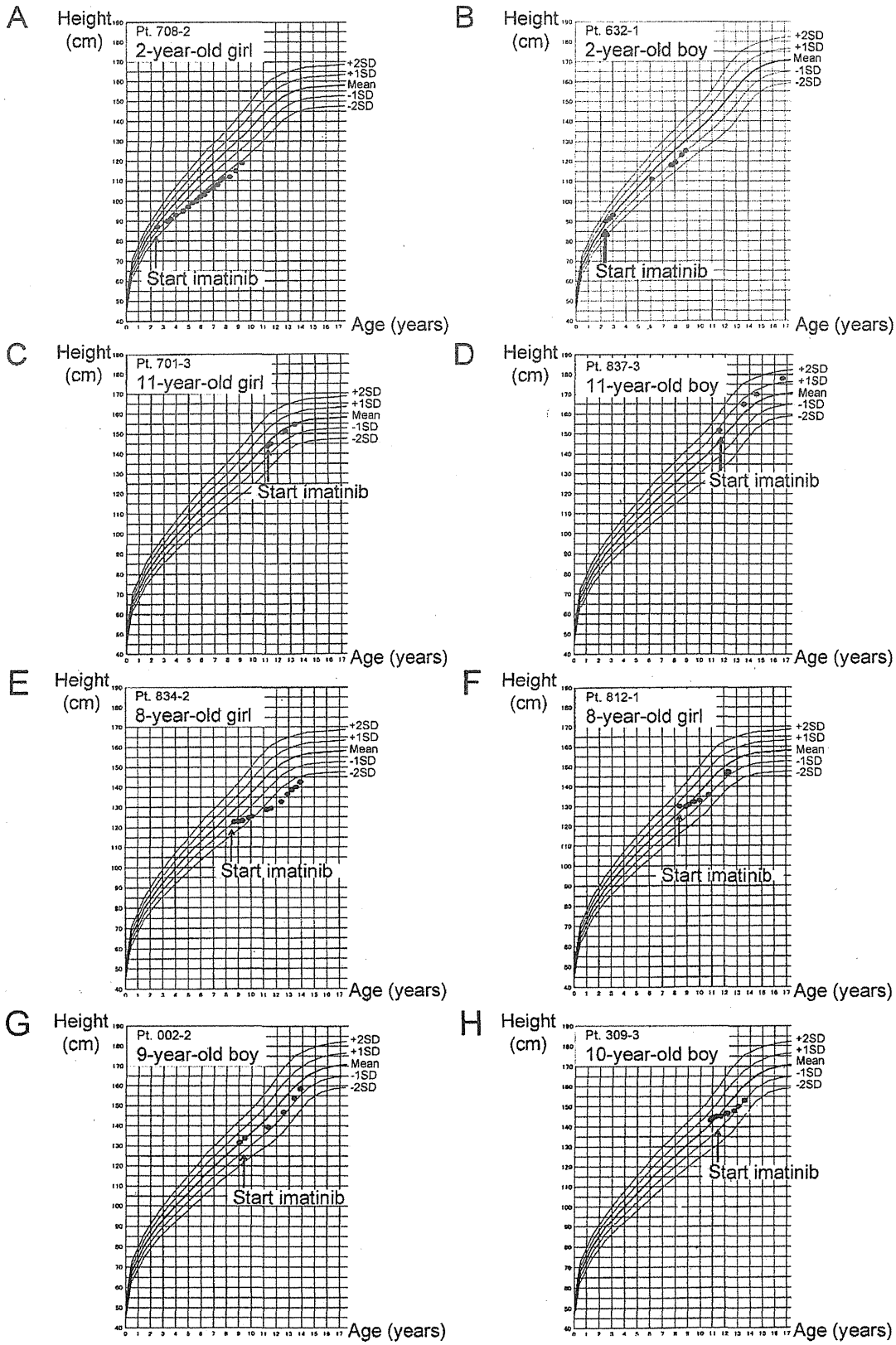

Figure 2. **A** and **B,** Representative height growth chart at the start of imatinib treatment of prepubertal children, and **C** and **D,** pubertal children. Growth impairment was observed in children at prepubertal age, but imatinib had little affect on growth in children at pubertal age. Impaired 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.

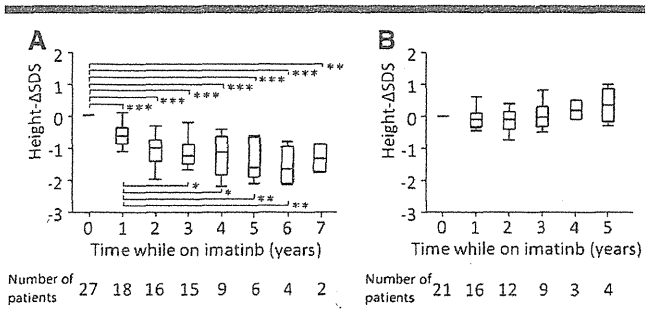

Figure 3. Height-ΔSDS during imatinib treatment of **A**, prepubertal (girls < 9 years, boys < 11 years) or **B**, pubertal children (girls ≥ 9 years, boys ≥ 11 years) in relation to age at the start of treatment. Annual height-ΔSDS is determined by subtracting height-SDS at each annual time point closest 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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ORIGINAL ARTICLE

Social outcomes and quality of life of childhood cancer survivors in Japan: a cross-sectional study on marriage, education, employment and health-related QOL (SF-36)

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Abstract Social outcomes and quality of life (QOL) of childhood cancer survivors (CCSs) remain unknown in Japan. We investigated these outcomes in young adult CCSs compared to those of their siblings in Japan, and analyzed the association between social outcome and SF-36 health survey subscale scores. Between 2007 and 2009, we performed a cross-sectional survey using self-rating questionnaires. We estimated social outcomes and health-related QOL by performing the SF-36 in each group: CCSs with or without stem cell transplantation (SCT)/radiotherapy (RT) and their siblings. Adjusted odds ratios for outcomes of interest were estimated using logistic regression analysis. Questionnaires from 185 CCSs and 72 CCS's siblings were analyzed. There were no differences in educational attainment or annual income. The SF-36 subscale scores of CCSs with SCT and RT were significantly lower than those of siblings in physical functioning (PF) ($p < 0.001$ and 0.003, respectively) and general health (GH) (both $p = 0.001$). Lower PF scores correlated with recurrence ($p = 0.041$) and late effects ($p = 0.010$), and poor GH scores with late effects ($p = 0.006$). The CCSs had made efforts to attain educational/vocational goals; however, a significant proportion of CCSs who had experienced late effects remain at increased risk of experiencing diminished QOL.

Keywords Childhood cancer survivors · Marriage · Education · Employment · Health-related QOL · SF-36

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1 Introduction

As a result of advances in treatment, 70–80% of children diagnosed with cancer become long-term survivors. In Japan, the estimated number of pediatric cancer survivors is upward of 50,000, or approximately one in 700 adults between the ages of 20 and 39 years. Although an increased number of children with cancer have been cured, many survivors experience various health problems or late effects as a result of their treatments [1, 2]. In addition to various physical problems in childhood cancer survivors (CCSs) [3], social outcomes vis-à-vis marriage, education and employment are apparently affected by these late effects, either directly or indirectly. An increasing number of studies have focused on the social outcomes of CCSs [4–12].

A Swedish population-based study [4] revealed that central nervous system (CNS) tumor survivors had poorer social outcomes compared to the general population, whereas outcomes for non-CNS cancer survivors were similar to those of the general population. On the other hand, the results of the Childhood Cancer Survivor Study (CCSS) suggest that CCSs generally have high school graduation rates similar to those in the general population, but they are slightly less likely to attend college; they are also more likely to be unemployed and not married as young adults [5]. Johannsdottir et al. [6] also outline important differences in social outcomes (i.e., employment and parenthood) between CCS and controls early in adult life.

The health-related quality of life (QOL) of CCSs has been studied extensively using the 36-item Short Form Health Survey (SF-36). Reulen et al. [13] demonstrated the validity and reliability of the SF-36 when used with CCSs, but they point out that ceiling effects should be recognized for researchers in using the SF-36 with CCSs. Maunsell et al. [14] show that QOL differences between CCSs and controls are small, and for the most part are probably not clinically important. In their study, survivors' scores on most subscales of the SF-36 were similar to those of controls, despite experiencing some difficulties in their daily activities [15].

