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Effect of thyroidectomy on circulating angiogenic cytokines in papillary thyroid carcinoma and benign goiter: Potential for new biomarkers?

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ABSTRACT

Background: Circulating angiogenic factors have been associated with clinical outcomes of papillary thyroid carcinoma, although they may also be released in the context of benign multinodular goiter. We sought to investigate the effect of thyroidectomy on the activity and importance of multiple circulating angiogenic factors in papillary thyroid carcinoma and benign multinodular goiter.

Methods: Between May 2015 and December 2016, patients scheduled for total thyroidectomy for papillary thyroid carcinoma or benign multinodular goiter were offered to enroll in this study. Serum levels of angiopoietin-2, fibroblast growth factor-2, hepatocyte growth factor, platelet-derived growth factor-BB, placenta growth factor, heparin-binding epidermal growth factor, and vascular endothelial growth factor-A and -C were collected preoperatively and 2 weeks postsurgery. These levels were measured by enzyme-linked immunosorbent assay and compared with those of 35 healthy control subjects.

Results: Sixty patients with a median age of 52 years, 37 of whom were females, were included: 36 had papillary thyroid carcinoma, and 24 had benign multinodular goiter. In both benign multinodular goiter and papillary thyroid carcinoma, preoperative, circulating angiogenic factors levels were increased with respect to controls ($P < .0001$), and a decrease after total thyroidectomy was observed in the levels of angiopoietin-2 ($P < .0001$), fibroblast growth factor-2 ($P < .0001$), hepatocyte growth factor ($P < .001$), and heparin-binding epidermal growth factor ($P < .01$ each). Only patients with papillary thyroid carcinomas, however, showed decrease in the postoperative levels of platelet-derived growth factor-BB and vascular endothelial growth factor-A ($P = .001$ each).

Conclusion: Results from this study raise the potential for vascular endothelial growth factor-A and platelet-derived growth factor-BB to be used as biomarkers of the effectiveness of treatment of papillary thyroid carcinoma. These results warrant further investigation and may have potential prognostic implications.

Roberto Ria and Francesco Prete contributed equally to this work.

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Introduction

Thyroid cancer is the most common endocrine malignancy and accounts for 3% to 4% of all newly diagnosed cancers annually. Over the past 2 decades, the incidence of thyroid cancer has increased, an increase attributable almost entirely to papillary thyroid cancer (PTC), which constitutes up to 90% of all cases.[1] PTCs usually grow slowly and are indolent, although they are associated with lymph node metastasis in 30% to 90% of cases.[2,3] Early and accurate diagnosis enables timely and effective treatment by surgery combined with postoperative radioiodine therapy. Inadequate fine needle aspiration biopsy, however, may contribute to nondiagnostic or indeterminate rates up to 20%.[4] Thus, considerable effort has been made to identify reliable markers for primary PTC.

Biomarkers based on genes that appear to be up-regulated in PTC, such as cytokeratin 19 or galectin-3, have shown poor positive predictive value or failed to distinguish benign nodules from PTC.[5] Also, traditional tissue assays are invasive and are sensitive to background tissue contamination and sampling errors.[6] Another area where markers are needed is confirmation of response to treatment, with persistent disease not removed at the initial operation, which represents the most common cause of recurrent, high-risk differentiated thyroid cancer.[6]

A serum assay is needed that is noninvasive, safe, and can be used potentially in screening, diagnosis, and follow-up, when tissue is not easily accessible. Serum markers based on a single genetic mutation, albeit valuable, may address only a fraction of PTCs.[7] Angiogenesis is an essential event for progression of solid tumors and is promoted by circulating angiogenic factors (CAngFs) released by the tumor and inflammatory cells in their microenvironment.[8]

Angiotensin-2 (Ang-2), fibroblast growth factor-2 (FGF-2), hepatocyte growth factor (HGF), heparin-binding epidermal growth factor (HBEGF), platelet-derived growth factor-BB (PDGF-BB), placenta growth factor (PLGF), and vascular endothelial growth factor (VEGF)-A and -C are the major cytokines involved in angiogenesis and lymphangiogenesis in cancer and have been shown to be highly expressed in PTC tumor tissue[9–14] and can be detected in plasma or serum.[15] To investigate their role as potential serum biomarkers in subjects with PTC, we examined the levels of these CAngFs as indicators of the pathogenic process and of response to therapeutic intervention, postulating that their serum levels should be (1) measurable by serum enzyme-linked immunosorbent assay (ELISA) analysis, (2) increased in patients with PTC, and (3) decreased by total thyroidectomy.

