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Chromosome abnormalities in advanced stage T-cell lymphoblastic lymphoma of children and adolescents: a report from Japanese Paediatric Leukaemia/Lymphoma Study Group (JPLSG) and review of the literature

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Summary

T-cell acute lymphoblastic leukaemia (T-ALL) and T-cell lymphoblastic lymphoma (T-LBL) are combined into one category as T lymphoblastic leukaemia/lymphoma in the current World Health Organization (WHO) classification. However, there is still ongoing discussion on whether T-ALL and T-LBL are two separate entities or represent two variant phenotypes of the same disease. Cytogenetic analysis has been used to identify the molecular background of haematological malignancies. To compare the distribution of chromosomal abnormalities of T-ALL and T-LBL, large series of cytogenetic data are required, but are absent in T-LBL in contrast to the abundant data in T-ALL. Among 111 T-LBL cases in our clinical trial, we obtained complete cytogenetic data from 56 patients. The comparison between our cytogenetic findings and those from three published T-LBL studies revealed no significant difference. However, meta-analysis showed that translocations involving chromosome region 9q34 were significantly more common in T-LBL than in T-ALL. In particular, four out of the 92 T-LBL cases, but none of the 523 paediatric T-ALL cases, showed translocation t(9;17)(q34;q22–23) ($P = 0{\cdot}0004$). Further studies are needed for the possible linkage between abnormal expression of genes located at 9q34 and/or 17q22–23 and the unique 'lymphoma phenotype' of T-LBL.

Keywords: T-cell lymphoma, child, non-Hodgkin lymphoma, cancer cytogenetics, leukaemia.

In children and adolescents, precursor T lymphoblastic neoplasms have been classified into two diseases: T-cell acute lymphoblastic leukaemia (T-ALL) and T-cell lymphoblastic lymphoma (T-LBL). Although the current World Health Organization (WHO) classification designates both malignancies as T lymphoblastic leukaemia/lymphoma (Borowitz & Chan, 2008), there is continuing discussion on whether T-ALL and T-LBL are two separate entities or whether they represent two different clinical presentations of the same disease. They show overlapping clinical, pathological and immunophenotypic features. In general, the word 'lymphoma' is used if there is a bulky mass in the mediastinum or elsewhere, with less peripheral blood and bone marrow (BM) involvement. Most study groups distinguish between leukaemia and lymphoma on the basis of the extent of BM involvement: patients with <25% lymphoblasts in the BM are diagnosed with lymphoblastic lymphoma; in cases

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of 25% or more BM blasts, the diagnosis is leukaemia. While this distinction may appear somewhat arbitrary, a notable observation is that T-LBL patients with large mediastinal masses frequently exhibit little, if any, evidence of tumour dissemination and BM involvement, but the molecular background for this difference is unknown.

Chromosomal analysis has been widely used as a primary step that is required to narrow down the responsible genes that define a disease entity. For instance, discovery of Ph chromosome led to the identification of the chimeric *BCR/ABL1* gene, which is responsible for and defines chronic myeloid leukaemia. Compared with T-ALL, chromosomal abnormalities in T-LBL are not well defined. Reports in the literature and current textbooks claim that the typical chromosomal aberrations reported in T-ALL can also be found in T-LBL (Borowitz & Chan, 2008). However, there are no large series of cytogenetic data on T-LBL (Burkhardt, 2010).

This study aimed to fill the gap regarding cytogenetic data in T-LBL and compare the cytogenetic findings of T-ALL and T-LBL, which may lead to identification of the molecular background behind phenotypical differences between the two disease entities.

Study patients

From November 2004 to October 2010, 154 eligible children (aged 1–18 years) with newly diagnosed advanced stage LBL (Murphy stages III and IV) (Murphy, 1980) were entered in the Japanese Paediatric Leukaemia/Lymphoma Study Group (JPLSG) ALB-NHL03 study (UMIN000002212, http://www.umin.ac.jp/ctr/index-j.htm). Patients with primary immunodeficiencies, Down syndrome and T-cell diseases as second malignancies were excluded. The ethics committee of each participating institute approved the study protocol.

Cytogenetic analysis

Cytogenetic analysis was performed on cell suspensions obtained from 31 tumour/lymph nodes, 19 pleural effusions and six bone marrow samples. The methods of chromosome preparation for cytogenetic analysis are described elsewhere (Sanger *et al*, 1987; Horsman *et al*, 2001). Karyotypes are described according to the International System for Human Cytogenetic Nomenclature (ISCN) (Shaffer & Tommerup, 2005). Only those cases with abnormal cytogenetic study results, defined as two or more cells with the same structural abnormality or the same numerical gain, three or more cells with the same numerical loss or isolated cells with disease-associated abnormalities, were eligible for inclusion in this study.

Statistical methods

Two-tailed Fisher's exact test was used to analyse the patients' characteristics and the frequency of each chromosome abnormality. Significant differences in the analysis of he frequency of each chromosome abnormality were determined by the two-tailed Fisher's exact test with Bonferroni correction comparison. The P value threshold for inclusion of a new variable was chosen to be $P < 0{\cdot}003$ in this analysis (0·05/17, after Bonferroni correction). A review of T-LBL and T-ALL karyotypes reported in the literature was obtained from a PubMed search and information on chromosome abnormalities and gene fusions was obtained from Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (http://cgap.nci.nih.gov/Chromosomes/Mitelman).

Results

Patient characteristics

A total of 154 children were enrolled on JPLSG ALB-NHL03 protocols; 111 cases were T-LBL. Among 111 T-LBL cases, the study population for the current analysis included 56 patients for whom complete cytogenetic data were obtained. With respect to presenting features, patients with reviewed and accepted cytogenetic data were similar to both those without accepted cytogenetic data and the entire cohort of concurrently enrolled T-lineage LBL patients (Table S1).

Frequency of chromosomal abnormalities

Multiple chromosome abnormalities were identified in 31 patients (45%). Structural chromosome abnormalities were identified in 29 patients (52%), and numerical chromosome abnormalities were identified in 18 patients (32%). Ploidy results included pseudodiploid in 14 patients (25%), hypodiploid in three patients (5%), hyperdiploid with 47–50 chromosomes in 10 patients (18%), hyperdiploid with more than 50 chromosomes in four patients (7%) and diploid in 25 patients (45%) (Table S2).

All of the hypodiploid cases had 43–45 chromosomes; none had a near-haploid karyotype. Of the four cases with more than 50 chromosomes, two had near-tetraploid karyotypes. The frequencies of ploidy groups in this series are compared with those reported in other series of karyotyped T-LBL patients and paediatric T-ALL (Table S2). Structural chromosome abnormalities were identified in 29 patients (52%). In the current study, seven patients (13% of those with abnormal karyotypes) exhibited a rearrangement at one or more of the chromosome bands (7p15, 7q32–36 and/or 14q11–13) that are the locations of T-cell receptor chain genes. Rearrangements in the 14q11–13 region, in which the T-cell receptor α/δ chain genes are located, were present in three patients (5%) of the karyotypically abnormal cases in this series (Table S2). Structural abnormalities involving chromosome region 9q34 were identified in nine patients (16%). Translocations involving chromosome region 9q34 were identified in three patients (5%) (t(9;17)(q34;q22), t(7;9)(q34;q34) and t(2;9)(q23;q34)). In comparison between cytogenetic findings in the current data and combined data of three published reports (Burkhardt

et al, 2006; Lones *et al*, 2007; Uyttebroeck *et al*, 2007; Table S1), the frequencies of numerical and structural cytogenetic abnormalities in T-LBL and T-ALL had no significant difference (Table S2).