Many reports including meta-analyses or systematic reviews of social outcomes [16] and QOL [17, 18] among CCSs have been published; however, the association between social outcomes and SF-36 scores remains to be elucidated [12, 19]. We have already reported that both stem cell transplantation (SCT) and radiotherapy (RT) are closely associated with the late effects of CCSs [20, 21] and that no significant differences are found between CCSs and siblings in terms of depression and anxiety, but CCSs have significantly more posttraumatic stress symptoms and greater posttraumatic growth [22]. In this article, we investigated the social outcomes and QOL of young adult CCSs with or without SCT/RT compared to those of their siblings in the same population, and analyzed the association between social outcomes and SF-36 subscale scores.

2 Patients and methods

2.1 Study design and participants

We performed a cross-sectional survey involving self-rating questionnaires vis-à-vis the social outcomes and QOL of CCSs, compared to those of the siblings [20, 23]. The study was conducted between 1 August 2007, and 31 March 2009. The subjects were divided into three groups: the CCS with or without SCT/RT, and siblings. The last group was considered as a control that matched with the CCSs with regard to genetic capabilities and environmental similarity. The CCS and their siblings were recruited from the participating hospitals listed in the supplemental appendix 1.

The eligibility criteria for CCSs and their siblings were as follows: (1) the subjects were 16 years old or older at the time of the survey, (2) CCSs had been diagnosed with cancer at 18 years of age or younger, (3) CCSs had been in continuous remission for more than 5 years since cancer diagnosis without any additional need for anticancer therapy, (4) they had been informed about their diagnoses, and (5) informed consent was provided by both CCSs/siblings and their guardians. If CCSs had two or more siblings, we selected the subject with the nearest age to the CCSs among the siblings. The exclusion criteria were as follows: (1) the attending physicians believed that the survey would cause an undesirable effect on CCSs, (2) the subjects had some underlying disease besides cancer that affected their social outcome or QOL, or (3) the subjects were unable to answer the questionnaires by themselves.

2.2 Methods

After obtaining appropriate informed consent, the CCSs were provided with an anonymous questionnaire by the attending pediatricians and asked to return it within post-one month. The patients' clinical records were reviewed to analyze cancer-related variables, including the diagnosis, birth year and month, age at diagnosis, age at therapy completion, time since diagnosis, treatment variables and the late effects of CCSs observed at the time of the survey. We used an encrypted numbering system for dispatching data to the principal investigator, to maintain the confidentiality of patient information. Late effects were defined as adverse events that were grade 2 (i.e., symptomatic or needing some intervention) or higher, according to the

Common Terminology Criteria for Adverse Events, v. 3 (CTCAEv3), which was originally developed by the National Cancer Institute (Japanese CTCAE v. 3.0 by JCOG and JSCO, http://www.jcog.jp/).

2.3 Measurement of variables

The questionnaire consisted of 220 items, with three items involving free writing. We evaluated seven background items (Q1), two truth-telling-related items (Q2), seven lifestyle-associated items (Q3), nine items related to medical visits to the hospital (Q4), four general health-related items (Q5), six past operation and drug usage history items (Q6), seven daily habit items (Q7), nine pregnancy and delivery history items (Q8), 72 subjective physical dysfunctions items (Q9), 36 SF-36-related items (Q10), 64 psychosocial problems-related items (Q11) and three free-writing items (Q12).

In this article, we focus on Q3 and Q10. Q3 contained seven items relating to lifestyle, marital status, educational achievements, current employment, work status, working ability (frequency of absence) and annual income in the last year. Q10, comprising 36 SF-36 items, was often used to measure health-related QOL outcomes [24]. The SF-36 is a generic self-report measure that evaluates eight subscales that represent different aspects of well-being, with respect to eight physical and mental health dimensions in Table 1: physical functioning (PF), bodily pain (BP), role limitations caused by physical health problems (RP), role limitations caused by personal or emotional health problems (RE), general mental health (MH), social functioning (SF), vitality (VT) and general health perception (GH). It also involves two summary scales: the mental component score (MCS) and the physical component score (PCS). Multi-item subscales are scored on a 0–100 percentage scale, with higher scores representing higher levels of functioning and health. Data were presented as *T* scores, with a mean score of 50 and a standard deviation (SD) of 10. *T* scores were dichotomized, in which a *T* score below the population score (i.e., the respective nation's norm, while matching for both age and gender in 2007 [25]) indicated a respondent as having reported poor health-related QOL (HR-QOL). Interpretation guidelines link SF-36 subscales and summary scores to the probability of outcomes, allowing scores to be used as predictors of morbidity (physical and mental) and health-care utilization. SF-36 and summary scores have been extensively tested for reliability and validity [26]. The Cronbach's alpha coefficient of SF-36 was found to be 0.79 (CCSs only) and 0.71 (CCSs and siblings) in this study.

In terms of marital status, subjects were categorized as married, never married and others (i.e., divorced or remarried), while educational achievement was classified as follows: lower than high school, high school graduate, college or vocational school graduate, and university or graduate school graduate. Further, employment status was classified as follows: company desk workers ("white collar"); part-time workers; those with medical jobs; industrial workers ("blue collar"); homemakers; those who were unemployed, including those on job training; and others. In terms of annual income, each subject was classified into one of five categories: less than 1 million Japanese yen (JPY), 1–2 million JPY, 2–3 million JPY, 3–5 million JPY and 5 million JPY or more.

2.4 Ethical issues

The study was performed in accordance with the Declaration of Helsinki and was approved by the ethics committee of the principal investigator's institution (Y. Ishida, Ehime University Graduate School of Medicine and St. Luke's International Hospital). The study was also approved by the local ethics committees of all the participating hospitals, prior to initiation.

2.5 Statistical analysis

We estimated the prevalence of outcomes among CCSs with or without SCT/RT and the siblings group. Three primary outcomes were assessed: (1) social outcomes and (2) general QOL according to SF-36 scores between each pair groups (i.e., CCSs and siblings, CCSs with SCT and CCSs without SCT, CCSs with RT and CCSs without RT), and (3) the association between social outcomes and SF-36 scores (for the CCS group only). We performed χ^2 tests or a Fisher exact test (for any cells with expected counts <5) within categorical predictors, and the *t* test or Kruskal–Wallis methods for continuous variables. As for cross-table comparisons, we used adjusted standardized residuals to evaluate the difference between the observed and expected values; the columns which gave more than 1.96 of adjusted standardized residual were considered as significant [27]. The adjusted odds ratios (ORs) for adverse outcomes were estimated by employing logistic regression analysis. As adjusted variables, we selected independent, significant risk factors such as SCT, solid tumors, recurrence and duration after therapy completion, as shown in our previous article. To avoid multi-collinearity, we assessed associations between predictors in a pairwise fashion. Data were analyzed through the use of SPSS software, v. 18.0 (SPSS IBM Japan Inc., Tokyo, Japan).

We planned a study of independent CCSs and siblings, with five CCSs per sibling. The results of a previous study [3] indicate that the probability of chronic health conditions among siblings is 0.35. If the true probability of chronic health conditions among CCSs is 0.60, we would need to

Table 1 Information of the SF-36 subscales and summary scores [25]

Name of subscale	No. of items	Summary of contents
Physical component score (PCS)		
Physical functioning (PF)	10	Extent to which health limits physical activities such as self-care, walking, climbing stairs, bending, lifting, and moderate and vigourous exercises
Role limitations caused by physical health problems (RP)	4	Extent to which physical health interferes with work of other daily activities, including accomplishing less than that required, limitations in the kind of activities or difficulty in performing activities
Bodily pain (BP)	2	Intensity or pain and effect of pain on normal work, both inside and outside the home
General health perception (GH)	5	Personal evaluation of health, including current health, health outlook and resistance to illness
Mental component score (MCS)		
Vitality (VT)	4	Feeling energetic and full of pep versus feeling tired and worn out
Social functioning (SF)	2	Extent to which physical health or emotional problems interfere with normal social activities
Role limitations caused by personal or emotional health problems (RE)	3	Extent to which emotional problems interfere with work or other daily activities, including decreased time spent on activities, accomplishing less and not working as carefully as usual
General mental health (MH)	5	General mental health, including depression, anxiety, behavioral–emotional control, general positive affect

study 180 case patients and 36 control patients to be able to reject the null hypothesis that the outcome rates for CCSs and siblings are equal with a power of 0.8 ($\beta = 0.2$) and a type I error probability (α) of 0.05. We therefore used an uncorrected χ^2 statistic to evaluate this null hypothesis. In addition, the number needed to analyze nine determinants via multivariate logistic regression methods—to determine the risk factors for late effects—was estimated as 180 for CCSs.

