ARTICLE

Late thyroid complications in survivors of childhood acute leukemia. An L.E.A. study

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ABSTRACT

Thyroid complications are known side effects of irradiation. However, the risk of such complications in childhood acute leukemia survivors who received either central nervous system irradiation or hematopoietic stem cell transplantation is less described. We prospectively evaluated the incidence and risk factors for thyroid dysfunction and tumors in survivors of childhood acute myeloid or lymphoid leukemia. A total of 588 patients were evaluated for thyroid function, and 502 individuals were assessed for thyroid tumors (median follow-up duration: 12.6 and 12.5 years, respectively). The cumulative incidence of hypothyroidism was 17.3% (95% CI: 14.1-21.1) and 24.6% (95% CI: 20.4-29.6) at 10 and 20 years from leukemia diagnosis, respectively. Patients who received total body irradiation (with or without prior central nervous system irradiation) were at higher risk of hypothyroidism (adjusted HR: 2.87; *P*=0.04 and 2.79, *P*=0.01, respectively) as compared with transplanted patients who never received any irradiation. Patients transplanted without total body irradiation who received central nervous system irradiation were also at higher risk (adjusted HR: 3.39; *P*=0.02). Patients irradiated or transplanted at older than 10 years of age had a lower risk (adjusted HR: 0.61; *P*=0.02). Thyroid malignancy was found in 26 patients (5.2%). Among them, two patients had never received any type of irradiation: alkylating agents could also promote thyroid cancer. The cumulative incidence of thyroid malignancy was 9.6% (95% CI: 6.0-15.0) at 20 years. Women were at higher risk than men (adjusted HR: 4.74; *P*=0.002). In conclusion, thyroid complications are frequent among patients who undergo transplantation after total body irradiation and those who received prior central nervous system irradiation. Close monitoring is thus warranted for these patients. *Clinicaltrials.gov identifier: NCT 01756599*.

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Introduction

As high cure rates have led to an increase in long-term survivors treated for acute childhood leukemia, a mounting number of studies have focused on the long-term health status of such patients.[1] It has long been known that the side effects which can occur include late thyroid complications, such as thyroid dysfunction[2-9] and thyroid cancer.[10-13] Thyroid dysfunction is rather frequent and has been mostly reported as a complication of irradiation, particularly for solid tumors, such as brain cancers[10,14] and Hodgkin lymphoma.[15,16] In leukemia survivors, thyroid complications have been mainly described as a consequence of central nervous system or total body irradiation and hematopoietic stem cell transplantation. However, the conclusions of many published studies may have been biased by the small numbers of patients included, the heterogeneity of the study cohorts (e.g., different disease types and treatment modalities), and the methodology used (e.g., questionnaires, retrospective studies). Concerning patients who receive central nervous system radiation, the possible effect of scatter radiation on the thyroid is poorly understood. Moreover, little is known regarding the risk of thyroid complications following busulfan-containing conditioning regimens and hematopoietic stem cell transplantation.[17,18] Lastly, few studies have assessed both thyroid dysfunction and thyroid malignancies simultaneously.

We therefore aimed to describe the incidence of and risk factors for thyroid dysfunction and thyroid malignancy in a large cohort of long-term survivors of childhood acute leukemia. As chemotherapy alone usually does not lead to an increased risk of late thyroid complications,[4,7,16,19] our study was focused on survivors of childhood acute leukemia who received either central nervous system irradiation (who could be vulnerable to the scatter effects of this type of radiation on the thyroid gland) or underwent hematopoietic stem cell transplantation (in whom the thyroid could be damaged both by the direct effect of total body irradiation and the possible effect of high-dose alkylating agents). The current study was based on data from the French *Leucémie de l'Enfant et de l'Adolescent* (L.E.A.) prospective cohort. All patients underwent systematic and repeated hormonal and ultrasound evaluations of any thyroid complications at predefined dates, thus minimizing potential bias while providing estimates of cumulative incidences over time.

Methods

We assessed thyroid dysfunction and tumors in patients from the L.E.A. cohort. The L.E.A. program was implemented in 2004 to prospectively evaluate the long-term health status, quality of life and socio-economic status of childhood acute leukemia survivors enrolled in French treatment programs from 1980 to present, in 13 cancer centers. Further details of the program have been described elsewhere.[20,21] The eligibility criteria for this study were the following: (i) provision of written informed consent to participation in the L.E.A. program between 2004 and 2012, and (ii) having received central nervous system irradiation and/or undergone hematopoietic stem cell transplantation with a myeloablative conditioning regimen as part of the treatment. All patients (or their parents) provided written informed consent to participation in the study, which was approved by the French National Program for Clinical Research and the National Cancer Institute and by our local institutional review boards.

The data were collected during specific medical visits at predefined dates, initially every 2 years during a 10-year post-transplantation follow-up period and subsequently every 4 years (for details, see the *Online Supplementary Methods*).

From 2004 to 2012, it was recommended that all patients undergoing a new L.E.A. evaluation who received either a hematopoietic stem cell transplant or central nervous system irradiation were systematically assessed for thyroid function and tumors. Participants in the thyroid function analysis had at least one assessment of hormone levels, while participants in the thyroid tumor evaluation had at least one ultrasound scan during their follow-up.