We compared the cytogenetic findings in the current study with the published reports from the three largest-scale studies on T-LBL (Burkhardt *et al*, 2006; Lones *et al*, 2007; Uyttebroeck *et al*, 2007; Table S3) and those from the two largest-scale studies on T-ALL combined (Heerema *et al*, 1998; Schneider *et al*, 2000; Table S3) (Table I). The frequencies of almost all of the cytogenetic abnormalities in T-LBL and T-ALL had no significant difference, but translocation involving chromosome region 9q34 was significantly more common in T-LBL than in T-ALL ($P = 0{\cdot}0004$, Table S3) and translocation t(9;17) was also more common in T-LBL (4%, 4/92) than in T-ALL (0%, 0/523, $P = 0{\cdot}0004$) (Table I).

The current study included a patient with translocation t(9;17)(q34;q22). As far as we could tell from the consulted published reports, all T-LBL patients with translocation t(9;17) presented with a mediastinal mass and without any bone marrow involvement (Kaneko *et al*, 1988; Shikano *et al*, 1992) (Table II).

Table I. Comparison of cytogenetic findings between T-LBL and T-ALL.

	T-LBL		T-ALL		
	n	%	*n*	%	*P* value
Total	92		523		
Normal karyotype†	36	39	219	42	0·6478
Abnormal karyotype	56	61	304	58	0·6478
Hypodiploid	4	4	20	4	0·9999
Pseudodiploid	30	33	204	39	0·2000
Hyperdiploid(47–50)	18	20	64	12	0·0328
Hyperdiploid(>50)	4	4	16	3	0·5217
Any translocation	26	28	177	34	0·3367
Any del chrome.	19	21	160	31	0·0328
Any der chrome.	4	4	58	11	0·0583
del(6q)	6	7	69	13	0·0833
Loss of 9p	10	11	44	8	0·5487
Any 14q11–13 abnormality	10	11	72	14	0·5100
Any 7q32–36 abnormality	7	8	35	7	0·8220
Any translocation including 9q34	8	9	7	1	0·0004*
t(7;10)	1	1	2	0	0·3855
t(10;11)	1	1	8	2	0·9999
t(9;17)	4	4	0	0	0·0004*

†Includes one Klinefelter syndrome, and one inv(9) without other abnormality in current report.
The *P* value threshold for inclusion of a new variable was chosen to be 0·003 (0·05/17, after Bonferroni correction). *$P < 0{\cdot}003$.
T-LBL: current study (JPLSG ALB-NHL03) combined with three published reports(Burkhardt *et al*, 2006; Lones *et al*, 2007; Uyttebroeck *et al*, 2007).
T-ALL: combined two published reports (Heerema *et al*, 1998; Schneider *et al*, 2000).

Discussion

This is the largest study involving cytogenetic analysis of T-LBL and the first study to directly compare cytogenetic findings of T-LBL and T-ALL. The frequencies of almost all of the cytogenetic abnormalities in both entities were found to have no significant difference, but translocation involving chromosome region 9q34 was significantly more common in T-LBL than in T-ALL. The current study included a patient with unique translocation t(9;17)(q34;q22). Interestingly, four out of the 92 T-LBL cases, but none of the 523 paediatric T-ALL cases, showed this translocation ($P = 0{\cdot}0004$) (Table I). Translocation t(9;17) has been reported in several haematological diseases, such as precursor B-cell ALL (Coyaud *et al*, 2010), acute myeloid leukaemia (Mrózek *et al*, 2001), chronic myeloid leukaemia (DeAngelo *et al*, 2004), chronic lymphocytic leukaemia (Michaux *et al*, 2005), diffuse large B-cell lymphoma (Hammond *et al*, 1992) and follicular lymphoma (Aamot *et al*, 2007), but these breakpoints, 9q34 and 17q22–23, are limited in the cases of T-LBL (http://cgap.nci.nih.gov/Chromosomes/Mitelman). These results imply a linkage between abnormal expression of genes located at 9q34 and/or 17q22–23 and the unique phenotypes of the T-LBL mentioned above.

Cytogenetic analysis has been used to identify the molecular background of haematological malignancies. To compare the distribution of chromosomal abnormalities of T-ALL and T-LBL, large series of cytogenetic data are required, but are absent in T-LBL in contrast to the abundant data in T-ALL. Three recent series of cytogenetic data on paediatric T-LBL have been published, reporting the cytogenetic findings in 13, 11 and 12 paediatric T-LBL cases (Burkhardt *et al*, 2006; Lones *et al*, 2007; Uyttebroeck *et al*, 2007). Thus, this study can play a role to fill the gap of cytogenetic data on T-LBL.

Translocation involving chromosome region 9q34 was found to be significantly more common in T-LBL than in T-ALL (Table I). Among genes located in the 9q34 region, *SET*, *PKN3*, *ABL1*, *NUP214* and *NOTCH1* have previously been implicated in malignancy, with *SET*, *ABL1*, *NUP214* and *NOTCH1* being implicated in leukemogenesis (Ellisen *et al*, 1991; van Vlierberghe *et al*, 2008; Hagemeijer & Graux, 2010).

An oncogenic *SET-NUP214* fusion gene has been reported in a case of acute undifferentiated leukaemia with a reciprocal translocation t(9;9)(q34; q34) (von Lindern *et al*, 1992) and NK adult acute myeloid leukaemia as a result of a cryptic deletion of 9q34 (Rosati *et al*, 2007). van Vlierberghe *et al* (2008) identified the *SET-NUP214* fusion gene in three patient samples out of 92 paediatric cases of T-cell leukaemia. *SET-NUP214* may contribute to T-ALL pathogenesis by inhibition of T-cell maturation through the transcriptional activation of the *HOXA* genes (van Vlierberghe *et al*, 2008). However, the frequency of this mutation in T-LBL is unknown.

NOTCH1, previously termed *TAN1*, was discovered as a partner gene in T-ALL with a translocation t(7;9)(q34;q34.3), and was found in <1% of T-ALLs (Ellisen *et al*, 1991). Several

Table II. Clinical characteristics and detailed karyotype data in T-LBL patients with t(9;17).

	Age (years)	Sex	Tumour site	Stage	BM blast %	Karyotype
Kaneko *et al* (1988)	14	F	Mediastinum	III	0	46,XX,t(9;17)(q34;q23)
	15	M	Mediastinum	III	0	46,XY,-9,del(6)(q13q21),t(9;17)(q34;q23),+der(9)t(9;17)(q34;q23)
	10	M	Mediastinum	III	0	47,XY,+19,t(9;17)(q34;q23)
Shikano *et al* (1992)	14	F	Mediastinum	III	0	46,XX,t(9;17)(q34;q23)
	7	M	Mediastinum	III	0	49,XY-l,+der(l)t(l;?)(p36;?),t(9;17)(q34;q23),+14,+marl,+mar2
	5	F	Mediastinum	III	0	47,XX,t(9;17)(q34;q23),+der(17)t(9;17)(q34;q23)
Burkhardt *et al* (2006)	ND	ND	ND	ND	ND	46,XX,del(6)(q1?2q1?6),t(9;17)(q34;q22)
	ND	ND	ND	ND	ND	47,XX,t(9;17)(q34;q22),+20
Lones *et al* (2007)	8	M	Mediastinum	III	0	47,XY,t(9;17)(q3?4;q2?3),+20
Current study	7	M	Mediastinum	III	0	46,XY,t(9;17)(q34;q22)

ND, no data available.

study groups reported *NOTCH1* mutations in 31–62% of T-ALL patients (Weng *et al*, 2004; Breit *et al*, 2006; van Grotel *et al*, 2006; Zhu *et al*, 2006; Malyukova *et al*, 2007; Asnafi *et al*, 2009; Gedman *et al*, 2009; Park *et al*, 2009). In contrast, only two studies reported *NOTCH1* mutation analyses in T-LBL: Park *et al* (2009) reported *NOTCH1* mutations in six out of 14 paediatric T-LBL patients (43%), and Baleydier *et al* (2008) reported mutations in six out of nine paediatric T-LBL (66%), with 32 adult patients with *NOTCH1* mutations in 16 cases (54% in all patients) (Baleydier *et al*, 2008). According to these reports, the frequencies of *NOTCH1* mutation were not significantly different between T-LBL and T-ALL.