3 Results

The demographic data of the participants are shown in Table 2. Among the CCSs, 189 returned the questionnaires (response rate 72%). Of these, four subjects were excluded because two of the four had an underlying disease besides cancer that affected their QOL, one questionnaire had been completed by the patient's mother and one CCS was 20 years old at diagnosis. We also excluded two questionnaires from siblings, because they were 14 and 15 years of age at the time of survey. The mean age at diagnosis was 8.3 years (SD 4.8) for female CCSs and 8.5 years (SD 5.0) for male CCSs. The proportion of those aged 16–19 years was a little smaller in the siblings group than in the CCSs group. With regard to the primary cancers involved, acute lymphoblastic leukemia comprised 43.9% of the CCSs, followed by acute myeloid leukemia/myelodysplastic syndrome (13.3%) and lymphoma (12.3%). A total of 128 cases of primary cancers were hematological, followed by brain tumors (10 cases), bone/soft tissue sarcoma (18 cases) and other solid tumors (29 cases). As for treatment of the primary cancer, 98% of the CCSs received chemotherapy, 61%, RT, 38% surgery; and 25% hematopoietic SCT. Among the CCSs, one or more late effects were found in 56%, two or more late effects in 17% and three or more in 6%.

The current social outcomes between each pair groups are shown in Table 3. The proportion of subjects living with a partner was higher and that living with parents was lower significantly in the sibling group, because the marriage rate within the female sibling group was high (36%). The marriage rate was especially high in the younger than 24 years of age group for siblings; however, the marriage rate was quite similar in the 25 years or more age group. There were also no large differences in educational attainment; the CCSs revealed a higher proportion of high school level and the CCS with SCT showed a higher proportion of university/graduate school level. The unemployment rate tended to be a little high in the CCSs, especially CCSs with SCT or RT compared to the siblings. The proportion of company desk workers ("white collar") was significantly higher in the sibling group compared to the CCSs. Of particular importance was the high proportion of CCSs holding medical jobs: 15% for females and 7% for males. Finally, there were no large differences in working

Table 2 The demographical data of participants

	Total CCS ($n = 184$)	Siblings ($n = 72$)	t test or χ^2 (p value) CCS versus siblings	CCS with SCT ($n = 46$)	CCS without SCT ($n = 138$)	t test or χ^2 (p value) SCT versus no SCT	CCS with RT ($n = 113$)	CCS without RT ($n = 72$)	t test or χ^2 (p value) RT versus no RT
Gender (female)	108 (58%)	42 (58%)	0.995	27 (59%)	81 (58%)	0.960	68 (60%)	40 (56%)	0.534
Age at diagnosis (median)	8.3 ± 4.8 (8)			10.1 ± 4.4 (10)	7.7 ± 4.8 (7)	0.003	8.6 ± 4.8 (8)	7.9 ± 4.9 (7)	0.350
0–5 years of age	60 (32%)	–		10 (22%)	50 (36%)[a]	0.036	37 (33%)	23 (32%)	0.256
6–10 years of age	50 (27%)	–		10 (22%)	40 (29%)		26 (23%)	24 (33%)	
≥11 years of age	75 (41%)	–		26 (57%)[a]	49 (35%)		50 (44%)	25 (35%)	
Age at survey (median)	23.1 ± 4.9 (22)	24.9 ± 5.1 (24)	0.001	22.9 ± 4.8 (22)	23.2 ± 5.0 (22)	0.659	24.1 ± 5.0 (23.5)	21.6 ± 4.5 (21)	0.001
16–19 years of age	47 (25%)[a]	7 (10%)	0.040	11 (24%)	36 (26%)	0.566	21 (19%)	26 (36%)[a]	0.026
20–24 years of age	75 (40%)	19 (41%)		19 (41%)	56 (40%)		46 (41%)	29 (40%)	
25–29 years of age	38 (21%)	12 (26%)		12 (26%)	26 (19%)		27 (24%)	11 (15%)	
≥30 years of age	25 (14%)	4 (9%)		4 (9%)	21 (15%)		19 (17%)	6 (8%)	
Duration after therapy cessation									
0–4 years	5 (3%)	–		3 (7%)	2 (1%)	0.003	4 (4%)	1 (1%)	0.255
5–9 years	50 (27%)	–		19 (41%)[a]	31 (22%)		28 (25%)	22 (31%)	
10–14 years	57 (31%)	–		15 (33%)	42 (30%)		31 (27%)	26 (36%)	
≥15 years	73 (40%)	–		9 (20%)	64 (46%)[a]		50 (44%)	23 (32%)	
Primary cancer									
Solid tumors	57 (31%)	–		46 (33%)	11 (24%)	0.242	80 (71%)	48 (67%)	0.553
Hematological	128 (69%)	–		93 (67%)	35 (76%)		33 (29%)	24 (33%)	
Treatment									
Operation	70 (38%)	–		14 (30%)	56 (40%)	0.232	40 (35%)	30 (42%)	0.391
Anthracyclines	152 (82%)	–		41 (89%)	111 (80%)	0.154	93 (82%)	59 (82%)	0.951
Alkylating agents	155 (84%)	–		45 (98%)	110 (79%)	0.003	101 (89%)	54 (75%)	0.010
Etoposide	76 (41%)	–		32 (70%)	44 (32%)	<0.001	50 (44%)	26 (36%)	0.273
Radiation	113 (61%)	–		39 (85%)	74 (53%)	<0.001	100%	0%	–
SCT	46 (25%)	–		100%	0%	–	39 (35%)	7 (10%)	<0.001
Recurrence	33 (18%)	–		18 (39%)	15 (11%)	<0.001	28 (25%)	5 (7%)	0.002
Late effects	103 (56%)	–		36 (78%)	67 (48%)	<0.001	77 (68%)	26 (36%)	<0.001
Only 1 late effects	61 (33%)	–		13 (28%)	48 (35%)	0.416	40 (35%)	21 (29%)	0.379
2 or more late effects	42 (23%)	–		23 (50%)	19 (14%)	<0.001	37 (33%)	5 (7%)	<0.001

Age was expressed as mean value ± standard deviation (median value)

CCS childhood cancer survivors, *SCT* stem cell transplantation, *RT* radiation

[a] Adjusted standardized residual ≥+1.96

Table 3 Current social outcome status between each pair groups (i.e., CCS and siblings, CCS with SCT and without SCT, CCS with RT[4] and without RT[4])

