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Serum osteoprotegerin and soluble receptor activator of nuclear factor κB ligand levels in patients with a history of differentiated thyroid carcinoma: a case-controlled cohort study

Massimo Giusti[a,*], Francesca Cecoli[a], Laura Fazzuoli[a], Vincenzina De Franchis[b], Enrica Ceresola[a], Diego Ferone[a], Michele Mussap[b], Francesco Minuto[a]

[a]*UO Clinica Endocrinologica, Azienda Ospedaliera Universitaria "San Martino," I-16000 Genoa, Italy*
[b]*UO Laboratorio Analisi, Azienda Ospedaliera Universitaria "San Martino," I-16000 Genoa, Italy*

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Abstract

Overt hyperthyroidism is associated with changes in bone metabolism, whereas the effect of levothyroxine (L-T4) load in patients with differentiated thyroid carcinoma (DTC) is controversial. The aim of our study was to evaluate osteoprotegerin (OPG) and soluble receptor activator of nuclear factor κB ligand (RANK-L) in patients with DTC with suppressed endogenous thyrotropin due to L-T4 regimen. A cohort of 80 subjects with DTC (68 women and 12 men; age range, 27-81 years) was studied. A cohort of 55 subjects with a history of partial or total surgery for nonmalignant thyroid pathology served as a control group. Groups were matched for sex, age, and body mass index. Per-week dosage of L-T4 was significantly higher in patients with DTC than in controls ($P < .001$). More elevated free T_4 concentrations ($P < .001$) and more suppressed thyrotropin and thyroglobulin levels ($P < .001$) were found in subjects with DTC than in controls. No difference in serum or urinary parameters related to bone metabolism or dual-energy x-ray absorptiometry was noted between the groups. Overall, OPG levels were similar in both groups but were significantly ($P = .03$) lower in postmenopausal women with DTC than in postmenopausal control women. Only control women showed lower OPG levels in premenopausal than in postmenopausal ($P = .002$) conditions. Overall, RANK-L levels were significantly higher ($P = .03$) in subjects with DTC than in controls. In both groups, OPG and RANK-L levels were unrelated to each other. A significant positive correlation was seen between OPG levels and age in both subjects with DTC ($P < .001$) and controls ($P < .001$). Serum RANK-L correlated negatively with age in subjects with DTC ($P = .05$). Although there were several differences in L-T4 dosages, OPG and RANK-L levels were similar in patients with a history of DTC and those with a history of nonmalignant thyroid diseases. The correlation between circulating OPG and RANK-L levels was not significant. The increase in OPG with age indicates its protective role in bone loss. The cause of bone loss after long-term L-T4 load will be more extensively studied.

1. Introduction

New interest in bone biology has been aroused by the discovery of osteoprotegerin (OPG) [1,2] and the receptor activator of nuclear factor κB (RANK)/RANK ligand (RANK-L) system [3,4], which are thought to explain the mechanisms of crosstalk between osteoblasts and osteoclasts. OPG is generally considered to be a soluble receptor secreted by several types of cells, including osteoblasts [1,5]. Thyroid follicular cells also seem to produce OPG, and this synthesis may be modulated by thyroid autoimmunity [6]. OPG works as a decoy receptor for RANK-L and is thus an inhibitor of osteoclastogenesis [7]. In addition, OPG neutralizes the apoptosis-inducing factor tumor necrosis factor (TNF)-related apoptosis-inducing ligand [8]. Experimental studies in mice have revealed that OPG knockout animals develop osteoporosis, whereas OPG gene overexpression or OPG treatment in healthy animals leads to osteopetrosis [1,2,9]. Many cell types express RANK-L, including osteoblasts [10]. The cell-bound form of RANK-L is the most common, although there is also a soluble form created by the cleavage of a truncated ectodomain by a protease and primary secretion from cells [4,7]. By binding to RANK, RANK-L promotes osteoclast differentiation, activation, survival, and adherence to bone surface [11,12]. Several experimental data have shown the role of the

* Corresponding author. Tel.: +39 010 353 7953; fax +39 010 353 8977.
E-mail address: magius@unige.it (M. Giusti).

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RANK/RANK-L system in favoring osteoclastogenesis in vitro [13,14] and in inducing osteoporosis and hypercalcemia in vivo [4]. Moreover, RANK-L knockout mice have shown a pathologic increase in bone mass and impaired tooth eruption because of a lack of mature osteoclasts [15].

In humans, recent studies have indicated that OPG can be considered a marker of cardiovascular risk [16] and that the RANK/RANK-L system and OPG are involved in several diseases, such as osteoporosis (both in the elderly and glucocorticoid-induced [17]), bone metastasis [18], and rheumatoid arthritis [19]. On the other hand, a pilot study in which OPG was administered to postmenopausal women showed that it might have a therapeutic role in reducing bone loss [20]. Overt hyperthyroidism is another condition in which bone loss is observed [21]. Amato et al [22] reported an increase in serum OPG levels in patients with hyperthyroidism, which was seen to reverse as bone metabolism markers normalized on treating the disease, even in the presence of persistent bone damage. An increase in OPG has also been found in subclinical and overt hypothyroidism before substitutive therapy, probably as a consequence of vascular damage [23,24].