ABL1 fusion genes have been identified that provide proliferation and survival advantage to lymphoblasts. *NUP214-ABL1*, *EML1-ABL1*, *BCR-ABL1* and *ETV6-ABL1* chimeric genes have been reported. The most frequent one in T-ALL is the *NUP214-ABL*1 fusion gene, which has been identified in 6% of cases, in both children and adults (Graux *et al*, 2009). In addition, using an oligonucleotide microarray, *ABL1* overexpression was identified in 8% of cases in T-ALL (Chiaretti *et al*, 2007). Our review of these published reports indicated that the frequency of ABL1 mutation in T-LBL is unknown.

Raetz *et al* (2006) analysed the gene expression profiles of ten T-ALL BM samples and nine T-LBL samples using a microarray. They identified 133 genes for which the expression levels differed between T-LBL and T-ALL. *ZNF79* (encoding zinc finger protein 79) and *ABL1*, both located in chromosome region 9q34, were included in these genes and showed at least twofold higher overexpression in T-LBL than that in T-ALL. Additionally, *MED13* (previously termed *THRAP1*), which is located in 17q22-q23, also showed at least twofold higher overexpression in T-LBL than that in T-ALL (Raetz *et al*, 2006). Taking these findings together, it is possible that *ZNF79*, *ABL1* or *THRAP1* as well as other genes at 9q34 and 17q22–23 are involved in the 'lymphoma phenotype' such as a bulky mass in the mediastinum and minimal BM involvement. These findings need further study to determine if this linkage constitutes a unique 'lymphoma phenotype'.

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Authorship

MS designed the study, prepared the data file, performed the analysis, interpreted data and wrote the manuscript. SS is a lead principal investigator for the JPLSG ALB-NHL03 study. AN contributed to pathological diagnosis. YH contributed to chromosome analysis. YO is a principal investigator contributing a patient to this study. AMS contributed to statistical analysis. KH received a research grant from the Ministry of Health, Labour and Welfare of Japan. MT is a chairperson of JPLSG. TM is a chairperson of JPLSG lymphoma committee. SS, KH, MT and TM were primarily responsible for the study design, data analysis and interpretation of the data. All authors approved the final manuscript.

Disclosure

The authors declare no competing financial interests.

Supporting Information

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

Table S1. Respective clinical characteristics with and without karyotype data in 111 T-LBL patients in the current study.

Table S2. Comparison of cytogenetic findings in T-LBL between current study and combined data of three published reports.

Table S3. Published data of cytogenetic findings in T-LBL and T-ALL.

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Distinct Impact of Imatinib on Growth at Prepubertal and Pubertal Ages of Children with Chronic Myeloid Leukemia

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Objective To determine the extent of growth impairment resulting from imatinib treatment in children with chronic myeloid leukemia (CML).

Study design Clinical records of 48 chronic-phase CML children administered imatinib as the first-line therapy between 2001 and 2006 were analyzed retrospectively. Cumulative change in height was assessed using the height height-SDS and converted height data from age- and sex-adjusted Japanese norms.

Results A decrease in height-SDS was observed in 72.9% of children, with a median maximum reduction in height-SDS of 0.61 during imatinib treatment. Median follow-up time was 34 months (range, 10-88 months). Growth impairment was seen predominantly in children who started imatinib at a prepubertal age compared with those who started at pubertal age. Growth velocity tended to recuperate in prepubertal children with growth impairment, as they reached pubertal age, suggesting that imatinib had little impact on growth during puberty.

Conclusions Growth impairment was a major adverse effect of long-term imatinib treatment in children with CML. We report the distinct inhibitory effect of imatinib on growth in prepubertal and pubertal children with CML. We should be aware of growth deceleration in children, especially in young children given imatinib before puberty and subjected to prolonged exposure. *(J Pediatr 2011;159:676-81).*

Since the introduction of imatinib, the treatment of chronic myeloid leukemia (CML) has changed from cure by allogeneic stem cell transplantation to maintenance of the best achievable treatment response (hematologic, cytogenetic, and molecular responses). Various side effects, including nausea, vomiting, diarrhea, skin rash, edema, elevated liver enzyme values, and cytopenia, are known to be common during imatinib treatment, but generally are mild to moderate.[1] However, the long-term side effects of imatinib therapy remain unknown, and its effects on growth are a major concern when treating children. Growth deceleration has been reported in 3 children as well as in a cohort given imatinib.[2-5] The present study was conducted to evaluate the effect of imatinib on growth in children and adolescents with CML.

Methods

In Japan, imatinib was approved and became available for treatment of CML in December 2001. The Japanese Pediatric Leukemia/Lymphoma Study Group's CML Committee reviewed records of 99 Japanese children under age 18 years diagnosed with chronic-phase CML between 2001 and 2006. Among these children, 76 who received imatinib as first-line therapy were eligible for the study. Concurrent hydroxyurea administration was permitted. Exclusion criteria were as follows: (1) reached final height at the time of diagnosis (n = 3); (2) afflicted by a chronic disease (eg, schistorrhachis) or on any treatment that could affect growth (n = 4); and (3) a follow-up period of <10 months while receiving imatinib (n = 21). Forty-eight children (21 girls, 27 boys) met these criteria and were enrolled in the study. The study design was approved by the Keio University School of Medicine's Ethics Committee.

BSA	Body surface area
CML	Chronic myeloid leukemia
GH	Growth hormone
PDGF	Platelet-derived growth factor

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Height–Growth Evaluation

As part of the medical examination, height was measured by experienced medical workers at the start of imatinib treatment and at follow-up visits. Height data were converted to numbers with SDs using age- and sex-adjusted Japanese norms to give SDSs. Growth while on imatinib therapy was assessed using cumulative change in height-SDS (ΔSDS) from the start of imatinib treatment to the annual follow-up time points. Minimum height-ΔSDS was determined as the lowest value of annually calculated height-ΔSDS in each patient. Average dose of imatinib d_{ave} (mg/m^2) for an individual during the administration period (i) from 1 through n during l-year treatment was calculated using the following formulas:

$$\overline{d} = \frac{\sum_{i=1}^{n} d_i m_i}{\sum_{i=1}^{n} m_i}, \overline{BSA} = \frac{\sum_{j=1}^{l} BSAj}{\sum_{j=1}^{l} k_j}, \text{ and } d_{ave} = \frac{\overline{d}}{\overline{BSA}},$$

where d is the dose of imatinib, m is the number of days of imatinib administration, and BSA is body surface area (BSA). BSA in the jth year (BSA_j) was calculated from data obtained at the observation time point closest to the jth full-year point within 6 months. The value of k_j is 1 if BSA_j is available at the jth year and 0 otherwise. The data after reaching final height were censored for 2 patients. The final height was defined as the maximum height measured when height increase velocity slowed to <1 cm per year. In this study, age threshold equivalent to the onset of puberty was defined as 9 years for girls and 11 years for boys, as generally agreed upon by pediatricians.

