0.9)	3 (2.7)	1 (0.9)	3 (2.7)	6 (5.3)	2 (1.8)
Risk group								
SR	262 (34.7)	234 (89.3)	0 (0.0)	3 (1.1)	0 (0.0)	0 (0.0)	20 (7.6)	5 (1.9)
IR	313 (41.5)	282 (90.1)	1 (0.3)	1 (0.3)	1 (0.3)	3 (1.0)	22 (7.0)	3 (1.0)
HR	179 (23.7)	153 (85.5)	3 (1.7)	4 (2.2)	3 (1.7)	3 (1.7)	12 (6.7)	1 (0.6)

TABLE II. Relationship Between Patient Characteristics and CNS Status

	CNS-positive group, n (%)	CNS-negative group, n (%)	*P* value
Overall	22 (2.9)	723 (95.9)	
Immunophenotype			
B lineage	19 (2.9)	637 (95.9)	0.80
T	3 (3.3)	86 (95.6)	
Gender			
Boy	13 (3.0)	411 (96.0)	0.83
Girl	9 (2.8)	312 (95.7)	
Age (years)			
1–10	14 (2.3)	592 (96.3)	0.03
10 or older	8 (5.8)	131 (94.2)	
WBC (/L)			
$<100 \times 10^9$	16 (2.4)	636 (96.4)	0.03
100×10^9 or more	6 (6.4)	87 (92.6)	
Cytogenetics			
BCR-ABL	4 (23.5)	13 (76.5)	*
MLL rearrangement	2 (11.8)	15 (88.2)	
E2A-PBX1	3 (7.9)	35 (92.1)	
TEL-AML1	1 (1.3)	76 (97.4)	
High hyperdiploid	2 (1.1)	179 (98.4)	
Pseudodiploid	2 (2.7)	72 (96.0)	
High hypodiploid	1 (3.3)	29 (96.7)	
Normal	6 (2.4)	236 (95.5)	
Day 8 blast (/μL)			
Zero	5 (2.0)	240 (96.4)	0.02
1–999	9 (2.3)	380 (96.9)	
PGR (0–999)	14 (2.2)	620 (96.7)	
PPR (1,000 or more)	8 (7.1)	103 (91.2)	
Risk group			
SR	3 (1.1)	254 (96.9)	<0.01
IR	6 (1.9)	304 (97.1)	
HR	13 (7.3)	165 (92.2)	

*Poor cytogenetics (BCR-ABL and MLL rearrangement) versus others: $P < 0.01$, E2A-PBX1 versus others: $P = 0.06$, favorable cytogenetics (high hyperdiploid and TEL-AML1) versus others: $P = 0.03$.

combined) 1,709 days after diagnosis, whereas 22 of the 723 patients (3.0%) in the CNS-negative group had CNS relapse (isolated 12, BM-combined 10). The CNS-positive group showed an inferior outcome compared with the CNS-negative group (4-year EFS was 68.2 ± 9.9% and 78.2 ± 1.6%, respectively; $P = 0.09$) (Fig. 2A). The 4-year cumulative incidence of any CNS relapse was not different between the CNS-positive and CNS-negative group: 0% and 3.4 ± 0.7% ($P = 0.59$), respectively (Fig. 2B).