	Total CCS ($n = 184$)	Siblings ($n = 72$)	χ^2 (p value) CCS versus siblings	CCS with SCT ($n = 46$)	CCS without SCT ($n = 138$)	χ^2 (p value) SCT versus no SCT	CCS with RT[4] ($n = 112$)	CCS without RT[4] ($n = 72$)	χ^2 (p value) RT versus no RT
Living style									
Living alone	37 (20%)	18 (25%)	0.031	7 (15%)	30 (22%)	0.819	22 (20%)	15 (21%)	0.456
Living with parents	116 (63%)[a]	32 (44%)		31 (67%)	85 (62%)		70 (63%)	46 (64%)	
Living with partner	23 (13%)	18 (25%)[a]		6 (13%)	17 (12%)		13 (12%)	10 (14%)	
Others	8 (4%)	4 (6%)		2 (4%)	6 (4%)		7 (6%)	1 (1%)	
Marital status									
Never married	158 (86%)[a]	54 (75%)	0.090	40 (87%)	118 (86%)	0.844	98 (87%)	60 (86%)	0.444
Married	24 (13%)	17 (24%)[a]		6 (13%)	18 (13%)		15 (13%)	9 (13%)	
Divorced or re-married	1 (0.5%)	1 (1%)		0	1 (1%)		0	1 (1%)	
Marriage rate									
≤24 years of age	2 (2%)	4 (10%)	0.014	0	2 (4%)	0.413	0	2 (4%)	0.112
25–29 years of age	8 (23%)	7 (33%)	0.328	2 (17%)	6 (26%)	0.612	3 (12%)	5 (56%)	0.011
≥30 years of age	14 (56%)	6 (55%)	0.732	4 (100%)	10 (48%)	0.053	12 (63%)	2 (33%)	0.199
Educational achievement									
Lower than high school	7 (4%)	2 (3%)	0.169	0	7 (5%)	0.126	3 (3%)	4 (6%)	0.033
High school	61 (33%)[a]	14 (19%)		14 (30%)	47 (34%)		31 (27%)	30 (42%)[a]	
College/vocational School	51 (28%)	24 (39%)		10 (22%)	41 (30%)		39 (35%)[a]	12 (17%)	
University/graduate school	66 (36%)	32 (45%)		22 (48%)[a]	44 (32%)		40 (35%)	26 (36%)	
Current job									
Student	72 (39%)	24 (33%)	0.011	22 (48%)	50 (36%)	0.694	35 (31%)	37 (51%)[a]	0.099
Company (white collar)	27 (15%)	18 (25%)[a]		5 (11%)	22 (16%)		17 (15%)	10 (14%)	
Part-time job	14 (8%)	8 (11%)		3 (6%)	11 (8%)		12 (11%)[a]	2 (3%)	
Medical job	20 (11%)[a]	0		5 (11%)	15 (11%)		13 (12%)	7 (10%)	
Industry (blue collar)	14 (8%)	3 (4%)		3 (6%)	11 (8%)		11 (10%)	3 (4%)	
Homemaker	15 (8%)	9 (13%)		3 (6)	12 (9%)		9 (8%)	6 (8%)	
Unemployed	7 (4%)	0		3 (6%)	4 (3%)		6 (5%)	1 (1%)	
Others	16 (9%)	10 (14%)		2 (4%)	14 (10%)		10 (9%)	6 (8%)	
Working ability									
No. of days/month	156 (89%)	62 (94%)	0.446	37 (86%)	19 (90%)	0.822	97 (89%)	59 (88%)	0.964
1–2 days/month	13 (7%)	3 (5%)		4 (9%)	9 (7%)		8 (7%)	5 (8%)	
More than 1–2 days/week	7 (4%)	1 (1%)		2 (5%)	5 (4%)		4 (4%)	3 (5%)	

Table 3 continued

	Total CCS ($n = 184$)	Siblings ($n = 72$)	χ^2 (p value) CCS versus siblings	CCS with SCT ($n = 46$)	CCS without SCT ($n = 138$)	χ^2 (p value) SCT versus no SCT	CCS with RT[4] ($n = 112$)	CCS without RT[4] ($n = 72$)	χ^2 (p value) RT versus no RT
Annual income in the last year (JPY)									
<1 million	111 (61%)	40 (58%)	0.586	32 (71%)	79 (58%)	0.276	61 (55%)	50 (70%)[a]	0.098
1–2 million	33 (18%)	9 (13%)		5 (11%)	28 (20%)		27 (24%)[a]	6 (9%)	
2–3 million	21 (12%)	11 (16%)		3 (7%)	18 (13%)		13 (12%)	8 (11%)	
3–5 million	15 (8%)	7 (10%)		5 (11%)	10 (7%)		9 (8%)	6 (9%)	
≥5 million	2 (1%)	2 (3%)		0	2 (2%)		1 (1%)	1 (1%)	

CCS childhood cancer survivors, *JPY* Japanese yen, *SCT* stem cell transplantation, *RT* radiation

[a] Adjusted standardized residual >+1.96

ability or annual income among each group; the CCSs with RT achieved a little lower annual income compared to the CCSs without RT because of a high proportion of students.

The current social outcome status of the CCSs with SCT or RT according to the number of late effects is shown in Table 4. No difference was found with respect to living style, marriage rate and annual income between CCSs lacking any late effects and CCS with only one late effect; however, CCSs with two or more late effects showed extremely low marriage rates (0 and 3%, respectively). A high unemployment rate (from 9 to 5%) was found in CCSs with any late effects in SCT and RT groups.

Figure 1 shows a box plot analysis of the SF-36 subscales and the summary scores among the CCSs with or without SCT and the siblings group. Ceiling effects were found to be high in the PF, RP, BP, SF and RE subscales, for both the CCSs and siblings (supplemental appendix 2). The distributions of each subscale score were much skewed and non-parametric methods using Kruskall–Wallis showed that there was a statistically significant difference in the PF ($p < 0.001$) and GH subscales ($p = 0.001$) between the CCSs with SCT and siblings. A statistically significant difference was also found in the J-PCS and PF subscales between the CCSs with SCT and without SCT, and in the GH subscales between the CCS without SCT and siblings. Figure 2 shows a box plot analysis of the SF-36 subscales and the summary scores among the CCSs with or without RT and the siblings group. A statistically significant difference in the PF ($p = 0.003$) and GH subscales ($p = 0.001$) between the CCSs with SCT and siblings was found. On comparison of the CCSs with the age-matched general population, a statistically significant difference was found in the J-MCS, PF, BP and RE subscales between the CCSs and the nation's standard reference values [25] (supplemental appendix 2).

We created dichotomous variables from each subscale score, to determine whether each subject showed lower SF-36 subscale scores compared to Japan's national norm standards in 2007 [25]. We explored risk factors associated with the lower PF and GH subscale scores of the CCSs, using logistic regression analysis (Table 5). Lower PF scores were associated with recurrence [OR 2.80; 95% confidence interval (CI) 1.04–8.33; $p = 0.041$] and late effects (OR 3.33; 95% CI 1.33–8.33; $p = 0.010$); also, lower GH scores were associated with late effects (OR 2.81; 95% CI 1.35–5.85; $p = 0.006$).

4 Discussion

We found that the long-term social outcome of the CCS group was almost similar to that of siblings in Japan. In line with the Erice statement [28], the majority of survivors

Table 4 Current social outcome status of cancer survivors with or without late effects in the SCT or RT groups

