



## Subclinical hypothyroidism in children and adolescents after hematopoietic stem cells transplantation without irradiation

Supklinički hipotireoidizam posle transplantacije matičnih ćelija hematopoeze kod dece i adolescenata koji nisu dobijali radioterapiju

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### Abstract

**Background/Aim.** Although total body irradiation (TBI) was considered to be the primary cause of thyroid dysfunction following hematopoietic stem cells transplantation (HSCT), a significant prevalence of subclinical hypothyroidism after HSCT with chemotherapy-only conditioning regimens has been observed in several studies. The aim of this study was to assess changes in thyroid stimulating hormone (TSH) levels in children after HSCT, without the use of irradiation at any time in the course of the treatment. **Methods.** Our cohort consisted of 41 children and adolescents who underwent autologous or allogeneic HSCT and were available for follow-up for at least one year after transplantation. Irradiation was not performed in any of the subjects, neither during pretransplantation therapy, nor during conditioning. The median duration of follow-up was 2.9 years. The indications for HSCT were hematologic malignancy (41.5%), solid malignant tumor (34.1%), and other disorders (24.4%). The thyroid status of all the subjects was assessed prior to HSCT and after follow-up period. **Results.** Thyroid dysfunction after HSCT was present in 27 (65.8%) subjects. Subclinical hypothyroidism was the most common abnormality, presenting in 23 (56.1%) patients, primary hypothyroidism was present in one (2.4%) patient, while 3 (7.3%) subjects had low free T4 with normal TSH values. Significantly ( $p < 0.01$ ) higher elevations in TSH levels were present in the patients who received chemotherapy for the underlying disease prior to HSCT. **Conclusion.** Our findings emphasize the need for long-term monitoring of thyroid function following HSCT, regardless of whether or not irradiation was used.

### Key words:

hematopoietic stem cell transplantation; child; adolescent; hypothyroidism; radiotherapy.

### Apstrakt

**Uvod/Cilj.** Iako se smatra da je radioterapija posle transplantacije matičnih ćelija hematopoeze (TMČH) glavni uzrok poremećaja tireoidne funkcije, u više istraživanja utvrđena je značajna prevalencija supkliničkog hipotireoidizma posle TMČH kada u sklopu kondicioniranja nije korišćena radioterapija, već isključivo hemioterapija. Cilj ovog istraživanja bio je procena promene u nivoima tireostimulišućeg hormona (TSH) posle TMČH kod dece koja nisu zračena tokom lečenja. **Metode.** Ispitivana grupa dece sastojala se od 41 deteta i adolescenata kojima je učinjena autologna ili alogena TMČH i koji su praćeni najmanje godinu dana posle transplantacije. Radioterapija nije primenjena kod ispitanika, ni tokom pretransplantacione terapije, niti tokom kondicioniranja. Prosečno vreme praćenja iznosilo je 2,9 godina. Indikacije za TMČH bile su: hematološko maligno oboljenje (41,5%), solidni maligni tumori (34,1%) i druga oboljenja (24,4%). Tireoidna funkcija svih ispitanika procenjena je pre TMČH i na kraju perioda praćenja. **Rezultati.** Tireoidna disfunkcija posle TMČH utvrđena je kod 27 (65,8%) ispitanika. Najčešći poremećaj funkcije bio je supklinički hipotireoidizam, kod 23 (56,1%) ispitanika, primarni hipotireoidizam kod jednog (2,4%) bolesnika, dok su 3 (7,3%) ispitanika imala niske nivoe slobodnog tiroksina i normalne vrednosti TSH. Značajno ( $p < 0.01$ ) veća tendencija povećanja koncentracija TSH uočena je kod bolesnika koji su dobijali hemioterapiju u sklopu lečenja osnovne bolesti pre TMČH. **Zaključak.** Nalazi učinjenog ispitivanja ukazuju na neophodnost dugoročnog praćenja tireoidne funkcije posle transplantacije matičnih ćelija hematopoeze, bez obzira na primenu radioterapije.

### Ključne reči:

transplantacija hematopoeznih matičnih ćelija; deca; adolescenti; hipotireoidizam; radioterapija.

