Cytokine profile in patients with differentiated thyroid cancer

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Multiple cytokines released in tumor microenvironment can promote anticancer effects, or carcinogenesis and tumor growth. Although cytokines mainly act locally, the changes in their circulating levels may reflect the interactions between tumor and inflammatory cells during the disease course. The aim of this study was to analyze the serum cytokine profile in patients with differentiated thyroid cancer (DTC) and to identify cytokines those could be associated with tumor progression/metastasis. Serum concentrations of thirteen cytokines were measured in control subjects and DTC patients before, three and seven days after radioactive iodine therapy, using multiplex cytokine detection systems for Th1/Th2/Th9/Th17/Th22 cell-related cytokines. Most cytokines were not detected in serum samples from control subjects, while detectable levels of the cytokines were measured in some, but not all DTC patients. The serum levels of the following cytokines: interleukin IL-17A, IL-10 and IL-13 were significantly increased in DTC patients with metastasis. At the same time, the concentrations of several cytokines (IL-12p70, IL-17A, IL-5, IL-1β) and tumor necrosis factor (TNF-α) were positively correlated with thyroglobulin (Tg) levels. The histological type of tumor, hypothyroidism, and intensity of oxidative stress were not associated with cytokine levels in patients’ sera. Radioactive 131-I therapy reduced serum levels of the majority of examined cytokines, but these differences did not reach statistical significance. In conclusion, the study indicates that the increase of the levels of several Th2/Th17 cells and proinflammatory cytokines in the serum of patients with DTC is associated with tumor progression/metastasis. Thus, the increase in this specific cytokine constellation might be an indicator of the malignant disease progression.

Keywords: Cytokine, Metastasis, Oxidative stress, Serum, Thyroglobulin, Thyroid cancer

Multiple cytokines and growth factors released in tumor microenvironment modulate tumor progression and metastasis¹. Although cytokines mainly act in the local microenvironment, changes in circulating levels of cytokines have been detected in patients with various malignancies, including malignant melanoma², pancreatic cancer³, colorectal cancer⁴, cervical cancer⁵, lung cancer⁶, breast cancer⁷, esophageal cancer⁸ and hepatocellular carcinoma¹⁰. Cytokine concentrations in serum samples from patients with differentiated thyroid cancers have been measured only in a few studies¹¹⁻¹⁵. Ozata et al.¹¹ showed no significant differences in serum concentrations of IL-6 and TNF-α between thyroid cancer patients before radioactive iodine therapy and control subjects, while Kammoun-Krichen et al.¹² found that IL-1β serum levels were lower in a group of patients with thyroid cancer when compared to individuals with other thyroid diseases and controls. Three recently published studies¹³⁻¹⁵ showed the increased level of IL-17 in patients with thyroid cancer. The aim of this study was to evaluate cytokine profiles in serum of patients with differentiated thyroid cancer (DTC) before, three and seven days after radioactive iodine therapy. Cytokine concentrations measured in patients before radioactive iodine therapy were analyzed in relation to the intensity of oxidative stress, histological type of the tumor, thyroglobulin level and the presence/absence of metastasis. Also, cytokine concentrations were correlated with levels of TSH to elucidate the possible association of hypothyroidism with cytokine release in the serum of patients with DTC.
Materials and Methods

Study population

The study included eighteen patients with DTC, among whom fourteen (77.8%) had papillary carcinoma, and four (22.2%) had follicular carcinoma. The mean age of the patients was 52.6 ± 18.5 years with a predominance of female subjects (13 or 72.2%). Eight patients had no clinical evidence of metastasis, while 10 patients had metastasis (6 patients had metastasis in the lymph nodes only and 4 patients had distant metastases in bone or lungs at the same time). The mean concentration of thyroglobulin (Tg) in patients before therapy was 10.3 ± 12.1 ng/mL. Four to six weeks after surgical (total) thyroidectomy, and 10 days after a low iodine diet, patients were treated at the Nuclear Medicine Department of the Clinical Center Kragujevac according to EANM guidelines, with fixed nominal activities of 3.7 GBq (100 mCi) (8 patients) or 5.5 GBq (150 mCi) (10 patients) of orally administered sodium iodide (131-I). At the time of 131-I administration, all 18 patients were hypothyroid after thyroid hormone withdrawal. The mean concentration of thyroid-stimulating hormone (TSH) was 176.1 ± 135.7 mIU/L and thyroid antibodies were negative. The patients had neither thyroid nor systemic autoimmune disorder. They received no immunomodulatory drugs. After the 131-I therapy, the patients received no corticosteroids or other medications that could affect the tested parameters.

