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Thyroid nodule with arteriovenous malformation: under-recognized cause of increased vascularity

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Summary. Background: Head and neck arterio-venous malformations (AVM) are not frequent lesions and no thyroid cases have been reported to date; as hypervascular nodular lesions, they can be misdiagnosed as malignant.

Findings: We present two patients with palpable thyroid nodules with suspicions of malignancy based on the hypervascular imaging findings. Histologically, these lesions were well-defined adenomatous nodules with multiple interconnected blood vessels of variable size, many of them dilated and arranged predominantly at the periphery of the lesions. These findings characterize thyroid AVM in the background of adenomatous nodules. Age-matched euthyroid benign non-infiltrative follicular lesions without vascular component, adenomatous hyperplastic nodules (37) and follicular adenomas (21), during the same period (2 years) were retrieved to evaluate vascular markers. Compared with the non-nodular tissues and controls, the hyperplastic nodules with vascular malformation displayed significant mRNA overexpression for *VEGF-A, PDGF-A, PDGF-B,* and *eNOS*.

Conclusions: Vascular lesions of thyroid gland are rare and they can present as palpable nodules revealing well-defined edges, zonal blood vessel distribution and up-regulation of VEGF-related pathway and eNOS. These findings can help identify the true nature of these lesions.

Key words: Thyroid, Hyperplastic nodule, Increased vascularity, Arteriovenous malformation, Hemangioma, Vascular markers, Gene expression

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Introduction

Thyroid nodules are discrete lesions within the thyroid gland and represent a common clinical problem. Clinically, they present as lesions radiologically distinct from the surrounding parenchyma, which must be distinguished from palpable lesions with no distinct radiologic abnormalities. Such abnormalities do not meet the strict definition for thyroid nodules. Non-palpable nodules detected by ultrasound (US) or other anatomic imaging studies are termed incidentally discovered nodules or "incidentalomas". Non-palpable nodules have the same risk of malignancy as palpable nodules with the same size (Hagag et al., 1998).

The term "palpable nodules of the thyroid gland" is used for clinically discovered nodules. Although the majority of such lesions are benign, the diagnostic procedures still have to distinguish benign from malignant nodules using clinical assessment, imaging (US) and cytological evaluation by fine needle aspiration (FNA) (Cooper et al., 2009; Faquin, 2009). It is recommended that only nodules bigger than 1 cm should be evaluated, since they have a greater potential to be clinically significant cancers. Nodules smaller than 1 cm only require evaluation in case of suspicious US findings, associated lymphadenopathy, or history of head and neck irradiation or thyroid cancer in one or more first-degree relatives (Papini et al., 2002; Kwak et al., 2009).

Abreviations. AVM, Arteriovenous malformation; FNA, Fine needle aspiration; US, Ultrasound; Angiogenesis-related markers: eNOS, Endothelial nitric oxide synthase; PDGF, Platelet-derived growth factor; VEGF, Vascular endothelial growth factor; VEGFR, Vascular endothelial growth factor receptor; Thyroid-specific differentiation markers: NIS, Na-I simporter; Tg, Thyroglobulin; TPO, Thyroid peroxydase; TSHR, Thyroid stimulating hormone receptor

We present two cases of hypervascular thyroid nodules with inconclusive thyroid FNA that demonstrated a vascular malformation maintained by the expression of vascular markers within the nodular parenchymatous lesion.

Materials and methods

Selection and description of patients

We present two euthyroid women (36 and 57 years old) with no cervical irradiation or family history of thyroid diseases. The patients had a history of several attempts of inadequate FNA (at least two in each patient).

Radiological and pathological examination

Patients were evaluated by ultrasound and subjected to US-guided FNA with inconclusive findings that resulted in diagnostic thyroid lobectomy. Specimens were fixed in 10% buffered formalin and routinely embedded in paraffin.

Histological evaluation included the predominant growth pattern, type of cells and nuclear features (size, shape, chromatin distribution and nucleolus). Atypical features included the presence of solid or trabecular architecture comprising epithelial cells with one or more of the following nuclear features: overlapping, uneven size, irregular borders, pale chromatin, and prominent nucleoli. Vascular or capsular invasion was evaluated after examining at least one block per 5 mm of maximum tumor dimension; each block contained 2-3 pieces to sample the whole tumor-parenchyma transition.