Thyroid function was assessed by monitoring thyroid stimulating hormone (TSH) and free thyroxine (T4) by immunoassay with a high molecular weight ligand or labeled antibody.[22] Uncompensated peripheral hypothyroidism was diagnosed in cases of elevated TSH levels (> 5 mIU/L) and low T4 levels (< 10 pmol/L), while compensated peripheral hypothyroidism was diagnosed in cases of elevated TSH levels and normal T4 levels. Central hypothyroidism was defined by low T4 (<10 pmol/L) and normal/low TSH levels.

Thyroid tumors (nodules or micronodules) were evaluated using thyroid ultrasound scans, which were carried out to assess tumor size and characteristics. In the case of suspicious nodules, fine needle aspiration, nodule biopsy and/or thyroidectomy were performed. Malignancy and cancer histopathology subtype were defined based on the histopathology findings.

Statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA) and Intercooled Stata 9.0 for Windows. The χ^2 and Fisher exact tests were used to compare qualitative variables. Quantitative variables were compared using the Mann-Whitney U-test. The prevalence rates of thyroid dysfunction and tumors were assessed with a 95% confidence interval (CI).

Cumulative incidence rates of hypothyroidism and thyroid cancers over time were estimated using the Kaplan-Meier method, with 95% confidence intervals, and compared using the log rank test. The proportional hazards assumption was assessed *via* examination of the log-minus-log survival plot of each cofactor. For the analysis of cumulative incidence rates of hypothyroidism, patients were censored when they underwent thyroidectomy, regardless of the underlying cause.

Hazard ratios (HR) and the associated 95% CI were estimated using Cox proportional hazard models; *P* values <0.05 are considered statistically significant.

Results

Patients' characteristics: comparison between participating and eligible but not-participating patients

Among 665 eligible patients from 13 French centers, 588 (88.4%) were evaluated for thyroid function during the years 2004 to 2012 and 502 (75.5%) for thyroid tumors (*Online Supplementary Figure S1*). The patients' characteristics and treatment modalities are shown in *Online Supplementary Table S1*. The percentages of transplanted patients were 75.7% among the group in which thyroid function was evaluated (of whom 48.1% were transplanted after relapse) and 76.3% in the group in which thyroid tumors were evaluated (49.6% were transplanted after relapse). The median (interquartile range, IQR) duration of follow-up from diagnosis to last hor-