The control group consisted of 18 healthy subjects, 13 (72.2 %) females and 5 (27.8 %) males, with a mean age of 41.9 ± 9.5 yrs (range 31-80 yrs). All control subjects were evaluated for thyroid function and thyroid antibodies. The mean concentration of TSH was 1.7 ± 0.4 mIU/L, and thyroid antibodies were negative. Individuals who were diabetic, hypertensive or suffered from liver disease, renal failure, malignant diseases and dysproteinemia/dyslipoproteinemia were not included in the study.

Patients and control subjects were not exposed to ionizing radiation or any other confounding factor within 3 months of sample collection. They received no salicylates or other agents that could influence the oxidative state for 3 weeks before the study. All subjects provided written consent according to the Declaration of Helsinki.

Sample collection and cytokine measurement

After venipuncture, blood samples (5 mL) from patients and control subjects were collected in tubes (Vacutainer). Blood samples were taken from control subjects once, while from DTC patients were taken three times (before, 3 days and 7 days after radioactive 131-I therapy). Within an hour the blood was centrifuged at 1400 × g for 15 min, and serum samples were collected in vials and frozen at −70°C until analysis. Serum cytokine levels in DTC patients and controls were determined in one step and included IL-12p70, IFN-γ, IL-17A, IL-2, IL-10, IL-9, IL-22, IL-6, IL-13, IL-4, IL-5, IL-1β and TNF-α, using a commercial flowcytometric kit (Human Th1/Th2/Th9/Th17/Th22 13plex, FlowCytomix Multiplex (ebioscience Cat. No. BMS817FF) on a FC500 Beckman Coulter Flow Cytometer according to the manufacturer’s instructions. Collected data were analyzed using FlowCytomix™ Pro 3.0 Software.

Measurement of malondialdehyde

For measurement of malondialdehyde (MDA) concentration one volume of serum sample collected as mentioned above was mixed thoroughly with two volumes of a stock solution of 15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid and 0.25 M hydrochloric acid. The mixture was heated in a bath of boiling water for 5 min. After cooling, the precipitate was removed by centrifugation at 1000 × g for 5 min. Absorbance in the supernatant was determined spectrophotometrically at 535 nm using an Ultrospec 2000 spectrophotometer (Pharmacia Biotech, England).

A standard curve was prepared by acid hydrolysis of 1,1,3,3-tetramethoxypropane. MDA values were expressed as nmol/mL using a molar extinction coefficient of 1.56 × 10^5 M^-1 cm^-1.

Statistical analysis

The commercial program SPSS version 20.0 was used for statistical analysis of the results. Data are presented as the mean ± standard deviation (SD) and median values with range (minimum-maximum) depending on distribution. Differences between the patient and control groups were evaluated by the t-test or U-test, whereas within the patient group the paired t-test or Wilcoxon test was used, depending on the data distribution. The observed variables were compared by the bivariate correlation test and determination of Pearson/Spearman coefficients. The multivariate analysis of variance (MANOVA) was also used, as an omnibus test, to determine whether
there are any differences between the groups of patients considering the histopathological type of tumor and the concentration of MDA, Tg, and TSH. P values less than 0.05 were considered statistically significant.

**Results**

We determined cytokine concentrations in serum samples from DTC patients before, three and seven days after radioactive iodine therapy and compared them with those in control subjects. The influence of the histological type of tumor, presence/absence of metastasis, thyroglobulin level, hypothyroidism and intensity of oxidative stress on the serum cytokine concentration was also evaluated. The results are shown in Tables 1-4 and Fig. 1.

Table 1 is displayed the detection limit for the cytokine concentrations in serum samples of DTC patients before radioactive iodine therapy and control subjects.