In this study,we measured the serum levels of these 8 CAngFs in patients with either PTC or benign multinodular goiter (BG), respectively, before and after total thyroidectomy, and compared CAngFs levels with clinicopathologic factors.

Methods and materials

Between May 2015 and December 2016, adult patients with primary PTC or BG were recruited prospectively for this study. The study protocol was approved by the Research Ethics Committee of the University of Bari Medical School. All patients gave informed consent in accordance with the Declaration of Helsinki. We included patients with differentiated thyroid cancer or nontoxic nodular goiter confirmed on histopathology after total thyroidectomy and normal platelet count. Exclusion criteria were pregnancy, distant metastases, other nonthyroidal malignancies, diabetes mellitus, hypertension, heart failure, and known autoimmune or inflammatory diseases outside of the thyroid gland, including rheumatoid arthritis; these conditions represent potential biologic bias in VEGF measurements.[16] Because up to one-third of PTCs in the literature appear to be associated with Hashimoto's thyroiditis[17] and greater risk pathologic variants of PTC are known to be associated with Graves' disease,[18] cases of thyroid inflammatory disease were included to include presentations of PTC encountered typically in the clinical practice, which are the focus of this study. Patients undergoing thyroidectomy for benign thyroid pathologies were sex- and age-matched to the patients with primary PTC.

The preoperative workup included measurement of thyroid function and autoantibodies as well as serum concentrations of calcium, phosphorus, and magnesium. Thyroid scintigraphy, ultrasonographic color Doppler imaging for determination of thyroid volume, and chest and neck X-ray and computed tomography were also performed when needed.

De-identified clinicopathologic data of all patients were entered into our Endocrine Surgery database, including medical history, preoperative investigations including fine-needle aspiration cytologic examination, intraoperative findings, pathologic results (PTC variant and size in mm, number of foci, extrathyroidal extension, presence and number of lymph node metastasis), treatment received, and follow-up outcomes. In PTC, central compartment dissection was performed in the presence of enlarged or suspicious lymph nodes for histologic confirmation and staging purposes; routine prophylactic lateral neck dissection was not performed.

The prognostic scoring systems of MACIS (Metastasis, Age, Completeness of Excision, Invasiveness, and Size), the 2015 American Thyroid Association,[6] the AMES (Age, distant Metastasis, Extrathyroidal invasion, and Size), and the UICC (International Union Against Cancer) pTNM (Tumor-Node-Metastasis) staging systems were used to assign tumor risk profiles and stratify PTCs into risk groups. Peripheral blood samples for all cytokines under investigation were obtained within a day before elective total thyroidectomy and 2 weeks postoperatively.

This study included a control group of 35 healthy subjects who gave consent to serum sampling. The inclusion criteria were a normal ultrasonography of the neck, normal levels of thyroid stimulating hormone (TSH) and platelet count, no thyroid disease or any other malignancies, no evidence of chronic or acute diseases, and no evidence of endocrine pathology. Exclusion criteria were the same as the study groups.

Serum sampling and storage

Blood samples were collected into tubes containing ethylenediaminetetraacetic acid (EDTA) during workup for diagnosis and 2 weeks postoperatively. Blood samples were obtained as part of routine preoperative workup or early postoperative routine clinical evaluations. All blood specimens were bar-coded at the time of sampling, and patient identifiers were removed before specimens were delivered to the technicians conducting ELISA analysis. Within an hour of collection, plasma was separated by centrifugation (2,000 rpm for 20 minutes at 4°C) and aliquoted into multiple cryovials. Serum in the upper layer was stored at −80°C for future analysis.