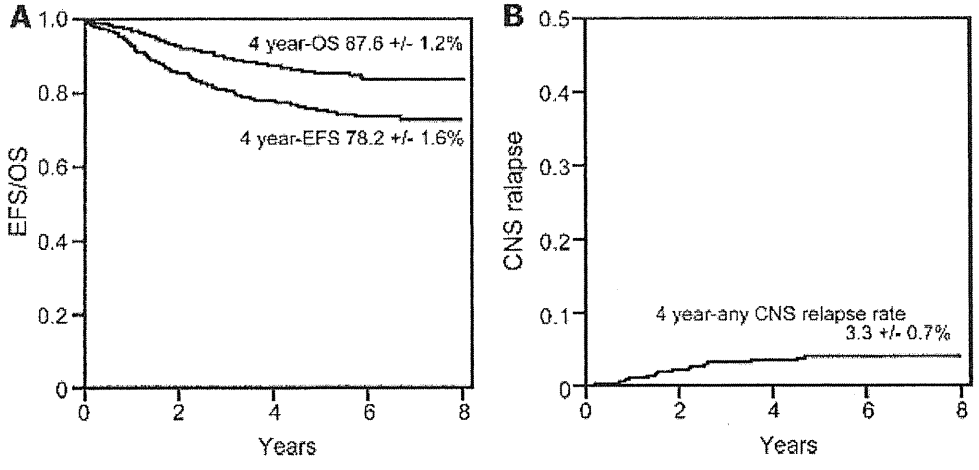

Fig. 1. (**A**) Kaplan–Meier estimate of overall survival, event-free survival of all evaluable patients (n = 754). (**B**) Cumulative incidence of all CNS relapses of patients after attaining first complete remission (n = 737).

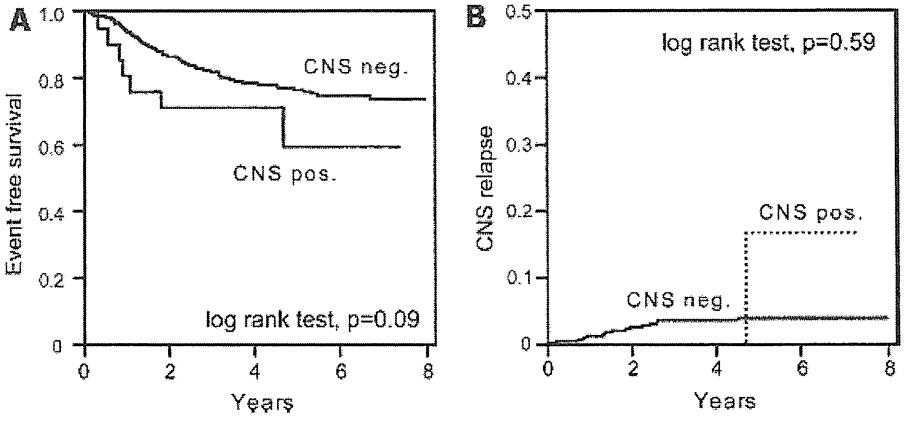

Fig. 2. The comparison of outcome between the CNS-positive (n = 22) and CNS-negative group (n = 723). (**A**) Event-free survival in patients in the CNS-positive group tended to be worse than that in patients in the CNS-negative group, 4-year EFS was 68.2 ± 9.9% and 78.2 ± 1.6%, respectively ($P = 0.09$). (**B**) The 4-year cumulative incidence of all CNS relapses was not different between the CNS-positive and CNS-negative group, 0% and 3.4 ± 0.7% ($P = 0.59$). One of the 22 patients in the CNS-positive group relapsed both in bone marrow and CNS 1,709 days after diagnosis, whereas 22 of 723 patients in the CNS-negative group had CNS relapse (isolated 12, BM-combined 10).

Comparison of the Treatment Results With Other Studies

Table III shows comparisons of the proportion of initial CNS status, EFS rate, and CNS relapse rate between the TCCSG studies [12] adopting day 8 IT strategy and the representative study groups employing the initial IT on day 1; the BFM group [10] and the St. Jude Children's Research Hospital (SJCRH) [2,17]. The initial LP/IT done on day 8 in remission induction therapy resulted in the decrease in the proportion of CNS-2, CNS-3, and TLP+; however, the frequency of CNS-1s and TLP− was almost the same between BFM-95 and TCCSG studies. EFS and CNS relapse rate of the L99-15 study (78.2% and 3.3%) improved from the L89-12 study (68.7% and 5.1%) in spite of limiting the indication of pCRT (8.6% and 80%, respectively) [12,18].