In Table 1 is displayed the detection limit for the cytokine concentrations in serum samples of DTC patients before radioactive iodine therapy in relation to the histological type of tumor.

<table>
<thead>
<tr>
<th>Cytokine, lower limit</th>
<th>Range</th>
<th>Detectable cases, n (%)</th>
<th>Range</th>
<th>Detectable cases, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-12p70 (pg/mL) (&gt;1.5)</td>
<td>0.0 – 5.6</td>
<td>10 (55.6)</td>
<td>0.0-1.1</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>IFN-γ (pg/mL) (&gt;1.6)</td>
<td>0.0-4.9</td>
<td>4 (22.2)</td>
<td>0.0-0.0</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>IL-17A (pg/mL) (&gt;2.5)</td>
<td>0.0-9.6</td>
<td>10 (55.6)</td>
<td>0.0-4.3</td>
<td>3 (16.7)</td>
</tr>
<tr>
<td>IL-2 (pg/mL) (&gt;16.4)</td>
<td>0.0-114.4</td>
<td>12 (66.7)</td>
<td>0.0-0.0</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>IL-10 (pg/mL) (&gt;1.9)</td>
<td>0.0-3.0</td>
<td>6 (33.3)</td>
<td>0.0-0.2</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>IL-9 (pg/mL) (&gt;1.5)</td>
<td>0.0-0.4</td>
<td>0 (0.0)</td>
<td>0.0-0.0</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>IL-22 (pg/mL) (&gt;43.3)</td>
<td>0.0-438.1</td>
<td>14 (77.8)</td>
<td>0.0 – 538.2</td>
<td>15 (83.3)</td>
</tr>
<tr>
<td>IL-6 (pg/mL) (&gt;1.2)</td>
<td>0.0-2.1</td>
<td>4 (22.2)</td>
<td>0.0-0.3</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>IL-13 (pg/mL) (&gt;4.5)</td>
<td>0.0-47.2</td>
<td>10 (55.6)</td>
<td>0.0-19.5</td>
<td>1 (5.6)</td>
</tr>
<tr>
<td>IL-4 (pg/mL) (&gt;20.8)</td>
<td>0.0-25.5</td>
<td>4 (22.2)</td>
<td>0.0-0.0</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>IL-5 (pg/mL) (&gt;1.6)</td>
<td>0.0-14.9</td>
<td>10 (55.6)</td>
<td>0.0-0.0</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>IL-1β (pg/mL) (&gt;4.2)</td>
<td>0.0-13.5</td>
<td>8 (44.4)</td>
<td>0.0-2.9</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>TNF-α (pg/mL) (&gt;3.2)</td>
<td>0.0-8.4</td>
<td>6 (33.3)</td>
<td>0.0-1.5</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

Table 2 shows the serum concentrations of seven cytokines in DTC patients before radioactive iodine therapy in relation to the histological type of the tumor and the presence/absence of any proven metastasis. The histological type of DTC was not

