Serum hepatocyte growth factor is increased in Hashimoto’s thyroiditis whether or not it is associated with nodular goiter as compared with healthy non-goitrous individuals

R.M. Ruggeri1, S. Sciacchitano2,3, A. Vitale2, P. Cardelli2, M. Galletti1, E. Vitarelli4, G. Barresi4, S. Benvenga1, F. Trimarchi1, and M. Trovato4

1Sezione di Endocrinologia, Dipartimento Clinico-Sperimentale di Medicina e Farmacologia, Università di Messina, Messina, Italy; 2S. Pietro Ospedale Fatebenefratelli- Associazione Fatebenefratelli per la Ricerca (FAF), Rome, Italy; 3Dipartimento di Medicina Sperimentale e Patologia, II Facoltà di Medicina, Università “La Sapienza”, Rome; 4Dipartimento di Patologia Umana, Università di Messina, Messina, Italy

ABSTRACT. Background: Some growth factors and cytokines are known to cooperate with TSH in thyroid nodular growth, but few data are available on their circulating levels in Hashimoto’s thyroiditis (HT). Aim: To evaluate in HT patients whether thyroid nodules are associated with variations in serum levels of hepatocyte growth factor (HGF) and interleukin-6 (IL-6). Subjects and methods: Serum levels of HGF and IL-6 were measured by enzyme-linked immunosorbent assay in 176 euthyroid subjects, subdivided into 4 groups: A) HT patients with nodular goiter (no. = 42); B) non-goitrous HT patients (no. = 36); C) non-HT patients with nodular goiter (no. = 48), and D) healthy subjects without thyroid disease (no. = 50). Results: The highest concentrations of serum HGF were found in patients with nodular goiter, irrespective of the presence of associated HT (groups A and C). Nevertheless, in group A serum HGF levels were significantly higher than in group C (860.8±333.6 pg/ml vs 691.5±156 pg/ml, p<0.01). Moreover, though serum HGF levels in group B (578.3±217 pg/ml) were lower than in group A, they were significantly higher than in healthy controls (group D, 512.7±170.4 pg/ml, p<0.001). Serum IL-6 levels were similar in the two HT groups (A and B), and increased with respect to groups C and D. Conclusions: Serum HGF is increased in HT, especially associated to thyroid nodules, as compared with healthy non-goitrous individuals.

INTRODUCTION
Hashimoto’s thyroiditis (HT) is the worldwide most prevalent autoimmune thyroid disease (AITD) and is characterized by variable behavior with respect to thyroid cell proliferation besides clinical presentation (1). In HT it is possible to observe true nodular lesions, along with the frequent “pseudo-nodular” areas (1, 2). These nodules may show the cytological features of follicular proliferation or, more frequently, colloid or hyperplastic nodule features. The association with malignant transformation has been reported (3, 4). However, HT could not be considered a risk factor for thyroid malignancy, due to there not being univocal evidence (5, 6). The proportion of HT patients harboring both uninodular or multinodular goiter remains relevant in moderately iodine-deficient areas (1).

It is well known that the main physiological stimulator of follicular growth is TSH, but the list of the growth factors and cytokines that can favor the development of goiter is wide (7-9). Nevertheless, only few studies have focused on the role of different growth factors in HT associated with nodular goiter.

Recently, we reported the immunohistochemical expression of the hepatocyte growth factor (HGF) in HT-associated nodular goiter specimens and demonstrated that it was more frequent and intense than that observed in non-HT goiter specimens (10). It is well known that HGF, initially recognized as a potent mitogen for hepatocytes, exerts mitogenic and anti-apoptotic activities in various cell types, including follicular thyroid cells (11, 12) and previous data demonstrated that HGF is expressed in colloid and hyperplastic nodules (but not in normal thyroid tissue) and is over-expressed in papillary thyroid carcinomas (PTC) (13-16). To the best of our knowledge, no data is currently available regarding the serum concentration of HGF in HT patients, the only available data existing for thyroid tumors (17).

Interleukin 6 (IL-6) represents another possible candidate in inducing nodular growth in HT. This pleiotropic cytokine, mainly synthesized from Th2 cells, regulates the cell growth and differentiation (18). IL-6 expression has been reported in normal thyroid epithelial cells, as well as in colloid nodules, follicular adenomas, PTC, and also in AITD (19-23). Moreover, we have previously demonstrated that IL-6 expression by follicular thyroid cells in the context of AITD correlates directly with the extent of lymphoid infiltration (23). Nevertheless, the serum IL-6 levels in HT patients have been measured only in few studies (24).