TABLE III. Comparison of Treatment Results With Other Groups

	TCCSG		BFM	SJCRH	
	99-15	89-12[12]	BFM-95[10]	Total XIIIA[17]	Total XV[2]
	Apr 1999–Jun 2003, n (%)	Jun 1989–Aug 1992, n (%)	Apr 1995–Jun 1999, n (%)	Dec 1991–Aug 1994, n (%)	Jun 2000–Oct 2007, n (%)
Total	754	418	2,021	165	498
CNS status					
CNS-1	669 (88.7)	324 (77.5)	1,605 (79.4)	101 (61.2)	359 (72.1)
CNS-1s	4 (0.5)	3 (0.7)	6 (0.3)	NA	NA
CNS-2	8 (1.1)	2 (0.5)	103 (5.1)	42 (25.5)	102 (20.5)
CNS-3	4 (0.5)	1 (0.2)	52 (2.6)	6 (3.6)	9 (1.8)
TLP+	6 (0.8)	2 (0.5)	135 (6.7)	16 (9.7)	28 (5.6)
TLP−	54 (7.2)	27 (6.5)	111 (5.5)	NA	NA
ND	9 (1.2)	59 (14.1)	9 (0.4)	NA	NA
Response to PSL					
PGR	641 (85.0)	332 (84.5)	1,807 (91.4)	NA	NA
PPR	113 (15.0)	61 (15.5)	170 (8.6)	NA	NA
1st Relapse site					
Isolated CNS	12 (1.6[1])	14 (3.3[1])	33 (1.6[1])	2 (1.2[1])	11 (2.2[1])
Combined CNS	11 (1.5[1])	4 (1.0[1])	38 (1.9[1])	3 (1.8[1])	4 (0.8[1])
BM	109	67	NA	13	17
BM + other	3	8	NA	NA	NA
Other	14	9	NA	NA	1
All relapses	149 (19.8[1])	102 (24.4[1])	269 (13.3[1])	18 (10.9[1])	33 (6.6[1])
Treatment result					
EFS	78.2 ± 1.6% at 4 years	68.7 ± 2.4% at 6 years	79% at 5 years	80.2 ± 9.2% at 5 years	85.6 ± 2.9% at 5 years
Any CNS relapse[2]	3.3%	5.1%	4.3%	3.2%	3.9%

NA, not applicable. [1]The proportion of patients who relapsed among all eligible patients. [2]Cumulative incidence of any CNS relapse.

Prognostic Factors

We analyzed the patient characteristics and CNS relapse (Table IV). In a univariate analysis, the only statistically significant predictive factor for CNS relapse was day 8 leukemic blast count. The patients with Day8NoBlast had a significantly lower risk for CNS relapse, whereas there was no difference in the incidence of CNS relapse between PGR and PPR. CNS relapse was not associated with older age, T-cell immunophenotype, hyperleukocytosis, specific genetic alterations (BCR-ABL, MLL rearrangement, and E2A-PBX1), and the presence of CNS leukemia at diagnosis. Multivariate analyses of the risk for CNS relapse also revealed that Day8NoBlast was a statistically significant independent factor, whereas age, initial WBC count, immunophenotyping, or initial CNS status were not.

DISCUSSION

Since the outcome of patients with TLP+ was shown to be unfavorable [2,9–11], many efforts to minimize TLP+ have been made, including correction of thrombocytopenia, use of deep sedation, or general anesthesia, and cautious procedure by the most skilled clinicians [7,19]. Although the design of the present study was not a randomized fashion, our results indicate that the frequency of patients with TLP+ was reduced considerably (below 1%) by performing the initial LP after 7-day monotherapy of PSL, which was consistent with our previous L89-12 study [12]. Similarly, the frequencies of CNS-2 and CNS-3 also decreased (1.1% and 0.5%, respectively) as compared to those of the BFM and SJCRH studies adopting day 1 IT strategy (Table III). Since it was demonstrated in rhesus monkeys that intravenously administered PSL penetrated into the CSF [20],