Gender	SCT group ($n = 46$)				RT group ($n = 77$)			
Late effects	Absent ($n = 10$)	Only 1 ($n = 13$)	2 or more ($n = 23$)	χ^2 (p value)	Absent ($n = 36$)	Only 1 ($n = 39$)	2 or more ($n = 36$)	χ^2 (p value)
Living style								
Living alone	0	2 (15%)	5 (22%)	0.126	7 (19%)	8 (21%)	7 (19%)	0.089
Living with parents	6 (60%)	8 (62%)	17 (74%)		18 (50%)	23 (59%)	28 (78%)[a]	
Living with partner	3 (30%)	3 (23%)	0		7 (19%)	6 (15%)	0	
Others	1 (10%)	0	1 (4%)		4 (11%)	2 (5%)	1 (3%)	
Marital status								
Never married	7 (70%)	10 (77%)	23 (100%)[a]	0.028	29 (81%)	33 (82%)	35 (97%)[a]	0.074
Married	3 (30%)	3 (23%)	0		7 (19%)	7 (18%)	1 (3%)	
Educational achievement								
Lower than high school	0	0	0	0.489	1 (3%)	1 (3%)	1 (3%)	0.342
High school	3 (30%)	3 (23%)	8 (35%)		5 (14%)[a]	14 (35%)	12 (33%)	
College/vocational school	1 (10%)	5 (39%)	4 (17%)		17 (47%)	13 (33%)	9 (25%)	
University/graduate school	6 (60%)	6 (39%)	11 (48%)		13 (36%)	12 (30%)	14 (39%)	
Current job								
Student	5 (50%)	3 (23%)[a]	14 (61%)	0.161	10 (28%)	8 (20%)	17 (47%)[a]	0.286
Company (white collar)	2 (20%)	1 (8%)	2 (95)		6 (17%)	7 (18%)	4 (11%)	
Part-time job	0	2 (15%)	1 (4%)		2 (6%)	8 (20%)[a]	2 (6%)	
Medical job	1 (10%)	1 (8%)	3 (13%)		3 (8%)	5 (12%)	5 (14%)	
Industry (blue collar)	0	3 (23%)	0		4 (11%)	5 (12%)	2 (6%)	
Homemaker	1 (10%)	2 (15%)	0		4 (11%)	3 (7%)	2 (6%)	
Unemployed	0	1 (8%)	2 (9%)		1 (3%)	2 (5%)	2 (6%)	
Others	1 (10%)	0	1 (4%)		6 (17%)	2 (5%)	2 (6%)	
Working ability								
No. of days/month	8 (89%)	7 (64%)[a]	22 (96%)	0.082	33 (97%)	32 (84%)	31 (86%)	0.275
1–2 days/month	1 (11%)	2 (18%)	1 (4%)		1 (3%)	3 (8%)	4 (11%)	
More than 1–2 days/week	0	2 (18%)[a]	0		0	3 (8%)	1 (3%)	
Annual income in the last year (JPY[a])								
<1 million	6 (60%)	10 (77%)	16 (73%)	0.247	17 (47%)	22 (56%)	21 (60%)	0.534
1–2 million	1 (10%)	2 (15%)	2 (9%)		11 (31%)	11 (28%)	5 (14%)	
2–3 million	0	0	3 (14%)		4 (11%)	3 (8%)	6 (17%)	
≥3 million	3 (30%)[a]	1 (8%)	1 (5%)		4 (11%)	3 (8%)	3 (9%)	

JPY Japanese yen, *SCT* stem cell transplantation, *RT* radiation

[a] Adjusted standardized residual >+1.96

become relatively well adjusted in adulthood; indeed, there is a proportion exhibiting extraordinary resilience. However, compared to siblings, a significant proportion of CCSs are at an increased risk of developing conditions that require medical, psychological or social care because SCT and RT are closely associated with various late effects reported previously [20, 21]. Our study showed that the marriage rate of the CCSs in 24 years of age or younger patients was a little lower than that of their siblings, and that little difference existed in educational achievement between the CCSs and their siblings [9, 15]. A limitation of our study was that the mean and median ages of the participants were only 23–24 years; this is too young an age to evaluate the total marriage rate, as the average marriage age has been increasing recently (i.e., in 2008, the Japanese national mean age of marriage was 30.2 years for males and 28.5 years for females). By using an analysis of stratification by age, the marriage rate became almost the same in the 25 years or more age group for both females and males.

On the other hand, there were small differences in employment status and annual income among each group

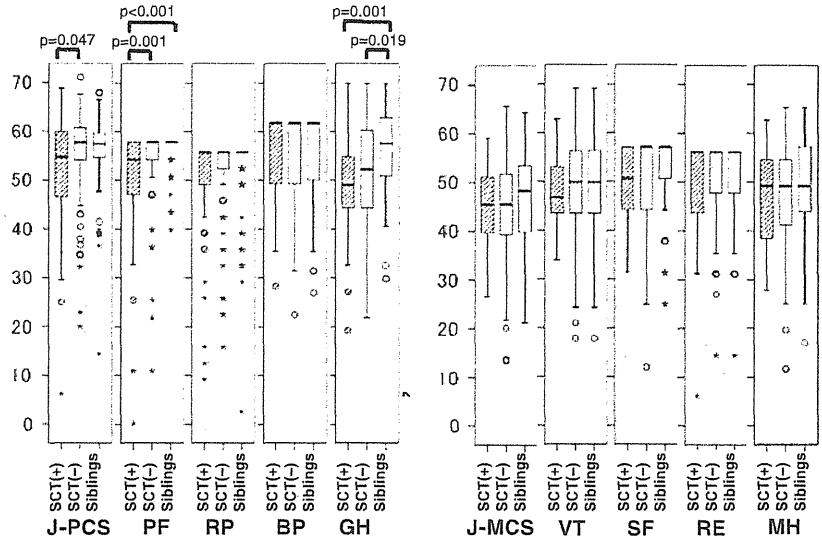

Fig. 1 Box and whisker plot of SF-36 subscale scores according to stem cell transplantation. The *bottom* and *top* of the *box* are the 25th and 75th percentile, respectively, and the thick band near the middle of the box is the 50th percentile (the median). The *ends of the whiskers* represent the lowest datum still within 1.5 interquartile range (IQR) of the lower quartile, and the highest datum still within 1.5 IQR of the upper quartile. The *open circles* are outliers between 1.5 and 3 IQR from the end of a box, and the *asterisks* are extreme values beyond 3 IQR from the end of a box. Kruskal–Wallis test reveal that SF-36 subscales scores of childhood cancer survivors (CCSs) with stem cell transplantation (SCT; *hatched bars*) are significantly lower than those of siblings (*open bars*) in PF and GH subscales, respectively. The J-PCS and PF scores in CCSs with SCT are also significantly lower than those in CCS without SCT (*dotted bars*). The GH scores of CCSs without SCT are significantly lower than those of siblings. All *p* values are adjusted by pairwise multiple comparison

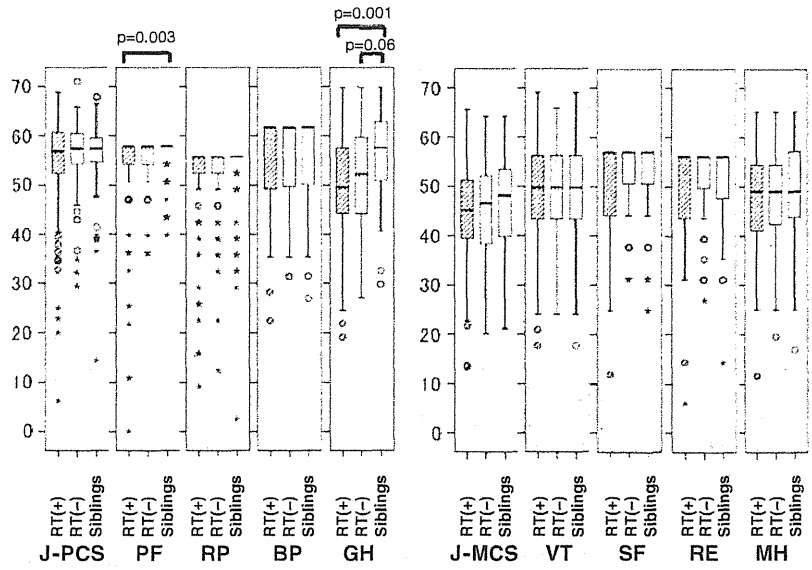

Fig. 2 Box and whisker plot of SF-36 subscale scores according to radiotherapy. Kruskal–Wallis test reveals that SF-36 subscale scores of childhood cancer survivors (CCSs) with radiotherapy (RT; *hatched bars*) are significantly lower than those of siblings (*open bars*) in PF and GH subscales, respectively. The GH scores of CCSs without RT are significantly lower than those of siblings. All *p* values are adjusted by pairwise multiple comparison

in our study despite that both SCT and RT had increased late effects for CCSs [20, 21]. The most important issue was that the proportion of CCSs with two or more late effects who were getting married was quite low. This finding accords with those of previous reports [5, 7]. In our study, the proportion of unemployment tended to be a little high (4%) in the CCSs, especially CCSs with SCT or RT compared to the siblings. A higher unemployment rate (from 9 to 5%) was found in the CCSs with any late effects. The small but significant portion of CCSs experiencing employment difficulties are of great concern [16]; in fact, meta-analysis [16] showed that CCSs were nearly twice as likely to be unemployed than healthy controls (OR 1.85; 95% CI 1.27–2.69) and that survivors in the USA had an overall threefold risk of becoming unemployed, whereas no such risk was found for European survivors. This is very

Table 5 Risk factors associated with lower subscale scores of SF-36 in cancer survivors