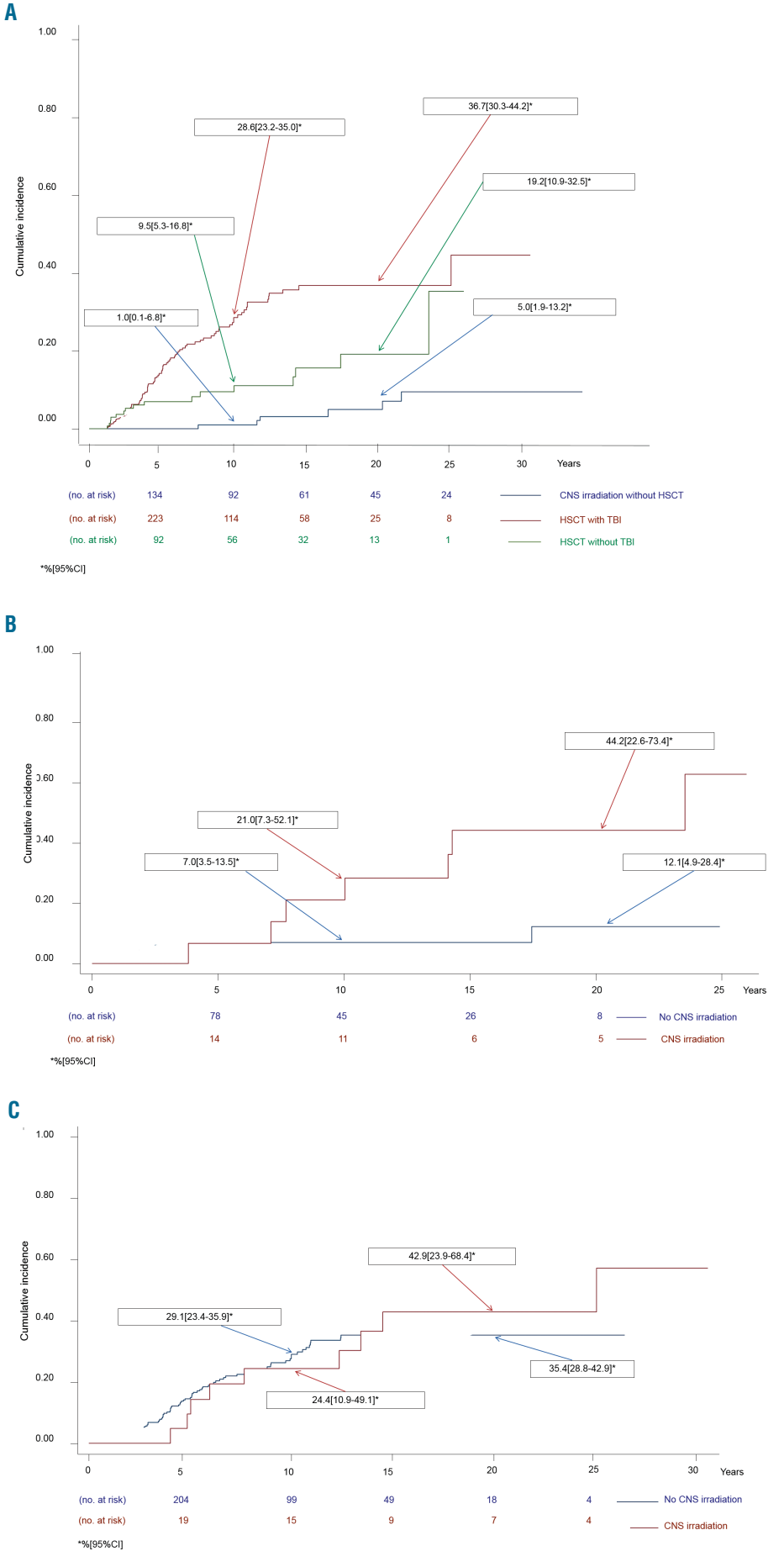

Figure 1. Cumulative incidences of hypothyroidism. (A) Cumulative incidence of hypothyroidism; impact of transplantation and irradiation type. ($P<10^{-3}$). (B) Cumulative incidence of hypothyroidism in patients treated with hematopoietic stem cell transplantation without total body irradiation (n=135): comparison between patients who received prior central nervous system irradiation and patients who did not (P=0.005). (C) Cumulative incidence of hypothyroidism among patients treated with hematopoietic stem cell transplantation who received total body irradiation as part of their conditioning: comparison between patients who were exposed to central nervous system irradiation and those who were not (P=non significant). HSCT: hematopoietic stem cell transplantation; CNS: central nervous system. TBI: total body irradiation.

monal assessment and last thyroid ultrasound scan was 12.6 (IQR: 6.4-18.8) and 12.4 (IQR: 6.4-19.1) years, respectively. Patients in the participating groups had more frequently experienced leukemia relapse and were more likely to have received total body irradiation before transplantation; they were also less likely to have received central nervous system irradiation than eligible patients who did not participate in the study. Gender, leukemia subtype, age at diagnosis, at central nervous system irradiation and at hematopoietic stem cell transplantation, central nervous system irradiation doses and fields were not different between the participating and non-participating patients.

Thyroid dysfunction

Prevalence and cumulative incidence

The most common thyroid dysfunction was hypothyroidism, which was observed in 105/588 patients (17.9%). Primary hypothyroidism was reported in 99 cases (94.3%), while central hypothyroidism was observed in six cases (5.7%). Hypothyroidism was uncompensated in 34 cases and compensated in 62 patients. A total of 98/105 patients received L-thyroxine substitution. The cumulative incidence of hypothyroidism for all patients was 17.3% (95% CI: 14.1-21.1) at 10 years and 24.6% at 20 years (95% CI: 20.4-29.6). The median age at diagnosis of hypothyroidism was 12.6 (IQR: 7.8-15.2) years. The median delay between irradiation or transplantation and hypothyroidism diagnosis was 3.3 (IQR 1.4-6.4) years. One patient developed hyperthyroidism (Grave disease).

Risk factors for hypothyroidism

We found that the type of irradiation had a marked impact on the risk of hypothyroidism (Figure 1A), which was higher in cases of total body irradiation-based conditioning regimens than in cases of transplantation without total body irradiation or cases of central nervous system irradiation without transplantation [cumulative incidences at 20 years were 36.7% (95%CI: 30.3-44.2), 19.2% (95% CI: 10.9-32.5) and 5.0% (95% CI: 1.9-13.2) for each group, respectively, $P<10^{-3}$]. Among the patients who underwent stem cell transplantation without total body irradiation, the risk was higher in cases of prior central nervous system irradiation than in cases without prior central nervous system irradiation [cumulative incidences at 20 years: 44.2% (95% CI: 22.6-73.4) *versus* 12.1% (95% CI: 4.9-28.4), respectively; $P=0.005$] (Figure 1B). In contrast, the incidence of hypothyroidism among patients who received a total body irradiation-based conditioning regimen was not affected by prior central nervous system irradiation (Figure 1C).

In a univariate analysis, we did not find any impact of acute leukemia subtype (lymphoid *versus* myeloid), transplantation type (autologous *versus* allogeneic), or central nervous system irradiation field (cranial *versus* craniospinal) among patients treated with central nervous system irradiation (Table 1). Of note, the risk of hypothy-

Table 1. Univariate analysis: risk factors for hypothyroidism (n=588).