TABLE IV. Relationship Between Patient Characteristics and CNS Relapse

	CNS relapse, n (%)	no CNS relapse, n (%)	*P* value
Overall	23 (3.1)	714 (96.9)	
Immunophenotype			
B lineage	21 (3.2)	631 (96.8)	0.67
T	2 (2.4)	83 (97.6)	
Gender			
Boy	16 (3.8)	402 (96.2)	0.21
Girl	7 (2.2)	312 (97.8)	
Age (years)			
1–10	21 (3.5)	582 (96.5)	0.23
10 or older	2 (1.5)	132 (98.5)	
WBC (/L)			
$<100 \times 10^9$	23 (3.6)	624 (96.4)	0.07
100×10^9 or more	0 (0.0)	90 (100.0)	
Cytogenetics			
BCR-ABL	0 (0.0)	15 (100.0)	*
MLL rearrangement	1 (6.3)	15 (93.8)	
E2A-PBX1	1 (2.7)	36 (97.3)	
TEL-AML1	2 (2.6)	74 (97.4)	
High hyperdiploid	4 (2.2)	177 (97.8)	
Pseudodiploid	2 (2.8)	69 (97.2)	
High hypodiploid	1 (3.6)	27 (96.4)	
Normal	9 (3.7)	237 (96.3)	
Day 8 blast (/μL)			
Zero	2 (0.8)	241 (99.2)	**
1–999	18 (4.6)	371 (95.4)	
PGR (0–999)	20 (3.2)	612 (96.8)	
PPR (1,000 or more)	3 (2.9)	102 (97.1)	
Risk group			
SR	6 (2.3)	253 (97.7)	0.35
IR	13 (4.2)	296 (95.8)	
HR	4 (2.4)	165 (97.6)	
CNS status			
CNS-1	17 (2.6)	636 (97.4)	***
CNS-1s	0 (0.0)	4 (100.0)	
CNS-2	1 (14.3)	6 (85.7)	
CNS-3	0 (0.0)	4 (100.0)	
TLP+	0 (0.0)	6 (100.0)	
TLP−	5 (9.3)	49 (90.7)	
ND	0 (0.0)	9 (100.0)	
CNS-positive group	1 (4.8)	20 (95.2)	
CNS-negative group	22 (3.1)	685 (96.9)	

*Poor cytogenetics versus others: $P = 0.97$, favorable cytogenetics versus others: $P = 0.37$; **Day 8 Blast Zero versus more than 1: $P = 0.01$, PGR versus PPR: $P = 0.87$; ***CNS-positive group versus CNS-negative group: $P = 0.67$.

it was possible that PSL administered before the initial CNS status was estimated decreased leukemic blasts in the CSF and, as a consequence, CNS diseases at diagnosis might have been underestimated in our study [13]. However, the CNS relapse rate in the CNS-negative group, which might contain patients with overlooked CNS diseases, remained at a 3% level without additional reinforcement of CNS-directed therapy (Fig. 2B).

Most study groups reinforce CNS-directed therapy in patients with CNS-positive using cranial irradiation and addition of IT chemotherapy, as they are thought to be at a greater risk for CNS and other relapse [7]. The disadvantage of cranial irradiation has been recognized [4–6]. Further, IT chemotherapy is accompanied by neurotoxicities [21,22]. In this study, we successfully reduced the number of patients who needed CNS-directed intensification to 2.1% of all patients.

The CNS-positive group in this study represents patients whose leukemic blasts remained in the CSF after PSL monotherapy and were thought to be at high risk for CNS relapse. However, only one patient in the CNS-positive group relapsed in the CNS concurrently with the BM, and CNS relapse rate in the CNS-positive group was comparable with that in the CNS-negative group. We conclude that the intensity of CNS directed therapy in both groups was appropriate. EFS was worse in the CNS-positive group than the CNS-negative group. Most events in the CNS-positive group were BM relapses. This seems to be due to the fact that patients in the CNS-positive group bore unfavorable features including older age, higher WBC count, higher frequency of poor risk cytogenetics, and more resistant to PSL monotherapy.

