JBR–BTR, 2011, 94: 185-190.

PROGNOSTIC VALUE OF VASCULARITY INDEX FOR THE DIAGNOSIS OF AUTOIMMUNE THYROID DISEASE

I. Banaka[1], G. Kaltsas[1], St. Antoniou[1], G. Kanakis[1], A. Zilos[1], C.S. Baltas[2], D. Thomas[1]

Aim: **to determine thyroid vascularity in healthy subjects and patients with autoimmune thyroid disease (AITD), and assess its sensitivity and specificity for the diagnosis of AITD.**
Methods: **High-sensitivity color flow Doppler sonography (HSCFDS) was used to estimate the thyroid intraparenchymal vascularity in 31 euthyroid patients with Hashimoto's thyroiditis (HD), 33 hypothyroid patients with HD, 13 hyperthyroid patients with Graves' disease, and in 34 healthy controls. Images obtained from the ultrasound unit were further processed with a widespread, available imaging analysis program and the predictive value of the maximum vascularity index (VI) was used for further statistical analysis.**
Results: **Compared to healthy controls, patients with AITD had higher mean VI of both the right and the left thyroid lobe (TL) ($P < 0.001$). The sensitivity of left TL VI values greater than 5.57% (the best cut-off value of the Receiver Operating Characteristics-ROC curve) for the diagnosis of AITD was 80.8% and the specificity was 85.3%. Right TL VI values greater than 14.75% had 84.6% sensitivity and 86.2% specificity for the differential diagnosis among patients with HT or GD.**
Conclusions: **Measurement of right and left TL vascularity index using HSCFDS is a high specific tool, particularly where there is a high clinical suspicion of an autoimmune process.**

Key-words: Thyroid, US – Thyroiditis.

Color flow Doppler sonography (CFDS) has become an important non-invasive diagnostic method for the evaluation of thyroid vascularity and function (1). One of its main uses is the ability to distinguish hyperthyroidism from other low radioiodine uptake causes of thyrotoxicosis (1). In conditions related to hyperfunctioning thyroid gland such as Graves' disease (2), toxic multinodular goiter (3) and autonomous adenoma (4) the vascular signals are increased, whereas they are decreased or normal in cases of thyrotoxicosis factitia (5), and destructive processes of thyroid gland, such as subacute thyroiditis or type-II amiodarone-induced thyrotoxicosis (6). Along with CFDS the echomorphological pattern is also used to verify the presence of autoimmunity due to a diffuse reduction in thyroid echogenecity (echointensity) and an irregular echo pattern often associated with this disorder (7, 8).

Several qualitatively classifications of intrathyroidal vascular patterns distinctive to specific pathological entities have been proposed, with that of Vitti's being the most widely used in clinical practice (9). This classification describes easily and quickly the intraparenchymal thyroid blood flow, but remains highly subjective and is not sufficient enough to evaluate subtle variations of thyroid vascularity. Several techniques for semi-quantitative measurements of intrathyroidal blood flow have been applied for the differentiation of painless thyroiditis from Graves' disease (10), and benign from malignant thyroid nodules (11). Similar techniques have been used for measurements of thyroid vascularity in healthy subjects (12), and in patients with subacute thyroiditis (13). Currently there are no available data regarding the predictive value of CFDS assessed thyroid vascularity for the diagnosis of autoimmune thyroid disease (AITD) *per se* and potential correlations with clinical or biochemical parameters.

The main purpose of this study is to determine thyroid vascularity with a semi-quantitative, widespread available method in healthy subjects and in patients with AITD [Hashimoto's thyroiditis (HT) and Graves' disease (GD)], and to assess its sensitivity and specificity for the diagnosis of AITD and for the differential diagnosis between patients with HT and GD. Secondary goals are to correlate thyroid vascularity with clinical and biochemical indices of thyroid autoimmunity, and to compare its sensitivity and specificity with other gray-scale sonographic findings.

Materials and methods

Subjects selection

Between August 2008 and November 2009, a total of 1086 consecutive subjects from the outpatient clinic of the Department of Endocrinology and Metabolism, with unknown thyroid functional status, were screened. The study was approved by the local Bioethical Committee and informed consent was obtained from all participants. All subjects included in the study underwent a detailed physical examination, and a complete medical and family history was recorded. From these subjects, 616 were excluded due to acute or chronic underlying diseases and co-morbidities and/or residence in iodine-deficient areas. All remaining 470 subjects from the same iodine-replete area, Athens and its suburbs (14, 15), were further evaluated with thyroid sonography and with free triiodothyronine (fT_3), free thyroxine (fT_4), thyrotropin (TSH), thyroglobulin (anti-Tg) and thyroid peroxidase (anti-TPO) autoantibodies, and thyroid stimulating hormone receptor antibodies (TRAb) levels measurements; when indicated, thyroid scintigraphy was performed. Although we did not include food questionnaire in the study participants, all subjects used commercial iodized salt. Subjects with simple goiter, non-autoimmune

From: 1. Endocrine Unit, Department of Pathophysiology, National and Kapodistrian University of Athens, Athens, Greece, 2. Radiology Imaging Department, General Hospital of Athens "G. Gennimatas", Athens, Greece.
Address for correspondence: Dr D. Thomas, Endocrine Unit, Department of Pathophysiology, National and Kapodistrian University of Athens, Mikras Asias 75, 15575, Athens, Greece. E-mail: thomasproge@endo.gr

Table I. — Epidemiological characteristics and laboratory findings of the patients, at the start of the study.