Cytokine measurement

Blood samples were analyzed for serum levels of CAngFs by ELISA. Before analysis, plasma samples were thawed slowly in an ice bath, and all analyses were done from a 1-off defrost sample. CAngFs were measured by using Q-Plex Array Human Angiogenesis Antigen (Quansys Biosciences, Logan, UT) allowing simultaneous quantification of the following cytokines in simple samples: Ang-2, FGF-2, HGF, HBEGF, PDGF-BB, PLGF, and VEGF-A and -C according to the manufacturer's instructions. Levels of cytokines were

Table I
Patient demographics and clinicopathologic data

Pathology	*N* (%) mean ± SD (range) Primary PTC	*N* (%) mean ± SD (range) Benign goiter	*N* (%) mean ± SD (range) Controls	*P*
	36 (58.3)	24 (41.7)	35	
Sex	M 7 (29.2)	M 20 (55.6)	M 18 (51.4)	.238
Age	51.5 ± 13 (24–75)	55.9 ± 11 (39–77)	51 ± 10 (34–68)	.635
Graves' disease	4 (11.1)	4 (16)	-	.820
Hashimoto's disease	6 (16.6)	4 (16)	-	
Stage (TNM 8th)			-	
I	28 (80)			
II	7 (20)			
Mean tumor size (cm)	0.8 ± 0.7 (0.1–3.5)			
Multicentric	7 (19.4)			
Extrathyroidal extension	9 (25)			
LN metastasis	5 (14.3)			
Variant				
Classic	7 (20)			
Classic + follicular	3 (8.6)			
Follicular	19 (54.2)			
Sclerosing	3 (8.6)			
Trabecular	3 (8.6)			
MACIS score				
<5	19 (52.8)			
≥5–5.99	11 (30.5)			
≥6–6.99	6 (16.7)			
≥7	-			
AMES score				
Low risk	27 (75)			
High risk	9 (25)			

LN, lymph node; *SD,* standard deviation.

quantified through Q-View Software (Quansys Biosciences). For all CAngFs, we analyzed triplicate samples and used the mean results in biomarker analysis.

Statistical analysis

Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS for Windows, version 22; IBM Corporation, Armonk, NY). Continuous data are presented as mean (± standard deviation, standard error of the mean). Student's *t* test was used to evaluate differences between CAngFs levels (in pg/mL) in serum samples of patients and control subjects. The Wilcoxon signed-rank test was used to evaluate differences between pre- and postsurgery cytokine serum levels. The Wilcoxon-Mann-Whitney rank unpaired test or the analysis of variance Kruskal-Wallis test was used to compare differences in preoperative cytokine levels between groups. Spearman rank correlation test was used to evaluate the correlation between cytokine variation. Linear regression was used to evaluate the relation between each cytokine variation with age and preoperative clinical parameters. *P* values <.05 were considered as statistically significant.

Results

Serum samples obtained from the 60 participants were categorized into 36 primary PTCs and 24 BGs based on final histology. Baseline demographic and disease characteristics of participants are reported in Table I. Patients undergoing total thyroidectomy for benign thyroid pathologies were sex- and age-matched to the primary PTC patients. The 35 patients in the control group were also comparable with the study group according to sex and age (Table I).

Figure 1 shows the scatter distribution of cytokines pre- and postsurgery for each patient. The levels of angiogenic cytokines in the serum samples of patients with both neoplastic and benign thyroid pathology were all significantly greater with respect to the control group. The serum levels of all CAngFs demonstrated a statistically significant decrease after total thyroidectomy, with the exception of PLGF in PTC patients and of PLGF, PDGF-BB, and VEGF-A in patients with BG (Table II). Subgroup analysis of preoperative and postoperative CAngFs levels by excluding patients with known pre-existing inflammatory disease of the thyroid (Hashimoto's thyroiditis, Graves' disease—Table III) showed the same pattern of postoperative decrease in cytokine levels observed in the 2 full groups of the study, while also showing that preoperative levels of PDGF-BB and VEGF-A were statistically significantly greater in patients with PTC with respect to benign nodular goiters (Fig 2, Table III).