Gene Expression of Vascular and Differentiation Markers

Gene expression analysis was determined by real time quantitative RT-PCR in a 7900HT Fast Real Time PCR System (Applied Biosystems, Foster City, CA, USA) after RNA extraction and cDNA synthesis, as previously described (Sponziello et al., 2011; Talat et al., 2011). Briefly, specific Assays-on-Demand gene expression products (Applied Biosystems) for each gene were selected to evaluate mRNA levels of angiogenesis-related genes (*VEGF-A; VEGFRs 1, 2, and 3; PDGF-A and -B; PDGFR-*α and *-*β*; eNOS*) and thyroid-specific markers of differentiations (*TSHR; NIS; Tg; TPO*) in each of the adenomatous hyperplastic nodules and in control groups including: contralateral non-nodular tissues, along with age-matched euthyroid adenonomatous nodules (37) and follicular adenomas (21) without vascular malformation received during the same period of time (2 years). The ß-actin gene was used as endogenous reference (Pre-Developed TaqMan Assay Reagents; P/N 4326315E; Applied Biosystems). Results, determined by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), were normalized to corresponding calibrator samples. Quantitative RT-PCR results were expressed as mean ± SD, and the statistical significance assessed by the Mann-Whitney nonparametric test; P values <0.05 were regarded as statistically significant. Data analysis was performed by means of StatView 5.0.1 software (SAS Institute Inc., Cary, NC, USA).

Results

Radiological and pathological findings

Both patients presented with palpable cold nodules (2 and 4.5 cm, respectively), revealing well-defined solid lesions on US studies (Figs. 1, 2). A significant increase of intranodular vascularity was observed using Doppler US (Figs. 1, 2). FNA smears revealed high cellularity with numerous clustered and isolated follicular cells showing minimal nuclear abnormalities in both cases. A small amount of colloid was identified in the background. These appearances were regarded as follicular-patterned lesion/neoplasm and diagnostic surgical excisions were performed.

In both index patients, the affected thyroid lobe and isthmus were excised and revealed single well-circumscribed solid lesions (2 cm and 4.5 cm of maximum dimension, respectively) without necrotic areas. After formalin fixation and paraffin embedding, 5 μm-thick serial sections were made for each specimen. No tumor displayed nuclear features typical of papillary cancer. No distant metastases were present at the time of diagnosis or during follow-up (66 months and 90 months).

Histologically, the lesions were non-encapsulated and revealed normo- and micro-follicular growth patterns (Figs. 1, 2). There was no evidence of parenchymatous, lymphovascular or perineural invasion. A remarkable proliferation of irregular and angulated blood vessels was observed in the background of fibrous stroma, predominantly at the periphery of the adenomatous nodules and in the inter-lobular compartment (Figs. 1, 2). Some of these vascular structures showed abrupt transition between thick- and thin-walled blood vessels, along with areas of extravasated red blood cells and siderophages, suggesting acute and chronic haemorrhage respectively. No desmoplastic reaction, high nuclear grade or necrosis was observed.

Gene expression findings

Compared with the non-tumor tissues from the same patients and the benign proliferative controls, the hyperplastic nodules with vascular malformation displayed significantly higher mean mRNA levels for all the growth factors *VEGF-A, PDGF-A* and *PDGF-B*, the receptor *VEGFR1, 2*, and the *eNOS* and not significantly different mean levels of *VEGFR3* and *PDGFR-*α and *-*β (Fig. 3). No *TSHR* mutations were identified in these nodules (Fig. 3). Euthyroid adenomatous hyperplastic nodules and follicular thyroid adenomas did not reveal