	Control group	AITD type-1	AITD type-2	AITD type-3A
n	34	31	33	13
Age (years) (sd)	41.3 (17.7)	44.5 (13.1)[a]	40.4 (13.1) [a]	41.3 (12.4)[a]
Sex (Female/Male)	25/9[b]	26/5[b]	29/4[b]	10/3[b]
BMI (sd)	25.65 (3.63)	25.85 (4.09)[c]	25.76 (3.94)[c]	25.18 (3.44)[c]
TSH (μU/ml) (sd)	2.12 (0.94)	2.37 (0.9)	11.84 (22.76)	0.025 (0.037)
Anti-Tg positive (%)	0	51.61	54.54	53.84
Anti-TPO positive (%)	0	90.3	93.9	69.23
TSI positive (%)	0	0	0	69.23
Mean thyroid volume (cc) (range)	8.8 (3.5-19.8)	11 (5.3-19.1)	11.8 (1.02-33.3)	20.7 (5.4-73.1)

[a,c]statistically non-significant from controls (Kruskal-Wallis non-parametric analysis of variance, p = 0.703 and analysis of variance-AN.O.VA., p = 0.96 respectively).
[b]statistically non-significant from controls (Chi-square test, p = 0.464).
AITD: autoimmune thyroid disease.

thyroiditis, non-autoimmune hyperthyroidism, and thyroid nodular disease, were further excluded. Finally 77 patients, all of Greek ethnic origin, with high levels of anti-Tg and/or anti-TPO and/or TSI due to autoimmune thyroid disease (AITD) were selected for further studies. The 77 patients were categorized further into 3 groups: clinically euthyroid patients with Hashimoto's thyroiditis (AITD type-1) (n = 31) and TSH between normal ranges (0.27-4.2 μU/ml), hypothyroid patients with Hashimoto's thyroiditis (AITD type-2) (n = 33), with TSH levels greater than 4.22 μU/ml, with or without clinical symptoms of hypothyroidism consistent with hypothyroidism, and patients with clinically and biochemically (TSH levels less than 0.1 μU/ml) Graves' disease (AITD type-3A) (n = 13). The epidemiological and laboratory parameters of the 77 patients are shown in (Table I).

An age and sex-matched group (Table I) of 34 healthy Greek volunteers, without any previous medical or family history of thyroid disease or any other acute or chronic diseases were selected for comparisons. All asymptomatic controls were from the same iodine replete area of Athens, and had the same hormonal and sonographic investigations as the patient's group.

Study protocol

Anti-Tg and anti-TPO autoantibodies were measured in the same laboratory using a two-site immunoluminometric assay (Diasorin, LIASON analyzer, normal range: < 100 IU/ml and < 25 IU/ml respectively). Only individuals with high anti-Tg and/or anti-TPO titers that were confirmed in a second test were classified within the AITD group. TRAbs were measured using commercial kit (Diasorin Inc., Stillwater, MN, USA, cut-off value: 10%). Thyroid stimulating hormone (TSH) levels were measured using a one step sandwich assay (RIAgnosthTSH: CIS Bio International, Gif-Sur-Yvette, Cedex, France, normal range: 0.25-4 mU/l). Free triiodothyronine and fT4 levels were measured by electrochemiluminescence immunoassay (E170 analyzer, Roche, Diagnostics, Mannheim, Germany), with reference values 3.9-6.9 pmol/l and 11-23 pmol/l respectively.

Following the diagnosis, all patients with AITD type-2 were treated with levo-thyroxine, with target serum TSH values in the normal or low-normal range (1.47 ± 0.34 μU/ml). Patients with AITD type-3 were initially treated with adequate doses of antithyroid drugs until euthyroidism was obtained and maintained with titration of the antithyroid drug dosage; only three patients were treated with a block (methimazole) and replace (levo-thyoxine) regimen. Control subjects and patients with AITD type-1 were regularly followed up at six-month intervals with physical examination, endocrine testing, and thyroid ultrasonography.