Mean preoperative serum levels of CAngFs were not different between benign and malignant cases dependent on age, sex, histologic variants of cancer, tumor size, multifocality, or duration of disease. Statistically significant differences in preoperative VEGF-C levels between PTCs and BGs were found when considering tumor extension beyond capsule, lymph node metastases (in central compartment in 4 cases, lateral compartment in 1), and the greater risk groups (Table IV). At univariate logistic regression, the difference between preoperative to postoperative levels of VEGF (pg/mL) was related to increasing odds of having PTC diagnosed (odds ratio 1.002, 95% confidence interval 1.001–1.004, $P = .006$). No differences were found between the cytokine levels in BG subtypes (Hashimoto's thyroiditis, nodular goiter, Graves' disease).

Discussion

Angiogenic activity is essential to the growth and progression of thyroid cancer,[14] and in recent years there have been many efforts to investigate and present suitable biomarkers of thyroid angiogenesis,[5,9] because dysregulation of angiogenic cytokines could result in cancer initiation, progression, and metastasis. To the best of our knowledge, this is the first study using ELISA to

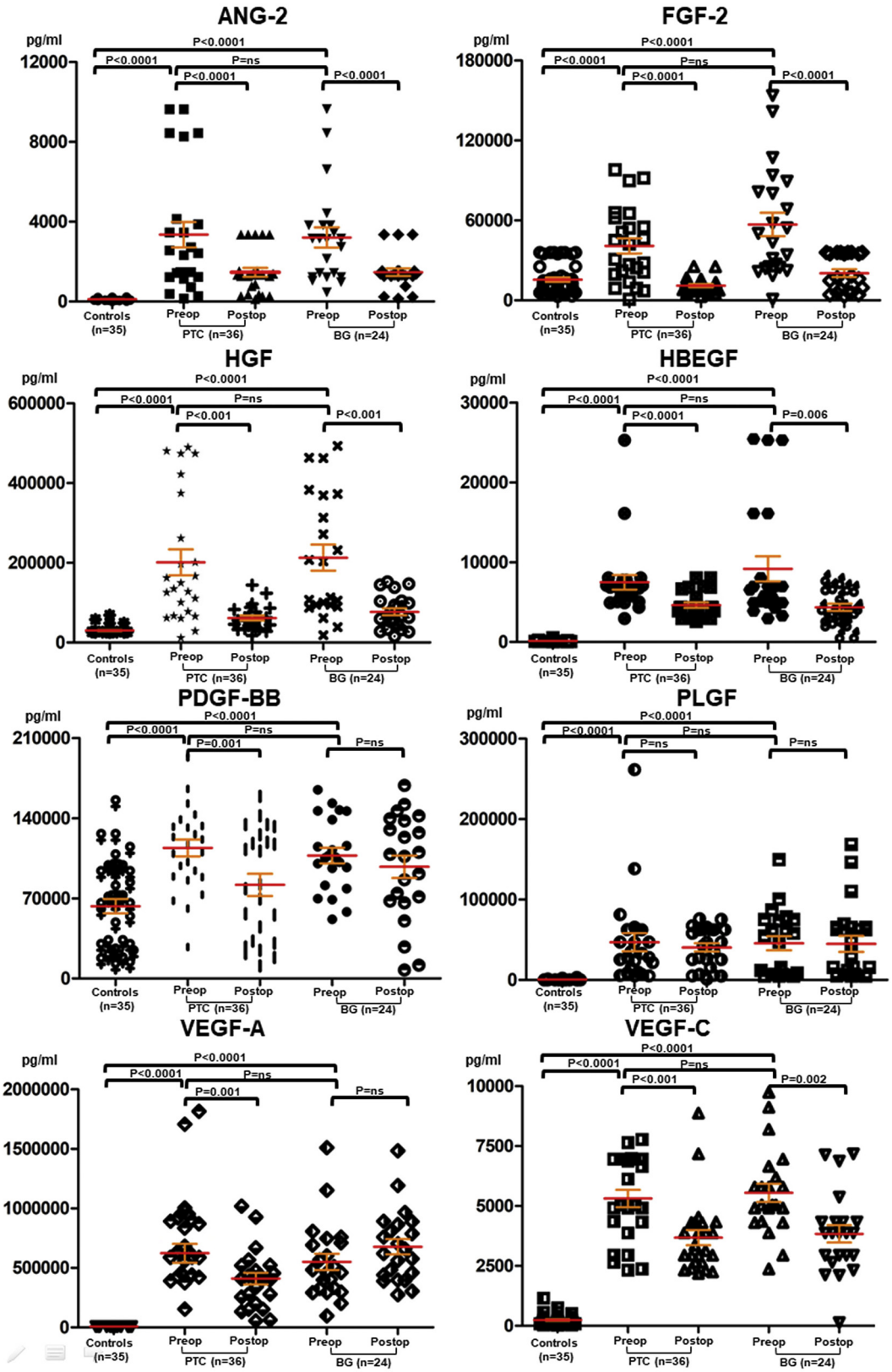

Fig 1. Analysis of the serum levels of CAngFs in preoperative and postoperative samples of patients undergoing total thyroidectomy for PTC or BG. CAngFs plasma levels of patients with neoplastic and non-neoplastic thyroid diseases were greater with respect to controls ($P < .0001$ for all cytokines in both groups). Decreases after total thyroidectomy were noted in serum levels of ANG-2, FGF-2, HGF, HBEGF, PDGF-BB, VEGF-A, and VEGF-C ($P < .001$ each), but not in PLGF in patients with PTC. Decreases after surgery were found in the levels of ANG-2, FGF-2, HGF, HBEGF, and VEGF-C ($P < .006$ each), but not of PLGF, PDGF-BB, and VEGF-A in patients with BG. The middle red horizontal lines indicate the mean value of measurements, and the error bars indicate standard error of mean. P = *ns,* non significant. (Color version of figure is available online.)