	Number (%)	*P* value	10-year cumulative incidence (%)	[95% CI]	20-year cumulative incidence (%)	[95% CI]
Gender						
Male	52 (15.3)		15.7	[11.9-20.7]	19.8	[15.1-25.7]
Female	53 (21.4)	0.16	19.3	[14.4-25.7]	30.5	[23.5-39.0]
Leukemia type						
Acute myeloid leukemia	16 (11.0)		12.5	[7.6-20.0]	15.5	[9.0-25.9]
Acute lymphoid leukemia	89 (20.1)	0.08	18.6	[14.9-23.1]	27.0	[22.0-32.7]
Age at transplantation/irradiation						
< 10 years	74 (20.5)		19.2	[15.1-24.2]	27.3	[22.0-33.6]
≥ 10 years	31 (13.7)	0.19	14.2	[9.9-20.7]	19.3	[13.2-27.8]
Hematopoietic stem cell transplantation type						
Autologous	26 (28.6)		23.2	[15.6-33.6]	29.4	[20.6-40.8]
Allogeneic	73 (20.6)	0.71	23.0	[18.1-28.9]	33.6	[26.4-42.2]
Treatment modality						
HSCT without TBI, without prior CNS irradiation	9 (7.5)		7.0	[3.5-13.5]	12.1	[4.9-28.4]
CNS irradiation, no HSCT	6 (4.2)		10	[0.1-6.8]	5.0	[1.9-13.2]
HSCT with TBI, with prior CNS irradiation	9 (42.9)	**<10⁻³**	24.4	[10.9-49.1]	42.9	[23.9-68.4]
HSCT with TBI, without prior CNS irradiation	74 (25.6)		29.1	[23.4-35.9]	35.4	[28.8-42.9]
HSCT without TBI, with prior CNS irradiation	7 (46.7)		21.0	[7.3-52.1]	44.2	[22.6-73.4]
CNS irradiation type						
Cranial irradiation	13 (10.7)		4.7	[2.0-10.9]	12.1	[6.6-21.9]
Cerebrospinal irradiation	8 (15.1)	0.49	6.6	[2.2-19.2]	19.2	[9.4-37.0]
CNS irradiation dose						
18 Grays	11 (8.2)		4.5	[1.9-10.5]	11.5	[6.2-20.8]
24 Grays	8 (22.2)		6.8	[1.7-25.0]]	23.9	[11.4-46.0]
Other	3 (37.5)	0.07	25.0	[6.9-68.5]	25.0	[6.9-68.5]

CNS: central nervous system; HSCT: hematopoietic stem cell transplantation; TBI: total body irradiation. In bold: statistically significant value.

roidism tended to be higher among patients who received central nervous system irradiation with 24 Grays than in those who received only 18 Grays, although the difference was not statistically significant (*P*=0.07).

Patients were then stratified into five groups according to the therapeutic modalities they had received: (i) transplantation without total body irradiation, without prior central nervous system irradiation [n=120 (20.4%)]; (ii) central nervous system irradiation, no transplantation [n=143 (24.3%)]; (iii) transplantation after total body irradiation with prior central nervous system irradiation [n=21 (3.6%)]; (iv) transplantation after total body irradiation, without prior central nervous system irradiation [n=289 (49.1%)]; and (v) transplantation without total body irradiation, with prior central nervous system irradiation [n=15 (2.6%)].

The multivariate analysis confirmed that transplantation and irradiation modalities played a role in the development of hypothyroidism. The adjusted hazard ratio was 2.87 (95% CI: 1.03-7.99) for patients who received total body irradiation and prior central nervous system irradiation (*P*=0.04), 2.79 (95% CI: 1.28-6.1) for those treated with total body irradiation without prior central nervous system irradiation (*P*=0.01), and 3.39 (95% CI: 1.23-9.35) for those who received a transplant without total body irradiation but who had had prior central nervous system irradiation (*P*=0.02), as compared with the reference population (patients transplanted without total body irradiation, without prior irradiation). In contrast, the adjusted hazard ratio was 0.27 for patients who received only central nervous system irradiation (and no transplantation) (*P*=0.02).