The aim of this study was to evaluate in a large number of euthyroid HT patients whether serum levels of HGF and/or IL-6 are different in the presence or the absence of thyroid nodules associated with the autoimmune disorder, as compared with non-HT patients with nodular goiter and healthy subjects without evidence of nodular goiter.
MATERIALS AND METHODS

Patients and control subjects

We enrolled 176 consecutive patients with overt or suspected thyroid disease referred to our outpatient clinic. All patients met the following criteria: 1) euthyroidism at the time of sampling; 2) no levothyroxine (L-T4) therapy or drugs affecting thyroid function. Exclusion criteria were: body mass index (BMI) > 35, diabetes mellitus, metabolic syndrome, coronary artery disease or peripheral arterial occlusive disease, neoplastic or chronic non-thyroidal inflammatory disorders (including autoimmune diseases).

Each subject received a careful medical evaluation, including recording of past medical history, and physical examination. Particularly, several different parameters related to the metabolic syndrome (fasting glucose, total cholesterol, triglycerides, blood pressure) and liver enzymes were analyzed, as well as smoking habits.

In all patients, we routinely performed thyroid ultrasonography (US) and measured serum free T4 (FT4), free T3 (FT3), TSH, thyroglobulin antibody (TgAb) and thyroid peroxidase antibody (TPOAb) levels. All patients with US evidence of thyroid nodules underwent US-guided fine needle aspiration biopsy (FNAB) of one or more selected nodules, and only patients with cytological features of colloid goiter were included in the study. The few subjects with follicular proliferation or indeterminate cytological features were excluded. Serum aliquots were collected from each subject and kept frozen at −20 C for HGF and IL-6 assays.

HT was diagnosed based on the currently accepted US criteria (heterogeneous echo-structure with diffuse or patchy hypoechogenicity) and the presence of thyroid autoantibodies.

On the basis of the clinical, US, and laboratory evidences, the patients were subdivided into 4 study groups. Group A (or HT patients with nodular goiter) included 42 HT patients with thyroid nodules (2 men and 40 women; age range: 18-80 yr, mean±SD: 45.8±13.5), group B (or non-goitrous HT patients) consisted of 36 HT patients without thyroid nodules (1 man and 35 women, age range: 13-69, mean±SD: 38.8±12.2), while group C (or non-HT patients with nodular goiter) consisted of 48 patients affected by nodular goiter without any evidence of HT (7 men and 41 women, age range: 19-71, mean±SD: 44.6±14.9). Finally, group D included 50 healthy control subjects (10 men and 40 women, age range 21-71, mean±SD: 40±11.9), without clinical, US, and biochemical evidence for thyroid disorders. The characteristics of the 4 groups are illustrated in Table 1. As shown in Table 1, the 4 study groups did not differ as to BMI, fasting glucose, cholesterol, and triglycerides, blood pressure and smoking habits (p>0.05).

Methods

Serum levels of HGF were measured by competitive enzyme-linked immunosorbent assay (ELISA) calibrated against a highly purified, recombinant human pro-HGF, according to the manufacturer’s instructions (R & D System, Minneapolis, USA). The limit of detection of the ELISA was 40 pg/ml. Serum concentrations of IL-6 were assayed by ELISA (Eurogenetics U.K. Ltd, Middlesex, UK), using recombinant human cytokine as standard. The lower limit of detection for IL-6 was 0.5 ng/ml. The assay was designed to measure the total amount of free and bound cytokine in serum. The between-batch coefficients of variation (CV) of the assays were <13%.

Table 1 - Clinical, biochemical, and sonographic characteristics of the 4 groups of patients.