The effect of subclinical hyperthyroidism on bone mineral density (BMD) is controversial [25-27], but could be significant in patients with differentiated thyroid carcinoma (DTC) who receive suppressive long-term doses of levothyroxine (L-T4) after total thyroidectomy [28,29]. Some authors [30] have shown that L-T4 suppressive therapy for at least 1 year in premenopausal women with DTC causes a reduction in BMD of the femoral neck, femoral trochanter, and Ward's triangle, whereas others [31,32] were unable to exclude the possibility that long-term suppressive doses of L-T4 do not decrease BMD. On the other hand, a clinical study in a cohort of patients with DTC did not observe an increased risk of developing low bone mass or record a higher prevalence of vertebral fractures, at least when the patients were treated with relatively low doses of L-T4 [33]. More recently, Reverter et al [26] reported that the proportion of premenopausal or postmenopausal women with DTC with normal BMD, osteopenia, and osteoporosis was similar to that found in healthy control women matched for body mass index (BMI) and menopausal status. Indeed, this study seems to indicate that L-T4 treatment does not affect skeletal integrity in women with DTC [26].

Owing to technical problems in the assay, data on soluble RANK-L levels in human subjects are very scant [10]. To our knowledge, no data are available on RANK-L in patients who have undergone thyroid surgery for malignant or benign disease.

Our study was designed to evaluate several parameters of bone metabolism in a cohort of patients with DTC, its main focus being on OPG and RANK-L levels as markers of bone activity in this clinical condition. As yet, no "normal" values of OPG and RANK-L have been established [10]; in this study, we used a cohort of matched subjects with a history of partial or total surgery for nonmalignant thyroid pathology as a control group. In both groups of subjects our aim was to correlate OPG and RANK-L levels with several clinical and biochemical parameters.

2. Materials and methods

2.1. Subjects

The study was conducted on a cohort of 80 outpatients (age, 55 ± 13 years [mean ± SD]; range, 27-81 years) during follow-up for DTC diagnosed less than 1 to 23 years earlier. Sixty-eight were women, aged on average 56 ± 13 years (range, 27-81 years), and 12 were men (age, 55 ± 12 years; range, 32-79 years). Histology revealed papillary cancer, follicular variant of papillary cancer, follicular cancer, insular cancer, and medullary carcinoma in 59, 11, 5, 4, and 1 subjects, respectively. Total thyroid ablation by near-total thyroidectomy and subsequent radiometabolic therapy was our standard treatment. Only 5 patients did not undergo radioiodine therapy, for the following reasons: subtotal thyroidectomy (n = 1), no indication (n = 3), and refusal (n = 1). All subjects were on an L-T4 regimen at the time of examination. Only one patient was on combined L-T4/liothyronine therapy. Women with DTC were subdivided, according to pituitary-gonadal function, into premenopausal (n = 17) and postmenopausal (n = 45) women. A cohort of 55 outpatients (age, 59 ± 15 years; range, 32-85 years) under follow-up for benign thyroid diseases diagnosed from less than 1 to 51 years earlier, which had been treated by thyroid surgery, from nodulectomy to near-total thyroidectomy, and under L-T4 regimen, served as a control group. Fifty-one control subjects were women (age, 59 ± 16 years; range, 32-85 years; n = 16, premenopausal; n = 35, postmenopausal) and 4 were men (age, 58 ± 11 years; range, 39-73 years).

There was no significant difference in the number of subjects on osteoporosis medications (bisphosphonate, oral calcium, and vitamin D supplementation) between the 2 groups. A history of hyperthyroidism before thyroidectomy was found in 6% and 37% of subjects with DTC and controls, respectively. A history of primary hyperparathyroidism was concomitant in 8% of patients with DTC and in 2% of controls.

Written informed consent was obtained from all patients.

2.2. Protocol

Clinical examination comprised pharmacological history, neck palpation and ultrasonography, and BMI evaluation. Biochemical evaluation, which was performed in the fasting condition in the morning, after 12-hour abstinence from smoking, comprised serum OPG, RANK-L, free thyroid hormones, thyrotropin (TSH), thyroglobulin (Tg), parathyroid hormone (PTH), osteocalcin, total calcium, phosphorus, creatinine, and alkaline phosphatase (ALP) measurements. Anti-Tg autoantibody was evaluated to identify sera in which

a Tg recovery test was necessary to obtain reliable Tg values. Two samples from 24-hour acidified urine collection and morning diluted urine spot collection were obtained to determine 24-hour urine calcium and calcium/creatinine, hydroxyproline (HP)/creatinine, pyridinoline (PD)/creatinine and deoxypyridinoline (DPD)/creatinine ratios. Serum and urine were frozen at −70°C before assays, which were performed within 6 months of collection.