Thyroid ultrasonography

Thyroid ultrasonography was carried out using a high-resolution apparatus (Logic-Book XP, General Electric Co, USA) equipped with a 6-11 MHz broadband linear array probe. A single operator who was unaware of the diagnosis performed the scans. Patients were examined by gray-scale and color Doppler examination in supine position, with the neck in hyperextension. Although all participants underwent a second and a third thyroid sonogram six and twelve months later respectively, only the measurements of the first examination were used for the purpose of the study.

In gray-scale ultrasonography, the maximum diameter of the long-axis and depth were estimated in longitudinal plane, while short-axis in transverse images. The maximum volume (cm^3) of each lobe was calculated using the approximate formula of the ellipsoid $\pi/6$ x [length (cm)] x [width (cm)] x [depth (cm)] (16). Measurements of thyroid volume, echogenecity and echostructure were made by gray-scale histogram analysis (17). Following sonographic evaluation of the thyroid echogenecity, all the participants were categorized into two groups: with normal or reduced (from slightly reduced-grainy echo texture to levels below that of echoes coming from muscles) echo intensity. Furthermore, all the participants were categorized into two groups: with homogeneous or irregular echo pattern of the thyroid gland.

High-sensitivity color flow Doppler sonography was used to estimate the intraparenchymal blood flow pattern. Normally, the vascularity of the thyroid tissue itself was minimal. Pulse repetition frequency was adjusted to 2.3 KHz, wall filters to 102 Hz, and Doppler frequency to 5 MHz. We tried to adjust the noise by initially increasing the color gain to a level that showed noise, and

Table II. — Predictive value of vascularity index, reduced echogenecity and irregular echo pattern for the diagnosis of autoimmune thyroid disease.

	Sensitivity (%)	Specificity (%)	Sensitivity X Specificity	Youden index*	Positive predictive value (%)	Negative predictive value (%)
right TL VI > 7.44%	71.8	91.2	0.6548	0.63	96.49	59.25
left TL VI > 5.57%	80.8	85.3	0.6892	0.66	92.53	65.9
reduced echogenecity	75.32	82.35	0.62	-	90.6	59.57
irregular echo pattern	88.31	67.64	0.597	-	86.08	71.87

*sensitivity + specificity-1.

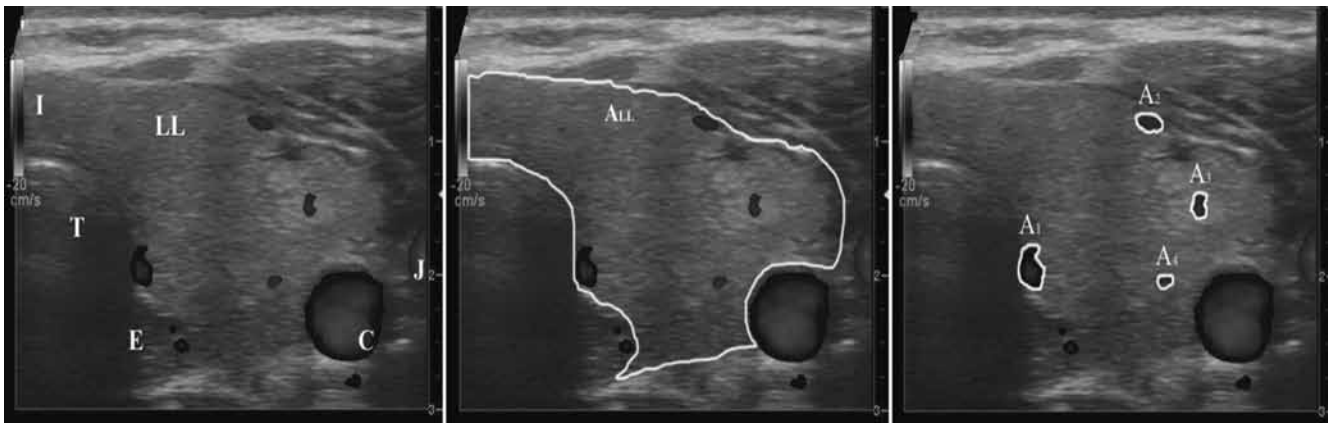


Fig. 1. — A: Transverse section of the left thyroid lobe (LL), as shown in color flow Doppler sonogram. I: isthmus, T: trachea, E: esophagus, C: left carotid artery, J: left jugular vein. Panel B: the boundaries of the left thyroid lobe were manually outlined and the number of pixels was counted by the program (A_{LL} = 75760 pixels). Panel C: the boundaries of all vessels spots were manually outlined, the number of colour pixels of each spot (A_1 = 788 pixels, A_2 = 400 pixels, A_3 = 340 pixels, A_4 = 153 pixels) was counted by the program and the total number of colour pixels were calculated as following: $A_{vessels} = A_1+A_2+A_3+A_4$ = 1681pixels. Vascularity index (VI) was further calculated by dividing the number of colour pixels of vessels spots ($A_{vessels}$) to the left lobe pixels (A_{LL}): $VI = A_{vessels}/A_{LL}$ = 0.0222 or 2.22%.

then decreasing until the noise disappeared. Because of variations in arterial flow during the cardiac cycle, and the number and size of vessels that appear on static images multiple sampling of at least 6 sections of each thyroid lobe (TL) was performed and the section with the maximum vascularity was used for further analysis.