Systemic chemotherapy is important to control CNS disease [7]. Especially, high-dose MTX, dexamethasone, and intensive use of asparaginase are recognized as valuable components that act not only in the PB and the BM but also in the CSF [2,7,20,23–25]. We employed these components in consecutive studies except the use of dexamethasone in remission induction treatment, because the precedent L95-14 study did not show any superiority of dexamethasone to PSL [12,14,15,18,26]. We can successfully limit the use of cranial irradiation replacing by high-dose MTX and IT chemotherapy [18]. In spite of restricting the indication for pCRT (from 80 to 8.6%), the EFS and CNS relapse rate in the L99-15 study improved from those in the L89-12 study [12,18]. Furthermore, although the proportion of the initial CNS involvements of the TCCSG L99-15 was small, cumulative incidence of CNS relapse was not inferior to that of the BFM-95 and SJCRH studies (Table III).

The proportion of PPR in this study was higher than that in the BFM study. This was probably because we did not administer IT chemotherapy on day 1, which also had systemic effects [27]. There was the possibility that a subset of patients with PPR would need less intensive treatment if IT chemotherapy had been given at the time of initiation of remission induction therapy. Among these patients who responded poorly to PSL monotherapy, we could discriminate patients who would be cured by less intensive treatment by utilizing a more sensitive assay for detecting minimal residual disease after induction or consolidation treatment [28]. In contrast, there were patients whose leukemic blasts in the PB became undetectable by 7-day PSL monotherapy. These patients with no leukemic blasts on day 8 had an excellent outcome, as previously reported [15]. Furthermore, those patients were also had a lower risk of CNS relapse in this analysis. We could select patients who might be cured with less intensive systemic and CNS-directed chemotherapy by employing a 7-day PSL monotherapy phase.

In conclusion, the number of patients who were CNS-positive in our study, adopting a day 8 LP/IT strategy, was lower than that in other representative study groups employing the initial IT on day 1, whereas the number of PPR might be increased utilizing this strategy. Because CNS relapse rate in this study was comparable to that in those other study groups, delay of the initial LP/IT is a realistic strategy to safely perform the initial LP/IT and to minimize the patients who need intensified CNS-directed therapy.

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Assessment of Late Cardiotoxicity of Pirarubicin (THP) in Children With Acute Lymphoblastic Leukemia

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Background. Pirarubicin (tetrahydropyranyl-adriamycin: THP) is a derivative of doxorubicin with reportedly less cardiotoxicity in adults. However no studies of cardiotoxicity in children treated with THP have been reported. This study was performed to assess the THP-induced cardiotoxicity for children with acute lymphoblastic leukemia (ALL). ***Patients and Methods.*** This study comprised 61 asymptomatic patients aged from 7.6 to 25.7 years old. Median follow-up time after completion of anthracycline treatment was 8.1 years (range: 1.7–12.5). The cumulative dose of THP ranged from 120 to 740 mg/m^2 with a median of 180 mg/m^2. Patients underwent electrocardiogram (ECG), echocardiography, the 6-min walk test (6MWT), and measurements of serum brain natriuretic peptide (BNP) before and after exercise. ***Results.*** All subjects showed normal left ventricular function assessed by echocardiography. Ventricular premature contraction in Holter ECG and reduced exercise tolerance in the 6MWT were detected in 2/46 (3.3%) and 5/41(12.2%), respectively. Abnormal BNP levels were detected in 6/60 (10%) both before and after exercise. The cumulative dose of THP was significantly correlated with BNP levels after exercise ($r = 0.27$, $P = 0.03$), but not with any other cardiac measurements. Further analysis revealed that subjects with a high cumulative dose $\geq$300 mg/m^2 had significantly higher BNP levels after exercise compared with subjects with a low cumulative dose <300 mg/m^2 ($P = 0.04$). ***Conclusions.*** No significant cardiac dysfunction was detected in long-term survivors who received THP treatment. The use of post-exercise BNP level to indicate high cardiotoxicity risk should be verified by further study. Pediatr Blood Cancer 2011; 57:461–466. © 2011 Wiley-Liss, Inc.

