Correlation between, Clinical, Biochemical, Color Doppler Ultrasound Thyroid Parameters, and CXCL-10 in Autoimmune Thyroid Diseases

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Abstract. Objective: the aim of present study is to determine possible contributions of INF-γ inducible chemokine CXCL-10 in the thyroid color doppler ultrasound (CDU) parameters typical of autoimmune disorders. Methods: we studied a consecutive series of 25 patients with autoimmune thyroid disease and 10 healthy control subjects. All subjects underwent a thyroid CDU examination by the same investigator, who was unaware of the laboratory values at the time of the examination. Moreover, all subjects underwent a clinical evaluation, CXCL-10 and thyroid hormonal assessment. Results: CXCL-10 levels were significantly higher in patients with autoimmune diseases and as well as in subjects with an increased thyroid vascularization at CDU. Moreover, CXCL-10 levels were significantly (p<0.05) correlated with inferior thyroid arteria peak systolic velocity (ITA-PSV; r = 0.376) and with thyroid volume even after adjustment for confounding factors. No difference was observed between vascular thyroid pattern at CDU and thyroid circulating hormones while, ITA-PSV was significantly associated with TSH (Adj. r = –0.373; p<0.05). Conclusions: our data seem to suggest that CXCL-10 could play an important role in the intra-thyroid angiogenesis modulation, explaining, at least partially, CDU findings typical of thyroid autoimmune diseases. Moreover we confirmed previous reports considering ITA-PSV as the best CDU parameters in the differential diagnosis of thyroid autoimmune disorders.

Key words: Thyroid color doppler ultrasound, Autoimmune thyroid diseases, Chemokines, CXCL-10

GRAVES’ disease (GD) and Hashimoto’s thyroiditis (HT) represent different expression of autoimmune thyroid diseases, the diagnosis of which is based on clinical findings and laboratory data [1]. A diffuse low echogenicity at ultrasound has been reported in autoimmune thyroid disease as results of lymphocytic infiltration and disruption of tissue architecture [2–5]. This echographic pattern may be useful as a diagnostic tool in some cases but in several cases it is similar in GD and HT [6]. Color doppler ultrasound (CDU) has added a new dimension to diagnostic thyroid sonography [7, 8]. Ralls et al. [9] reported for the first time that a diffusely increased thyroid blood flow is pathognomonic of untreated GD. It was later possible to demonstrate that HT can also be present, even in the initial clinical phase, a CDU qualitative pattern similar to that of hyperthyroidism, attributable to the phlogosis, angiogenetic factors, and to high levels of circulating TSH [6, 10, 11]. Given this similarity, the CDU pattern of diffuse hypervascularity could no longer be considered specific to hyperthyroidism. In the last few years the measurement of peak systolic velocity in the inferior thyroid artery (ITA) has been considered to be a promising and useful CDU parameter in the differential diagnosis of diffuse thyroid disease and in follow up of Graves’ disease during medical therapy [7, 12].
The mechanisms responsible for initiating thyroid autoimmunity and promoting the progression of diseases remain partially unknown [13]. It is well known that T-lymphocytes play a central role in the induction of the autoimmune responses as well as in the tissue damage [14, 15]. Two different forms of T helper (Th) cell-mediated-specific immune responses have been described [16]. Th1 cells are involved in the pathogenesis of organ specific autoimmune disorders, while Th2 cells modulated antibody responses by B-cells [16]. It has been suggested that both GD and HT are characterized, at least in the early stage, by a Th1 autoimmune response [17, 18] while patients with GD switch to Th2 responses during the course of disease [19].

Chemokines are a group of peptides of low molecular weight that not only induce the chemotaxis of different leukocyte subtypes but they also play a role in the tumoral growth, angiogenesis and organ sclerosis [17, 20]. At present more than 50 chemokines have been described, which have been classified into four major families [20]. To date only two of these families have been extensively studied, namely, the CC and CXC chemokines. The CXC chemokines inducible by interferon-INF-γ (CXCL9/Mig, CXCL10/IP-10, CXCL11/I-TAC), are associated with Th1-mediated immune responses and interact exclusively with the two alternatively sliced variants of the CXC3 receptor, named CXCR3-A and CXCR3-B [17, 21]. It has been recently demonstrated that serum CXCL10 concentrations are increased in thyroid autoimmune diseases such as GD and HT [17, 19, 22].

The aim of the present study was to determine possible contributions of CXCL10 in the CDU thyroid parameters typical of autoimmune disorders.