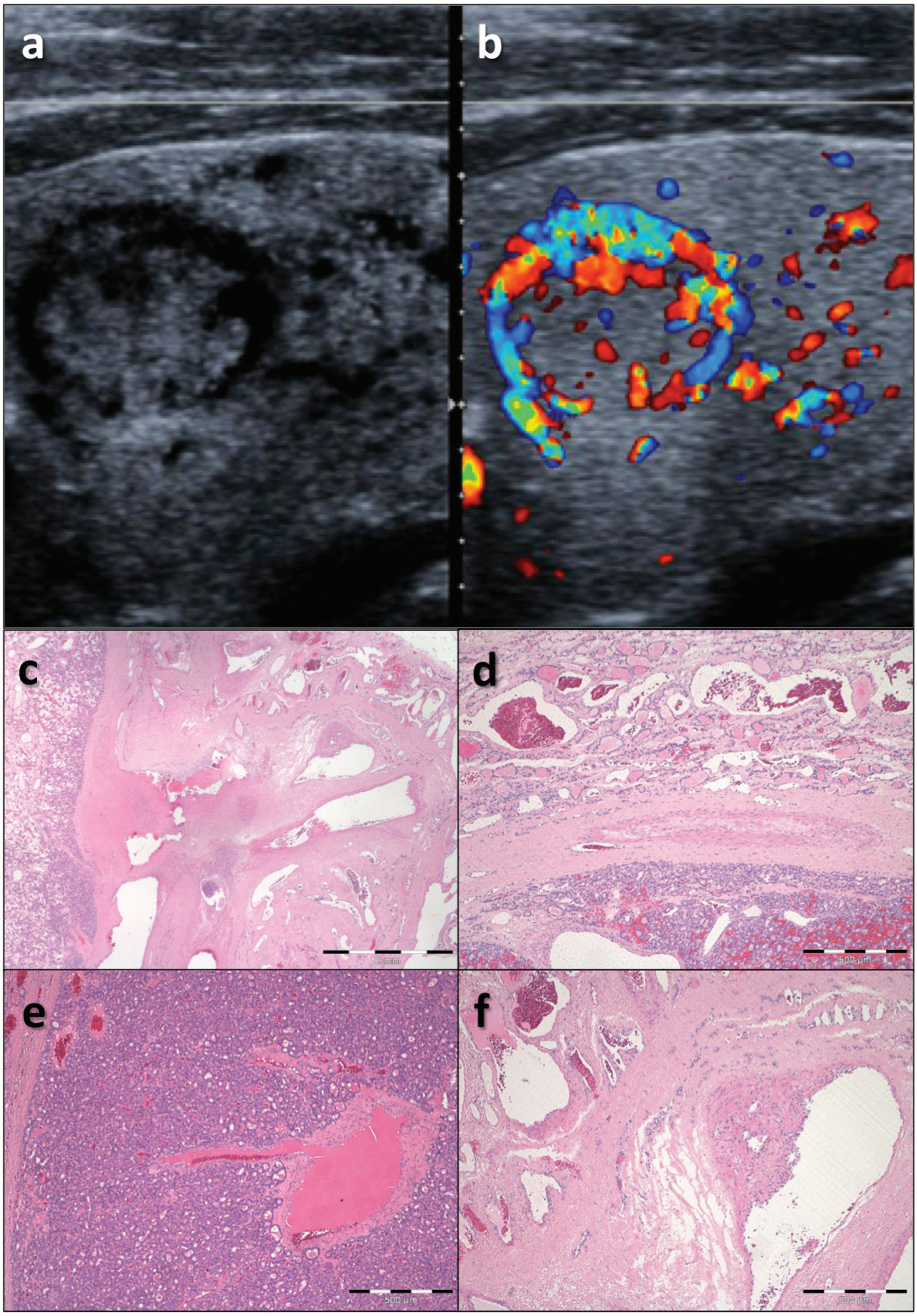

Fig. 1. a. Sonogram study showing a well-defined heterogeneous tumor. **b.** Doppler sonogram shows an increased vascular density, predominantly at the periphery. **c.** An irregular vascular proliferation within a fibrous stroma is present at the periphery of the thyroid nodule (hematoxylin-eosin). **d.** Irregular blood vessels are admixed with the follicular structures of the nodule (hematoxylin-eosin). **e.** Microfollicular pattern predominates in this hyperplastic nodule (hematoxylin-eosin). **f.** Dilated arterial and venous vascular structures are present within the internodular fibrous septa (hematoxylin-eosin). c, x 12.5; d-f, x 40