<table>
<thead>
<tr>
<th>Cytokine (pg/mL)</th>
<th>Papillary (n = 14)</th>
<th>Follicular (n = 4)</th>
<th>P value</th>
<th>Metastasis + (n = 10)</th>
<th>Metastasis – (n = 8)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (pg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-17A</td>
<td>4.0 ± 3.7</td>
<td>3.0 ± 2.4</td>
<td>0.714</td>
<td>6.4 ± 2.3</td>
<td>1.5 ± 1.8</td>
<td>0.016</td>
</tr>
<tr>
<td>IL-2</td>
<td>43.6 ± 39.3</td>
<td>45.5 ± 60.7</td>
<td>0.956</td>
<td>43.7 ± 36.7</td>
<td>44.7 ± 52.6</td>
<td>0.976</td>
</tr>
<tr>
<td>IL-10</td>
<td>1.2 ± 1.4</td>
<td>0.9 ± 0.8</td>
<td>0.725</td>
<td>2.1 ± 0.7</td>
<td>0.3 ± 0.6</td>
<td>0.003</td>
</tr>
<tr>
<td>IL-13</td>
<td>18.5 ± 20.3</td>
<td>8.9 ± 7.0</td>
<td>0.337</td>
<td>27.2 ± 19.4</td>
<td>5.8 ± 6.7</td>
<td>0.042</td>
</tr>
<tr>
<td>IL-5</td>
<td>6.8 ± 6.1</td>
<td>5.0 ± 10.3</td>
<td>0.905</td>
<td>8.6 ± 6.5</td>
<td>4.3 ± 6.6</td>
<td>0.423</td>
</tr>
<tr>
<td>IL-1β</td>
<td>5.7 ± 5.4</td>
<td>2.8 ± 2.2</td>
<td>0.407</td>
<td>7.6 ± 5.5</td>
<td>2.4 ± 2.2</td>
<td>0.093</td>
</tr>
<tr>
<td>TNF-α</td>
<td>3.5 ± 3.3</td>
<td>1.7 ± 1.2</td>
<td>0.401</td>
<td>4.6 ± 3.3</td>
<td>1.5 ± 1.4</td>
<td>0.094</td>
</tr>
</tbody>
</table>
associated with cytokine concentrations. However, the concentrations of the following cytokines: IL-17A ($P = 0.016$), IL-10 ($P = 0.003$) and IL-13 ($P = 0.042$) were significantly higher in patients with metastasis than in patients without metastasis.

According to the serum values of Tg, DTC patients were divided into two groups: Tg concentrations below 5 ng/mL (10 patients) and Tg concentrations above 5 ng/mL (8 patients). Multivariate analysis showed a statistically significant ($P = 0.020$) difference in the cytokines concentration between two group of patients.

DTC patients with Tg values above 5 ng/mL had significantly higher the concentrations of following cytokines: IL-12p70 ($P = 0.001$), IL-17A ($P = 0.009$), IL-13 ($P = 0.004$), IL-5 ($P = 0.016$), IL-1β ($P = 0.010$) and TNF-α ($P = 0.011$) (Table 3). Additionally, the concentrations of all of these cytokines except for IL-13 were positively correlated with Tg levels (Table 4).

The mean level of MDA in the serum of DTC patients with Tg values above 5 ng/mL had significantly higher than in controls (2.5 ± 0.7 nmol/mL vs 1.7 ± 0.3 nmol/mL, $P = 0.010$). But, there was no correlation of any cytokine concentration with MDA level in the peripheral circulation (Table 4). There was also no correlation with TSH values. There was a significant ($P < 0.001$) difference in the concentration of TSH in severely hypothyroid DTC patients (176.11 ± 135.72 mIU/L) and euthyroid controls (1.67 ± 0.42 mIU/L). However, there was no correlation in the concentration of any of the examined cytokine with TSH level in patients’ serum (Table 4).

Fig. 1 shows the changes in the serum cytokine concentrations in DTC patients before, 3 and 7 days after radioactive iodine therapy. Although the mean

![Image](https://example.com/image1.png)

**Fig. 1** — Cytokine concentrations (pg/mL) in serum samples of DTC patients before (0 day), three days (3 day) and seven days (7 day) after radioactive iodine therapy.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Correlation with MDA</th>
<th>Correlation with TSH</th>
<th>Correlation with Tg</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-12p70</td>
<td>$r = 0.161, P = 0.680$</td>
<td>$r = -0.173, P = 0.657$</td>
<td>$r = 0.817, P = 0.007$</td>
</tr>
<tr>
<td>IL-17A</td>
<td>$r = -0.003, P = 0.994$</td>
<td>$r = -0.181, P = 0.641$</td>
<td>$r = 0.720, P = 0.029$</td>
</tr>
<tr>
<td>IL-2</td>
<td>$r = 0.204, P = 0.598$</td>
<td>$r = -0.382, P = 0.310$</td>
<td>$r = 0.641, P = 0.063$</td>
</tr>
<tr>
<td>IL-10</td>
<td>$r = 0.111, P = 0.777$</td>
<td>$r = -0.243, P = 0.528$</td>
<td>$r = 0.494, P = 0.177$</td>
</tr>
<tr>
<td>IL-22</td>
<td>$r = 0.074, P = 0.849$</td>
<td>$r = -0.165, P = 0.672$</td>
<td>$r = 0.492, P = 0.179$</td>
</tr>
<tr>
<td>IL-13</td>
<td>$r = 0.140, P = 0.719$</td>
<td>$r = -0.114, P = 0.771$</td>
<td>$r = 0.655, P = 0.055$</td>
</tr>
<tr>
<td>IL-5</td>
<td>$r = 0.287, P = 0.454$</td>
<td>$r = 0.070, P = 0.859$</td>
<td>$r = 0.870, P = 0.002$</td>
</tr>
<tr>
<td>IL-1β</td>
<td>$r = 0.178, P = 0.646$</td>
<td>$r = -0.237, P = 0.539$</td>
<td>$r = 0.714, P = 0.031$</td>
</tr>
<tr>
<td>TNF-α</td>
<td>$r = 0.150, P = 0.699$</td>
<td>$r = -0.283, P = 0.460$</td>
<td>$r = 0.679, P = 0.044$</td>
</tr>
</tbody>
</table>