**Materials and Methods**

A consecutive series of 25 patients (2 males and 23 females) with thyroid autoimmune disease and 10 normal control subjects were studied. Patients with mental retardation, or coming from foreign countries, as well as patients with concomitant clearly distinguishable thyroid nodule were excluded. All patients provided their informed consent and the study. Characteristics of the sample are summarized in Table 1. The site at which the study was performed was the Endocrinology Unit at the University of Florence. Among 25 patients with autoimmune thyroid disease, 8 were affected by Graves’ disease and 17 by Hashimoto’s thyroiditis. The diagnoses were established on commonly accepted clinical laboratory instrumental parameters [18].

Patients were studied prior to the beginning of any thyroid treatment. All patients underwent a complete physical examination. Blood samples were drawn in the morning, after an overnight fast, for determination of TSH (by electrochemilluminescent method, Modular Roche, Milan, Italy); free-T$_3$ (FT$_3$) and free-T$_4$ (FT$_4$) (by radioimmunometric assay Amersham, Bioscience, Littele Chalfont, UK); auto-antibodies against thyroid peroxidase (TPO-Ab), thyroglobulin (Tg-Ab) and TSH receptor (TR-Ab) (by radioreceptor assay Radim, Italy); and CXCL10 (by quantitative sandwich immunoassay method, R&D System, Minneapolis.

**Table 1.** Physical and biological characteristics of patients with Graves’ disease, Hashimoto thyroiditis and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Hashimoto’s Thyroiditis</th>
<th>Graves’ disease</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>31.3 ± 5.7</td>
<td>37.0 ± 16.1</td>
<td>46.3 ± 20.5</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>25.3 ± 3.9*</td>
<td>22.3 ± 3.6</td>
<td>20.4 ± 1.7</td>
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<tr>
<td>Thyroid volume (ml)</td>
<td>81 [63–105]**</td>
<td>115 [70–140]*</td>
<td>233 [109–538]</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td>1.2[1.03–1.08]**</td>
<td>3.7 [2.06–8.9]***</td>
<td>0.01 [0.01–0.04]</td>
</tr>
<tr>
<td>FT3 (pmol/l)</td>
<td>3.9 [2.8–4.9]***</td>
<td>4.3 [3.9–4.7]***</td>
<td>13.2 [10.9–14.9]</td>
</tr>
<tr>
<td>FT4 (pmol/l)</td>
<td>15 [13.3–17.2]***</td>
<td>14.1 [13.0–15.9]***</td>
<td>43.2 [36.0–51.6]</td>
</tr>
<tr>
<td>Tg-Ab (U/l)</td>
<td>20 [16.2–20]**</td>
<td>49 [21.5–99.0]</td>
<td>98 [44.7–782.5]</td>
</tr>
<tr>
<td>TPO-Ab (U/l)</td>
<td>10 [10–20]**</td>
<td>471 [180.8–877.3]</td>
<td>800 [446.3–2500]</td>
</tr>
<tr>
<td>TR-Ab (U/l)</td>
<td>NA</td>
<td>NA</td>
<td>8.4 [4.6–32.3]</td>
</tr>
<tr>
<td>CXCL10 (pg/ml)</td>
<td>60 [46.8–73.3]***</td>
<td>74 [43.7–123]**</td>
<td>233 [179.8–316.3]</td>
</tr>
</tbody>
</table>

BMI = body mass index. Data are expressed as mean ± S.D when normally distributed, median [quartiles] when non-normally distributed. NA = not available.

*p<0.05; **p<0.005; ***p<0.0001 vs Graves’ disease

*p<0.005; **p<0.0001 vs Hashimoto thyroiditis
USA). All patients underwent a thyroid color doppler ultrasound (CDU) examination by the same investigator, who was unaware of the laboratory values at the time of the examination. The examination was performed with Corevision SSA-350A (Toshiba Medical Systems-Toshiba Corporation) using a linear multifrequency Probe (8 MHz, central frequency: range 8–10 MHz) provided by the manufacturer; the same Probe was used for color doppler and pulsed doppler, using 5.2 MHz frequency.

The thyroid gland was first examined in gray scale, its size measured and the volume calculated by the ellipsoid model \((\text{width} \times \text{length} \times \text{thickness} \times 0.52)\) for each lobe as previously described [23]. In each case color doppler evaluations were performed using the same standard pre-set: PRF: 5 KHz with color gain adjusted to maximum level to allow minimum noise. The vascular density in the thyroid gland was graded as has been described by others [6]. Pattern 0: blood flow limited to the peripheral thyroid arteries, while parenchymal flow was absent. Pattern I: presence of mildly increased parenchymal blood flow. Pattern II: clearly increased color flow with diffuse homogenous distribution. Pattern III: markedly increased color flow with diffuse homogenous distribution including the so-called thyroid inferno. The flow velocity (PSV) determinations were performed at the level of inferior thyroid arteria (ITA) as previously described [7,12].