The patients' age at transplantation or irradiation also

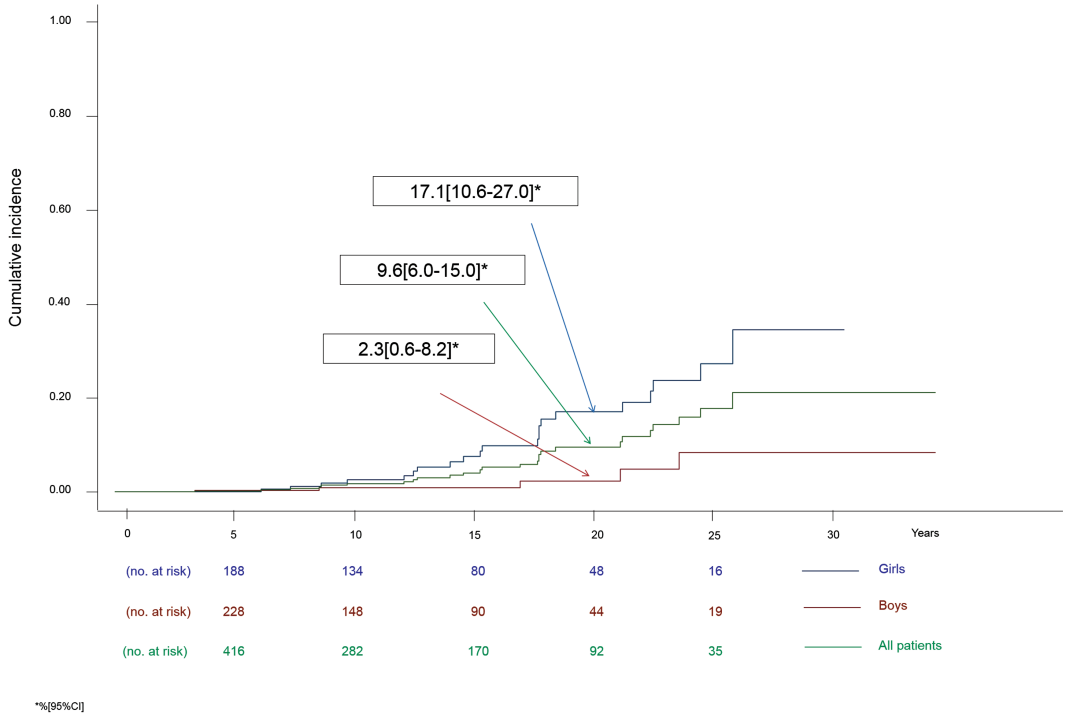

Figure 2. Cumulative incidence of thyroid cancer. The figure shows the cumulative incidence of thyroid cancer in the whole cohort (green line), and a comparison of the incidence between females (blue line) and males (red line). *P*<0.001.

Table 2. Risk factors for hypothyroidism: multivariate analysis (n=588).

Variables	N.	%	Adjusted HR	95% CI	*P* value
Age at transplantation/irradiation					
< 10 years	361	61.4	1		
≥ 10 years	227	38.6	**0.61**	**0.40-0.93**	**0.02**
Gender					
Male	340	57.8	1		
Female	248	42.2	1.43	0.97-2.11	0.07
Leukemia type					
Acute lymphoid leukemia	442	75.2	1		
Acute myeloid leukemia	146	24.8	0.62	0.33-1.14	0.13
Treatment modality					
HSCT without TBI, without prior CNS irradiation	120	20.4	1		
CNS irradiation, no HSCT	143	24.3	**0.27**	**0.09-0.81**	**0.02**
HSCT with TBI, with prior CNS irradiation	21	3.6	**2.87**	**1.03-7.99**	**0.04**
HSCT with TBI, without prior CNS irradiation	289	49.1	**2.79**	**1.28-6.10**	**0.01**
HSCT without TBI, with prior CNS irradiation	15	2.6	**3.39**	**1.23-9.35**	**0.02**

CNS: central nervous system; HSCT: hematopoietic stem cell transplantation; TBI: total body irradiation. In bold: statistically significant values.

had a marked impact on hypothyroidism. Patients irradiated or transplanted at older than 10 years of age had a lower risk of hypothyroidism (adjusted hazard ratio: 0.61; *P*=0.02). The results of the multivariate analyses are summarized in Table 2.

Risk factors for primary hypothyroidism

We also performed the same univariate and multivariate analyses exclusively considering patients presenting with primary hypothyroidism, excluding six patients with central hypothyroidism for whom we could not diagnose a potential primary form of the disease. The results were very similar to those reported in the previous section (including both primary and central hypothyroidism). In the univariate analysis, patients who received total body irradiation and those who underwent transplantation without total body irradiation but with prior central nervous system irradiation had a higher risk of primary hypothyroidism ($P<10^{-3}$). This was confirmed by the multivariate analysis (for both univariate and multivariate analyses, see *Online Supplementary Tables S2 and S3*).

Thyroid tumor

Prevalence and cumulative incidence

A total of 162 patients (32.3%) were found to have thyroid nodules or micronodules [median age at nodule diagnosis: 21.1 (IQR: 15.7-27.8) years]. Of those 162 patients, 56 (34.6%) underwent cytological examination: 26 (46.4%) had papillary carcinoma, 28 had benign adenoma and two had dystrophic lesions without evidence of malignancy. The cumulative incidence of thyroid cancer was 1.7% at 10 years (95% CI: 0.8-3.9) and reached 9.6% (95% CI: 6.0-15.0) at 20 years (Figure 2). The median age at diagnosis of thyroid cancer was 20.5 (IQR: 17.1-24.1) years. Patients with thyroid cancer were treated with total or subtotal thyroidectomy and radioactive iodine therapy. No patients died from thyroid cancer.

Risk factors for thyroid cancer

In the univariate analysis, the only significant variable that had an impact on the incidence of thyroid cancer was gender; women were at a higher risk of developing thyroid cancer than were men [cumulative incidence at 20 years: 2.3% (95% CI: 0.6-8.2) and 17.1% (95% CI: 10.6-27) for men and women, respectively, *P*<0.001] (Figure 2). Leukemia subtype, age at transplantation or irradiation, type of stem cell transplant (autologous *versus* allogeneic), central nervous irradiation type (cranial or craniospinal) or dose (18 *versus* 24 Grays) had no impact on thyroid cancer (Table 3). However, we did observe a trend towards a higher cumulative incidence among patients who received total body irradiation, the cumulative incidence of thyroid cancer at 15 years being 8.3% and 5.5% for patients who received total body irradiation with and without central nervous system irradiation, respectively, 3.8% for patients who underwent transplantation without any irradiation, and 2.1% for those who received only central nervous system irradiation without transplantation.