For the evaluation of thyroid vascularity, images from each TL were retrieved from the ultrasound unit and converted into high-resolution tagged image file format (TIFF). All images were further processed with a widespread, available for downloading from public domain, imaging analysis program (ImageJ, 1.42q, N.I.H., USA, http://rsd.info.nih.gov/ij). The vascularity index (VI = total pixels of vessels spots of the lobe/total image pixels of the lobex100 %) was calculated for all images of each TL (Fig. 1) and the highest VI (%) was used for statistical analysis. Thyroid echogenicity was also measured with histogram analysis, using the same program and the same technique. Tissue echo levels were automatically calibrated to the value of 255 for the white pixels and 0 for the black pixels. The mean echo level of all pixels of the parenchyma of each thyroid lobe and of the ipsilateral strap muscle was counted and the hypogenecity index (HI) was calculated, according to the formula:

HI = mean echo level of all pixels of strap muscle/mean echo level of thyroid lobe. Conventionally, we considered that hypogenecity was present if only HI was greater than 1.

The time needed for imaging processing and for quantifying the vascularity of each thyroid lobe was 10-20 minutes.

Statistical analysis

Paired t-test was used to compare mean VI (%) of the right and the left thyroid lobe, in controls, patients with AITD type-1, 2, and 3. Following the application of Levene's test for homogeneity of variances, non-parametric analysis of variance (Kruskal-Wallis one way AN.O.VA) was used to compare VI (%) among controls and patients with AITD type-1, 2, and 3, and among controls and seropositive patients for anti-TPO or anti-Tg and seropositive patients for both anti-TPO and anti-Tg. The same statistical test was used to compare the mean age among controls and patients with AITD type-1, type-2 and type-3. Post hoc comparisons were made using Mann-Whitney U test with a downward adjustment of the level to compensate for multiple comparisons. Spearman's (r_s) correlation coefficient was calculated for the correlation of VI (%) with age, Basic Metabolic Index (BMI), TSH levels, hypogenecity index (HI), and total volume of thyroid gland among control subjects and patients with AITD type-1, 2, and 3. Chi-square test and parametric analysis of variance

were used to compare the females/males ratio and mean basic metabolic index (BMI) between healthy controls and patients with AITD type-1, type-2 and type-3. Receiver operating characteristic (ROC) curve for VI (%) (a plot of the sensitivity of VI or true positive rate to the false negative rate) was developed as a means for pretest probability of AITD. The best cut-off value of the ROC plot was determined to maximize the Youden index (sensitivity+specificity-1). Calculations were performed using the Number Cruncher Statistical System (NCSS/PASS 2000, Dawson Edition) and Statistical Software for Social Sciences (SPSS, v.13.0). $p < 0.05$ was considered statistically significant.