<table>
<thead>
<tr>
<th></th>
<th>Group A (HT patients with nodular goiter)</th>
<th>Group B (Non-goitrous HT patients)</th>
<th>Group C (Non-HT patients with nodular goiter)</th>
<th>Group D (Healthy controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of patients</td>
<td>42</td>
<td>36</td>
<td>48</td>
<td>50</td>
</tr>
<tr>
<td>Sex</td>
<td>Female 2 Male 40</td>
<td>Male 1 Female 35</td>
<td>Male 7 Female 41</td>
<td>Male 10 Female 40</td>
</tr>
<tr>
<td>Age</td>
<td>mean±SD 45.8±13.5 (range) (18-80)</td>
<td>38.8±12.2 (range) (13-69)</td>
<td>44.6±14.9 (range) (19-71)</td>
<td>40±11.9 (range) (21-71)</td>
</tr>
<tr>
<td>BMIa (kg/m²)</td>
<td>27.7±4</td>
<td>26.5 (13-69)</td>
<td>27.4±7 (19-71)</td>
<td>27±5 (21-71)</td>
</tr>
<tr>
<td>Total cholesterola (mg/dl)</td>
<td>198±47</td>
<td>196±39</td>
<td>203±50</td>
<td>194±37</td>
</tr>
<tr>
<td>Triglyceridesa (mg/dl)</td>
<td>95±53</td>
<td>94±44</td>
<td>102±63</td>
<td>91±60</td>
</tr>
<tr>
<td>Blood pressurea (mmHg)</td>
<td>126±16</td>
<td>120±15</td>
<td>129±16</td>
<td>126±14</td>
</tr>
<tr>
<td>TSH (mIU/l)a</td>
<td>1.35±0.89</td>
<td>1.98±1.08</td>
<td>1.06±0.85</td>
<td>1.61±0.85</td>
</tr>
<tr>
<td>FT4 (pg/ml)a</td>
<td>3.29±0.63</td>
<td>3.54±2.51</td>
<td>3.33±0.54</td>
<td>2.95±0.50</td>
</tr>
<tr>
<td>FT3 (pmol/l)a</td>
<td>16.8±3.24</td>
<td>14.62±4.24</td>
<td>18.56±2.89</td>
<td>18.84±2.65</td>
</tr>
<tr>
<td>Tg-Ab (IU/l)b</td>
<td>367 (120-1693)</td>
<td>418 (114-1347)</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>TPO-Ab (IU/l)b</td>
<td>772 (44-6400)</td>
<td>749 (87-9329)</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Thyroid volume (ml)a</td>
<td>19.41±10.5</td>
<td>9.17±3.52</td>
<td>20.87±4.1</td>
<td>10.9±3.4</td>
</tr>
</tbody>
</table>

*Data are mean±SD, except TgAb and TPO Ab which are median and, in parenthesis, range Normal values are specified under Material and Methods.

1) Tg-Ab were positive in 25/42 patients of group A and 24/36 patients of group B. TPO-Ab were positive in 30/42 patients of group A and 29/36 patients of group B. c 26 patients were receiving drugs for arterial hypertension: 5 in group A; 7 in group B; 10 in group C; 4 in group D. #5 patients had hypercholesterolaemia and were being treated with hydroxymethylglutaryl coenzyme A reductase inhibitors: 1 in group A; 1 in group B; 2 in group B; 1 in group D.

BMI: body mass index; TgAb: thyroglobulin antibody; TPO Ab: thyroid peroxidase antibody.
Serum FT₄, FT₃, and TSH concentrations were measured by electrochemiluminescent assay (commercial kits by Boehringer-Mannheim, Germany; normal values in our laboratory: 10.3-24.6 pmol/l and 21.8-4.2 pg/ml for FT₄ and FT₃, respectively; 0.4-4.0 mIU/l for TSH). Serum TPOAb and TgAb were determined using an immunoradiometric method (kit by CIS, Gif-sur-Yvette, France; normal values: <10 U/ml for TPOAb and <100 U/ml for TgAb).

A real-time 2D apparatus (Esaote SpA, Italy) with a 7.5 MHz linear transducer was used to perform thyroid US. The volume of thyroid lobes by 2D was calculated according to the ellipsoid formula (π/6 × height × width × depth).

Statistical analysis
Data are expressed as means±SD. The normal distribution, variance and two-tailed Student t test with Yates correction for continuity have been tested by the Primer statistical program. The association between two variables was analyzed by the Pearson’s correlation coefficient of Microsoft Excel program. The level of statistical significance was set at p<0.05.

RESULTS
Serum levels of HGF
Average values of serum HGF in the four groups of patients are shown in Figure 1. We found serum HGF levels significantly higher in both groups of patients with nodular goiter, irrespective of the associated HT (group A: 860.8±333.6 pg/ml; group C, 691.5±156 pg/ml), in comparison with the healthy population (group D, 512.7±170.4 pg/ml, p<0.001). However, serum HGF values in HT patients with nodular goiter (group A) were slightly but significantly higher than in non-HT patients with nodular goiter (group C, p<0.01). Mean values of serum HGF in non-goitrous HT patients (group B, 578.3±217 pg/ml) were lower than those measured in both group A and C patients (p<0.001). Nevertheless, they were significantly higher than those measured in healthy controls without thyroid disease (group D, p<0.001).