Data were expressed as mean ± SD when normally distributed, and as median [quartiles] for parameters with non-normal distribution, unless otherwise specified. Differences among more than two groups were assessed by a Kruskal-Wallis test. Unpaired two-sided Student’s \(t\) tests were used for comparison of means of normally distributed parameters; when distribution could be normalized through logarithmic transformation, the same test was applied to logarithmically transformed data. In all other cases, Mann-Whitney \(U\) test was used for comparisons between groups. Correlations were assessed using Spearman’s or Pearson’s method whenever appropriate. Stepwise multiple linear regression was applied for multivariate analysis, categorizing yes/no parameters as dummy 0/1 variables, whenever appropriate.

All statistical analysis was performed on SPSS for Windows 12.0.

Results

No difference considering age was observed between Graves’ (GD), Hashimoto (HT), and control (CG) groups (Table 1). Patients with GD showed a significantly \((p<0.05)\) lower BMI and higher thyroid volume when compared to the rest of the sample while no differences were observed between HT and CG although thyroid volume tends to be higher in HT without raising significance \((p = 0.059; \text{Table 1})\). No correlation between thyroid volume and BMI was observed (data not shown).

Patients with autoimmune thyroid diseases showed an increased thyroid vascularization, as assessed by CDU, when compared to the control group (see, Fig. 1). Among thyroid disease groups a significantly \((p<0.005)\) higher thyroid color flow pattern was observed in GD-patients when compared to HT-patients (Fig. 1). A significant \((r = 0.494; p<0.005)\) correlation between thyroid color flow pattern and thyroid volume was observed. In particular, patients with type II or III thyroid color flow pattern showed a higher thyroid volume when compared to the rest of the sample (173 [87–293] vs. 86 [65.5–122] ml; \(p<0.005\)). Patients with type II or III thyroid vascular pattern showed a significantly \((p<0.005)\) lower logarithmically transformed TSH levels and higher logarithmically transformed FT3, FT4, Tg-Ab and TPO-Ab values when compared to the rest of the sample (data not shown). A strong correlation between thyroid vascular pattern and PSV of ITA (inferior thyroid arteria) was observed \((r = 0.637; p<0.0001)\). In particular pattern II and III were characterized by higher PSV when compared to pattern 0 and I (67.1 [35.5–80.1] vs. 18 [16–26] cm/sec; \(p<0.0001\)). Patient with Graves’ disease showed a higher PSV when compared to HT and CG (76.5 [68.5–83.8] cm/sec, 25 [16.5–29.5] cm/sec and 17 [16–29.5] cm/sec for GD, HT and CG respectively; \(p<0.0001\) for GD-patients vs. the rest of the sample). ITA-PSV was significantly \((p<0.05)\) correlated to thyroid hormone levels \((r = −0.520; 0.578; 0.549 \text{ for logarithmically transformed TSH, FT3, FT4 respectively})\), and thyroid autoimmunity markers \((r = 0.378 \text{ and } r = 0.357 \text{ for logarithmically transformed Tg-Ab and TPO-Ab respectively})\). Among GD-patients no significant correlation was observed between TR-Ab and PSV (data not shown).

CXCL10 levels were significantly higher in patients with autoimmune diseases when compared to control group (107.7 [55.5–204.3] vs. 60.1 [46.8–73.3] pg/ml
respectively; p<0.05). CXCL10 levels were significantly correlated to thyroid hormone levels (r = –0.348; 0.779; 0.683 for logarithmically transformed TSH, FT3 and FT4 respectively; all p<0.001) and thyroid autoimmunity markers (r= 0.399; and 0.484 for logarithmically transformed Tg-Ab and TPO-Ab respectively; all p<0.005). Among GD-patients CXCL10 levels were not correlated to logarithmically transformed TR-Ab (data not shown). Higher CXCL10 levels were observed in patients with an increased thyroid vascularization (p<0.001 among thyroid color flow pattern groups at Kruskall Wallis). In particular, patients with pattern II and III showed significantly higher CXCL10 levels when compared to the rest of the sample (191 [46.9–263.3] vs 69.8 [55.3–93.5] pg/ml; p<0.05). Moreover, CXCL10 levels were significantly (p<0.05) correlated with ITA-PSV (r = 0.376) and with logarithmically transformed thyroid volume (see also Fig. 2). The correlation between CXCL10 levels and thyroid volume retained significance (Adj r = 0.571; p<0.0001) even after correction for thyroid vascular pattern and presence of Graves’ disease. Separate stepwise linear regression analysis was performed in order to define the relative contribution of different parameters to thyroid PSV and vascular pattern. Considering thyroid hormones as putative factors, logarithmically transformed PSV was significantly associated with logarithmically transformed TSH (Adj r = –0.371; p<0.05) while no significant association was observed between thyroid hormone parameters and thyroid vascular pattern after correction for type of thyroid disease (data not shown). When thyroid autoimmunity markers were considered, PSV was significantly correlated to logarithmically transformed CXCL10 (B = 0.32 ± 0.12; p<0.05) but not to logarithmically transformed Tg-Ab and TPO-Ab. A higher vascular pattern was significantly correlated to CXCL10 (Adj r = 0.73; p<0.001) even after correction for all thyroid autoimmunity markers. Finally, among thyroid echografic parameters PSV was significantly correlated to thyroid logarithmically transformed volume (B = 0.503 ± 0.15; p<0.001) but not to thyroid vascular pattern.