Key words: BNP; cardiotoxicity; childhood ALL; pirarubicin

INTRODUCTION

During the past 30 years, the use of anthracyclines (AC) for the treatment of childhood cancers has significantly improved survival outcomes [1,2]. However, the therapeutic potential of these agents is limited by their cardiotoxicity: acute cardiotoxicity occurs immediately after treatment, early-onset chronic cardiotoxicity presents within 1 year after treatment, and late-onset chronic cardiotoxicity appears after a prolonged asymptomatic period with a latency of one or more years following AC therapy [3–5].

In children, late-onset cardiotoxicity is more common than acute or early-onset toxicity [6–11]. In an effort to reduce overall cardiotoxicity, various AC derivatives have been studied [5]. Pirarubicin (tetrahydropyranyl-adriamycin: THP) is a derivative of doxorubicin (DOX) with reportedly low cardiotoxicity in adult patients [12–20]. However, these reports were limited to acute cardiotoxicity immediately after THP treatment, and there are no available data of late-onset cardiotoxicity in both adult and childhood patients [21,22]. Since the 1990s, the Japanese Childhood Cancer and Leukemia Study Group (JCCLSG) has employed THP in the treatment of acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphomas, and recently, it reported long-term patient outcomes, finding a very low incidence of congestive heart failure among survivors [23–25]. This finding led to assessment of the incidence of subclinical cardiac abnormalities among these survivors, because many previous studies had shown a considerable proportion of asymptomatic childhood cancer survivors who had received AC therapy with possible abnormalities of cardiac function or myocardial biomarkers [26–31]. That is, the importance of longer follow up has become apparent with the increasing numbers of asymptomatic cancer survivors at risk of cardiac dysfunction late in life.

In this study, THP-induced late cardiotoxicity was evaluated for asymptomatic children who received THP therapy in three consecutive JCCLSG studies (ALL911/ALL941/ALL2000). The results showed that THP-induced late cardiac dysfunction was not detected in any subjects, but careful observation may be necessary for subjects who show elevated biomarker levels following the exercise test.

PATIENTS AND METHODS

Study Population

The 33 member institutes of the JCCLSG participated in three consecutive ALL trials, and the total number of long-term survivors was 825 (161 in ALL911, 381 in ALL941, and 283 in ALL2000). This study was performed on subjects from the 7 of these hospitals which had follow-up systems for long-term survivors with the collaboration of cardiologists. In each institute, survivors who had clinical heart failure, as defined by the New York Heart Association classification (NYHA, class III-IV) [32] or cardiovascular disease were excluded. Prior written informed consent was obtained from patients or legal guardians. Finally, 61 patients (9 in ALL911, 48 in ALL941, 4 in ALL2000) were enrolled in this study (Table I). Since many survivors from ALL911 (1991–1993) are now adults with no time to participate the study, and those from ALL2000 (2000–2003) have had a very short follow-up duration, 80% of patients consisted of survivors

Additional Supporting Information may be found in the online version of this article.

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Conflict of interest: Nothing to declare.

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TABLE I. Characteristics of Patients

Sex—male:female		30:31
Age at onset (years old)		5.7 ± 3.5
Age at evaluation (years old)		14.7 ± 3.5
Follow-up period (years)		7.2 ± 2.8
Treatment protocol		
ALL 911	Total	9
	LR	2
	IR	3
	HR	4
ALL 941	Total	48
	LR	7
	IR	21
	HR	17
	HHR	3
ALL 2000	Total	4
	IR	1
	HR	3
Total dose of THP (mg/m^2)		299 ± 192 (120–740; 180)[a]
Total dose of anthracyclins converted to THP (mg/m^2)		346 ± 206 (135–812; 207)[a]

Data are expressed as mean ± SD. HHR, high-high-risk; HR, high-risk; IR, intermediate -risk; LR, low-risk. [a]The number of parenthesis shows the range and median value.

from the ALL941 (1994–1999) study. Ages ranged from 7.6 to 25.7 years old with a median of 14.7, and the median follow-up time after completion of AC therapy ranged from 1.7 to 12.5 years with a median of 8.1. Ten age-matched healthy controls were also recruited (6 males and 4 females; mean age 13.8 ± 2.4 years old). They had normal cardiac function and had not received any treatment affecting the heart, kidneys, or fluid balance before the study.