Statistical Analyses

Statistical differences in height-SDS between 2 time points—at the commencement of imatinib treatment and at final follow up—within the cohort were assessed using the Wilcoxon signed-rank test. Statistical differences between the 2 subgroups classified according to minimum height-ΔSDS were assessed using the Mann-Whitney U test. The statistical differences among the 3 subgroups classified according to the average imatinib dose were evaluated using the Steel-Dwass test. The statistical differences among all annually calculated height-ΔSDS values during imatinib therapy in prepubertal and pubertal children at the commencement of imatinib treatment were assessed using the Tukey-Kramer honestly significant difference test.

Results

The median age at diagnosis was 9 years (range, 2-15 years). The median average imatinib dose was 287 mg/m^2 (range, 161-543 mg/m^2), and median follow-up was 34 months (range, 10-88 months). The overall median height of the 48 children was nearly normal at the start of imatinib treatment (median height-SDS, 0.01; range, −2.30 to 1.50), but was decreased significantly at the final measurement, with a median height-SDS of −0.85 (range, −2.80 to 1.30) ($P < .001$, Wilcoxon signed-rank test), indicating that imatinib adversely affected growth (**Figure 1**, A and B). Height <−2 SD at the last follow-up was observed in 6 children (12.5%), excluding 1 child whose height was <−2 SD at the start of imatinib treatment. A decrease in height-SDS of >0.5 SD was observed in 25 children (52.1%), including 16 (33.3%) with a decrease of >1 SD during imatinib treatment. The median minimum annually calculated height-ΔSDS during follow-up was −0.61 (range, −2.20 to 0.60) (**Figure 1**, C).

We next divided the study cohort according to their minimum height-ΔSDS into 2 subgroups: <−0.5 (n = 25) and ≥−0.5 (n = 23). Sex distribution, average imatinib dose, and proportion of patients with hydroxyurea administration were comparable between the 2 subgroups (**Table**). The greatest significant difference observed between the 2 subgroups was age at initiation of imatinib treatment. The proportion of prepubertal children was significantly higher in the minimum height-ΔSDS <−0.5 subgroup than in the ≥−0.5 subgroup. In contrast, the ≥−0.5 subgroup consisted mainly of children at pubertal age at the start of imatinib treatment.

To evaluate the relationship between administered imatinib dose and growth impairment, we divided the cohort according to the average administered dose for each individual and

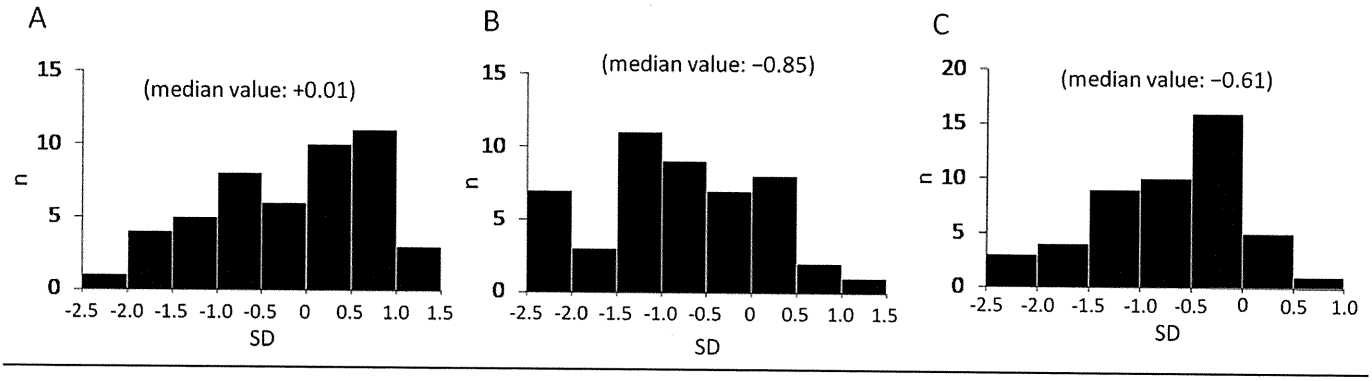

Figure 1. Change in height-SDS during imatinib treatment. Height-SDS is shown at **A,** the commencement of imatinib treatment and **B,** at the last follow-up. **C,** Minimum height-ΔSDS during imatinib treatment. The median value is indicated above each plot. n, number of patients.

Table. Patient characteristics

	Minimum height-ΔSDS		
	<−0.5 (n = 25)	≥−0.5 (n = 23)	*P* value
Age at the commencement of imatinib			
Median, years	7	12	<.001
Range, years	2-12	4-15	
Prepubertal age, n (%)*	23 (92.0)	4 (17.4)	<.001
Pubertal age, n (%)†	2 (8.0)	19 (82.6)	<.001
Male sex, n (%)	14 (56.0)	13 (56.5)	.9808
Duration of imatinib treatment, months, median (range)			
Prepubertal age*	42 (19-88)	14 (10-22)	.009
Pubertal age†	41 (21-60)	26 (10-61)	.406
Average imatinib dose, mg/m^2			
Median	293	282	.272
Range	161-543	197-376	
Hydroxyurea administration, n (%)	2 (8.0)	3 (13.0)	.577

*Prepubertal age: males, <11 years; females, <9 years.
†Pubertal age: males, ≥11 years; females, ≥9 years.

recommended pediatric doses for treating chronic-phase CML (260-340 mg/m^2)[6] into 3 subgroups: <260 mg/m^2 (n = 17), 260-340 mg/m^2 (n = 19), and >340 mg/m^2 (n = 12). The median minimum height-ΔSDS of these 3 subgroups was −0.6 (median dose, 222 mg/m^2), −0.48 (median dose, 293 mg/m^2), and −0.85 (median dose, 360 mg/m^2), respectively, indicating no significant difference among the 3 subgroups.

Representative growth charts of children at various ages at the start of imatinib treatment are shown in **Figure 2**. Growth impairment was particularly significant in children who were prepubertal at the start of imatinib treatment (**Figure 2**, A and B), and only mild growth impairment or no impairment was seen in most of the children who were pubertal at the start of imatinib treatment (**Figure 2**, C and D). However, the prepubertal children with growth impairment regained growth velocity as they reached pubertal age (**Figure 2**, E-H).

Mariani et al[2] reported a 9-year-old boy who demonstrated impaired growth shortly after the start of imatinib treatment but experienced catch-up growth with the onset of puberty. Thus, to evaluate whether children at pubertal age evade growth deceleration, we dichotomized the study cohort into 2 subgroups: children who started imatinib at prepubertal age (n = 27) and those who did so at pubertal age (n = 21). In the former group, height-ΔSDS began to decline during the first year of imatinib treatment, resulting in significant deceleration in growth. In the latter group, height-ΔSDS remained steady through imatinib treatment, suggesting that imatinib has little effect on growth in pubertal children (**Figure 3**).