Table II
Levels of circulating angiogenic cytokines in PTC, BG, and healthy control subjects. Statistical significance for within-group comparison of preoperative and postoperative concentrations.

Cytokine	Controls pg/mL Mean ± SEM (range)	PTC ($n = 36$)			BG ($n = 24$)		
		Diagnosis pg/mL Mean ± SEM (range)	Postsurgery pg/mL Mean ± SEM (range)	*P*	Diagnosis pg/mL Mean ± SEM (range)	Post-surgery pg/mL Mean ± SEM (range)	*P*
ANG-2	118 ± 83 (12–219)	3,320 ± 451 (158–9,647)	1,356 ± 143 (118–3,354)	<.0001	3,147 ± 466 (464–9,638)	1,370 ± 187 (143–3,210)	<.0001
FGF-2	1,212 ± 16 (294–3,596)	3,974 ± 424 (86–9,804)	1,024 ± 96 (294–2,542)	<.0001	5,361 ± 845 (86–15,389)	1,801 ± 206 (328–1,828)	<.0001
HGF	276 ± 13 (255–686)	1,959 ± 248 (119–4,896)	546 ± 43 (179–1,474)	<.001	2,144 ± 310 (185–4,931)	760 ± 85 (156–1,520)	<.001
HBEGF	115 ± 13 (27–531)	734 ± 62 (294–2,528)	461 ± 29 (256–803)	<.0001	908 ± 165 (216–2,542)	408 ± 44 (85–783)	.006
PDGF-BB	63 ± 6 (10–153)	116 ± 6 (30–195)	80 ± 8 (11–159)	.001	106 ± 6 (52–164.760)	98 ± 9 (7.489–168.643)	.568
PLGF	272 ± 89 (84–3,093)	485 ± 78 (50–2,617)	385 ± 43 (12–760)	.379	497 ± 81 (51–1,498)	467 ± 92 (49–1,686)	.732
VEGF-A	74 ± 3 (47–77)	638 ± 58 (154–1,708)	411 ± 43 (56–1,020)	.001	543 ± 64 (97–1,510)	671 ± 61 (276–1,484)	.097
VEGF-C	179 ± 35 (31–1,147)	5008 ± 254 (2,651–7,776)	3,489 ± 228 (2,189–8,877)	<.001	5,095 ± 350 (2,349–8,327)	3,695 ± 346 (139–7,173)	.002

SEM, standard error of the mean.

perform cumulative profiling of 8, prominent, serum angiogenic cytokines among patients with PTC, BG, and cancer-free control subjects, respectively, before and after total thyroidectomy.