2.3. Assays

Serum OPG and RANK-L were assayed by means of a commercial sandwich enzyme-linked immunosorbent assay method (Biomedica Medizinprodukte, Vienna, Austria). The manufacturer reports a median value for OPG of 1.8 pmol/L observed in 1134 subjects aged 19 to 96 years. For soluble RANK-L, median values of 0.37 and 0.46 pmol/L are reported in 635 female and 395 male adult subjects, respectively. In our laboratory the detection limits were 0.1 and 0.08 pmol/L for OPG and RANK-L. Intra- and interassay variations expressed as coefficient of variation (CV) were 5% and 7% for OPG and 3% and 6% for RANK-L. Serum Tg was assayed up to February 2006 by immunoradiometric assay (IRMA) (Medipan Diagnostic, Selchow, Germany) and then by chemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). Both assays were standardized against the certified reference for human Tg (CRM 457) of the Community Bureau of References of the European Commission. The lower detection limits of the 2 methods are 0.3 and 0.1 μg/L, and functional sensitivity is 0.5 μg/L or less for both methods. In our laboratory the intra- and interassay CVs were 5% and 8% for both methods. On the basis of the functional sensitivity of the methods, we selected 0.5 μg/L as the cutoff value discriminating undetectable from detectable Tg levels. Thyroglobulin antibodies were measured by commercial assay (Dia Sorin, Saluggia, Italy). A concentration of 100 mIU/L IgG to Tg was taken as the cutoff value. Sera positive for Tg autoantibody (>100 mIU/L) were further processed by means of a Tg recovery test to define the right Tg level. Serum free thyroid hormones and TSH were measured by using ultrasensitive chemiluminescence immunoassay (Roche Diagnostics). Reference ranges are 0.3 to 4.2 mIU/L for TSH, and 3.9 to 6.8 and 12.0 to 22.0 pmol/L for free triiodothyronine (f-T3) and free thyroxine (f-T4), respectively. PTH was analyzed by chemiluminescence immunoassay (Immulite 2000, Diagnostic Products, San Juan Capistrano, CA). Intra- and interassay CVs were 4% and 6%, and assay sensitivity was 5 ng/L. In our laboratory the reference range of PTH is 15 to 65 ng/L. Osteocalcin was measured by chemiluminescence immunoassay (Nichols Advantage, San Juan Capistrano, CA). The reference range is 0.5 to 7.0 μg/L, and intra- and interassay CVs were 5% and 8%. Serum creatinine (reference range, 44-115 μmol/L), calcium (2.12-2.70 mmol/L), phosphorus (0.80-1.45 mmol/L), and total ALP (98-280 U/L) levels were determined by Modular Roche. Urinary calcium concentration was measured by standard procedures in 24-hour samples (reference range, 1.25-7.50 mmol per 24 hours) or in spot urine samples and expressed as molar ratio to creatinine (reference range, 0.0-0.2). Urinary HP, PD, and DPD were measured by high-performance liquid chromatography (BioRad, Milan, Italy). Methods were optimized using the Center for Disease Control and Prevention (Atlanta, GA) standards. Data are expressed as molar ratio of creatinine. In our laboratory, the reference ranges of HP/creatinine, PD/creatinine, and DPD/creatinine are 6 to 22 mmol/mol, 25 to 63 nmol/mmol, and 6 to 13.5 nmol/mmol, respectively. Coefficients of variation were, on average, 4% for HP, 6% for PD, and 7% for PDP. Bone mineral density (grams per square centimeter) in lumbar spine (L2-L4) and total hip was measured in the anteroposterior direction by dual-energy x-ray absorptiometry (DXA) using Hologic instruments (QDR 1500 and QDR 4500, Bedford, MA). DXA was performed only in 67 subjects with DTC and 26 controls. The so-called standard deviation scores, which indicate the deviation from normal values, were calculated by the following equations: T score = (BMD measured − BMD young normal population)/SD; z score = (BMD measured − BMD sex- and age-matched population)/SD.