Factors	PF scores		χ^2 (p value)	Logistic regression analysis[a]	
	Lower[a] ($n = 51$)	Higher ($n = 132$)		Adjusted odds ratio (95% CI)	p value
Gender (female)	24	83	0.052	0.59 (0.28–1.27)	0.177
Age at Dx (years)					
0–5	13	45	**0.044**	0.40 (0.15–1.09)	0.074
6–10	10	41		0.41 (0.16–1.08)	0.070
≥11	28	46		Ref	
Tx off (years)					
≥15	16	56	0.170	0.88 (0.35–2.22)	0.787
≤14	35	76		Ref	
Solid tumors	23	33	**0.008**	1.85 (0.53–6.46)	0.334
Hematological	28	99		Ref	
Radiation	34	78	0.346	0.72 (0.30–1.73)	0.464
Stem cell transplantation	21	25	**0.002**	1.96 (0.78–4.88)	0.150
Operation	28	40	**0.001**	1.49 (0.45–4.95)	0.513
Recurrence	17	16	**0.001**	**2.80 (1.04–8.33)**	**0.041**
Late effects	41	61	**<0.0001**	**3.33 (1.33–8.33)**	**0.010**
Factors	GH scores		χ^2 (p value)	Logistic regression analysis[a]	
	Lower[a] ($n = 107$)	Higher ($n = 76$)		Adjusted odds ratio (95% CI)	p value
Gender (female)	64	43	0.662	1.48 (0.77–2.87)	0.240
Age at Dx (years)					
0–5	37	21	0.148	1.31 (0.55–3.16)	0.543
6–10	24	27		0.56 (0.26–1.24)	0.155
≥11	46	28		Ref	
Tx off (years)					
≥15	40	32	0.519	0.64 (0.29–1.38)	0.255
≤14	67	44		Ref	
Solid tumors	33	23	0.933	0.65 (0.21–1.96)	0.439
Hematological	74	53		Ref	
Radiation	71	41	0.09	1.10 (0.54–2.23)	0.792
Stem cell transplantation	32	14	0.078	1.11 (0.48–2.60)	0.809
Operation	41	27	0.700	1.26 (0.43–3.63)	0.675
Recurrence	25	8	**0.026**	1.64 (0.60–4.52)	0.335
Late effects	71	31	**0.001**	**2.81 (1.35–5.85)**	**0.006**

[a] After data were presented as T scores with a mean score of 50 and a standard deviation (SD) of 10, T scores were dichotomized, in which a T score below the population score (respective nation's norm matching both age and gender in 2007) classified a respondent as having reported poor HRQOL

important, because the national health-care and social support systems must address these groups of CCSs in Japan. The Children's Cancer Association of Japan (http://www.ccaj-found.or.jp/english/) is now providing assistance and job training to CCSs, and an effective job-training system for CCSs will continue to be warranted in the future.

In our study, the validity and reliability of applying the SF-36 to CCSs in Japan were supported by Cronbach's alpha coefficient. Reulen et al. [13] demonstrated that the occurrence of ceiling effects should be recognized. In our study, a ceiling effect was observed in PF, BP and SF in more than half of the CCSs; it was found to be highest in the RP (66.1%) and RE (61.7%) subscales. These results were quite similar to those pertaining to British CCSS and siblings. The Kruskal–Wallis test showed a statistical significant difference between CCSs with SCT/RT and siblings in the RP and GH subscales. In the CCSS study, the CCSs score was worse than that of siblings with respect to the overall physical ($p < 0.001$), but not the emotional

aspects of HR-QOL. Nonetheless, effect sizes were small, other than in VT [29]. In a Canadian study, three clinical characteristics—having had CNS or bone cancer, more than one treatment series, and two organs dysfunction—were independently associated with poorer QOL in the physical dimensions [14]. Only survivors with two organs with dysfunction reported poorer QOL in both the physical and psychosocial domains. In our study, multivariate analysis-revealed late effects were common risk factors for lower PF and GH subscale scores, neither SCT nor RT were risk factors for lower PF and GH subscale scores after adjusting.

The limitations of our study are as follows: (1) a limited number of subjects were analyzed, (2) patients with solid tumors were underrepresented, compared to those with hematological cancers, (3) a selection bias may have been presented, because patients were not recruited through random sampling and (4) some patients' siblings were inappropriate as controls because they experienced significant psychosocial distress during the patients' cancer experience. Nonetheless, our report fills a gap in the published literature—and usefully so, given the numerous articles in Japan that survey social outcomes and QOL of young adult CCSs.

5 Conclusions

Our study revealed that the long-term social outcome of the CCS group was almost similar to that of the control (i.e., their siblings), but a significant proportion of CCSs were at an increased risk of developing poor social outcomes and QOL, thus requiring psychological or social care if they had some late effects.

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ORIGINAL ARTICLE

Flow cytometric analysis of de novo acute lymphoblastic leukemia in childhood: report from the Japanese Pediatric Leukemia/ Lymphoma Study Group

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Abstract Although the antigen expression patterns of childhood acute lymphoblastic leukemia (ALL) are well known, little attention has been given to standardizing the diagnostic and classification criteria. We retrospectively analyzed the flow cytometric data from a large study of antigen expression in 1,774 children with newly diagnosed ALL in JPLSG. T- and B-lineage ALL accounted for 13 and 87% of childhood ALL cases, respectively. Cytoplasmic CD3 and CD7 antigens were positive in all T-ALL cases. More than 80% of T-ALL cases expressed CD2, CD5 and TdT. In B-lineage ALL, the frequencies of early pre-B, pre-B, transitional pre-B and B-ALL were 81, 15.5, 0.6 and 2.9%, respectively. More than 90% of early pre-B ALL cases expressed CD19, CD79a, CD22, CD10 and TdT. CD34 was expressed in three-fourths of early pre-B ALL cases. The frequencies of TdT and CD34 expression were lower in pre-B ALL than in early pre-B ALL. B-ALL showed less frequent expression of CD22, CD10, CD34 and TdT than other B-lineage ALL cases. Expression of CD13 and CD33, aberrant myeloid antigens, was significantly more frequently associated with B-lineage ALL than with T-ALL. Based on this retrospective study of antigen expression in 1,774 de novo childhood ALL cases in JPLSG, we propose standardized clinical guidelines for the immunophenotypic criteria for diagnosis and classification of pediatric ALL.

Keywords Acute lymphoblastic leukemia · Childhood · Flow cytometry · Immunophenotype

1 Introduction

Flow cytometric immunophenotyping of childhood acute lymphoblastic leukemia (ALL) plays an important role not

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only in the diagnosis and classification of B and T cell lineages, but also in predicting the outcome [1–8].

Childhood ALL is a heterogeneous group of diseases. Therefore, leukemic cells from patients with ALL express a variety of differentiation antigens that are also found on normal lymphocyte precursors at discrete stages of maturation. With the development of monoclonal antibodies specific for relatively lineage-restricted or hematopoietic cell antigens, it has been possible to demonstrate considerable phenotypic heterogeneity in the vast majority of ALL cases by using panels of those antibodies [1, 2, 9–12].

The immunophenotypic patterns of acute leukemia, especially ALL, are well known, and classification into major immunologic categories is also accepted [1, 2, 9–12]. However, little attention has been given to standardizing the criteria for concluding which antigens are present on childhood leukemic cells, especially in Japan.

Herein, we report for the first time the results of a large, retrospective study of antigen expression in 1,774 children, older than 1 year and younger than 19 years of age, with newly diagnosed ALL, who had been enrolled between 1997 and 2007 at hospitals affiliated to the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG). Based on these results, we have formulated guidelines for use of immunologic markers and proper interpretation of the results. It should be noted that this study did not investigate possible associations of antigen expression with the clinical, hematological and biological features or their prognostic importance, because the present study included patients for whom a complete set of these information and the immunophenotypic characteristics based on flow cytometry were not available due to several limiting factors associated with the registration system.