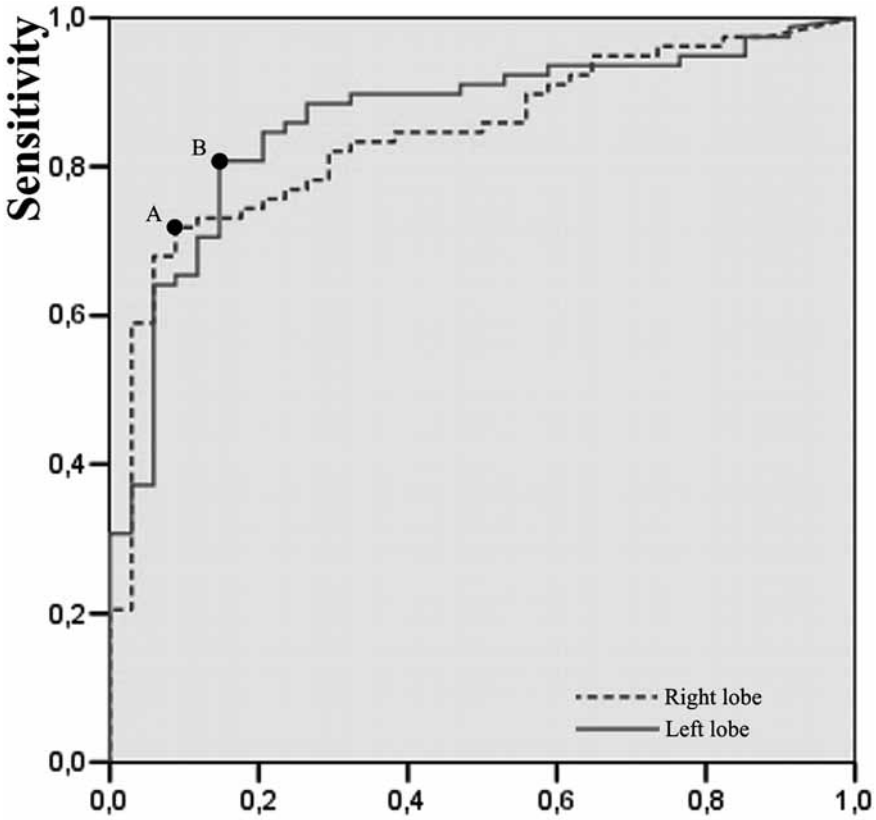
Results

No statistically significant differences were observed in mean VI (%) of the right and the left TL among controls (3.86 ± 3.91 vs 3.78 ± 3.4, P = 0.844), patients with AITD type-1 (9.58 ± 5.86 vs 10.26 ± 7.43, P = 0.5), patients with AITD type-2 (13.4 ± 13.49 vs 14.03 ± 16.42, P = 0.657), and patients with AITD type-3 (32.53 ± 15.45 vs 30.73 ± 14.27, P = 0.673). Patients with AITD type-1, AITD type-2, and AITD type-3 exhibited higher mean VI (%) measurements of both the right and the left TL compared to controls (8.22 ± 5.78, 13.49 ± 12.75, 26.42 ± 15.45, 3.63 ± 3.84, P < 0.001, and 9.49 ± 6.87, 13.8 ± 14.74, 24.67 ± 14.27, 3.45 ± 3.54, P < 0.001, respectively).

In patients with AITD, the area under the ROC curves for VI (%) of the right TL and left TL was 0.843 (P < 0.001) and 0.862 (P < 0.001) respectively. The best cut-off value of the ROC plot for the right and left TL VI was 7.44% and 5.57% respectively. The sensitivity and specificity for the diagnosis of AITD of right TL VI values greater than 7.44% were 71.8% and 91.2% respectively (point A, Fig. 2), whereas those of left TL VI values greater than 5.57% were 80.8% and 85.3% (point B, Fig. 2) respectively. For the differential diagnosis among patients with HT or GD, right TL VI (%) values greater than 14.75% (Youden index = 0.708) had 84.6% sensitivity and 86.2% specificity, while left TL VI (%) values greater than 16.8% (Youden index = 0.615) had 76.9% sensitivity and 84.6% specificity.

Compared to reduced echogenecity and irregular echo pattern, right TL VI values greater than 7.44% were more specific, but less sensitive for the diagnosis of AITD (Table II). Right and left TL VI was positively and significantly correlated with right and left HI ($r_s = 0.225$, $p = 0.016$ and $r_s = 0.302$, $p < 0.001$, respectively). Twenty-seven out of 31 euthyroid patients with Hashimoto's thyroiditis had increased right TL VI (> 5.57%) and 62.9% of them had right TL hypogenecity (HI > 1). Twenty-two out of 33 hypothyroid patients with Hashimoto's thyroiditis had increased right TL VI and 31.8% of them had right TL hypogenecity. All patients with Graves' disease had increased right TL VI, but only 23% of them had right TL HI > 1.

Statistically significant correlations were also found between right TL VI (%) and age ($r_s = -0.22$, $p = 0.02$), BMI ($r_s = -0.207$, $p = 0.029$), total thyroid volume ($r_s = 0.257$, $p = 0.006$), and between left TL VI (%) and age ($r_s = -0.185$, $p = 0.05$), BMI ($r_s = -0.225$, $p = 0.017$), and total thyroid volume ($r_s = 0.278$, $p = 0.003$).



	Vascularity index (%)	Sensitivity (%)	Specificity (%)
Right thyroid lobe	5.19	82.1	70.6
	7.44 (point A)	**71.8**	**91.2**
	9.44	59	97.1
Left thyroid lobe	3.83	88.5	70.6
	5.57 (point B)	**80.8**	**85.3**
	7.95	70.5	88.2

Fig. 2. — Receiver operating characteristic (ROC) curves for vascularity index of both right and left thyroid lobe showing the pretest probability of autoimmune thyroid disease.

The mean right TL VI (%) of controls was lower compared to patients who had only anti-TPO or anti-Tg, and those who had both anti-TPO and anti-Tg (3.63 ± 3.84, 13.26 ± 13.04, 13.91 ± 12.26, P < 0.001). Similarly, mean left TL VI (%) of controls was lower compared to patients who had only anti-TPO or anti-Tg, and those who had both anti-TPO and anti-Tg (3.45 ± 3.54, 14.07 ± 14.08, 13.68 ± 11.8, P < 0.001). No statistically significant differences in mean right TL VI (%) and left TL VI (%) were observed among patients who had both anti-TPO and anti-Tg, and patients who had anti-TPO or anti-Tg ($p = 0.587$ and $p = 0.727$, respectively).