Collectively, our data show a high frequency of growth impairment and >0.5 SD of cumulative decrease in height-SDS in children given imatinib for chronic-phase CML. This growth impairment was seen predominantly in young children who were started imatinib at prepubertal age.

Discussion

Imatinib is now a major option as the first-line therapy for childhood CML.[6-9] Thus, it is important for clinicians to be aware of its possible long-term effects. Imatinib inhibits several tyrosine kinases, including c-abl, c-kit, c-fms, and platelet-derived growth factor (PDGF) receptors.[7,10,11] Several studies in adults have suggested that inhibition of c-kit, c-fms, and PDGF receptors results in modulation of bone metabolism.[12-15] Inhibition of osteoclasts and osteoblasts may result in dysregulated bone remodeling.[11,15-17] Three recently published case reports indicated growth impairment as an adverse effect of long-term imatinib treatment in children.[2-4] In addition, a French group reported a significant decrease in height-SDS in 22 children, with a median difference of −0.37 (range, −1.09 to 0.14; $P < .0001$) during the first year of imatinib treatment.[5] Although the impact of imatinib on growth was noticeable in children in these previous studies, it has not yet been fully elucidated.

In our study of 48 children with chronic-phase CML, the severity of growth impairment was related to age at the start of imatinib treatment. Growth impairment was observed predominantly in children at prepubertal age compared with children at pubertal age. In children who started imatinib at prepubertal age, height-ΔSDS decreased during treatment, and in most cases, more than 2 years of continuous treatment was necessary to exhibit a reduction in height-SDS of >0.5 SD (**Figure 3**). Although 4 children who started imatinib at prepubertal age were included in the height-ΔSDS ≥−0.5 subgroup, these children were receiving imatinib for <2 years (**Table**), possibly indicating a high risk for developing severe growth impairment thereafter. We compared the distinct impact of long-term imatinib treatment on growth in prepubertal and pubertal children with CML.

Because the average imatinib dose varied among patients in our cohort, analysis was also performed according to the administered dose of imatinib. Although not significant, children exposed to imatinib doses >340 mg/m^2 showed a greater decrease in height-SDS compared with those exposed to lower doses, suggesting the need for further analysis to determine the correlation between imatinib dose and severity of growth impairment.

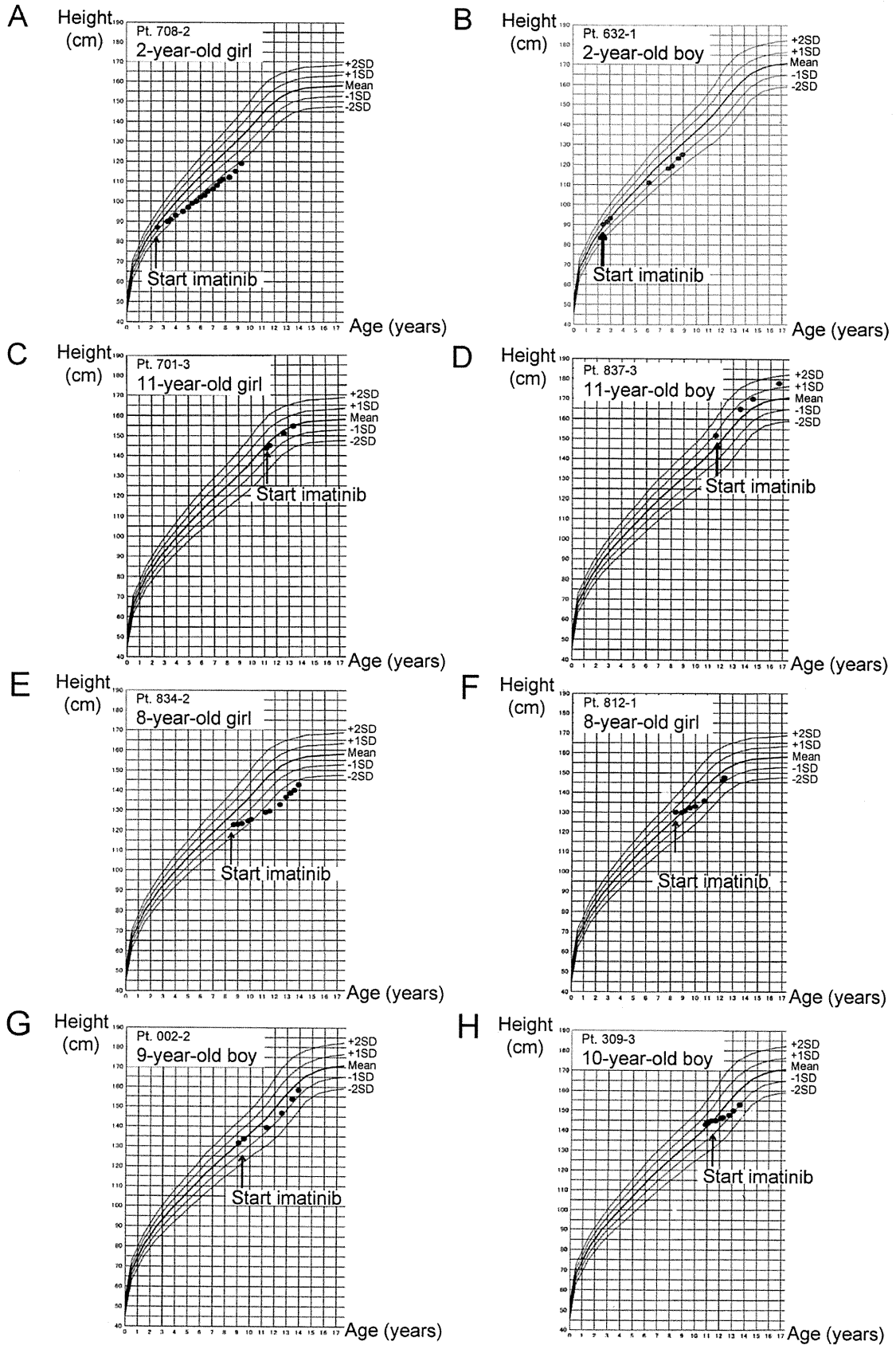

Figure 2. **A** and **B,** Representative height growth chart at the start of imatinib treatment of prepubertal children, and **C** and **D,** pubertal children. Growth impairment was observed in children at prepubertal age, but imatinib had little affect on growth in children at pubertal age. Impaired growth before puberty recovered as children reached pubertal age even during imatinib treatment. Catch-up growth was observed at **E** and **F,** approximately 11 years for girls, and **G** and **H,** 13 years for boys.

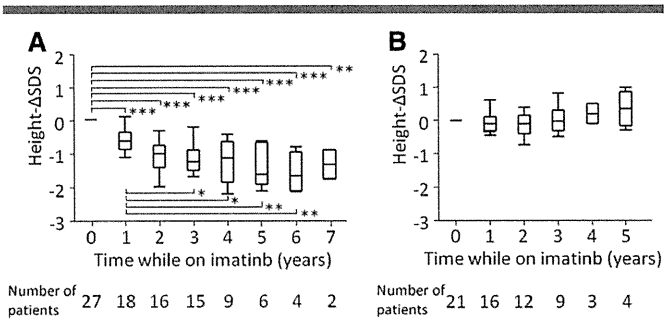

Figure 3. Height-ΔSDS during imatinib treatment of **A,** prepubertal (girls < 9 years, boys < 11 years) or **B,** pubertal children (girls ≥ 9 years, boys ≥ 11 years) in relation to age at the start of treatment. Annual height-ΔSDS is determined by subtracting height-SDS at each annual time point closest to each full-year point within ±6 months from the start of imatinib treatment. *$P < .05$; **$P < .01$; ***$P < .001$, Tukey-Kramer highly significant difference test.