After adjusting for other variables, the prognostic value of gender remained the only significant value (adjusted HR: 4.74 for women, *P*=0.002) (Table 4).

Table 3. Univariate analysis: risk factors for thyroid cancer (n=502).

	Number (%)	*P* value	15-year cumulative incidence (%)	[95%CI]	20-year cumulative incidence (%)	[95% CI]
Gender						
Male	5 (1.8)	**<10⁻³**	1.0	[0.2-3.9]	2.3	[0.6-8.2]
Female	21 (9.6)		7.5	[3.9-14.2]	17.1	[10.6-27.0]
Leukemia type						
Acute myeloid leukemia	6 (5.1)	0.40	1.6	[0.2-10.9]	12.3	[4.6-30.7]
Acute lymphoid leukemia	20 (5.2)		4.7	[2.5-8.7]	8.9	[5.3-14.9]
Age at transplantation/irradiation						
< 10 years	18 (5.9)	0.92	4.2	[2.0-8.7]	11.0	[6.5-18.3]
≥ 10 years	8 (4.1)		3.6	[1.3-9.8]	6.0	[2.2-15.4]
Hematopoietic stem cell transplantation type						
Autologous	7 (10.0)	0.39	7.1	[2.7-18.0]	9.4	[4.0-21.3]
Allogeneic	14 (4.5)		3.7	[1.5-9.0]	13.5	[6.9-25.5]
Treatment modality						
HSCT without TBI, without prior CNS irradiation	2 (2.0)	0.08	3.9	[0.6-24.3]	8.2	[2.1-29.2]
CNS irradiation, no HSCT	5 (4.2)		2.1	[0.5-8.2]	6.0	[2.2-16.0]
HSCT with TBI, with prior CNS irradiation	2 (10.5)		8.3	[1.2-46.1]	8.3	[1.2-46.1]
HSCT with TBI, without prior CNS irradiation	16 (6.3)		5.5	[2.6-11.5]	14.3	[7.9-25.2]
HSCT without TBI, with prior CNS irradiation	1 (10.0)		0.0		0.0	
CNS irradiation type						
Cranial irradiation	6 (5.7)	0.83	2.9	[0.7-11.3]	7.1	[2.7-18.3]
Cerebrospinal irradiation	2 (5.1)		2.7	[0.4-17.7]	2.7	[0.4-17.7]
CNS irradiation dose						
18 Grays	7 (6.1)	0.14	3.7	[1.2-11.2]	8.1	[3.3-19.2]
24 Grays	0 (0.0)		0.0		0.0	
Other	1 (11.1)		0.0		0.0	

CNS: central nervous system; HSCT: hematopoietic stem cell transplantation; TBI: total body irradiation. In bold: statistically significant value.

Correlation between hypothyroidism and thyroid cancer

To assess the relationship between hypothyroidism and thyroid cancer, we selected patients who were evaluated for both diseases (488 patients). We did not find any correlation between hypothyroidism and thyroid cancer either in the entire cohort of 488 patients (P=0.80) or in a selected population of patients who had received total body irradiation as part of their conditioning (P=0.78).

Discussion

We assessed the incidence of and risk factors for late thyroid complications (thyroid dysfunction and thyroid tumors) that can occur after treatment for acute leukemia during childhood. Few studies have focused on both forms of thyroid pathology. The high number of patients included and the prospective nature of this study enabled us to determine precisely the cumulative risk of thyroid complications over time among a homogeneous cohort of long-term survivors of childhood acute leukemia following treatment with cranial irradiation and/or hematopoietic stem cell transplantation.

Hypothyroidism was found to be frequent in our cohort (17.9%) and was mostly of the primary type. Compensated hypothyroidism was reported in the majority of cases. Of note, the prevalence of compensated hypothyroidism in the French general population is 1.9% and 3.3% for men and women, respectively; these prevalences increase with age.[23]

Our findings are consistent with those of previous comparable studies. A retrospective questionnaire-based study from the Childhood Cancer Survivor study including 3467 survivors of leukemia found hypothyroidism in 6.5% of cases, with prevalences ranging from 6.8% (patients treated with cranial irradiation) to 29.8% (patients receiving total body irradiation and prior central nervous system irradiation).[24] However, that study did not mention the cumulative incidence of hypothyroidism, which was 24.6% at 20 years (95% CI: 20.4-29.6) in our cohort. This cumulative incidence is much higher than that reported by Chow *et al.* (15-year cumulative incidence of hypothyroidism: 1.6%), probably because their study also included patients treated with chemotherapy alone, and because the methodology used may have undervalued the incidence of hypothyroidism (retrospective study, self-reported thyroid dysfunction).[4]