Intralaboratory Exercise Testing

Master two-step intralaboratory testing with triple exercise loads was performed on every subject. The electrocardiogram (ECG) tracing was recorded before, immediately following, and 1 min after exercise. An abnormal ECG response was defined as a horizontal or downsloping ST segment depression of 0.10 mV (1 mm) for 80 msec [33].

Natriuretic Peptide

Blood samples for measuring brain natriuretic peptide (BNP) before intralaboratory exercise testing were obtained during fasting in the morning, and further samples were obtained after the exercise test. 1.5 ml of blood was drawn into ice-chilled tubes containing ethylene-diamine-tetraacetic acid while the subjects were in a supine position. The blood was centrifuged at 4°C to separate plasma, and stored below −20°C until analysis. Plasma BNP concentrations were measured using chemiluminescent enzyme immunoassay kits (Shionogi BNP; Shionogi & Co., Ltd., Osaka, Japan) [34].

Heart Rate Variability

Holter ambulatory ECG was recorded for every subject to evaluate heart rate variability (HRV). The measurements of heart rate adopted in the present study were standard deviation of NN intervals (SDNN) and co-variance of NN intervals (CVNN).

Heart periods with arrhythmia were excluded from the HRV analyses.

Echocardiography

Echocardiograms were recorded for each subject from the parasternal and apical windows. Two-dimensionally guided M-mode echocardiography was performed, and the measurements were expressed as indices [35]. Variables of systolic functions included: left ventricular diastolic dimension (LVDd), left ventricular end-systolic dimension (LVDs), ejection fraction (EF) defined as (LVDd3 − LVDs3)/LVDd3, and fractional shortening (FS) defined as (LVDd − LVDs)/LVDd. FS < 28% and EF < 54% were considered abnormal [36]. The end-diastolic and end-systolic phases were defined as the beginning of the QRS wave of the ECG tracing and the point at which the second heart sound was recorded by the phonocardiogram, respectively. The variable of diastolic function was the ratio between early (E) and late or atrial (A) ventricular filling velocity (the E/A ratio) [37,38] by a pulsed Doppler measurement. The sample volume was placed between the mitral anulus and the leaflet tips where the greatest velocities were found. Cardiac dysfunction was defined by abnormal FS, and abnormalities of the other determinations were used as confirmatory evidence.

The 6-Minute Walk Test

The 6-min walk test (6MWT) was used to evaluate the functional capacity of the subjects. The field test was performed on a running track to measure the furthest distance a subject can walk. Normal values according to age and sex were defined by Geiger et al. [39].

Statistical Analyses

Regression analyses were used to study the correlation between cumulative THP dose on one side and cardiac function and biomarkers. The unpaired Student's *t*-test was used for the comparison of mean values. SPSS statistical analysis software (SPSS 12.0 J, SPSS Japan Inc., Tokyo, Japan) was used for all computations.

RESULTS

Cumulative dose of THP ranged from 120 to 740 mg/m^2 with a median of 180 mg/m^2. In addition to THP, subjects in ALL941 and ALL2000 received DOX. Thus, total cumulative doses of AC (THP + DOX) ranged from 135 to 812 mg/m^2 with a median of 207 mg/m^2 (Table I). To calculate this, the DOX/THP ratio used was 1:1.08 based on the molecular weight ratio.

The measurements of cardiac functions and the number of abnormal subjects are listed in Table II. ECG at rest was normal in all subjects. However, abnormal ST elevation on ECG was found after laboratory exercise testing in one subject (1.6%). The Holter recording was performed on 59 subjects, and abnormal findings with supra-ventricular premature contraction were detected in 2 (3.3%). These two did not show any other cardiac abnormal measurements. Heart rate variability was normal in all