## Introduction

The increasing number of performed autologous and allogeneic hematopoietic stem cells transplantations (HSCT) in the treatment of acquired and inborn disorders, along with a substantial decrease in early patient mortality has resulted in an increased frequency and importance of late effects of HSCT, especially in children<sup>1-7</sup>. The endocrine system is well-known to be highly sensitive to both cytotoxic drugs and radiation used in pretransplant conditioning regimens<sup>8-10</sup>. Disorders of thyroid function are the commonest endocrine complications of HSCT in childhood, most notably subclinical hypothyroidism which usually presents within one year after HSCT with reported incidence of up to 40%<sup>11-13</sup>. Although total body irradiation (TBI) was considered to be the primary cause for thyroid dysfunction following HSCT, these disorders have also been observed after HSCT with chemotherapy-only conditioning regimens<sup>14-17</sup>. The role of other factors associated with the development of hypothyroidism following HSCT remains unclear, including the influence of the underlying disorders, graft *versus* host disease or post-HSCT immune reconstitution<sup>11, 18</sup>. Data regarding this matter are scarce, and although several studies investigated hypothyroidism as a late complication of childhood HSCT without the use of TBI in conditioning, most of them were cross-sectional in design, with no record of the thyroid status of subjects prior to HSCT, and the patients who received TBI or thyroid irradiation treatment before conditioning for HSCT were not excluded<sup>11, 15</sup>.

The aim of this study was to assess the frequency of hypothyroidism and the factors associated with changes in thyroid stimulating hormone (TSH) levels in children and adolescents after HSCT, without the use of TBI or thyroid irradiation at any time in the course of the treatment.

## Methods

We studied a group of 41 children and adolescents who underwent autologous (n = 24) or allogeneic (n = 17) HSCT at the Institute of Mother and Child Health Care of Serbia "Dr Vukan Ćupić", and who were available for follow-up for at least one year after transplantation. There were 10 female and 31 male subjects, aged 2.7–20.5 years (median age 11.4 years). HSCT was performed at the mean age of  $8.2 \pm 5.3$  years, and median duration of follow-up was 2.9 (range 1.0–6.0) years. The mean age at the diagnosis of the underlying disease was  $7.2 \pm 5.3$  years, and the indications for HSCT were: hematologic malignancy (41.5%), namely acute myeloid leukemia (n = 7), chronic myelogenous leukemia (n = 2), acute biphenotypic leukemia (n = 1), Hodgkin lymphoma (n = 3) and Burkitt lymphoma (n = 4); solid malignant tumor (34.1%), namely neuroblastoma (n = 10), medulloblastoma (n = 2) and Ewing's sarcoma (n = 2); and other disorders (24.4%), namely aplastic anemia (n = 4), Wiskott–Aldrich syndrome (n = 2),  $\beta$ -thalassemia major, hemophagocytic lymphohistiocytosis, mixed connective tissue disease and Omenn syndrome (n = 4).

All the patients were clinically euthyroid and none were treated with L-thyroxin prior to HSCT. The thyroid status of all the subjects, namely TSH and T4/ free T4 (in regards to the analysis used at the time of HSCT), was assessed prior to HSCT and after the follow-up period. Normal thyroid status was defined by low or normal TSH with normal T4 or free T4, subclinical hypothyroidism by elevated TSH with normal T4 or free T4, primary hypothyroidism by high TSH concomitant with low T4 or free T4 and central hypothyroidism by low T4 or free T4 and low or normal TSH. T4 was measured by radioimmunoassay (RIA T4, INEP; laboratory normal range: 55.0–160.0 nmol/L), free T4 was measured by electrochemiluminescence immunoassay (ECLIA Cobas e411, Roche; laboratory normal range: 12.0–22.0 pmol/L) and serum TSH was measured using electrochemiluminescence immunoassay (ECLIA Cobas e411, Roche; laboratory normal range 0.27–4.20 mIU/L). Although for descriptive purposes TSH concentrations higher than 4.0 mIU/L were considered as elevated in our study, due to different physiologic reference intervals in children in regards to age and gender, changes in TSH levels pre- and post-HSCT were also calculated and analyzed<sup>19</sup>.

Irradiation was not performed in any of the subjects, neither during pretransplantation therapy, nor during conditioning. The therapy for underlying disease prior to HSCT in 34 (82.9%) patients consisted of chemotherapy, while the remaining seven (17.1%) patients did not receive chemotherapy prior to HSCT conditioning. All subjects received conditioning regimens without irradiation. As part of pretransplantation regimen, 14 (34.1%) patients received corticosteroids for more than 4 weeks, 10 (24.4%) received antithymocyte globulin. Nine (21.9%) patients were given methotrexate for prophylaxis of graft *versus* host disease.

The differences in the means of variables between the groups were tested using both parametric and nonparametric tests depending on the distribution of the variables. Comparisons were performed using Student's *t*-test in the case of normally distributed continuous variables or Mann–Whitney *U* test for non-normally distributed continuous variables. Paired samples *t*-test, Wilcoxon signed-rank test and McNemar's test were used to test the differences before and after the treatment. Spearman's correlation analysis was used to test the relationship between the TSH change and other numerical variables. Probability values of less than 0.05 were considered to be statistically significant, and values were expressed as frequencies or means  $\pm$  SD unless otherwise stated. SPSS version 15.0 (SPSS, Chicago, IL) was used for statistical analysis.