2.4. Statistical analysis

Data from subjects with DTC and from controls were analyzed by means of the Prism 4.0 software (GraphPad Software, San Diego, CA). To compare absolute and percentage data, the Mann-Whitney test and χ^2 test were used when appropriate. Correlation analyses between variables were carried out by Spearman correlation. All values quoted are means ± SEM. Data below the functional sensitivity of the assay were analyzed for statistical purposes by using the functional sensitivity value. Significance was taken as $P < .05$. At least 1 year after primary therapies, the best predictor of cure in patients with DTC was considered to be an undetectable Tg level (<0.5 μg/L) after recombinant human TSH (rhTSH) testing combined with negative neck ultrasonography findings. In accordance with the World Health Organization classification system, osteoporosis

Table 1
Serum levels of f-T3, f-T4, TSH, Tg, calcium, phosphorus, creatinine and ALP observed in the whole group of subjects with DTC and controls (mean ± SEM)

	Subjects with DTC	Control subjects	P
f-T3 (pmol/L)	4.38 ± 0.12	4.36 ± 0.13	NS
f-T4 (pmol/L)	20.5 ± 0.6	15.1 ± 0.6	<.001
TSH (mIU/L)	1.26 ± 0.37	2.50 ± 0.43	<.001
Tg (μg/L)	4.7 ± 2.1	69.5 ± 21.9	<.001
Calcium (mmol/L)	2.44 ± 0.02	2.50 ± 0.02	NS
Phosphorus (mmol/L)	1.10 ± 0.02	1.15 ± 0.03	NS
Creatinine (μmol/L)	70.6 ± 1.8	77.2 ± 2.7	<.05
ALP (U/L)	183.4 ± 6.6	172.1 ± 9.2	NS

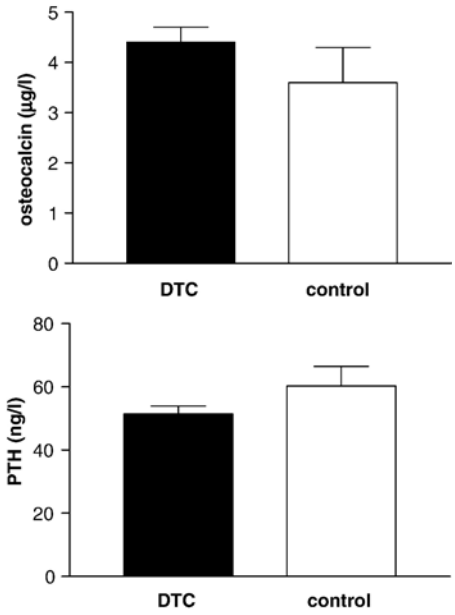

Fig. 1. Osteocalcin and PTH levels in the cohort of subjects with DTC and controls studied.

was defined as T scores lower than 2.5 in lumbar spine or total hip.

3. Results

The interval between diagnosis and treatment of the thyroid diseases was significantly shorter in patients with DTC (5.6 ± 0.6 years) than in controls (12.8 ± 1.8 years) ($P < .001$). The 2 groups of subjects were not significantly different in age even when analyzed according to sex. Body mass index was 26.1 ± 0.5 kg/m^2 in patients with DTC and 25.6 ± 0.5 kg/m^2 in controls. Both patients with DTC (Spearman coefficient of correlation, $r_S = 0.30$, $P < .01$) and controls ($r_S = 0.50$, $P < .001$) showed a significant positive correlation between BMI and age. Per-week dosage of L-T4 was significantly higher in patients with DTC (844 ± 22 µg/wk) than in controls (612 ± 39 µg/wk; $P < .001$). In patients with DTC, the weekly L-T4 dosage was not related to BMI ($r_S = -0.13$, not significant [NS]) or age ($r_S = 0.22$, NS), whereas a significant negative correlation was noted in controls between L-T4 dosage per week and both BMI ($r_S = -0.41$ $P < .05$) and age ($r_S = -0.59$, $P < .001$). Free T3, f-T4, TSH, Tg, serum calcium and phosphorus, creatinine, and ALP values are reported in Table 1. As expected, owing to the higher L-T4 dosage per week, patients with DTC showed more elevated f-T4 concentrations ($P < .001$) and more suppressed TSH levels ($P < .001$) than controls (Table 1). Thyroglobulin levels were significantly lower in patients with DTC than in controls ($P < .001$; Table 1). Detectable (baseline or after rhTSH testing) Tg values were found in 14% of patients with DTC after primary therapies, as an index of thyroid remnant or disease recurrence. In the control group, as a result of less radical thyroid surgery, no radioiodine ablation, and milder L-T4 suppressive therapy, Tg levels were undetectable only in 18% of subjects. The percentage of patients with undetectable Tg levels was significantly higher in subjects with DTC than in controls ($P < .001$). No difference in serum calcium and phosphorus, creatinine, and ALP levels was noted between the groups (Table 1). Osteocalcin and PTH levels were also similar in subjects with DTC and controls (Fig. 1). However, 23% of patients with DTC and 30% of control subjects showed slightly or markedly elevated PTH levels. Fig. 2 shows mean ± SEM