Serum levels of IL-6
Serum IL-6 levels were not different in the two HT groups (group A: 1.33±1.3 ng/l; group B: 0.98±1.13 ng/l, p=0.21). Both groups of HT patients showed significantly higher levels of serum IL-6 as compared to non-HT patients with nodular goiter (group C: 0.43±0.4 ng/l, p<0.001) and to healthy controls (group D: 0.30±0.18 ng/l, p<0.001).

No correlation was found between the serum levels of HGF and IL-6 in any group and even considering the 4 groups as a whole.

DISCUSSION
The biological mechanism underlying goiter nodule growth is still not completely understood. It is well established that many thyroid nodules are clonal in origin, i.e. true benign tumors, and that both clonal and polyclonal nodules may coexist in the same multinodular goiter (25, 26). The main physiological factor regulating thyroid cells proliferation is the TSH, but strong evidence exist that other growth factors may contribute to nodule formation, by acting in cooperation with TSH (7-9, 27).

The list of growth factors involved in favoring the development of nodular goiter is wide, including, among others, the IGF-I and its receptor, the basic fibroblast growth factor, members of the transforming growth factor β family, as well as HGF (7-9, 13-15, 27-31).

Several studies have described the production/ expression of different growth factors in benign and malignant thyroid nodules based on their direct detection in the thyroid tissue, using immunohistochemistry, in situ hybridization or PCR (10, 13-15, 27-31). Conversely, few data are available on the concentrations of these growth factors in the serum of patients affected by nodular goiter (17). The only data are from Vessely and co-workers, who measured the serum concentrations of several growth factors including HGF in a not large number of patients with thyroid nodules (14 patients with adenomas and 14 patients with PTC) and compared these con-
centrations with those in healthy people (only 8 subjects, not completely characterized). They found significantly higher serum levels of HGF in patients with thyroid adenomas and PTC as compared to healthy subjects. Moreover, serum HGF concentrations in PTC patients were higher (but not significantly) than in patients with thyroid adenomas (17). Finally, few studies have focused on the tissue expression and/or serum concentrations of growth factors and cytokines in those conditions in which nodular lesions are associated to HT (10).

In the present study, we measured the serum levels of HGF and IL-6, known to be involved in thyroid cell proliferation, in both nodular goitrous and non-goitrous HT patients and compared these values with those measured in non-HT patients with nodular goiter as well as in healthy subjects without thyroid disease.

We detected the highest serum levels of HGF in patients with nodular goiter, irrespective of the presence of associated HT. In fact, serum concentrations of HGF in both HT (group A) and non-HT (group C) patients with nodular goiter were significantly higher when compared to the healthy subjects (p<0.001). Moreover, serum HGF in HT patients with nodular goiter was significantly higher than in patients with non-HT associated nodular goiter (p<0.01). These observations are in agreement with the evidence and suggestions of our previous immunohistochemical studies. We reported therefore that HGF was expressed in specimens of nodular goiter, whereas it proved undetectable in normal thyroid tissue (9, 10). In addition, we have recently reported a more frequent and more intense immunohistochemical expression of HGF in HT patients with nodular goiter as compared to patients with non-HT associated nodular goiter (6).

Taken together, these evidences support the hypothesis of HGF involvement in the development of thyroid nodules, indicating that HGF is not only expressed in thyroid tissue, but it is also elevated in the serum of patients harboring nodules. Our data on nodular goiter associated with HT would suggest a possible (but not demonstrated here) role of HGF in inducing nodular growth in the context of HT.

Because of the archival nature of our previous immunohistochemical studies, we could not relate HGF immunostaining with the circulating levels of the growth factor. On the other hand, due to the benign nature of the nodular lesions in all the patients investigated in this study (all colloid nodules at cytology), none of them underwent surgery. Consequently, we could not evaluate HGF tissue expression on surgical specimens in any of the patients in which serum HGF had been measured. Even though it is known that the production of HGF, as well as of the other growth factors and cytokines, is ubiquitous in the organism and cannot be attributed to a single organ, its increased serum concentration should be considered. Significantly increased serum HGF levels in patients with thyroid nodules and HT, as compared to healthy subjects, would suggest a thyroid contribution to the finding, in agreement with other previous studies describing HGF production directly in the thyroid gland (13-15).

Finally, all HT patients showed serum IL-6 levels significantly higher than those of non-HT patients with nodular goiter or healthy controls, but no differences were found between nodular goitrous and non-goitrous HT patients. IL-6, therefore, could play some role in the development of HT, rather than in the nodular growth.

REFERENCES

Serum hepatocyte growth factor in Hashimoto’s thyroiditis