**Table 4** — Possible correlations of serum cytokine concentrations in DTC patients with MDA, TSH and Tg values.

### Table 3 — Cytokine concentrations in DTC patients with thyroglobulin concentrations below 5 ng/mL (Tg ≤ 5 ng/mL) and above 5 ng/mL (Tg > 5 ng/mL).

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Tg ≤ 5 ng/mL (n = 10)</th>
<th>Tg &gt; 5 ng/mL (n = 8)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-12p70</td>
<td>0.32 ± 0.72</td>
<td>3.80 ± 1.31</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-17</td>
<td>1.54 ± 1.82</td>
<td>6.43 ± 2.28</td>
<td>0.009</td>
</tr>
<tr>
<td>IL-2</td>
<td>21.81 ± 37.34</td>
<td>72.29 ± 35.82</td>
<td>0.079</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.54 ± 0.75</td>
<td>1.78 ± 1.26</td>
<td>0.109</td>
</tr>
<tr>
<td>IL-22</td>
<td>79.77 ± 70.79</td>
<td>229.19 ± 140.71</td>
<td>0.075</td>
</tr>
<tr>
<td>IL-13</td>
<td>3.15 ± 5.12</td>
<td>30.49 ± 13.81</td>
<td>0.004</td>
</tr>
<tr>
<td>IL-5</td>
<td>1.32 ± 2.91</td>
<td>12.30 ± 3.52</td>
<td>0.016</td>
</tr>
<tr>
<td>IL-1β</td>
<td>1.62 ± 2.09</td>
<td>8.63 ± 3.87</td>
<td>0.010</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.01 ± 1.24</td>
<td>5.21 ± 2.79</td>
<td>0.011</td>
</tr>
</tbody>
</table>
serum levels of some cytokines decreased after therapy in relation to pretreatment values, the differences did not reach statistical significance.

Discussion
The current study demonstrated that: i) the progression of thyroid cancer is followed by the increase of serum levels of several Th2/Th17 and proinflammatory cytokines; ii) the levels of cytokines in patients’ sera correlates with the concentration of Tg; iii) the histological type of tumor, hypothyroidism, and intensity of oxidative stress were not associated with cytokine levels in patients’ sera and iv) radioactive iodine therapy does not significantly influence serum cytokine levels.

Cytokines can promote anticancer effects, or carcinogenesis and tumor growth. Although cytokines mainly act in the local microenvironment, changes in the circulating levels of some cytokines have been detected in patients with various malignancies. However, the results are very diverse, depending on the type of cancer and the cytokine selected for measurement. Besides, in the majority of studies cytokines were determined after applying some form of specific therapy. In herein presented study serum cytokine levels were analyzed after surgical total thyroidectomy, before and up to seven days after radioactive iodine therapy. The concentrations of cytokines measured in the sera of DTC patients before 131-I therapy were very low, as earlier showed in patients with hepatocellular carcinoma and chronic myeloid leukemia. Our results are consistent with three recently published studies showing the increased level of IL-17 in patients with thyroid cancer. The increase in the levels of IL-17 was also recorded in patients with non-small cell lung cancer, colon cancer, and hepatocellular carcinoma. In these studies, local and systemic IL-17 immune response, an elevated level of IL-17 and/or prevalence of Th17 cells were associated with disease progression, advanced stage of the disease and/or pure prognosis. We also found a higher level of IL-17A in sera from patients with high Tg levels and DTC patients with proved metastasis.