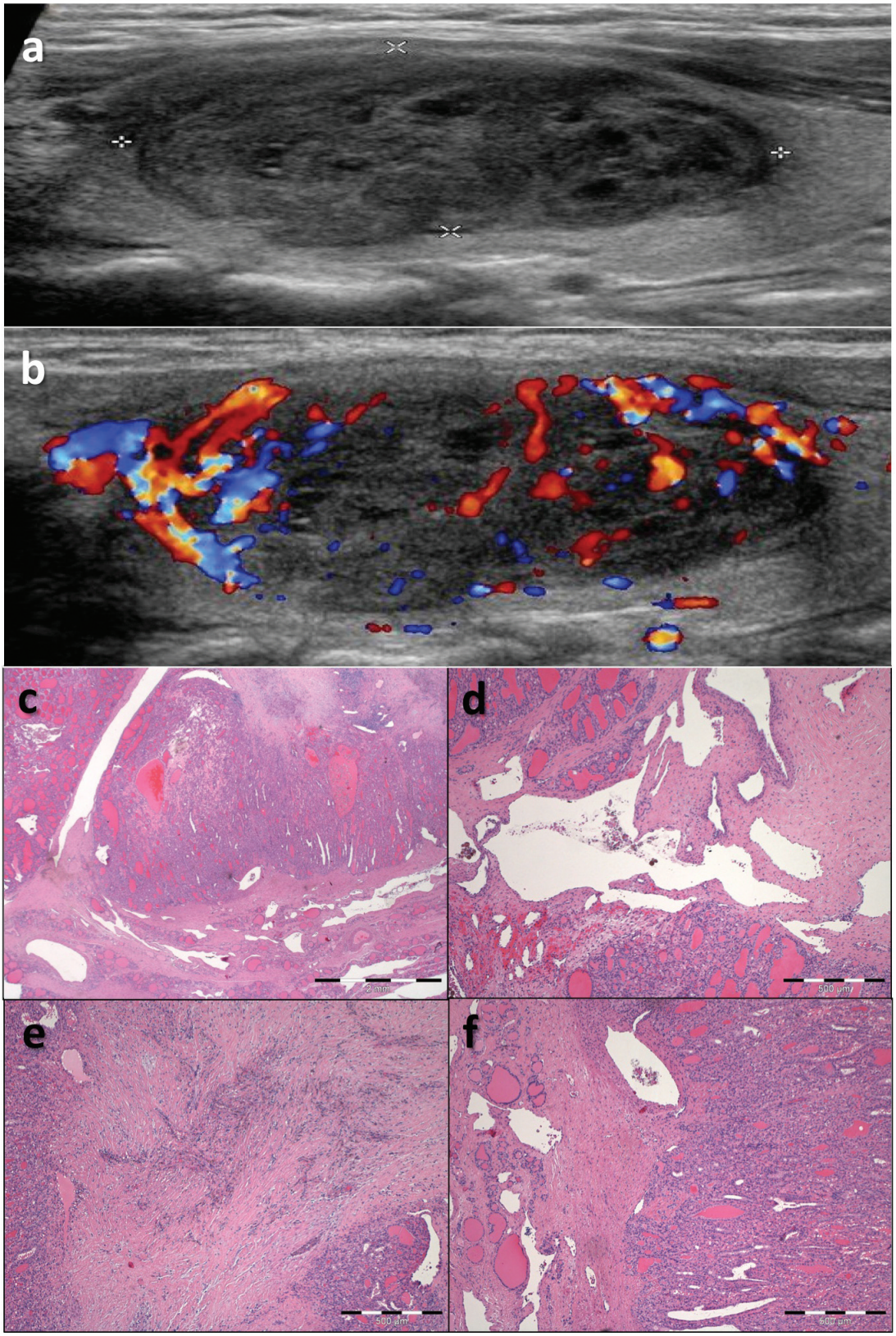

Fig. 2. a. Sonogram study showing a well-defined large lesion with heterogeneous appearances. **b.** Doppler sonogram study showing a highly increased vascularity within the tumor. **c.** Irregular vascular proliferation admixed within fibrous tissue is present at the periphery of the nodule (hematoxylin-eosin). **d.** Fibrous tissue surrounded by follicular structures and containing dilated arterial and venous blood vessels (hematoxylin-eosin). **e.** Fibrous septa with dilated blood vessels and containing hemosiderin-laden macrophages, suggestive of chronic hemorrhage (hematoxylin-eosin). **f.** Dilated vessels immerse in a fibrous tissue predominantly at the periphery of the follicular lession (hematoxylin-eosin). c, x 12.5; d-f, x 40

statistically significant differences with matched normal controls.

Discussion

Vascular lesions of thyroid gland are rare lesions that can present as palpable nodules, hypervascular on US studies. However, recognizing the zonal blood vessel distribution pattern and assessing the well-defined margins of the lesion can help identify the benign nature of AVM associated with adenomatous nodules.