Discussion

The findings of the present study suggest that left TL VI values greater than 5.57% exhibit 80.8% sensitivity

for the diagnosis of AITD whereas the sonographic appearance of autoimmune thyroiditis may vary, reflecting the phase and the severity of the disease process. For example, a diffuse hypoechoic pattern in a patient with lymphocytic thyroiditis has been shown to correlate with the replacement of the gland by lymphocytes and is highly predictive of either the existence or the future development of hypothyroidism (18). We calculated ROC curves for the mean VI (VI of right lobe+VI of left lobe/2, data not shown), but the Younden index was not as high as that of the left lobe. Thus, VI of the left lobe seems to be the best predictor of autoimmune thyroid disease in our study. The false negative values of VI could be attributed to the normal sonographic appearance of the thyroid gland in some patients, especially at early stages of AITD. Although the thyroid autoimmune process has already begun, thyroid tissue and vascularity may remain intact and thyroid echostracture changes may not be typical for AITD. It has been shown that sonographic appearance of thyroid gland may remain normal, even for 46 months following the diagnosis of Hashimoto's thyroiditis in children (19). Although similar data are not available for adult populations, it is probable that the 12-month follow-up period used in our protocol may be too short for the increased thyroid vascularity to become evident in some of the patients. Another reason for the low VI values in some of our patients could be the lack of hypervascularity found in patients with the atrophic form of thyroiditis (20). However, only a small proportion of the negative findings observed could be attributed to thyroid gland atrophy. Only one patient (1.3%) had heterogeneous and atrophic thyroid parenchyma, profound hypoechoic pattern, and coarse septations from fibrous band all consisting thyroid sonographic findings encountered in the atrophic form of thyroiditis (20). A further potential reason for a relatively low sensitivity of the VI in identifying AITD could be the inclusion of patients that have been receiving replacement therapy with levothyroxine. In order to avoid this confounding factor we have not included such patients in the studied population, because we had already observed that patients receiving levothyroxine for some years had lower mean values of VI, almost as low as healthy controls.

Our findings showed that right TL VI values greater than 7.44% have 91.2% specificity for the diagnosis of AITD. We observed that 3 out of the 34 healthy controls studied had VI (%) values greater than 7.44%, without any other sonographic findings suggestive of AITD and normal titres of thyroid autoantibodies. Although none of these subjects presented with any clinical or laboratory findings of AITD and still remain disease-free, seronegative thyroiditis could only be excluded if the follow-up period was longer (21). The possibility of increased VI (%) values in such subjects being an early sign of autoimmune thyroiditis remains hypothetical and needs to be addressed in a formal comparative prospective study. For our 34 healthy controls we found an overall mean VI equal to 3.55 %, similar to 3.67% found by Macedo TAA et al. in 84 healthy subjects (22). Increased vascularity in thyroid sonography could be diagnosed in patients with TSH secreting pituitary adenomas or resistance to thyroid hormone (23), acromegaly (24), and pregnancy (25); however patients with such diseases were initially excluded and no related signs, symptoms or typical laboratory findings were detected in any of our healthy controls or any of the patients at the start or during the study.

Although right TL VI values greater than 14.75% were sensitive and specific enough to differentiate patients with Graves' disease from those with Hashimoto's thyroiditis, in clinical practice we encountered a few difficulties. The two disorders are related in fundamental ways, as it has been proposed that GD develops on a background of thyroiditis. The progression from Graves' hyperthyroidism to chronic autoimmune thyroiditis and hypothyroidism is well-recognized (26) and the converse also occurs (27). Moreover, there are patients who have hypothyroidism one year, Graves' hyperthyroidism another, and hypothyroidism again later (28). Finally, ultrasonographic conversion from end-stage HD to Graves' hyperthyroidism may take some time to become evident, 30 months in a previously described patient (29). The follow-up period of our study was much shorter and in our opinion the VI at the time of diagnosis cannot provide further information for the conversion from one autoimmune disease to the other.

We compared the sensitivity and specificity of VI (%) to the corresponding of reduced echogenicity and irregular echo pattern and found the first to be more specific, but less sensitive for the diagnosis of AITD. The validity of using reduced thyroid echogenicity as a predictor of possible AITD in general has been found to be very good (18, 30). Pedersen OM et al. studied 452 patients and found the positive and negative predictive values of reduced thyroid echogenicity as an indicator of AITD to be 88.3% and 93%, respectively (31). Despite this, they found a higher rate of false negative values and they concluded that some patients presented with less severe markers of autoimmune process may have not evolve the typical sonographic findings. However, reduced thyroid echogenecity still remains one of the principal findings of AITD. We also observed that 88.3% of patients with AITD had irregular echo pattern, another important marker for early thyroid failure. The prognostic value of the evaluation of echogenecity and echo pattern has been found to be increased in the general population, as only a small minority of randomly selected subjects has increased levels of thyroid autoantibodies, when both sonographic characteristics are absent (32).