2 Methods

2.1 Patient samples

This is a retrospective analysis of 1,774 pediatric patients with newly diagnosed and untreated ALL. It excluded acute undifferentiated leukemia and true mixed-lineage leukemia, defined as co-expression of golden markers of two different lineages, e.g., MPO^+ and $CD79a^+$, or MPO^+ and $CD3^+$ [10]. The analyzed patients had been enrolled between 1997 and 2007 at hospitals affiliated to the Japan Association of Childhood Leukemia Study (JACLS), the Tokyo Children's Cancer Study Group (TCCSG) and the Japanese Children's Cancer and Leukemia Study Group (JCCLSG). These three study groups, combined with the Kyushu Yamaguchi Children's Cancer Study Group (KY-CCSG), constitute the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG). All patients were diagnosed with ALL according to the French–American–British (FAB) morphology, enzyme cytochemical analysis and immunologic phenotype based on flow cytometric analysis. Samples obtained from bone marrow or peripheral blood of patients were immediately transported in sodium heparin tubes overnight to the central reference flow cytometry laboratories of the JPLSG. Informed consent for reference laboratory studies was obtained using forms approved by the local institutional review boards.

2.2 Flow cytometry

Ficoll–Hypaque-enriched blasts were stained by two-color immunofluorescence using various combinations of monoclonal antibodies, conjugated to phycoerythrin (PE) or fluorescein isothiocyanate (FITC), against the following antigens: CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD13, CD14, CD15, CD19, CD20, CD22, CD33, CD34, CD38, CD41, CD42b, CD45, CD56, CD58, CD66c, CD117, glycophorin A, HLA-DR, immunoglobulin kappa (Igκ) and lambda (Igλ) light chains, T cell receptors ($\alpha\beta$ and $\gamma\delta$) on the surface of leukemic cells and cytoplasmic Igμ chain, CD3, CD22, CD79a and myeloperoxidase antigens, as well as nuclear TdT. For detection of cytoplasmic (cCD3, cCD22, CD79a and MPO) and nuclear TdT antigens, antibodies were added after permeabilization using an Intraprep Permeabilization reagent kit (Beckman Coulter Immunotech, Miami, FL, USA). Isotypical immunoglobulins were used as negative controls. Two-color flow cytometric immunophenotyping was performed on an FACScan (Becton–Dickinson, San Jose, CA, USA) or EPICS flow cytometer (Beckman Coulter, Fullerton, CA, USA) according to the manufacturer's directions. The analysis gate was set in the forward and side light-scattering positions with lymphoid morphology. Data were recorded by an observer blinded to the patient's clinical status and diagnostic features, except for the immunophenotype. An antigen was rated as "positive" if more than 20% of the gated cells showed specific labeling above that of controls, or if a positive subpopulation was distinctively identified even in less than 20% positive cases. In principle, the criteria recommended by the European Group for the Immunological Characterization of Leukemias and others [1, 9, 10] were used for immunophenotypic classification.

2.3 Statistical analysis

Statistical analysis was performed by taking into account gender, age and the presence or absence of myeloid antigens, i.e., CD13 and CD33. Differences in the distributions of variables between groups of patients were analyzed by Mann–Whitney's U test, Kruskal–Wallis test or the χ^2 test.

3 Results

3.1 Clinical features and FAB morphology

The clinical presenting features, which include gender and age, and the FAB morphology, are summarized in Table 1. The boys-to-girls ratio of the incidence and the median age in cases of T-lineage ALL were significantly higher than in cases of B-lineage ALL ($p < 0.001$). Among patients with B-lineage ALL, these clinical characteristics were statistically more frequent in cases of mature B-ALL than in other types of B-lineage ALL ($p < 0.05$). In FAB morphology,

Table 1 Characteristics and immunophenotypic profile of 1,774 de novo cases of acute lymphoblastic leukemia

	T-ALL	B-lineage ALL		
		Early pre-B	Pre-B[a]	Mature B
Number of cases	231	1250	248	45
Frequency (%)	13.0	70.5	14.0	2.5
Clinical features				
Gender (boy/girl) (%)	74/26	55/45	51/49	74/26
Median age (range)	8 (1–16)	4 (1–18)	5 (1–15)	10 (1–15)
FAB morphology				
L1/L2/L3 (%)	72/28/0	82/17.5/0.5	84/16/0	0/0/100
T-lineage markers				
CD1a	53.7	0.3	1.5	0.0
CD2	83.5	4.1	4.0	2.2
cCD3	100	0.0	0.0	0.0
sCD3	49.3	0.0	0.0	0.0
CD4	54.8	0.8	0.0	0.0
CD5	94.2	0.5	10.1	0.0
CD7	100	3.2	6.9	2.2
CD8	68.3	1.1	0.0	0.0
TCR$\alpha\beta$	29.4	6.3	8.5	0.0
TCR$\gamma\delta$	10.9	0.0	0.0	0.0
B-lineage markers				
CD19	0.0	99.6	98.8	100
CD20	0.0	19.2	23.6	88.9
cCD22	2.9	90.1	97.3	77.8
sCD22	1.8	70.3	87.6	60.5
CD79a	21.8	99.2	100	100
cIgμ	0.0	0.0	100	88.9
sIgμ	0.0	2.1	9.0	83.3
sIgκ or λ	0.0	0.0	0.0	100
Non-lineage specific markers				
TdT	84.4	97.0	83.8	13.0
CD10	31.6	91.2	93.5	77.8
CD34	37.3	74.6	44.5	7.0
HLA-DR	16.7	99.3	94.7	97.7
Myeloid markers				
MPO	0.0	0.0	0.0	0.0
CD13	20.7	36.0	22.7	14.3
CD14	0.0	0.6	0.0	0.0
CD33	15.2	31.6	15.0	2.2
CD41	0.0	0.8	3.3	0.0
CD66c	0.5	43.5	25.9	0.0
CD117	15.6	10.1	13.4	11.5
GlyA	0.0	0.0	0.0	0.0

Values indicate the proportion of positive cases (%)

c cytoplasmic, *s* surface

[a] Pre-B cases include transitional pre-B cases

the L3 subtype was detected in all cases of mature B-ALL and only in five cases of early pre-B ALL without $t(8;14)$ or its variants. The present study did not evaluate any further possible associations of immunophenotypic characteristics with other clinical, hematological or biological features or their prognostic importance because of several limiting factors associated with the registration system.

3.2 T-lineage ALL

T-lineage ALL accounted for 13% (231/1,774) of de novo childhood ALL (Table 1). Cytoplasmic CD3 and CD7 antigens were expressed in all T-ALL cases, which we were able to analyze. More than 80% of this subset expressed CD2, CD5 and the nuclear antigen, terminal deoxynucleotidyl transferase (TdT). Surface CD1a, CD3, CD4 and CD8 were detected in 49.3–68.3% of 231 cases of T-ALL. The HLA-DR antigen was not commonly expressed, and about 30% of the T-lineage ALL cases were $CD10^+$ and/or $CD34^+$. T cell receptor (TCR) proteins were heterogeneously expressed in T-lineage ALL. About 30% of the T-lineage cases expressing surface TCR chains expressed the $\alpha\beta$ form of TCR, whereas a minority, less than 15% of the T-lineage cases, expressed TCR$\gamma\delta$ proteins. Cytoplasmic CD79a and CD22, reliable markers for B-lineage ALL, were expressed in 21.8 and 2.9% of the T-lineage ALL cases, respectively. None of the T-ALL cases expressed CD19, CD20 or immunoglobulin molecules. Myeloid-associated antigen expression analysis found that CD13 and CD33 were expressed in 20.7 and 15.2% of the T-lineage ALL cases, respectively (Fig. 1). None of the T-ALL cases in this study expressed MPO or CD14. Early T cell precursor-ALL, a poor prognosis subgroup defined by its associated distinctive immunophenotype ($CD1a^-$, $CD8^-$, CD5 weak with stem-cell/myeloid markers) [13], was found in 3.7% of de novo T-ALL cases.