Primary hypothyroidism has been linked to exposure to thyroid radiation,[25] primarily in solid tumors such as Hodgkin lymphoma[15,16] and brain tumors,[10,14] although it was also found to be associated with cranial or craniospinal irradiation in patients with childhood acute leukemia.[4] Cranial irradiation in patients with acute lymphoblastic leukemia has long been suggested to play a role in hypothyroidism,[3,4,26] although a few authors have argued that the impact of prophylactic cranial radiation is controversial.[3,5,7,27] However, those earlier studies should be interpreted with caution because of the small numbers of patients included, the retrospective nature of the studies and/or the short follow-up periods. In the current study, our results support the hypothesis that the risk of hypothyroidism is significantly lower in patients who received only central nervous system irradiation (adjusted HR=0.27, P=0.02) compared with that in the reference population (i.e., patients treated with hematopoietic stem cell transplantation without any history of irradiation). This might be due to a low dose of radiation received by the thyroid gland (most patients who underwent central nervous system irradiation received a dose of 18 Grays). This also raises the question of the potential impact of high-dose alkylating agents (used in many preparative regimens, instead of total body irradiation) on the thyroid gland, as previously suggested.[17]

According to the literature, the risk of primary hypothyroidism seems to be strongly associated with the total irradiation dose received by the thyroid gland.[25] It has been suggested that the risk of hypothyroidism is higher in patients exposed to craniospinal irradiation than in those who undergo cranial irradiation.[4] We, however, did not find similar results, which may be due to the small number of patients who received craniospinal irradiation.

Several studies have focused on thyroid dysfunction after hematopoietic stem cell transplantation.[17,28-31] In a

Table 4. Multivariate analysis: risk factors for thyroid cancer.

Variables	N.	%	Adjusted HR	95% CI	*P* value
Age at transplantation/irradiation					
< 10 years	306	61.0	1		
≥ 10 years	196	39.0	0.89	0.37-2.12	0.79
Gender					
Male	284	56.6	1		
Female	218	43.4	**4.74**	**1.76-12.82**	**0.002**
Leukemia type					
Acute lymphoid leukemia	384	76.5	1		
Acute myeloid leukemia	118	23.5	1.47	0.52-4.18	0.47
Treatment modality					
HSCT without TBI, without prior CNS irradiation	99	19.7	1		
CNS irradiation, no HSCT	119	23.7	0.74	0.12-4.66	0.74
HSCT with TBI, with prior CNS irradiation	19	3.8	2.19	0.24-19.68	0.49
HSCT with TBI, without prior CNS irradiation	255	50.8	3.13	0.62-15.81	0.17
HSCT without TBI, with prior CNS irradiation	10	2	0.99	0.08-12.12	1

CNS: central nervous system; HSCT: hematopoietic stem cell transplantation; TBI: total body irradiation. In bold: statistically significant value.

large, prospective study from the Fred Hutchinson Cancer Research Center,[17] which included 791 patients transplanted for malignant or non-malignant disease during childhood, the prevalence of hypothyroidism was as high as 30%. In the present study, the 10-year cumulative incidence of hypothyroidism among transplanted patients was 23%, which is lower than in the aforementioned study. This could be due to the focus on acute leukemia survivors in our study, whereas Sanders *et al.* studied patients with a variety of pathologies. They also studied patients who had been treated with conditioning regimens including both busulfan and total body irradiation, which markedly increased the risk of thyroid dysfunction. In other studies concerning transplanted patients, based on smaller cohorts with various durations of follow-up, the prevalence of hypothyroidism ranged from 10.8% to 40%.[18,28-30] This varying prevalence may be due to the heterogeneity of the cohorts, in terms of both follow-up time and pathology (e.g., leukemia, lymphoma, and non-malignant).

As expected, in our study total body irradiation was strongly associated with an increased risk of hypothyroidism, compared with the risk in transplanted patients who did not receive any type of irradiation (i.e., total body irradiation or central nervous system irradiation) and in those who received only central nervous system irradiation (multivariate analysis) (Table 2). This observation is consistent with previously reported results.[14,24,29] More interestingly, and more rarely compared to other studies, our study also included a large number of patients treated with hematopoietic stem cell transplantation without the use of total body irradiation (n=135). We found that, in transplanted patients who did not receive total body irradiation, prior cerebral irradiation increased the risk of developing hypothyroidism (adjusted HR=3.39, *P*=0.02), which suggests that irradiation and alkylating agents may exert a cumulative effect. It is, therefore, important to monitor thyroid dysfunction closely in transplanted patients who receive a non-total-body-irradiation-based conditioning regimen with prior central nervous system irradiation as well as in those treated with total body irradiation. To our knowledge, this is the first study describing such results.

The patients included in our study were more likely to have received total body irradiation and less likely to have been treated with central nervous system irradiation than eligible non-included patients. This could have introduced a degree of bias in our results.