## Results

We found clinical and/or laboratory evidence of thyroid dysfunction after HSCT in 27 (65.8%) subjects. Subclinical hypothyroidism was the most common abnormality, presenting in 23 (56.1%) patients, primary hypothyroidism was present in one (2.4%) patient, while 3 (7.3%) subjects had low free T4 with normal TSH values. Among the 24 subjects with elevated TSH levels after HSCT (range 4.08–9.34

mIU/L), six had TSH > 4.0 mIU/L prior to HSCT (range 4.05–5.55 mIU/L).

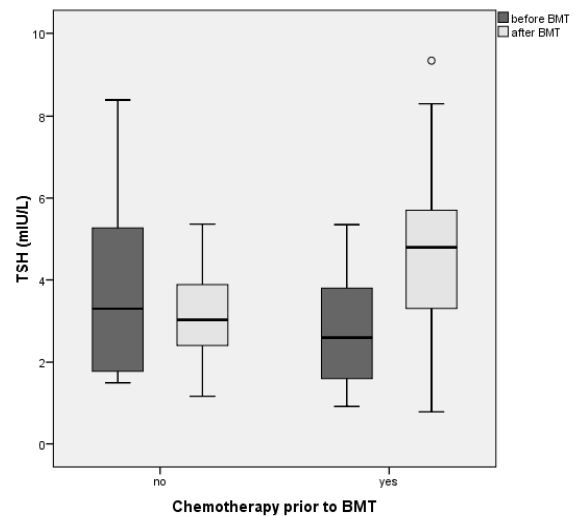
According to McNemar’s test, a statistically significant difference ( $p < 0.001$ ) was observed regarding the number of subjects with elevated TSH levels prior to HSCT ( $n = 8$ ) and after HSCT ( $n = 24$ ), and as shown in Table 1, mean TSH levels of all subjects after HSCT were significantly higher than mean TSH levels prior to HSCT.

No significant differences were observed in the changes of TSH levels in regards to gender, underlying disease or HSCT type (Table 1). Correlation analysis showed no correlation between the changes in TSH levels after HSCT and the age at diagnosis ( $\rho = 0,053$ ;  $p = 0,753$ ), age at HSCT ( $\rho = 0,135$ ;  $p = 0,411$ ) or the follow up period ( $\rho = -0,034$ ;  $p = 0,839$ ). Also, no correlation was found between TSH levels prior to HSCT and these parameters.

No significant differences were observed between all the groups shown in Table 1 in regards to TSH levels before HSCT, while higher post-HSCT TSH levels were observed with a borderline significance ( $p = 0.061$ ) in subjects who received chemotherapy prior to HSCT.

Although a greater elevation in the mean TSH levels after HSCT was observed in the patients who underwent autologous HSCT and those who received corticosteroids for more than four weeks, as a well as lower TSH rise in the subjects who received methotrexate and antithymocyte globulin (Table 1), these findings were not statistically significant. In nine patients who developed graft *versus* host disease, no significant differences were observed in either post-HSCT TSH levels or in relative changes in TSH levels after HSCT. However, analysis showed significantly ( $p = 0.003$ ) higher elevations in TSH levels in the patients who

received chemotherapy for the underlying disease prior to HSCT (Table 1, Figure 1).



**Fig. 1 – Thyroid-stimulating hormone (TSH) levels before and after hematopoietic stem cells transplantation (HSCT) in regards to chemotherapy prior to HSCT.**

**Discussion**

Compensated hypothyroidism defined as an elevation of TSH levels in the presence of normal thyroid hormone concentrations, is recognized as one of the most frequent complications of HSCT<sup>12, 20</sup>. According to the results of previous studies, the occurrence of hypothyroidism in children after HSCT has been observed in up to 58% of patients<sup>21</sup>. In

**Table 1**  
**Thyroid-stimulating hormone (TSH) levels before and after hematopoietic stem cell transplantation (HSCT) in regards to selected clinical parameters**