This study found that before thyroidectomy, CAngFs were all increased in patients with both PTC and BG with respect to control patients, consistent with the known role of these cytokines in thyroid growth. HGF and FGF are known to be potent mitogens for follicular thyroid cells, and the overexpression of FGF, HGF, and VEGF has been described in both nodular hyperplasia and PTC, in contrast to minimal production in normal thyroid tissue.[19,20] Ang-2 shows high expression in PTC tissues related to clinical staging.[9] In the reported literature, the expression of FGF2 and VEGF-C was found to be greater in PTC tissues than in normal tissues.[20] HBEGF, a mitogen and a potent chemotactic factor, mediates the invasion and metastasis of PTC.[10] PLGF plays critical roles in the pathologic angiogenesis of PTC through regulating matrix metalloproteinases.[11] PDGF-BB, recognized as the most potent lymphangiogenic factor, can bind with high affinity to PDGF receptor-α to promote lymphatic metastases in PTC.[12] Overexpressed by many cancer types of epithelial origin, PDGF-BB also affects cancer-related angiogenesis by up-regulating VEGF-A expression.[13] As reported previously, in this study greater preoperative levels of serum VEGF-C in PTCs correlated with lymph node metastases and a greater group ranking of cancer risk.[21] Preoperative levels of VEGF-C in PTC instead were not greater than in BG, possibly owing to the small proportion of cases presenting with lymph node metastases or cancers at an early stage.

The greater preoperative levels of all CAngFs in PTC and BG with respect to healthy control patients support the angiogenic and mitogenic role of CAngFs in both PTC and BG, including thyroid inflammation. All CAngFs that we analyzed were also reported to be increased in cancers of different origins, including gastric, liver, cervical, and lung cancers.[15] We found no differences in preoperative serum CAngFs levels between PTC and BG, but when cases of thyroid inflammation were excluded from the main study groups, preoperative serum levels of PDGF-BB and VEGF were found to be greater in PTC than in BG (Table III). Greater preoperative serum VEGF levels in PTC than in BG are consistent with previous studies[22]; VEGF production and secretion in both thyroid tissue and plasma samples appear to correlate directly with tumor stage, aggressive PTC behavior, and metastasis.[14]

Total thyroidectomy in both the main and subgroup analyses were associated with a postoperative decrease in the levels of PDGF-BB and VEGF in PTC but not in BG (Table II and III). In other studies, serum VEGF and VEGF-C that were increased in newly diagnosed PTC patients, decreased progressively to levels comparable to those of control subjects with benign disease 3 months after operative treatment, suggesting that a subsequent increase of either of these markers during follow-up might be regarded as a sign of tumor recurrence.[21] Postoperative decreases in the postoperative serum levels of PDGF-BB in PTC but not in BG has not been reported before. In this study, the postoperative levels of all CAngFs did not decrease to the respective levels of control subjects, probably owing to the timing of sampling that occurred 2 weeks postoperatively, when wound healing processes are still active; while other studies demonstrated that the decreases in VEGF and VEGF-C back to normal levels is likely a time-dependent process,[21] the accuracy of measuring CAngFs in the early postoperative period could be affected by the interaction of FGF, VEGF, VEGF-C, and PDGF