Two previous reports demonstrated a recovery in growth velocity, one after discontinuation of imatinib treatment[3] and another at the onset of puberty even during imatinib treatment.[2] In our study, among 27 children who started imatinib at prepubertal age, 8 children were followed up over the pubertal age range; catch-up growth occurred in 4 children as they reached pubertal age, even during imatinib treatment (**Figure 2**, E-H). Human growth is described by the infancy-childhood-puberty growth model, and growth in puberty is dependent on the synergism between sex hormones and growth hormone (GH).[18] In these 4 children, noticeable catch-up growth was observed at approximately 11 years in girls (**Figure 2**, E and F) and 13 years in boys (**Figure 2**, G and H), consistent with the age at onset of the pubertal growth spurt.[18] These data support the hypothesis that imatinib has little effect on growth of children at pubertal age. Although more follow-up is needed to determine whether this catch-up is complete or incomplete, at least incomplete catch-up growth may be expected in the remaining 4 boys, who were only 13 years or younger at the last follow-up. Our study was performed based on generally agreed-upon prepubertal and pubertal ages, and more detailed studies are needed to determine the relationship between pubertal development and growth impairment.

Vandyke et al[19] recently reported that the rapid acceleration of growth plate closure resulting from the inhibition of PDGF-β receptor signaling by imatinib caused rapid acceleration of growth plate closure. However, bone age detected by wrist and hand X-rays showed no acceleration in other studies,[2,3] and the mechanism associated with the growth inhibitory effect of imatinib remains uncertain. A recent juvenile mouse model study indicated that long-term imatinib treatment impaired the length growth of tubular bone predominantly in prepubertal animals.[20] Consistent with this mouse model, growth impairment due to imatinib may be mild during the age period when height growth is dependent on sex hormones. Thus, imatinib may have a negative effect on GH or its functions. Indeed, Hobernicht et al[21] recently reported a case demonstrating iatrogenically induced GH deficiency due to tyrosine kinase inhibitor therapy for CML. However, performing a GH provocative test in all cohorts proved to be challenging, and moreover, the follow-up period was not of sufficient length for the majority of our cohort to allow determination of later effects on growth. To clarify the potential growth impairment mechanism of long-term imatinib treatment, further study with an extended follow-up period is needed to evaluate the growth recovery that likely would occur concomitantly with pubertal maturation. Because impaired bone remodeling and GH deficiency are caused by inhibition of tyrosine kinase, which is not specific to imatinib,[1,21] careful monitoring of growth velocity, as well as bone metabolic markers and serum insulin-like growth factor 1, is recommended for children treated with tyrosine kinase inhibitors. ■

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Continuous and High-Dose Cytarabine Combined Chemotherapy in Children with Down Syndrome and Acute Myeloid leukemia: Report from the Japanese Children's Cancer and Leukemia Study Group (JCCLSG) AML 9805 Down Study

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Background. The aim of the JCCLSG AML 9805 Down study was to evaluate the effect of continuous and high-dose cytarabine combined chemotherapy on the survival outcome of acute myeloid leukemia (AML) with Down syndrome (DS). ***Procedure.*** From May 1998 to December 2006, DS patients with newly diagnosed AML were enrolled. Remission induction therapy consisted of two courses of pirarubicin, vincristine, and continuous-dose cytarabine (AVC1). The patients who achieved complete remission (CR) after two courses of AVC1 were subsequently treated with mitoxantrone and continuous-dose cytarabine (MC), etoposide and high-dose cytarabine (EC) and pirarubicin, vincristine, and continuous-dose cytarabine (AVC2). ***Results.*** Twenty-four patients were enrolled. All patients were younger than 4 years and diagnosed as having acute megakaryoblastic leukemia. Twenty-one patients achieved CR. Three patients died during remission induction therapy due to serious infection. No toxic deaths were observed during remission. All but one patient maintained CR without serious complications. The 5-year overall and event-free survivals were 87.5% ± 6.8% and 83.1% ± 7.7%, respectively. ***Conclusions.*** Continuous and high-dose cytarabine combined chemotherapy with reduced intensity would be effective in DS children with AML. Pediatr Blood Cancer 2011;57:36–40. © 2010 Wiley-Liss, Inc.

Key words: AML; Clinical trials; Down syndrome

INTRODUCTION

Down syndrome (DS) is one of the most common chromosomal abnormalities and is associated with an increased risk of leukemia [1]. The clinical and biological features of acute myeloid leukemia (AML) in DS children are quite different from those in children without DS: younger age, lower white blood cell count, and high incidence of acute megakaryoblastic leukemia [2,3]. Before the 1990s, most patients with AML with DS (AML-DS) received suboptimal therapy, resulting in poor outcomes. In 1992, high rates of event-free survival (EFS) with intensive AML treatment were reported from the pediatric oncology group (POG) [4]. After recognition of the favorable outcome of AML-DS patients treated with the AML protocol, recruitment to collaborative studies for AML-DS patients increased, but it became apparent that treatment-related toxicity was high in most series [5–7]. Since then, several collaborative groups have adapted their AML protocols for AML-DS by reducing the dosage of chemotherapeutic agents [6].

We report herein the results of the Japanese Children's Cancer and Leukemia Study Group AML 9805 Down study, which evaluated the feasibility, efficacy, and safety of continuous and high-dose cytarabine combined chemotherapy, which was adapted for DS patients by reducing dose intensity.

PATIENTS AND METHODS

Patients

Between May 1998 and December 2006, 24 AML patients with DS entered the Japanese Children's Cancer and Leukemia Study Group AML 9805 Down study after informed consent was obtained. Neonates with transient myeloproliferative disorder (TMD), defined as appearance of myeloid blasts within the first months of life, and those with spontaneous remission were not included. All children and adolescents less than 18 years of age with no prior treatment were eligible. The initial diagnosis of AML and its subtypes was determined according to the FAB classification by institution pathologists, with central review for most cases.

Therapy

The scheme of treatment for the JCCLSG AML 9805 Down study is shown in Table I. Remission induction therapy consisted of two courses of AVC1 (cytarabine (Ara-C) 100 mg/m^2/day continuous infusion on days 1–7, pirarubicin 25 mg/m^2 by 60 min infusion on days 2, and 4, and vincristine (VCR) 0.7 mg/m^2 on day 7).

Patients who achieved complete remission (CR) after two courses of AVC1 were subsequently treated with MC (Ara-C 100 mg/m^2/day continuous infusion on days 1–5 and mitoxantrone (MIT) 3.5 mg/m^2 by 60 min infusion days 2–4), EC (high-dose Ara-C 1 g/m^2 every 12 hr on days 1–5, and etoposide 66 mg/m^2 by 2 h infusion on days 2–4) and AVC2 (Ara-C 100 mg/m^2/day continuous infusion on days 1–5, pirarubicin 35 mg/m^2 by 60 min infusion on day 2, and VCR 0.7 mg/m^2 on day 5).

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Conflict of Interest: Nothing to report.