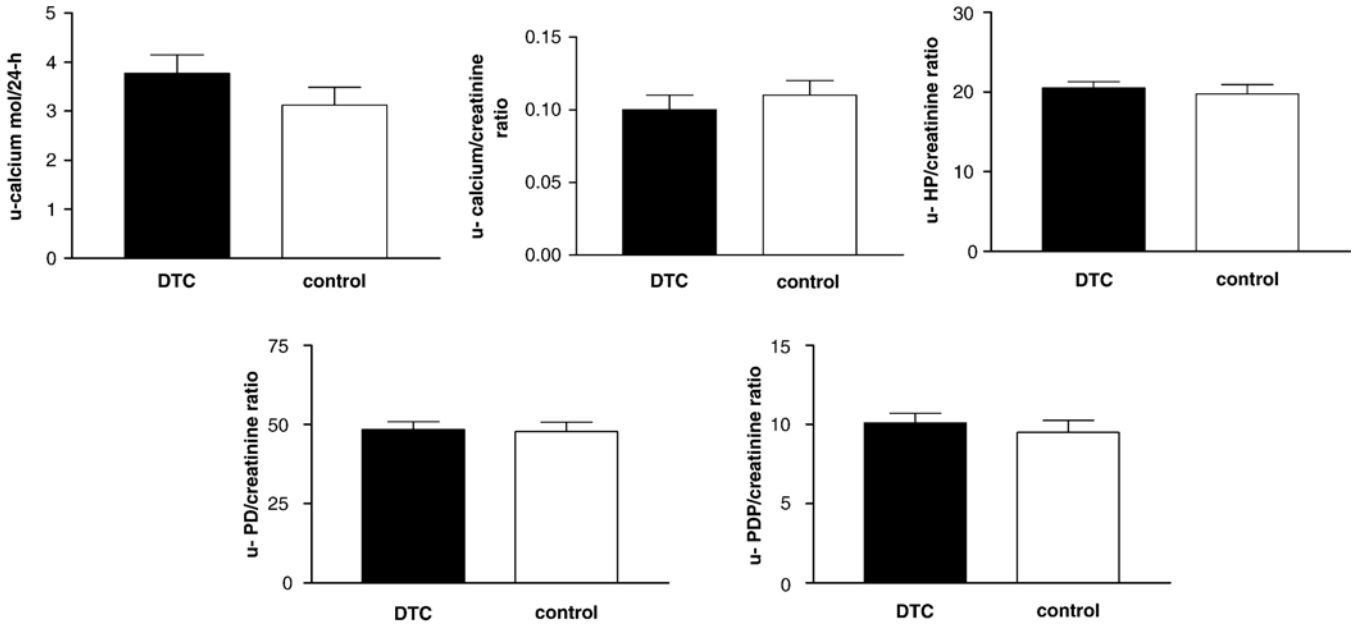

Fig. 2. Urinary levels of calcium and urinary calcium/creatinine, HP/creatinine, PD/creatinine, and DPD/creatinine ratios in the cohort of subjects with DTC and controls studied.

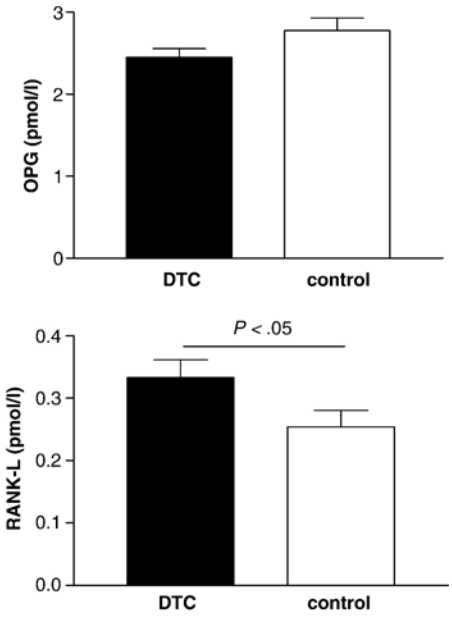

Fig. 3. OPG and RANK-L levels in the cohort of subjects with DTC and controls studied.

of the urinary parameters of bone metabolism evaluated. No difference was noted between subjects with DTC and controls (Fig. 2). DXA showed a similar percentage of patients with osteoporosis in the DTC (30%) and control (31%) groups.

On the whole, no difference in OPG levels was noted between DTC and control groups (Fig. 3). In premenopausal women (DTC, 2.4 ± 0.2 pmol/L; controls, 2.2 ± 0.1 pmol/L) and in men (DTC, 2.3 ± 0.4 pmol/L; controls, 1.9 ± 0.3 pmol/L), OPG levels were similar in both groups of subjects. Moreover, OPG levels were significantly ($P < .05$) lower in the subgroup of postmenopausal DTC (2.5 ± 0.1 pmol/L) than postmenopausal control (3.1 ± 0.2 pmol/L) women. Only control women showed lower OPG levels in premenopausal (2.2 ± 0.1 pmol/L) than in postmenopausal (3.1 ± 0.2 pmol/L; $P < .01$) conditions.