Parameters	n	TSH (mIU/L) <sup>a</sup>		p-value
		before HSCT	after HSCT	
Total	41	2.96 ± 1.58	4.32 ± 1.88	< 0.001 <sup>a</sup>
Gender				NS <sup>b</sup>
male	31	3.15 ± 1.58	4.46 ± 1.90	0.001 <sup>a</sup>
female	10	2.36 ± 1.57	3.92 ± 1.87	0.015 <sup>c</sup>
Indication for HSCT				NS <sup>b</sup>
hematologic malignancy	17	3.03 ± 1.39	4.77 ± 2.23	0.001 <sup>c</sup>
solid tumors and other	24	2.92 ± 1.75	4.00 ± 1.58	0.024 <sup>c</sup>
Type of HSCT				NS <sup>b</sup>
autologous	24	2.77 ± 1.20	4.52 ± 2.00	< 0.001 <sup>c</sup>
allogeneic	17	3.28 ± 2.08	4.04 ± 1.74	NS <sup>c</sup>
Chemotherapy prior to HSCT				0.003 <sup>b</sup>
no	7	3.93 ± 2.57	3.16 ± 1.36	NS <sup>c</sup>
yes	34	2.79 ± 1.33	4.56 ± 1.91	< 0.001 <sup>a</sup>
Corticosteroid use > 4 weeks				NS <sup>b</sup>
no	27	3.14 ± 1.73	4.29 ± 1.73	0.005 <sup>c</sup>
yes	14	2.57 ± 1.18	4.39 ± 2.23	0.005 <sup>a</sup>
Methotrexate				NS <sup>b</sup>
no	32	2.95 ± 1.63	4.51 ± 1.96	< 0.001 <sup>a</sup>
yes	9	2.68 ± 1.28	3.51 ± 1.50	NS <sup>c</sup>
Antithymocyte globulin				NS <sup>b</sup>
no	31	2.79 ± 1.29	4.39 ± 1.90	< 0.001 <sup>a</sup>
yes	10	3.52 ± 2.34	4.11 ± 1.94	NS <sup>c</sup>

All the values expressed as mean ± SD; NS – no significant difference ( $p > 0.05$ ); <sup>a</sup>analysis performed by *t*-test; <sup>b</sup>analysis performed by Mann-Whitney *U* test for group comparison of TSH change; <sup>c</sup>analysis performed by Wilcoxon signed-rank test.

our cohort, subclinical hypothyroidism as the most common abnormality of thyroid function after HSCT was discovered in 56.1% of the patients. Although this finding correlates well and is simple to compare to the results of other studies, having in mind that physiologic reference intervals for TSH in children differ significantly in regards to age and gender<sup>19</sup>, we focused on the dynamics of TSH change after HSCT in the present study. These analyses confirmed a significant increase in TSH in our cohort after HSCT, which is an important finding having in mind that previous studies in the prevalence of thyroid dysfunction after HSCT without irradiation investigated only post-HSCT TSH levels, with no data regarding thyroid status prior to HSCT<sup>11</sup>. Thus, the results of our study complement and further strengthen the results of other studies regarding thyroid dysfunction after HSCT with chemotherapy-only conditioning regimens.

Although thyroid dysfunction after HSCT was mainly linked to the use of TBI<sup>12, 22, 23</sup>, the results obtained in our cohort from subjects that were not exposed to any kind of irradiation therapy during treatment, show a high percentage of thyroid abnormalities after HSCT with chemotherapy-only conditioning regimens. This correlates with other reports that indicate that TBI or thyroid irradiation is not the only cause of thyroid dysfunction following HSCT<sup>11, 12, 14, 15, 24</sup>. Other proposed factors inducing thyroid injury either directly or indirectly by modifying immune processes include the underlying disease, the process of HSCT, immune reconstitution and chemotherapy<sup>12, 15, 18, 25</sup>. In our cohort, the children who received chemotherapy prior to HSCT conditioning had a significant rise in TSH levels after HSCT compared to other

children, who had a slight decrease in TSH levels after HSCT. Since these groups did not differ significantly in regards to TSH levels before HSCT, a statistically significant difference in both the change and the post-HSCT TSH levels between these groups, indicates that the previous chemotherapy represents a significant risk factor for hypothyroidism following HSCT with chemotherapy-only conditioning regimens.

The present study is partially limited by a relatively small number of subjects in our cohort. However, our results are strengthened by a well-defined sample of children and adolescents who underwent HSCT without the use of TBI or thyroid irradiation at any time in the course of the treatment. Also, to our knowledge, this is the first study to evaluate the pre- and post-HSCT dynamics of TSH levels following HSCT with chemotherapy-only conditioning regimens.

### Conclusion

Our results demonstrate a substantial rise in thyroid stimulating hormone levels following hematopoietic stem cells transplantation without the use of total body irradiation or thyroid irradiation. The most common thyroid disorder was subclinical hypothyroidism, and chemotherapy prior to hematopoietic stem cells transplantation conditioning was the most significant factor associated with the increase in thyroid stimulating hormone levels after hematopoietic stem cells transplantation. These findings emphasize the need for long-term monitoring of thyroid function following hematopoietic stem cells transplantation, regardless of whether or not irradiation was used during conditioning.

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