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TABLE I. Treatment Regimen of the JCCLSG AML9805 Down Study

	Regimen	Administration	Daily dose	Days
Induction				
AVC1	Cytarabine	IV (24 h)	100 mg/m^2	1–7
	Pirarubicin	IV (1 h)	25 mg/m^2	2–4
	Vincristine	IV	0.7 mg/m^2	7
	Methotrexate	IT	Age-adjusted[a]	1
	Cytarabine	IT	Age-adjusted[a]	1, (5, 10)[b]
	Hydrocortisone	IT	Age-adjusted[a]	1, (5, 10)[b]
Consolidation				
MC	Cytarabine	IV (24 h)	100 mg/m^2	1–5
	Mitoxantrone	IV (1 h)	3.5 mg/m^2	2–4
EC	Cytarabine	IV (2 h)	1 g × 2 /m^2	1–5
	Etoposide	IV (2 h)	66 mg/m^2	2–4
AVC2	Cytarabine	IV (24 h)	100 mg/m^2	1–5
	Pirarubicin	IV (1 h)	35 mg/m^2	2
	Vincristine	IV	0.7 mg/m^2	5
	Methotrexate	IT	Age-adjusted[a]	1
	Cytarabine	IT	Age-adjusted[a]	1
	Hydrocortisone	IT	Age-adjusted[a]	1

Recommended interval of each cycle was 4 weeks. [a]The doses were adjusted according to patient's age as follows: younger than 1 year, methotrexate (MTX) 5 mg, cytarabine (Ara-C) 10 mg, hydrocortisone (HDC) 10 mg; younger than 2 years, MTX 8 mg, Ara-C 20 mg, HDC 15 mg; younger than 3 years, MTX 10 mg, Ara-C 30 mg, HDC 20 mg; 3 years and older, MTX 12 mg, Ara-C 40 mg, HDC 25 mg. [b]For CNS-positive patients. The doses were adjusted according to patient's age as follows: younger than 1 year, cytarabine (Ara-C) 20 mg, hydrocortisone (HDC) 10 mg; younger than 2 years, Ara-C 30 mg, HDC 15 mg; younger than 3 years old, Ara-C 50 mg, HDC 20 mg; 3 years and older, Ara-C 70 mg, HDC 25 mg.

Prophylactic treatment for central nervous system (CNS) leukemia was performed by intrathecal injection of Ara-C, methotrexate, and hydrocortisone on the first day of AVC1 and AVC2. An absolute neutrophil count of more than 1,500/μL and a platelet count of more than 75,000/μL were the criteria for starting the first course of consolidation therapy, and an absolute neutrophil count of more than 1,500/μL and a platelet count of more than 100,000/μL were the criteria for starting the second course.

Definitions and Statistics

Evaluation of each treatment was performed on the 28th day. Treatment response was defined as follows: CR, less than 5% blasts in the bone marrow; partial remission (PR), less than 15% blasts; and no response (NR), more than 15% blasts or progressive disease at other sites.

CNS involvement was diagnosed if more than 5 leukocytes/μL were identified in the cerebrospinal fluid (CSF) in combination with detectable leukemic cells in the cytospin and/or with neurological symptoms (e.g., cranial nerve palsy).

EFS was calculated from the date of the first day of chemotherapy to last follow-up or to the first event (early death, resistant leukemia, relapse, or death from any cause). The EFS time of patients with an induction failure was calculated as zero. Toxicity was graded according to the Common Terminology Criteria for Adverse Events version 3.

Univariate comparisons of the survival data were performed using the log-rank test. The Statistical Analysis Software (SAS) computer program was used for the analysis. Follow-up data were actualized as of July 31, 2009.

RESULTS

Patient Characteristics

The relevant initial clinical and hematological data of the 24 patients in this study are shown in Table II. Males predominated, and all patients were younger than 4 years (median age, 17 months). The median white blood cell count was 6,500/μL (range 500–70,900/μL). All patients showed FAB M7 morphologically. No patients had CNS involvement. One patient had an extramedullary mass (skin) at initial diagnosis. Cytogenetic analysis of leukemic blasts was available for 22 patients. Favorable cytogenetics, such as inv (16) and t (8; 21), were not observed. Six patients had normal karyotypes with constitutional trisomy 21 only. The remainder had complex karyotypes with aneuploidy and translocation. GATA1 mutation was confirmed only in one patient.

Seven patients had a history of TMD. No patients of them received cytarabine therapy. Nine patients had documented congenital heart disease. Most patients had either surgically repaired defects or asymptomatic atrial septal defect or ventricular septal defect with normal function.

Overall Outcome

Overall, 21 (87.5%) of 24 patients achieved first remission. One patient relapsed with an isolated extramedullary mass after cessation of chemotherapy. The patient has been in third remission after chemotherapy, electron beam irradiation and cord blood cell transplantation following reduced intensity conditioning. The other 20 patients remain in first CR. Estimated 5-year OS and EFS were 87.5% ± 6.8% and 82.6% ± 7.9%, respectively (Fig. 1). No patients with secondary malignancy and severe cardiotoxicity were observed. Median follow-up period for all patients was 75 (range, 0–131) months.

Treatment-Related Mortality

Three deaths occurred that were not related to leukemia during induction therapy. Two of them occurred during the initial induction therapy, and the other occurred during second induction therapy.

TABLE II. Patients' Characteristic in the JCCLSG AML 9805 Down Study (N = 24)

Characteristic	No	%
Age, months		
Median	17	—
0–12	4	17
12–24	12	46
24–36	4	17
36–48	4	17
Sex		
Male	19	79
Female	5	21
History of TMD		
Yes	7	29
No	13	54
Unknown	4	17
Hepatomegaly		
Yes	10	42
No	12	50
Unknown	2	8
Splenomegaly		
Yes	10	42
No	12	50
Unknown	2	8
WBC, $\times 10^9$/L		
Median	6.5	—
Range	2.8–70.9	—
Hb, g/dL		
Median	8.1	—
Range	3.2–11.8	—
Plt, $\times 10^9$/L		
Median	26	—
Range	3–139	—
Cytogenetics		
Trisomy 8	5	21
Monosomy 7	4	17
Additional 21	2	8

Toxic Events

The incidence of grade 3 or 4 toxicity during induction and each intensification phase of therapy is shown in Table III. Three patients died during remission-induction therapy. One death was attributable to intracranial hemorrhage with disseminated intravascular coagulation, and the others were due to sepsis. The rate of induction death was 12.5%. No toxic deaths were observed during remission.

Prognostic Factors

Extramedullary invasion at initial diagnosis was a significant prognostic factor for 5-year EFS on univariate analysis ($P = 0.046$). Other factors, including sex, initial age, initial WBC, history of TMD, and chromosomal abnormality, were not significant.

DISCUSSION

The results of the JCCSLG AML 9805 Down study, which was conducted to evaluate the efficacy and safety of continuous and high-dose cytarabine combined chemotherapy with reduced intensity for AML-DS patients were presented. All patients enrolled in our study were younger than 4 years and had a phenotype of acute megakaryocytic leukemia (AMKL), which was consistent with previous reports for AML-DS. The number of patients was limited, but this regimen appears to be highly effective because there were no non-responders, and only one patient relapsed.

Contemporary clinical trials for AML-DS children are summarized in Table IV [5–11]. Treatment strategies for AML-DS are based on reduced intensity for AML non-DS, such as BFM and our study, or on a specifically designed strategy, such as the AT/DS study and the AML99 Down study in Japan [8,9]. The EFS of these studies, including the present study, has been between 80% and 90%.