Concerning proinflammatory cytokines, level of IL-6 was undetectable in most patients, while the level of IL-1β and TNF-α were increased in DTC patients with metastasis compared with controls. As a pleiotropic pro-inflammatory cytokine, IL-1β is produced by antigen presenting cells, it mediates acute immune responses and provides a link between the innate and adaptive immune responses. An excessive IL-1 β production has also been recorded in malignancies and associated with the tumor invasiveness and angiogenesis. TNF-α is one of the first cytokines found in cancer microenvironment, proved to be active in tissue remodeling and stromal development, which is of great importance in tumor growth and spreading. This cytokine is chronically produced during the course of the malignant disease and its overexpression in several tumor cell lines refers to greater tumor invasiveness. In two recently published studies is shown that TNF-α induces an increase of the membrane expression of chemokine receptor CCR6 in thyroid cancer cells and secretion of CXCL8, i.e. the chemokine receptor and ligand involved in the metastatic spread of thyroid cancer.

Besides, DTC patients with metastasis had the increased levels of two more cytokines: IL-13 and IL-10. The IL-13 belongs to the T-helper 2 type of cytokines. The literature data on the role of IL-13 in tumor growth and/or immunosurveillance are contradictory. It is assumed that the antitumor effect of IL-13 is associated with the accumulation of neutrophils and macrophages, although the action of IL-13 through eosinophils could not be excluded. Interleukin-10 was initially described as a Th2-derived cytokine but its production is later shown in almost all leukocytes, epithelial and tumor cell. The role of IL-10 in the pathogenesis of thyroid carcinoma is complex, and can be manifested at several levels. First, IL-10-1082 gene polymorphism is associated with papillary thyroid cancer. Second, an autocrine production of IL-10 is required for survival and growth of thyroid cancer cells. And finally, as an anti-inflammatory and immunosuppressive cytokine, IL-10 can contribute to the immune escape of neoplastic cells. It was published recently that IL-10 expression is related to aggressiveness and poor prognosis of patients with thyroid cancer.

The results of previous studies indicate a shift in Th cell profile towards Th2 cell subtype during cervical carcinogenesis. Recently, we have noted that PHA-stimulated peripheral blood cells of patients with DTC produce more Th2 cytokines than controls, while Mardente et al. observed a mixed Th1/Th2 type of intracellular cytokine content in peripheral blood lymphocytes of patients with thyroid cancer after non-specific in vitro stimulation. In this study, we have demonstrated the increase in
Th2/Th17 type cytokine in serum samples of DTC patients. But, in patients with metastasis and high Tg levels, the concentration of some pro-inflammatory (or Th1) cytokines was also increased.

It is well known that oxidative stress can lead to cell damage and death, while inflammation is a physiological response to acute cell/tissue damage. Moreover, oxidative stress can be an initiator of cytokine release. In one study with thyroid cancer patients, the increase of the level of oxidative stress before surgical treatment was recorded, followed by the decrease after the surgery. The level of the oxidative stress in our DTC patients after surgery and before radioactive iodine therapy was still significantly higher than in controls, as it was shown in an earlier published study. However, we failed to demonstrate a clear association between the oxidative stress and cytokine levels in patients with DTC.

Despite the increased serum level of MDA, a biological marker of oxidative stress, its serum level correlated with level of none of the cytokines measured in patients' serum.

Serum thyroglobulin is used as a surrogate marker for recurrence of well-differentiated thyroid cancer. Although serum factors (especially thyroglobulin antibodies) can affect Tg measurement, the serum level of Tg mainly reflects the magnitude of TSH receptor stimulation and the mass of differentiated thyroid tissue. All patients included in this study were hypothyroid, with very high serum values of TSH. Since Tg might be produced by thyroid follicular cells and/or by cells of differentiated thyroid carcinomas (papillary and follicular), Tg measured two months after surgery and before radioactive 131-I therapy indicate its production by remnant and/or metastatic thyroid tissue. Namely, the half-life of Tg in patients serum is approximately 65 h and the Tg produced before surgery will disappear from the circulation in about 1 month. Although we cannot exclude a production of Tg from remnant thyroid tissue, we assume that Tg measured in this study is mainly produced from differentiated cancer cells.