Vascular lesions of the thyroid gland are extremely rare forms of thyroid nodules that often escape preoperative diagnosis, most cases being cavernous hemangiomas (Maciel et al., 2011). Some lesions are described secondary to trauma or FNA, and they have been considered as a reactive vascular proliferation that follows the organization of a hematoma (Tsang and Duggan, 1992). There are only nine cases of primary thyroid hemangioma reported in the literature. In these cases, a male predominance has been observed with variable presentation age and clinical features. No AVM in the thyroid gland has been reported in the literature to date (Maciel et al., 2011).

AVM is a non-neoplastic vascular lesion characterized by the presence of arterio-venous shunts, which reveal two distinctive variants: deeply seated and superficial cutaneous (also called cirsoid aneurysm or acral arterio-venous tumors). AVM should not be confused with juvenile cutaneous (cellular) hemangiomas as they do not regress spontaneously. When these lesions involve multiple tissue planes, they are termed angiomatosis. Clinically, pain is a frequent symptom and superficial cutaneous changes mimicking Kaposi sarcoma can be seen (pseudo-Kaposi sarcoma or acroangiodermatitis). Although the presence of shunting can be confirmed by auscultation, angiography is an essential tool to confirm the diagnosis and to assess the extent of the disease. Microscopically, the lesions are characterized by large number of vessels of different

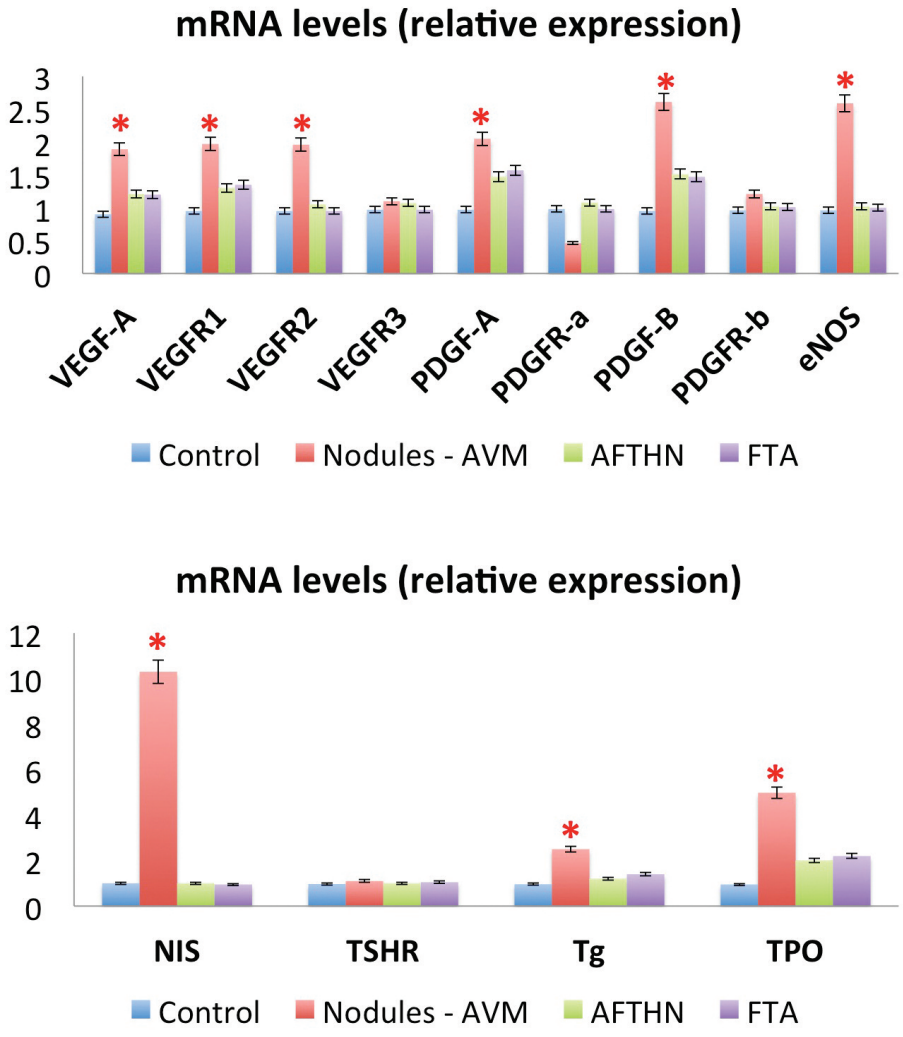

Fig. 3. Relative gene expression for each marker. AVM: Ateriovenous malformation; AFTHN: Adenomatous follicular thyroid hyperplastic nodules; FTA: Follicular thyroid adenomas *: Statistically significant differences in the expression between the nodule and the corresponding control.