Table III
Subgrop analysis excluding cases where thyroiditis is present

Cytokine	Controls pg/mL Mean ± SEM (range)	PTC ($n = 26$)			Multinodular goiter ($n = 16$)		
		Diagnosis pg/mL Mean ± SEM (range)	Postsurgery pg/mL Mean ± SEM (range)	*P*†	Diagnosis pg/mL Mean ± SEM (range)	Postsurgery pg/mL Mean ± SEM (range)	*P*†
ANG-2	118 ± 83 (12–219)	3005 ± 480 (159–9,647)	1,382 ± 193 (119–3,354)	.004	2,979 ± 542 (464–9,638)	1,409 ± 221 (157–3,210)	.001
FGF-2	1,212 ± 16 (294–3,596)	3,999 ± 550 (86–9,804)	942 ± 107 (294–2542)	<.001	4008 ± 752 (85.9–9,873)	1,597 ± 228 (328–3,463)	.002
HGF	276 ± 13 (255–686)	2,036 ± 313 (119–4,896)	547 ± 44 (179–1,107)	<.001	2,246 ± 395 (613–4,931)	765 ± 108 (156–1,520)	.001
HBEGF	115 ± 13 (27–531)	750 ± 84 (294–2,528)	477 ± 35 (256–803)	<.001	978 ± 232 (216–2,542)	408 ± 56 (85–783)	.023
PDGF-BB	63 ± 6 (10–153)	116 ± 7 (30–195)	85 ± 9 (11–159)	.014	96 ± 6 (52–149)	101 ± 12 (7–168)	.642
PLGF	272 ± 89 (84–3,093)	390 ± 47 (50–887)	347 ± 46 (12–811)	.388	506 ± 110 (51–1,498)	457 ± 114 (49–1,686)	.756
VEGF-A	74 ± 3 (47–77)	666 ± 60 (153–1,708)	407± 55 (56–1,020)	.004	496 ± 58 (202–1,151)	653 ± 58 (275–965)	.121
VEGF-C	179 ± 35 (31–1,147)	5,031 ± 289 (2,651–7,552)	3,360 ± 218 (2,189–7,173)	<.001	5,621 ± 431 (2,375–8,327)	3,746 ± 431 (139–7,173)	.005

SEM, standard error of the mean.
These subgroups are generated by excluding cases of thyroiditis from the main groups of the study (PTC and BG, respectively) previously presented in Panel A.
† Wilcoxon signed-rank test.

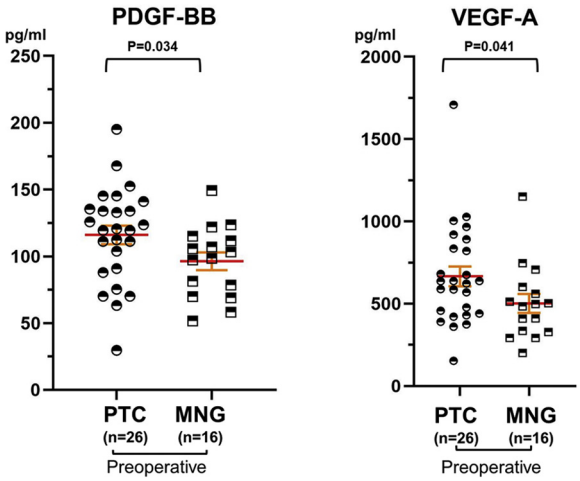

Fig 2. Subgroup analysis: CAngFs levels in preoperative serum samples of patients undergoing total thyroidectomy for PTC or benign multinodular goiter. Cases of thyroiditis have been excluded from both groups before analysis. A statistically significant difference was observed between PTC and BG in the preoperative serum levels of PDGF-BB (26 patients, 116 ± 7 and 96 ± 6 pg/mL, respectively) and VEGF-A (16 patients, 666 ± 60 and 496 ± 58 pg/mL, respectively). *MNG*, multi nodular goiter. (Color version of figure is available online.)

produced from other sources during the wound healing process or the presence of residual tumors.[23]

Other limitations to the evidence provided from this study include our small sample size, postoperative levels that have been read at a single time point, and ranges of serum CAngFs that overlap with levels in healthy control subjects. Low serum levels of VEGF that overlap with normal controls have been observed even in patients with distant metastases, because VEGF levels do not correlate with number of foci or with the extension of persistent disease or relapse.[24] Despite these limitations, our study showed that under the same experimental conditions the preoperative and postoperative profiles for PDGF-BB and VEGF in PTC are different from those in patients with BG.