Discussion

This is the first study which systematically analyzed the relationship between CXCL10 clinical biochemical and CDU thyroid vascular parameters in patients with autoimmune thyroid diseases. CXCL10 is a INF-γ inducible chemokine involved in the Th1 autoimmune responses. In autoimmune thyroid diseases CXCL10 are involved in the recruitment of Th1 lymphocytes which secrete INF-γ that in turn stimulates chemokine production by follicular cells, maintaining the autoimmune process [17, 24]. We report higher CXCL10 levels in patients with autoimmune diseases confirming our previous studies [19, 22]. Our data showed that CXCL10 levels were significantly higher in patients with higher vascular pattern. It has been previously demonstrated that angiogenetic factors such as basic fi-
broblast growth factor, placenta growth factor, and vascular endothelial growth factor (VEGF) are produced by thyroid epithelial cells [25]. Until recently, Itaka et al. [25] reported higher VEGF levels in patients with untreated TH and GD and a direct correlation between VEGF levels and intra-thyroidal vascular area suggesting an important role of VEGF in the intra-thyroid angiogenesis. Very interestingly, Boulday et al. [26] recently demonstrated a VEGF-induced augmentation of CXCL-10 in endothelial cells. It could be speculated that increases of VEGF could contribute to the increase of CXCL-10 in patients with thyroid autoimmune diseases explaining, at least in part, the correlation found between CDU vascular patterns and CXCL-10 levels. Moreover, the association between thyroid volume and CXCL-10 levels could reflect the degree of autoimmune reaction and thyroid infiltration by inflammatory cells. In fact, a higher volume of the gland means an higher number of infiltrating cells which are a big source of CXCL-10 [19]. Accordingly, our data showed higher CXCL-10 levels and a higher thyroid volume among subjects with GD when compared with HT subjects. Although a correlation between CXCL-10 and hyperthyroidism as well as severity of hypothyroidism has been reported previous studies demonstrated that the increase of CXCL-10 serum levels in thyroid autoimmune diseases are not the result of the thyroid function per se (see ref. n.27 for review). In fact, no significant changes in CXCL-10 levels were observed after correction of hypothyroidism with levo-tiroxine [28]. Furthermore, Diez et al. [29] demonstrated that Graves’ patients, stratified in relation to circulating thyroid hormone concentrations, showed similar serum levels of CXCL-10. The association between CXCL-10 and thyroid autoimmune diseases are related to the autoimmune process itself and the degree of plasma levels reflects the intensity of autoimmune reaction.

Our data also confirms previous reports [7] showing independence of thyroid vascular CDU pattern from thyroid hormonal status, in fact no correlation between CDU vascular pattern and thyroid hormone parameters was observed after adjustment for confounding factors.

Peak systolic velocity in the inferior thyroid artery has been considered the most useful CDU thyroid parameter in the differential diagnosis of thyroid autoimmune diseases [7]. Our results confirm these hypotheses. Higher ITA-PSV levels were observed only in GD when compared to the rest of the sample and they were inversely correlated to logarithmically transformed TSH even after adjustment for confounding factors.

In conclusion, this is the first study which systematically analyzed the contribution of CXCL10 chemokine in the analysis of thyroid vascular parameters in autoimmune thyroid disorders. Our data seems to suggest that CXCL10 has an important role in the intra-thyroid angiogenesis modulation explaining, at least in part, CDU findings typical of thyroid autoimmune diseases. Moreover we confirm previous reports considering ITA-PSV as the best CDU parameters in the differential diagnosis of thyroid autoimmune disorders.

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