size, which include veins and arteries with the former largely outnumbering the latter. Treatment of these lesions is difficult because of the degree of involvement, which also has to be determined by angiographic examination. Local recurrence is common because of difficulties in achieving complete excision (DeLellis et al., 2004).

No thyroid gland AVM has been reported to date, but a few cases have been described in head and neck, including mandible, parotid, submaxilar, and pineal gland (Stocks et al., 2007; Shailaja et al., 2012; Weil et al., 2012). Thyroid US is particularly useful in establishing the main lesion features, such as size and its solid or cystic nature; it is the study of choice in thyroid nodule assessment. US suspicion of malignancy is mainly based on findings like: large size, infiltrating margins, heterogeneity, presence of calcifications, and a highly vascularised pattern (Papini et al., 2002; DeLellis et al., 2004; Cappelli et al., 2006, 2007). The preoperative diagnosis is normally confirmed by FNA, which will determine the extent of the surgery. Although thyroid US provides reliable imaging parameters (Foschini et al., 2004; Mandel, 2004; Kwak et al., 2009), the diagnosis of vascular lesions can be very difficult even for experienced radiologists. Vascular lesions normally present as hypoechoic areas within the thyroid gland, without specific or distinctive characteristics. In this context, the presence of coarse calcifications is a reliable sign of hemangiomas. More specific studies such as magnetic resonance imaging, single photon angiography, patterns of distribution of B-flow imaging, and red blood cell scans may improve diagnostic ability (Brunese et al., 2008; Maciel et al., 2011).

The expression of VEGF receptors 1 and 2 genes was increased in the nodules, as described for follicular proliferative conditions (Viglietto et al., 1997; Talat et al., 2011). Thus, the VEGF-related pathway appears as a major effector for the vascularization of nodules, toxic adenomas and thyroid cancer. Our data also showed increased mRNA levels of both PDGF isoforms in nodules versus normal controls, not correlating with the expression of the correspondent receptors. A relationship between eNOS expression and vascular control during goiter formation and hyperthyroidism has already been reported, including cases of TSH receptor germline mutation (Colin et al., 1995). Our data also support a role for NO as a vascular relaxing factor in proliferative tissues. However, the activation of the NO pathway in concert with angiogenic factors has been described also in papillary thyroid carcinomas (Donckier et al., 2006). Investigation of the expression of follicular differentiation markers showed an expected increase in the mRNA levels of *NIS, TPO,* and *Tg*, consistent with the well-differentiated phenotype of these lesions. The lack of significant difference in the TSHR gene expression between nodules and controls would be expected, and the absence of TSHR mutations does not preclude alterations in this signal transduction pathway (cAMP or Ca-IP dependent) that can be activated by "internal feedback loops" involving adenylate cyclase, phosphodiesterase, or functional CREB expression (Celano et al., 2003). Finally, a role for environmental factors in addition to the genetic alterations may largely influence the gene expression profile (Arturi et al., 1998; Celano et al., 2003).

Our cases highlight the importance of assessing both the margins and shape of the lesion, oval-to-round well-defined limits suggesting benign behaviour of vascularized nodules, and the distribution-pattern of the blood vessels. The vascular proliferation is governed by VEGF-related pathway and eNOS. When blood vessels are distributed predominantly in the periphery of the nodule and within the nodule in a septal fashion, a benign lesion is more likely; this is particularly true in the absence of perilesional increase of vascularity (Cappelli et al., 2006, 2007; Kwak et al., 2009). Multiple FNA are frequently associated with patternless vessel distribution within the nodule and increased perinodular vascularization. Finally, we highlight the importance of considering the possibility of AVM in the thyroid gland during the evaluation of well-circumscribed nodules with hypervascular zonal pattern and a history at repeated attempts of thyroid FNA.

There is no financial disclosure or duality of interest to declare.
The protocol used in the study was approved by the Hospital Research Board and Ethical Committees and complied with their requirements.

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