Previous studies showed that distinct profiles of serum cytokine decline can be detected after effective treatment of other malignancies including breast cancer (VEGF), acute myeloid leukemia (Ang-2, FGF-2), multiple myeloma (FGF-2,VEGF, HGF), non-Hodgkin lymphomas, and other solid cancers.[25] Together with the results of this study, this evidence supports further research on serum CAngFs to investigate their potential use to confirm completeness of total thyroidectomy. To achieve reliable and clinically helpful biomarkers in thyroid cancer, it will be necessary to combine serum biomarkers, epigenetics, genomics, and proteomics.[8]

In conclusion, the present study analyzed the expression and biologic importance of CAngFs in patients who underwent total thyroidectomy for PTC and BG. The different profiles of the preoperative and post-thyroidectomy decreases in the levels of PDGF-BB and VEGF-A in PTC and BG respectively may suggest a different role of these 2 cytokines in the pathogenesis of cancer compared with benign thyroid disease. If validated, results from our study might have implications for the eventual use of serum VEGF or PDGF as biomarkers of the effectiveness of the operative treatment

Table IV
Correlation with clinicopathological variables

Variables	*n*	PDGF-BB (pg/mL) ± SEM	*P*	VEGF (pg/mL) ± SEM	*P*	VEGF-C (pg/mL) ± SEM	*P*
Age							
<45	11	103 ± 11	.144	623 ± 112	.873	4,616 ± 332	.347
>45	25	121 ± 6		643 ± 65		5,159 ± 327	
Sex							
F	20	125 ± 6	.083	643 ± 71	.911	5,188 ± 369	.437
M	16	116 ± 6		630 ± 91		4,783 ± 344	
Tumor size (mm)							
<20	34	117 ± 6	.288	620 ± 47	.206	5,015 ± 260	.919
>20	2	91 ± 28		931 ± 777		4,899 ± 1,714	
Multifocal							
No	29	119 ± 5	.299	653 ± 67	.570	5,135 ± 276	.320
Yes	7	104 ± 19		572 ± 77		4,486 ± 255	
Extrathyroidal extension							
No	27	115 ± 7	.871	597 ± 58	.207	4,526 ± 252	<.001
Yes	9	118 ± 6		761 ± 136		6,457 ± 408	
Lymph node metastasis							
No	30	115 ± 7	.731	609 ± 65	.323	4,692 ± 261	.001
Yes	5	121 ± 6		773 ± 71		6,949 ± 159	
pTNM stage							
I	33	116 ± 6	.828	618 ± 59	.247	4,824 ± 254	.014
II	3	112 ± 5		854 ± 91		7,038 ± 258	
MACIS score							
<5	19	118 ± 9	.788	580 ± 74	.296	4,374 ± 268	.026
5–5.99	10	110 ± 8		625 ± 85		5,703 ± 424	
6–6.99	7	119 ± 9		812 ± 162		5,739 ± 769	
7	-						
AMES							
Low risk	27	115 ± 7	.871	597 ± 58	.207	4,526 ± 252	<.001
High risk	9	118 ± 6		761 ± 136		6,457 ± 408	
ATA_2015							
Low risk	26	114 ± 7	.863	595 ± 61	.406	4,598 ± 251	.007
Intermediate risk	7	118 ± 8		789 ± 168		5,603 ± 677	
High risk	3	125 ± 10		654 ± 145		7,179 ± 304	

AMES, Age, Metastases, Extent, and Size risk classification; *ATA,* American Thyroid Association; *MACIS,* distant Metastasis, patient Age, Completeness of resection, local Invasion, and tumor Size; *SEM,* standard error of the mean.

in PTC or to be used as building blocks for further research to improve detection and treatment of refractory or recurrent PTC. Our future studies will be aimed at verifying the efficacy of these candidate markers in larger cohorts.

Conflict of interest/Disclosure

The authors declare no competing interests.

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Authors' Contributions

Conception and design: R.R., M.T.
Development of methodology: R.R.
Acquisition of data: A.M., G.D.M., V.F., F.P., A.P., L.I.S., A.G.
Execution of the experiments: I.S., A.G.S.
Analysis and interpretation of data: R.R., F.P., A.M.
Writing, review, and revision of the manuscript: R.R., F.P., A.V., M.T.

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