We also found similar VI (%) values in patients seropositive for both anti-TPO and anti-Tg, as compared to seropositive patients for anti-TPO or anti-Tg. Given that high levels of both anti-Tg and anti-TPO are closely related to autoimmune process and the development of goiter and hypothyroidism (21, 33), it could be proposed that seropositivity for both thyroid peroxidase and thyroglobulin autoantibodies represents a mechanism affecting mainly the gray-scale sonographic appearance and in a lesser extend the density of thyroid vessels in a sonographic section. The former was also supported by Vlachopapdopoulou et al, who observed that double seropositivity was associated with an acceleration of the time needed for the thyroid sonographic findings to change and become diagnostic for Hashimoto's thyroiditis (19).

In this study we used a semi-quantitative method for the evaluation of thyroid vascularity. Vascularity index represents the total area of vessels spots in each section of the thyroid lobe, but is not currently being used for the direct measurement of thyroid blood supply. Although the time needed for imaging processing and for

quantifying the vascularity of each thyroid lobe relatively short, an automated system would be more helpful in clinical practice. Nowadays, most of the high resolution apparatus have their own system for the measurements of the VI; however we preferred to use a wide spread available software for convenient reasons.

In conclusion, measurement of vascularity index is an easy, non-invasive, and reproducible method. Although it depends on operator experience, it can provide relatively prompt information on thyroid status and diagnose autoimmune thyroid disease with high specificity albeit relatively low sensitivity.