Soluble RANK-L levels were significantly higher ($P = .03$) in patients with DTC than in controls (Fig. 3). A similar result was observed between the premenopausal DTC (0.49 ± 0.07 pmol/L) and premenopausal control (0.20 ± 0.03 pmol/L; $P < .01$) women, but not in postmenopausal women (DTC, 0.27 ± 0.03 pmol/L; controls, 0.28 ± 0.04 pmol/L) or in men (DTC, 0.28 ± 0.02 pmol/L, controls, 0.18 ± 0.09 pmol/L). In subjects with DTC, RANK-L levels were higher in premenopausal (0.49 ± 0.07 pmol/L) than in postmenopausal (0.27 ± 0.03 pmol/L; $P < .01$) women. In controls, RANK-L levels were similar in both premenopausal (0.20 ± 0.03 pmol/L) and postmenopausal (0.28 ± 0.04 pmol/L) conditions.

No relationship was seen between OPG and RANK-L levels in either cohort of subjects. OPG levels displayed a significant positive relationship to age in both DTC ($r_S = 0.41$, $P < .001$) and control ($r_S = 0.59$, $P < .001$) subjects. Serum RANK-L was negatively related to age in subjects with DTC ($r_S = -0.22$, $P < .05$) but not in controls (Fig. 4). Other correlations are reported in Table 2. In subjects with DTC, a significant correlation was found between OPG and ALP, PD/creatinine, or DPD/creatinine, whereas in controls, OPG was related to BMI, weekly L-T4 dosage, phosphorus,

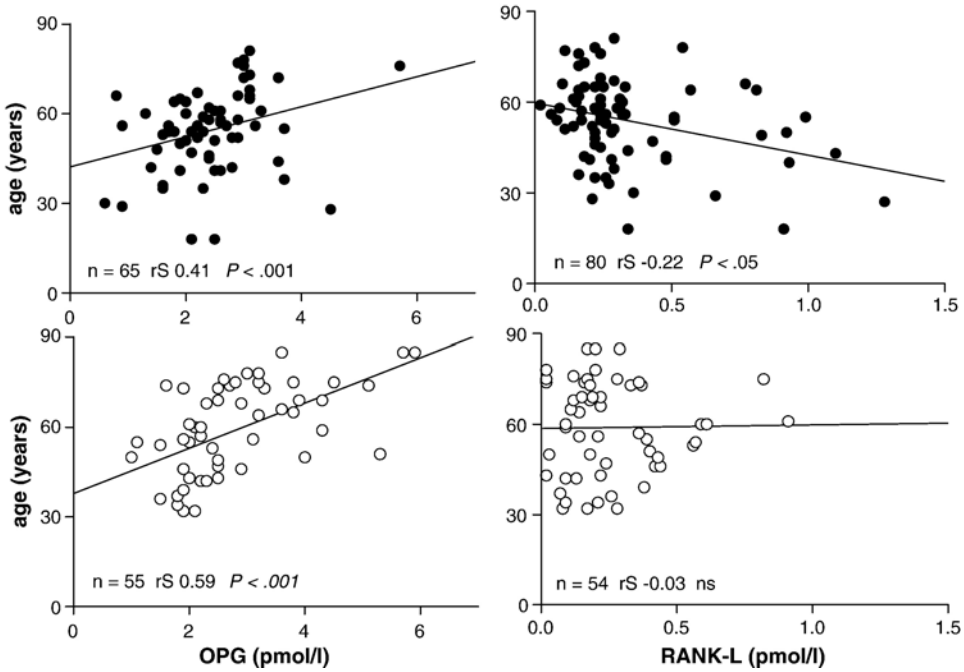

Fig. 4. Correlation between age and OPG or RANK-L levels in the cohort of subjects with DTC and controls studied. The number of pairs and significance for each analysis is reported.

Table 2
Correlation between OPG or RANK-L and clinical and biochemical parameters evaluated in subjects with DTC and controls