3.3 Early pre-B ALL

In this study, early pre-B ALL was found in 70.5% (1,250/1,774) of our de novo ALL cases (Table 1). Almost all of the early pre-B ALL cases were positive for CD19, cytoplasmic CD79a and cytoplasmic or surface CD22, but immunoglobulins were not detected. CD20, known to be a specific marker for early pre-B ALL, was detected in just 20% of the early pre-B ALL cases. More than 90% of the early pre-B ALL cases expressed CD10, TdT and HLA-DR, which are non-lineage specific antigens for B-lineage ALL. Moreover, CD34, a progenitor cell antigen, was expressed in 74.6% of the early pre-B ALL cases. CD66c, a member of the carcinoembryonic antigen family, was detected in nearly half of the early pre-B ALL cases. CD13 and CD33 antigens were expressed in 36.0 and 31.6% of the early pre-B ALL cases, respectively (Fig. 1). It is of note that neither cytoplasmic nor surface CD3 antigens were expressed in any B-lineage ALL (early pre-B, pre-B and B cell ALL) case in this series.

3.4 Pre-B ALL

According to the general consensus [1, 10, 14, 15], pre-B ALL blasts express cytoplasmic immunoglobulin μ heavy chains, but have no detectable surface immunoglobulins in B-lineage ALL. On the other hand, lymphoblasts of transitional pre-B ALL have both cytoplasmic and surface immunoglobulin μ heavy chains, without κ or λ light chains [1, 10, 15]. Since transitional pre-B ALL cases represented only 0.5% (9/1,774) of our de novo ALL cases, we analyzed these cases together with the pre-B ALL cases. This immunophenotype accounted for 14.0% (248/1,774) of our cases of newly diagnosed childhood ALL (Table 1) and expressed CD19, cCD22 and CD79a. Surface CD20 was detected in about a quarter of these pre-B

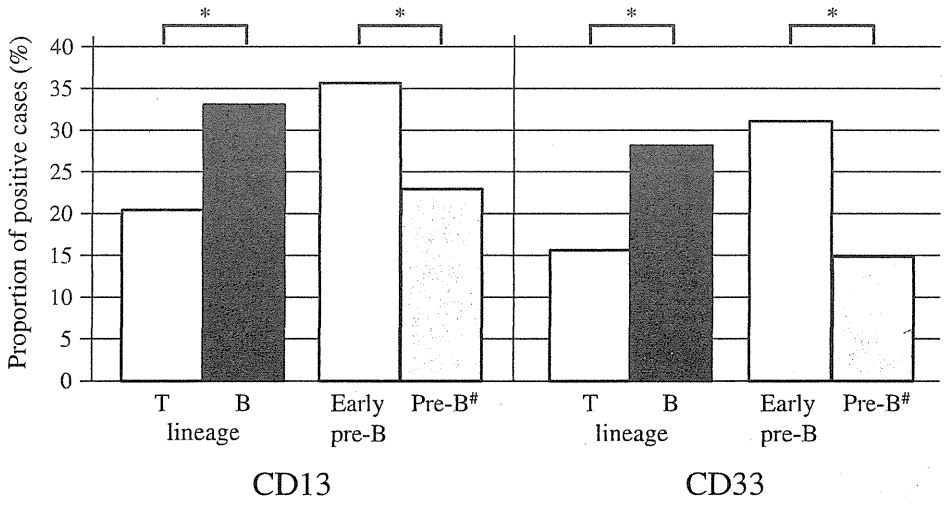

Fig. 1 Distribution of myeloid antigen (CD13 and CD33) expression. Acute lymphoblastic leukemia immunophenotypes: T-lineage ALL, B-lineage ALL, early pre-B ALL, pre-B ALL and B-ALL. *Values* indicate proportion of positive cases (%). #Pre-B cases include transitional pre-B cases. Expression was observed in all cases. *$p < 0.001$

ALL cases, and more than 90% expressed CD10 and HLA-DR. However, the frequencies of TdT and CD34 expression were 83.8 and 44.5%, respectively, which are lower than for early pre-B ALL cells. The expression frequencies of CD13 and CD33 were also lower than in the early pre-B ALL cases, at 22.7 and 15.0% ($p < 0.001$) (Fig. 1).

3.5 B cell ALL

B-ALL cells are characterized by L3 morphology, as defined in the FAB classification, and by surface membrane expression of immunoglobulin μ heavy chains (sIg) plus monotypic light chain [1, 9, 10]. In our present study, B-ALL cases accounted for 2.5% (45/1,774) of our de novo ALL cases (Table 1). The blasts of the B-ALL cases also expressed CD19, cCD79a, CD20 and HLA-DR. Both CD22 and CD10 were less frequently expressed in these cases than in other B-lineage ALL cases, including early pre-B and pre-B ALL. Although B-ALL cells are generally negative for expression of TdT and CD34, a few B-ALL cases with blasts that expressed TdT and/or CD34 have been reported [10, 16–19]. Moreover, Gluck et al. [20] diagnosed a B-ALL case that was L3 in the FAB classification with typical Burkitt's type translocation, but lacking sIg. In fact, we also identified a few cases with expression of TdT and/or CD34 and one case without sIg expression (positive for monotypic light chain) in this series. CD13 and CD33 antigens were expressed in some cases: 14.3 and 2.2%, respectively (Fig. 1).

4 Discussion

Immunophenotypic analysis of acute leukemia by flow cytometry has been used clinically as an indispensable tool for identification of the lineage association of leukemic cells and evaluation of the response to treatment [1, 2, 10–12, 21]. Recently, panels of monoclonal antibodies specific for lineage-associated antigens have been expanded. As a result, immunophenotyping of ALL has been applied to distinguish it from acute myeloid leukemia (AML) and to achieve more accurate phenotyping within ALL.

We retrospectively analyzed the flow cytometric data from a large study of antigen expression in 1,774 children with newly diagnosed ALL who were enrolled at hospitals affiliated to the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) between 1997 and 2007. Each central reference flow cytometry laboratory of the JPLSG made immunophenotypic diagnoses based on the criteria recommended by the European Group for the Immunological Characterization of Leukemias and others for childhood acute leukemia [1, 9, 10]. Although these criteria are actually similar to each other and standardized, they advocate some different subclasses in T- or B-lineage ALL. Additionally, ALL with myeloid antigen expression might be observed frequently in cases with mixed-lineage leukemia. However, the criteria for myeloid marker-positive childhood ALL and the clinical significance of these antigens also vary. We then formulated guidelines for the use of immunomarkers and proper interpretation of the results in childhood ALL, as summarized in Table 2.

T-lineage ALL, according to our analytical findings, is characterized by cytoplasmic or surface membrane expression of CD3 together with CD2, CD5, CD7 or CD8 (Table 2). Some of our T-ALL cells expressed CD79a or CD22 as a marker for B-lineage ALL. Although such T-ALL cases have been reported by other investigators [22, 23], none of our T-ALL cases satisfied the diagnostic criteria for B-lineage ALL described below. Recently, Campana et al. [13] reported diagnosis of early T cell precursor (ETP)-ALL, as a subgroup with a poor prognosis,

Table 2 Proposed immunophenotypic criteria for de novo cases of acute lymphoblastic leukemia

T-lineage ALL
1. $CD3^+$
2. Express CD2, CD5, CD7 or CD8
B-lineage ALL
Early pre-B ALL
Express at least two B-lineage markers (CD19, CD20, CD22 or CD79a)
Pre-B ALL[a]
1. Express at least two B-lineage markers (CD19, CD20, CD22 or CD79a)
2. Negative for surface membrane immunoglobulin κ or λ light chains
3. Express cytoplasmic and/or surface immunoglobulin μ heavy chains
B-ALL
1. Express at least two B-lineage markers (CD19, CD20, CD22 or CD79a)
2. Express surface membrane immunoglobulin κ or λ light chains
ALL with aberrant myeloid-associated antigen expression
My Ag^+ T-lineage ALL
1. $CD3^+$ and express CD2, CD5, CD7 or CD8
2. $CD79a^-$
3. MPO^- and express myeloid-associated markers (CD13, CD15, CD33 or CD65)
My Ag^+ B-lineage ALL
1. Express at least two B-lineage markers (CD19, CD20, CD22 or CD79a)
2. $CD3^-$
3. MPO^- and express myeloid-associated markers (CD13, CD15, CD33 or CD65)

[a] Pre-B ALL cases include transitional pre-B cases