The key drugs for the treatment of AML-DS are anthracyclines, cytarabine, and etoposide; it was also confirmed by in vitro studies that AMKL-DS blasts were significantly more sensitive to these drugs than non-DS AML cells [12]. AMKL-DS blasts are especially sensitive to cytarabine, possibly to the effect of the GATA1 mutations and trisomy 21 on the levels of cytarabine-metabolizing enzymes [13].

In the BFM 98 DS study, with a 3-year EFS of 89%, high-dose cytarabine (3 g/m^2) was used as intensification [6]. The authors reported that a high cure rate could be achieved in DS patients with therapy protocols including high-dose cytarabine. However, they also mentioned that it should be confirmed whether a dosage of 3 g/m^2 of cytarabine is necessary because of its toxicity. In

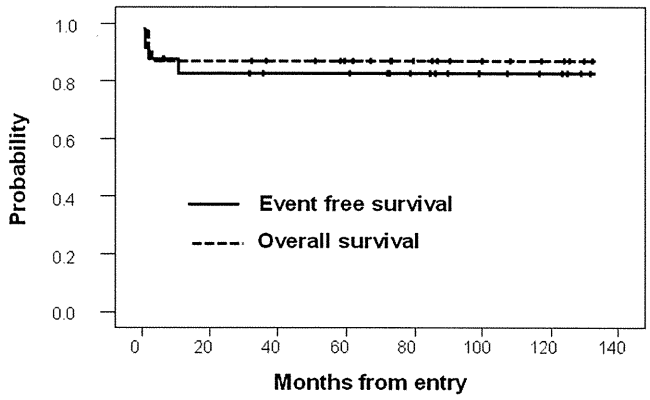

Fig. 1. Actuarial survival rate for the JCCLSG AML 9805 Down study. Of the 24 patients, 22 achieved CR. One patient relapsed. Two patients died during induction therapy. One patient died as a result of sepsis during the first CR. The 5-year overall survival (OS) was 87.5%, and the 5-year EFS was 82.6%.

TABLE III. Severe Adverse Events in the JCCLSG AML9805 Down Study (Grade III–IV)

Adverse events	AVC1-1 no.	n = 24 (%)	AVC1-2 no.	n = 22 (%)	MC no.	n = 21 (%)	EC no.	n = 21 (%)	AVC2 no.	n = 21 (%)
ALT/AST	5	23	2	9	0	0	1	5	0	0
Gastrointestinal	9	41	5	23	5	24	2	10	2	10
Renal	0	0	0	0	0	0	0	0	0	0
Cardiac	0	0	0	0	0	0	0	0	0	0
Pulmonary	1	5	0	0	0	0	0	0	0	0
Neurology	0	0	0	0	0	0	0	0	0	0
Pain	1	5	1	5	0	0	0	0	1	5
Fever/infection	14 (2)	64	9 (1)	41	11	52	15	71	7	33
Others	0	0	0	0	0	0	0	0	0	0

Number of patients who died.

our JCCLSG AML9805 Down study, 1 g/m^2 of cytarabine with etoposide was used for intensification. Serious non-hematological adverse effects, including infection, were not more frequent in this phase than in the other phase of this study (Table III). The dosage of 1 g/m^2 used in the present study may be sufficient for the treatment of AML-DS.

In the Japanese trial AML 99 Down study, the 4-year EFS was 83%, and treatment-related mortality was only 1.4%, which is much lower than that of recent reports for AML-DS [9]. However, relapse and induction failure were more frequent than in other reports with an intensive regimen. The regimen consisted of simple repeating of intermediate doses of pirarubicin and etoposide, so it is possible to reduce the rate of relapse and resistant disease using continuous and high-dose cytarabine combined chemotherapy, as in the JCCLSG AML9805 Down study.

As for other types of leukemia, risk-oriented therapy is proposed if any prognostic factors are identified in AML-DS. In the CCG 2891 study, patients with AML-DS who were older than 2 years had an increased risk of relapse [5]. However, in the BFM 98 DS study and in the Japanese AML 99 Down study, there was no difference in outcome between those 2 years or younger and those older than 2 years [6,9]. The present study also did not identify age older than 2 years as a risk factor, because all 7 patients older than 2 years survived without relapse after completing this protocol.

For cytogenetic factors, monosomy 7 is known to be a risk factor in children with AML [14,15]. In AML-DS, the presence of monosomy 7 adversely affected the outcome in the previous two Japanese trials, but not in the CCG 2891 study [5,8,9]. In the present study, four patients were found to have monosomy 7, and they all maintained remission. Continuous and high-dose cytarabine combined chemotherapy might affect intensification, which negates risk factors such as age and monosomy 7.

It is important to note that only one patient relapsed in the present study. Moreover, the cumulative doses of anthracycline and etoposide in this JCCLSG AML9805 Down study were lower than in other recent reports with intensive regimens for AML-DS. No patients had developed secondary cancer or cardiac insufficiency at the time of this analysis. The survival of DS patients has become longer, and it would be more important to decrease the late toxicity by reducing the cumulative doses of antileukemic drugs for AML-DS patients.

On the contrary, treatment-related mortality occurred in 3 of 24 patients (12.5%), which is more frequent than in other recent reports with intensive regimens for AML-DS. All three patients died from infection during the initial and second courses of this protocol. We could not identify any risk factors for toxicity in these patients, such as age or cardiac disease, compared with the patients who were successfully treated by this protocol. Serious non-hematological adverse effects, including infection, were more frequent during the remission induction phase than during the intensification phase. Induction therapy with combined continuous cytarabine might be toxic for AML-DS patients, although the induction rate is high. On the other hand, toxicity during the intensification phase including high-dose cytarabine was tolerable.

On the basis of the results of the previous Japanese trials and the present study, we have designed a risk-oriented therapy protocol for our next trial with AML-DS. Patients with M2, M3 marrow after induction therapy by pirarubicin, intermediate-dose cytarabine, and etoposide classified into a high-risk group will receive the continuous and high-dose cytarabine combined regimen of this JCCSLG AML9805 Down study.

TABLE IV. Comparison of Recent Clinical Trials for AML-DS

Study	Registry (year)	N	Daunorubicin (mg/m^2)	Ara-C (mg/m^2)	Etoposide (mg/m^2)	TRM (%)	OS (%)	EFS (%)
BFM98 for DS	1998–2003	67	220–240	23–29,000	950	5	91	89 (3y)
BFM93	NA	51	220–400	23,000	950	4	70	68 (3y)
NOPHO AML93	1988–2002	41	300	48,600	1,600	5	NA	85 (8y)
MRC AML10/12	1988–2002	46	670	10,600	NA	15	74	74 (5y)
CCG 2861/2891	1989–1999	160	320	15,800	1,600	4	79	77 (6y)
POG 9421	1995–1999	57	100	20,700	—	0	NA	79 (3y)
AT/Down	1987–1997	33	100–400	4,200	2,700	9	NA	80 (8y)
AML99 DS	2000–2004	72	250	3,500	2,250	1	84	83 (4y)
JCCLSG 9805DS	1998–2006	24	190	12,600	200	12.5	88	83 (5y)

TRM, treatment-related mortality; OS, overall survival; EFS, event-free survival; NA, not evaluated.

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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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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 $\geqq 300$ 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.