	Subjects with DTC			Control subjects		
	n	r_S	P	n	r_S	P
OPG (pmol/L)						
BMI (kg/m^2)	65	0.14	NS	52	0.28	<.05
L-T4 (μg/wk)	65	−0.04	NS	34	−0.40	<.05
f-T3 (pmol/L)	65	−0.07	NS	55	0.07	NS
f-T4 (p/mol/L)	65	0.15	NS	55	−0.26	NS
TSH (mIU/L)	65	−0.11	NS	55	−0.12	NS
Tg (μg/L)	64	0.24	NS	50	0.36	<.01
Calcium (mmol/L)	65	−0.01	NS	51	−0.12	NS
Phosphorus (mmol/L)	63	−0.02	NS	49	−0.29	<.05
Creatinine (μmol/L)	64	0.16	NS	49	0.25	NS
ALP (U/L)	65	0.24	<.05	49	0.09	NS
PTH (ng/L)	63	0.24	NS	54	0.19	NS
Osteocalcin (μg/L)	59	0.01	NS	43	0.49	<.001
Urinary calcium (mmol/24 h)	43	−0.06	NS	29	0.24	NS
Calcium-creatinine ratio	58	−0.09	NS	39	0.19	NS
HP/creatinine ratio	55	0.10	NS	38	0.27	NS
PD/creatinine ratio	53	0.28	<.05	30	0.41	<.05
DPD/creatinine ratio	53	0.27	<.05	30	0.32	NS
RANK-L (pmol/L)						
BMI (kg/m^2)	80	−0.14	NS	52	0.12	NS
L-T4 (μg/wk)	80	−0.08	NS	33	−0.18	NS
f-T3 (pmol/L)	80	0.02	NS	54	−0.01	NS
f-T4 (p/mol/L)	80	0.02	NS	54	−0.16	NS
TSH (mIU/L)	80	0.01	NS	54	−0.15	NS
Tg (μg/L)	78	−0.08	NS	50	−0.06	NS
Calcium (mmol/L)	79	0.01	NS	50	−0.04	NS
Phosphorus (mmol/L)	77	−0.07	NS	48	0.07	NS
Creatinine (μmol/L)	76	0.03	NS	48	−0.14	NS
ALP (U/L)	78	−0.22	NS	48	0.02	NS
PTH (ng/L)	78	0.08	NS	53	−0.14	NS
Osteocalcin (μg/L)	68	−0.06	NS	43	0.02	NS
Urinary calcium (mmol/24 h)	56	−0.06	NS	29	0.06	NS
Calcium-creatinine ratio	66	−0.22	NS	39	−0.03	NS
HP/creatinine ratio	61	−0.29	<.05	38	0.27	NS
PD/creatinine ratio	58	−0.15	NS	30	0.24	NS
DPD/creatinine ratio	59	−0.15	NS	30	0.26	NS

n indicates number of pairs.

osteocalcin, and PD/creatinine urinary ratio (Table 2). In subjects with DTC, RANK-L levels were significantly related to HP/creatinine urinary ratio only in patients with DTC (Table 2).

4. Discussion

Osteoprotegerin and RANK-L are critical factors in the regulation of osteoclastic activity and in maintaining bone remodeling. In vitro, OPG secretion from mature osteoblasts is stimulated by f-T3 and inhibited by active vitamin D_3 [34]. In humans, OPG seems to be involved in the etiopathogenesis of postmenopausal osteoporosis [20,35] and other metabolic diseases involving bone loss [36,37]. Moreover, it has recently been suggested that OPG could be linked to hyperthyroidism-induced bone loss [27,34]. Overt hyperthyroidism is characterized by accelerated bone turnover, which is caused by direct stimulation of bone cells by elevated thyroid hormone levels [21,38,39]. Interleukin 6 and interleukin 8 have been suggested to mediate the effects of thyroid hormones on bone metabolism [39,40]. Thyroid hormones regulate OPG secretion [34], and overt hyperthyroidism is accompanied by an increase in serum OPG levels [22]. Antithyroid drugs determine a significant reduction in OPG levels [22]. Increased OPG levels have also been found in subclinical or clinical hypothyroidism [23]. After initiation of L-T4 therapy, OPG levels decrease [23].

To date, methodological problems due to assay sensitivity and reproducibility in peripheral blood have rendered very few data available on soluble RANK-L in normal and pathologic conditions [10]. To our knowledge, the present data are the first recorded in subjects who have undergone lobotomy or subtotal, near-total, or total thyroidectomy for benign or malignant thyroid pathology matched for sex, age, menopausal status, BMI, medical treatments, BMD, and other biochemical parameters involved in bone metabolism. Differences in surgical thyroid removal and the need for

more vigorous TSH suppression in patients with DTC both justify the differences in L-T4 dosage and serum TSH levels observed between the groups.

In our study, OPG concentrations were similar in subjects who had undergone radical thyroidectomy plus radioiodine ablation for DTC currently in drug-induced hyperthyroxinemia and control subjects who had undergone different degrees of thyroid removal and were on substitutive L-T4 treatment. This finding seems to undermine the significance of the amount of OPG directly produced by the thyroid gland [6] as a portion of circulating OPG.

At present, the literature data indicate a positive correlation between OPG and age [41,42]; this phenomenon was also found in our subjects, regardless of whether they had a history of benign or malignant thyroid pathology. Moreover, in our study the OPG increase on passing from premenopausal to postmenopausal conditions was less marked in DTC than in control women, in contrast with what has been reported by Mazziotti et al [43]. The present study did not find any correlation between OPG levels and weekly L-T4 dosage (suppressive or substitutive), whereas some authors have reported that OPG levels are always higher in patients on L-T4 (suppressive) therapy for DTC than in healthy controls with similar gonadal function [43]. As in overt hyperthyroidism, there is no correlation between OPG levels and age [22]; we hypothesize that only sustained hyperthyroxinemia can, through an unknown mechanism, minimize the physiologic age-related OPG increase. It is unlikely that this phenomenon is directly due to thyroid hormone levels, as no correlation was observed in our study between these parameters and OPG; moreover, in hyperthyroid subjects, the correlation between OPG and thyroid hormones is weak [22].