References

1. Bogazzi F., Bartalena L., Brogioni S., et al.: Thyroid vascularity and blood flow are not dependent on serum thyroid hormone levels: studies in vivo by color flow Doppler sonography. *Eur J Endocrinol*, 1999, 140: 452-456.
2. Baldini M., Castagnone D., Rivolta R., Meroni L., Pappalettera M., Cantalamessa L.: Thyroid vascularization by color Doppler ultsasonography in GD disease. Changes related to different phases and to the long-term outcome of the diasease. *Thyroid*, 1997, 7: 823-828.
3. Boi F., Loy M., Piga M., Serra A., Atzeni F., Mariotti S.: The usefulness of conventional and echo Doppler sonography in the differential diagnosis of toxic multinodular goiters. *Eur J Endocrinol*, 2000, 143: 339-346.
4. Becker D., Bair HJ., Becker W., et al.: Thyroid autonomy with color-coded image-directed sonography: internal hypervascularization for the recognition of autonomous adenomas. *J Clin Ultrasound,* 1997, 25: 63-69.
5. Bogazzi F., Bartalena L., Vitti P., Rago T., Brogioni S., Martino E.: Color flow Doppler sonography in thyrotoxicosis factitia. J Endocrinol Invest, 1996, 19: 603-606.
6. Bogazzi F., Bartalena L., Brogioni S., et al.: Color flow Doppler sonogaphy rapidly differentiates type I and type II amiodarone-induced thyrotoxicosis. *Thyroid*, 1997, 7: 541-545.
7. Nordmeyer J.P., Shafeh T.A., Heckmann C.: Thyroid sonography in autoimmune thyroiditis. A prospective study on 123 patients. *Acta Endocrinol* (Copenh), 1990, 122: 391-395.
8. Vitti P., Rago T., Mancusi F., et al.: Thyroid hypoechogenic pattern at ultrasonography as a tool for predicting recurrence of hyperthyroidism after medical treatment in patients with Graves' disease. *Acta Endocrinol*, 1992, 126: 128-131.
9. Vitti P., Rago T., Mazzeo S., et al.: Thyroid blood flow evaluation by color-flow Doppler sonography distinguishes Graves' disease from Hashimoto's thyroiditis. *J Endocrinol Invest*, 1995, 18: 857-861.
10. Ota H., Amino N., Morita S., et al.: Quantitative measurement of thyroid blood flow for differentiation of painless thyroiditis from Graves' disease. *Clin Endocrinol*, 2007, 67: 41-45.
11. Lyshchik A., Moses R., Barnes S.L., et al.: Quantitative analysis of tumor vascularity in benign and malignant solid thyroid nodules. *J Ultras Med*, 2007, 26: 837-846.
12. Ying M., Douglas K.S., Yung D.M.C., Lee E.S.T.: A semi-quantitative approach to compare high-sensitivity power Doppler sonography and conventional power Doppler sonography in the assessment of thyroid vascularity. *Thyroid*, 2009, 19: 1265-1269.
13. Hiromatsu Y., Ishibashi M., Miyake I., et al.: Doppler ultrasonography in patients with subacute thyroiditis. *Thyroid*, 1999, 9: 1189-1193.
14. Doufas G., Mastorakos G., Chatziioannou S., et al.: The predominant form of non-toxic goiter in Greece is now autoimmune thyroiditis. *Eur J Endocrinol,* 1999, 140: 505-511.
15. Koutras D.A., Alevizaki M., Tsatsoulis A., Vagenakis A.G.: Greece is iodine sufficient. *Lancet*, 2003, 2: 405-406.
16. Murakami Y., Takamatsu J., Sakane S., Kuma K., Ohsawa N.: Changes in thyroid volume in response to radioactive iodine for Graves' hyperthyroidism correlated with activity of thyroid-stimulating antibody and treatment outcome. *J Clin Endocrinol Metab*, 1996, 81: 3257-3260.
17. Schiemann U., Avenhaus W., Gellner R., Gross M.: Standardized grey-scale ultrasonography in Hashimoto's thyroiditis: correlation of hypoechogenicity values to autoimmune activity. *Eur J Endocrinol*, 2002, 15: 46-47.
18. Marcocci C., Vitti P., Cetani F., Catalano F., Concetti R., Pinchera A.: Thyroid ultrasonography helps to identify patients with diffuse lymphocytic thyroiditis who are prone to develop hypothyroidism. *J Clin Endocrinol Metab*, 1991, 72: 209-213.
19. Vlachopapadopoulou E., Thomas D., Karachaliou F., et al.: Evolution of sonographic appearance of the thyroid gland in children with Hashimoto's thyroiditis. *J Pediatr Endocrinol Metab*, 2009, 22: 339-344.
20. Vitti P., Lampis M., Piga M., et al.: Diagnostic usefulness of thyroid ultrasonography in atrophic thyroiditis. *J Clin Ultrasound*, 1994, 22: 375-379.
21. Vanderpump M.P.J., Tunbridge W.M.G., French J.M., et al.: The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. *Clin Endocrinol*, 1995, 43: 55-68.
22. Macedo T.A.A., Chammas M.C., Jorge P.T., et al.: Reference values for Doppler ultrasound parameters of the thyroid in a healthy iodine-non-deficient population. *Brit J Radio*l, 2007, 80: 625-630.
23. Bogazzi F., Manetti A.L., Tomisti L., et al.: Thyroid color flow Doppler sonography: an adjunctive tool for differentiating patients with inappropriate thyrotropin (TSH) secretion due to TSH-secreting pituitary adenoma or resistance to thyroid hormone. *Thyroid*, 2006, 16: 989-995.
24. Bogazzi F., Manetti L., Bartalena L., et al.: Thyroid vascularity in patients with active acromegaly. *Clin Endocrinol (*Oxf), 2002, 57: 65-70.
25. Hari Kumar K.V., Vamsikrishna P., Verma A., Muthukrishnan J., Meena U., Modi K.D.: Evaluation of thyrotoxicosis during pregnancy with color flow Doppler sonography. *Int J Gynecol Obstet,* 2008, 102: 152-155.
26. Tamai H., Kasagi K., Takaichi Y., et al.: Development of spontaneous hypothyroidism in patients with Graves' disease treated with antithyroidal drugs: clinical, immunological, and histological findings in 26 patients. *J Clin Endocrinol Metab*, 1989, 69: 49-53.
27. Takasu N., Yamada T., Sato A., et al.: Graves' disease following hypothyroidism due to Hashimoto's disease: studies of eight cases. *Clin Endocrinol* (Oxf), 1990, 33: 687-698.
28. Kraiem Z., Baron E., Kahana L., Sadeh O., Sheinfeld M.: Changes in stimulating and blocking TSH receptor antibodies in a patient undergoing three cycles of transition from hypo to hyper-thyroidism and back to hypothyroidism. *Clin Endocrinol* (Oxf), 1992, 36: 211-214.
29. Champion B., Gopinath B., Ma G., El-Kaissi S., Wall J.R.: Conversion to Graves' hyperthyroidism in a patient with hypothyroidism due to Hashimot's thyroiditis documented by real-time ultrasonography. *Thyroid*, 2008, 18: 1135-1137.
30. Sostre S., Reyes M.M.: Sonographic diagnosis and grading of Hashimoto's thyroiditis. *J Endocrinol Invest,* 1991, 14: 115-121.
31. Pedersen O.M., Aardal N.P., Larssen T.B., Varhaug J.E., Myking O., Vik-Mo H.: The value of ultrasonography in predicting autoimmune thyroid disease. *Thyroid,* 2000, 10: 251-259.
32. Vejbjerg P., Knudsen N., Perrild H., et al.: The association between hypogenecity or irregular echo pattern at thyroid ultrasonography and thyroid function in the general population. *Eur J Endocrinol*, 2006, 155: 547-552.
33. Gordin A., Lamberg B.A.: Spontaneous hypothyroidism in symptomless autoimmune thyroiditis: a long term follow-up study. *Clin Endocrinol* (Oxf), 1981, 15: 537-543.