When our DTC patients were divided into two groups, a statistically significant difference in concentration of some cytokines was detected in sera from the patients with Tg values below and above 5 ng/mL. Additionally, there was positive correlation between Tg concentration and cytokine levels in patients' serum. To the best of our knowledge, this is the first study demonstrating the correlations between serum cytokine levels with thyroglobulin concentration. Since the proven metastasis and higher Tg levels indicate a progression of the malignant disease, it seems highly likely that the increase of cytokine levels in DTC patients was associated with the disease progression.

Although some studies indicate an influence of hypothyroidism on immune function, our results do not confirm that. Namely, the increase in serum cytokine concentrations presented here and elevated cytokine levels in supernatants of blood cells stimulated in vitro was not induced by hypothyroidism. The statistical analysis excluded any association between serum cytokine and TSH levels.

The data regarding cytokine secretion in patients treated with radioactive iodine are still rare. In one study, a significant increase of IL-6 two months after radioactive iodine therapy was found, while TNF-α level did not change after therapy. In patients with hepatocellular carcinoma iodine-125 implantation induced Th2/Th1 deviation. Also, in patients with Graves disease treated with radioactive 131-I a transient increase of Th2 cytokines (IL-4, IL-6, and IL-10) was obtained. The results of those studies are not consistent with the findings that ionizing radiation induces an activation of pro-inflammatory cytokine network, including IL-1β, IL-6, and TNF-α. In the recently published study we showed a statistically significant decrease of Th2 cytokine production (IL-4, IL-5 and IL-13) in PHA-stimulated whole blood culture 7 days after radioactive 131-I therapy, while 131-I therapy diminished serum concentrations of some cytokines, but these differences did not reach statistical significance.

The levels of cytokines measured in the sera of DTC patients are similar, but not fully consistent with the cytokine production in PHA-stimulated whole blood cells of the DTC patients in vitro. Namely, in vitro stimulation of blood cells before radioactive iodine therapy gives a statistically significant increase in production of Th2/Th9 cytokines, followed by their decrease after the therapy. On the other hand, serum samples of DTC patients before 131-I therapy have increased levels of Th2/Th17 cytokines and, with disease progression, some pro-inflammatory cytokines, while changes in cytokine levels after the therapy were not significant. The lack of full compliance of the results obtained in vitro and in vivo can be explained by the difference in study design, whereby both provide
certain information on the cytokine profile in patients with DTC. Namely, cytokine levels measured in supernatants of whole blood cultures in vitro give insight into the capacity of these cells to produce cytokines in response to nonspecific stimulation. On the other hand, cytokine concentrations in sera possibly reflect their production not only from blood cells but also non-blood cells, including thyroid cancer cells. Besides, the cytokine concentrations in sera may be influenced by many serum factors, e.g. soluble receptors, anti-cytokine antibodies or receptor antagonists.

In some studies, serum cytokine levels were analyzed in order to find an independent prognostic factor for the outcome of malignant disease. This was not the purpose of our study since the differentiated thyroid cancers have a very good prognosis. Here, we took advantage of the multiplex immunoassay in order to identify cytokine(s) that could be associated with tumor progression/metastasis. We showed that levels of several cytokines Th2/Th17 and proinflammatory cytokines were increased in patients with metastasis and correlated with the concentration of Tg. Given that the serum Tg values are considered to be a highly specific indicator of the efficacy of surgical treatment and radioactive iodine therapy in DTC patients, we assume that the serum levels of cytokines which correlate with serum Tg level could also be valuable markers of the disease progression/metastasis.

In conclusion, the increase of the levels of several Th2/Th17 and proinflammatory cytokines in sera of patients with DTC coincides with tumor progression/metastasis. The intensity of oxidative stress, histological type of tumor, hypothyroidism, and treatment with radioactive iodine cannot be directly associated with cytokine levels in patients’ serum.

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