In the L.E.A. program, only patients who either underwent stem cell transplantation or received cranial/craniospinal radiotherapy were screened for thyroid dysfunction, which could also be considered as a bias in the present study. However, this choice was based on numerous studies that have failed to demonstrate that standard-dose chemotherapy alone plays a role in promoting thyroid dysfunction.[4,16,19,32]

We found that age (<10 years) at transplantation or irradiation was another risk factor for the development of hypothyroidism (adjusted HR=0.61 for patients over 10 years of age, *P*=0.02), which correlates with previously reported findings.[17,29,30]

Nearly all of the patients diagnosed with hypothyroidism received replacement therapy with levothyroxine, both in cases of uncompensated and compensated hypothyroidism. This is in accordance with general practice in France, as high TSH levels could be associated with an increased risk of thyroid cancer in the general population.[33] Nevertheless, systematic replacement therapy for subclinical compensated hypothyroidism remains debated.[34]

We found only six cases of central hypothyroidism, all of which were in patients who received either total body irradiation or cranial irradiation. This is consistent with the reports of most studies in the field, which describe peripheral hypothyroidism as the most common thyroid disorder following treatment in leukemia survivors.[5,25,29,35] However, Sanders *et al.* reported 74 cases of central hypothyroidism among 791 patients,[17] which may be due to a high number of single doses of total body irradiation. It was previously shown that single-dose total body irradiation is associated with increased toxicity and notably greater thyroid toxicity.[29]

We also evaluated the occurrence of and risk factors for thyroid cancer. In the general population, the incidence of thyroid cancer is 1.5/100,000 men and 4.7/100,000 women.[36] Many previous studies have focused on thyroid cancer after treatment for childhood cancer.[13,30,37-39] The reported prevalences varied, which may be due to several factors, e.g., the studies were frequently retrospective, based on questionnaires, or included small numbers of patients. In contrast, our study was prospective and included a large number of patients. In our cohort, thyroid cancer was found in 26 (5.2%) of 502 patients who underwent systematic ultrasound examination of the thyroid. In all 26 cases, the histological subtype was papillary carcinoma. This subtype is the most frequent one, not only in the general population, but also following treatment for childhood malignancy.[11,13,37,38,40,41] Some authors found that being less than 10 years of age was associated with an increased risk of thyroid cancer,[11,13,38] although we did not find similar results. In contrast, we confirmed that women are at an increased risk of developing thyroid cancer (HR=4.74; *P*=0.002), which has been suggested by other studies[13] and observed in the general population.[36] Notably, thyroid cancer occurred at a very young age in our cohort [median age at thyroid cancer diagnosis: 20.5 (IQR: 17.1-24.1) years, median period from leukemia diagnosis: 16.1 (IQR: 11.5-21.1) years], whereas thyroid cancer is diagnosed much later in the general population, usually at 45-50 years of age.[42] As the median follow-up duration was only 12.4 (IQR: 6.4-19.1) years, we probably underestimate the prevalence of thyroid cancer in our population, with disease occurrence increasing with age. This underscores the need for long-term follow-up of this population.

Radiation therapy is a well-known risk factor for thyroid cancer,[13,43] even in cases of low-dose radiation. Notably, two patients among the 26 who developed a thyroid cancer in our cohort had never received any irradiation but had been transplanted after receiving a busulfan-based conditioning regimen. This is consistent with the results published by Cohen *et al.*, who found that among 32 patients who developed thyroid cancer after transplantation, seven had never been exposed to any radiation therapy.[13] The use of alkylating agents may, therefore, also promote the development of thyroid cancer, as suggested by previous studies.[18,39] We, therefore, suggest that all transplanted patients be carefully monitored for thyroid malignancy, irrespectively of the conditioning regimen applied. We did not evaluate whether thyroid ultrasound scan was the best way to

screen for thyroid malignancies. Systematic thyroid ultrasound scanning is not cost-effective for the general population. However, the incidence of thyroid malignancies among survivors of childhood cancer is high, and neck palpation is often insufficient to detect small suspicious nodules in these high-risk patients.[37] Furthermore, in the follow-up of irradiated individuals, several studies have shown that many large, ultrasound-detected thyroid nodules escape detection *via* palpation.[37,44] Lastly, thyroid ultrasound scanning is a simple and non-invasive method. Taken together, these arguments lead us to consider that thyroid ultrasound scanning is a valuable method for screening for malignancies, regardless of a lack of definitive data comparing this method with other screening modalities.

There was no central review of the thyroid ultrasound scans, which could be considered another weakness of this study. However, given the nature of this imaging method, it is difficult to perform a central review.

We did not find any relationship between hypothyroidism and thyroid cancer. Some authors have suggested a link between high TSH levels and the occurrence of thyroid cancer in the general population,[33,45] based on the understanding that TSH is a major growth factor for thyroid cells and that some animal models have indicated that TSH plays a role in the development of follicular cell-derived thyroid cancer. Nevertheless, this has never been confirmed for survivors of childhood cancer, which may be because of frequent systematic replacement therapy with levothyroxine in cases of elevated TSH levels.

In conclusion, our findings suggest that irradiated and transplanted long-term survivors of childhood acute leukemia should undergo careful and prolonged monitoring for thyroid dysfunction. Patients exposed to total body irradiation or central nervous system irradiation followed by transplantation are at the highest risk of thyroid complications. Clinicians should be aware that thyroid cancer is frequent among survivors of acute leukemia treated with central nervous system irradiation and/or hematopoietic stem cell transplantation and such patients therefore warrant early and prolonged systematic screening with ultrasound scans.

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