Recently, Regalbuto et al [44] found in a small group of patients with DTC that relative hyperthyroxinemia did not modify osteocalcin, bone ALP, or urine HP in comparison with control subjects. On the other hand, Mazziotti et al [43] found that PTH levels were more sustained in subjects with DTC than control subjects, with a statistically significant difference emerging only in postmenopausal women, in whom bone ALP was also more elevated. Our study documented no significant difference in PTH, osteocalcin, ALP, or urinary markers of bone remodeling between the 2 groups of subjects, whereas slight but significant differences in TSH levels were observed. In hyperthyroid subjects, Amato et al [22] reported a significant positive correlation between OPG levels and C-telopeptides of type 1 collagen (cross laps) and a negative correlation between OPG and bone ALP or BMD. In their study, medical treatment of the disease normalized OPG levels, and the differences in cross laps and bone ALP between hyperthyroid and control subjects disappeared [22].

In vitro data indicate that OPG secretion is under the stimulatory control of transforming growth factor β [45] and 17β-estradiol [46], whereas it is under the inhibitory control of PTH [47] and glucorticoids [48].

In vivo, in humans, a negative correlation between OPG and PTH or urinary PD seems to exist [49]. In postmenopausal women, an inverse correlation between OPG and serum markers of bone resorption (bone ALP and cross laps), but not urinary markers or PTH, has been found [50]. For both sexes, the relationship between 17β-estradiol and OPG is weak [49,50]. Serum OPG is correlated with BMD in postmenopausal women [50] but not in men [49].

In a cross-sectional study performed on a random sample of community-dwelling adults in Iceland, serum OPG levels were associated with a profile of bone formation markers, suggesting that OPG may protect against age-related bone loss [42]. In our study, OPG was positively related with markers of bone resorption in subjects with relative hyperthyroxinemia, whereas in control subjects a significant relationship was found with BMI, weekly L-T4 dosage, serum Tg, osteocalcin, and urinary PD. In particular, osteocalcin seemed to be the parameter most closely related to OPG in subjects with normal thyroid hormones; this is in line with the concept that OPG acts as a protective factor on bone [42].

Owing to the low circulating concentration of soluble RANK-L and technical problems in its assay, data on this cytokine have been collected in in vitro studies; consequently, little is known regarding the correspondence between peripheral levels and tissue activity [10]. However, in order to study OPG, even if in a speculative way, a corresponding evaluation of RANK-L is essential.

Our data, collected by means of a sensitive assay for soluble RANK-L, did not show a significant correlation between the 2 circulating cytokines in subjects who had undergone thyroid surgery for DTC or benign thyroid diseases. Slightly higher RANK-L levels were found in DTC than in control subjects, with an age-related trend in DTC opposite to that observed for OPG in our study and in the literature [41,42]. In contrast with the several correlations reported with OPG, we found only a significant negative correlation between soluble RANK-L and the urine marker of bone resorption HP. This finding further underlines the difficulty of studying RANK-L in vivo. On the other hand, the role on bone of RANK-L in vitro is strongly supported by the fact that RANK-L is modulated by several factors, such as proinflammatory cytokines, interleukins, prostaglandin, PTH, glucocorticoids, and active vitamin D_3 [47,48,51]. Further studies will be necessary to clarify the clinical meaning of soluble RANK-L in human subjects.

In conclusion, our knowledge of OPG and the RANK/RANK-L system in humans is still scant, and the future role of their evaluation in clinical practice remains to be clarified [10,52]. At present, population studies are required to investigate changes in these analyses in relation to specific diseases and therapies [52]. Our study was undertaken to define OPG and soluble RANK-L concentrations in patients who had undergone thyroid surgery, in whom lifelong L-T4 administration, sometimes at a supraphysiologic dosage, is needed. In this condition, the correlation between circulating

OPG and RANK-L levels was not significant; however, the positive correlation of OPG with age was striking, whereas that of RANK-L with age was negative and weaker and emerged only in patients with DTC. The increase in OPG with age and markers of bone turnover further confirms the protective role of the cytokine against bone loss [42]. The cause of bone loss after long-term sustained hyperthyroxinemia [21,25,28-30,53] will be more extensively studied in the light of new knowledge on the OPG and RANK/RANK-L systems. Finally, the controversial extrathyroid action of TSH on bone [54] must be considered in this new scenario. At present, however, short-term rhTSH administration in subjects with DTC does not seem to change OPG [43,55] and RANK-L [55] levels.

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