TABLE II. Measurements of Cardiac Functions

Tests	Measurements	Number of subjects	Results mean ± SD (range)	Number of abnormal subjects
ECG	At rest	61	Normal	0
	After exercise	61	ST elevation	1
Holter ECG	Arrhythmia	59	SVPC	2
	CVNN (%)	59	19.8 ± 3.2 (2.7–27.1)	0
Echocardiography	LVDd (mm)	61	43.9 ± 4.0 (36.1–52.0)	0
	LVDs (mm)	61	26.9 ± 3.4 (19.0–34.6)	0
	EF (%)	61	70.4 ± 6.2 (53.0–81.3)	1
	FS (%)	61	38.7 ± 4.6 (29.4–50.0)	0
	E/A ratio	48	2.08 ± 0.47 (1.43–4.0)	0
6MWT	Total (m)	41	563.4 ± 142.5	5
	Males (m)	18	650.4 ± 110.9 (362.0–904.5)	1
	Females (m)	23	495.4 ± 126.7 (252.0–699.6)	4
Laboratory exercise testing	BNP at rest (pg/ml)	60	13.3 ± 14.6 (2.0–70.8)	6
	BNP after exercise (pg/ml)	60	15.1 ± 15.4 (2.0–85.2)	6

6MWT, 6-min walk test; CVNN, co-variance of NN intervals; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; EF, ejection fraction; FS, fractional shortening; SVPC, supra-ventricular premature contraction.

TABLE III. Plasma BNP Levels in Patients and Controls

	BNP (pg/ml)		
	At rest	After exercise	Difference
Patients (n = 60)	13.3 ± 14.7	15.1 ± 15.5	1.8 ± 8.7
Control (n = 10)	10.7 ± 9.3	11.1 ± 10.5	0.4 ± 1.8
P	0.60	0.53	0.63

Values are expressed as mean ± SD.

subjects. Echocardiographic studies showed no cardiac dysfunction, and abnormal measurement was recorded in only one subject with a subnormal EF value of 53%. The 6MWT was performed on 41 subjects, and a significantly short distance as compared to the standard values adjusted to sex and age was recorded in 5 (one male and 4 females). The elevated plasma BNP levels defined as greater than the mean + 2 SD of the 10 healthy controls were >28.3 pg/ml (before exercise) and >31.1 pg/ml (after exercise), respectively. Based on this criterion, abnormal BNP levels were detected in six subjects whose values were elevated both at rest and after exercise. The mean BNP values before and after exercise in patients and control subjects are shown in Table III, revealing no significant difference between the patients and controls.

Overall, some abnormal cardiac measurements were detected in 14 subjects, and the type of abnormality and cumulative AC dose for each subject are shown in Table IV.

Table V shows the correlation between cumulative THP dose and various cardiac measurements. The cumulative dose showed a significant correlation with plasma BNP levels after exercise (Fig. 1), but not with any other cardiac measurements. Further analysis of the plasma BNP levels after exercise revealed that 21 subjects who received a high cumulative dose $\geq$300 mg/m^2 of THP had significantly higher BNP levels as compared with 39 other subjects who received a low cumulative dose <300 mg/m^2 (Table VI). This table also shows increments in BNP levels (ΔBNP) after exercise compared to base-line values (at rest) between the two groups. A significant rise in ΔBNP after exercise

TABLE IV. Cumulative Dose of Anthracyclins and Abnormal Cardiac Measurements

Case	Cumulative dose of anthracyclins (DOX/THP) (mg/m^2)	Exercise ECG	Holter ECG	6MWT	Plasma BNP	
					At rest	After exercise
1	25/180	−	+	−	−	−
2	0/180	−	−	+	−	−
3	25/160	−	−	−	+	+
4	75/600	−	−	−	+	+
5	75/730	+	−	−	−	−
6	75/150	−	+	−	−	−
7	75/120	−	−	+	−	−
8	75/740	−	−	−	+	+
9	75/590	−	−	−	+	+
10	15/160	−	−	−	+	+
11	0/135	−	−	−	+	+
12	25/180	−	−	+	−	−
13	25/180	−	−	+	−	−
14	75/740	−	−	+	−	−

+ and − denote positive and negative results for cardiac measurements, respectively. DOX, doxorubicin; THP, pirarubicin.