Abstract
Objectives: To report our experience in the categorization of thyroid pathology using the sonographic parameters of malignancy, elastography with strain ratio measurement, and the correlation of our findings with the Bethesda cytological classification.

Materials and methods: A prospective observational study that included 137 thyroid nodules was conducted between September 2012 and April 2013. We excluded 10 cases with Bethesda categories III-IV. Ultrasonography, power Doppler, Micropure imaging and elastography with strain ratio measurement were performed, as well as ultrasound-guided fine needle aspiration cytology (FNAC) (with a cytologist present during the procedure). The Bethesda classification was used. All studies were performed by the same operator using a Toshiba Apio 400 ultrasound unit. Statistical data were analyzed using IBM SPSS Statistics 20.

Results: We studied 127 nodules in patients with a mean age of 59±16 years; 82% were female; 120 nodules (94%) were classified as Bethesda II. The average strain ratio for Bethesda I-II nodules was 1.94±2.12 vs. 7.07±5.46 for Bethesda V-VI nodules (p:0,048). An elastography strain ratio $\leq 2$ (87 of 127 nodules) had a sensitivity of 85.7% and a specificity of 81.7% for predicting Bethesda associated with benign pathology, with a negative predictive value (NPV) of 99% and a positive predictive value of 15%.

Conclusion: The elastography strain ratio allowed ruling out malignant nodules with a strain ratio $\leq 2$ and a NPV of 99%, improving the selection of patients for FNAC. The increase in the elastography strain ratio was associated with a higher probability of malignant thyroid pathology, although no cutoff value could be established because of the low number of cases with Bethesda V-VI nodules.

Keywords
Thyroid nodules; Elastography; Strain ratio; Bethesda cytological classification

Introduction
Thyroid nodular disease is a very common medical condition and its frequency increases with the advancing age of the population. Most cases are benign. Conventional ultrasound is very sensitive for the detection of this entity, but may not always be able to diagnose its nature. This is reflected in the fact that no single ultrasound (US) criterion can attain enough sensitivity to determine the positive predictive value of malignancy. Combining US criteria of malignancy would increase specificity at the expense of lowered sensitivity. Fine needle aspiration (FNA), especially when guided by ultrasound, is considered as the most reliable diagnostic method for the evaluation of thyroid nodules. However, this requires an adequate selection of nodules for FNA, given the high prevalence of nodules detected by this technique. Furthermore, for nodules that undergo FNA, 10 to 20% of results are inadequate or indeterminate; therefore, the quest for highly accurate noninvasive methods for detecting thyroid
malignancy is ongoing. Elastography combines a variety of techniques used to measure parameters related to tissue stiffness, which in turn are used to characterize the different pathologies. Although this method was described two decades ago, it was only in recent years with the advent of new technologies that it emerged as a real-time application on ultrasound machines. In oncology, this technique has been used for the evaluation of various pathologies, such as cancer of the breast, prostate, lymph nodes, liver, cervix, salivary glands, pancreas and thyroid. To date, approximately 20 reports have been published on the use of ultrasound elastography for the evaluation of thyroid nodules. These series have included 34 to 309 nodules and have used FNA and conventional surgery as the reference standard. Even though results have been heterogeneous, elastography has shown a higher diagnostic accuracy than conventional ultrasound, to a large extent because the values obtained by this technique were supported by direct elastographic testing of thyroidectomy specimens, which have shown that the stiffness of malignant nodules is significantly higher than that of benign nodules and normal thyroid parenchyma.

The aim of this study is to report our experience in the categorization of thyroid pathology using the sonographic parameters of malignancy, elastography with strain ratio measurement, and the correlation of our findings with the Bethesda classification.

Materials and methods

Population

This was a prospective observational study that included 124 patients with 137 thyroid nodules evaluated between September 2012 and April 2013. The study was approved by the Ethics Committee at Hospital Militar Central. As the elastography ratio was correlated with the Bethesda cytological classification, we excluded 10 cases with Bethesda category III-IV, with diagnostic confirmation of malignancy performed by histology. Patients were referred for ultrasound-guided fine needle aspiration after endocrinologic evaluation.

Ultrasound equipment

Images were acquired using a Toshiba Apio 400 (Tokyo) ultrasound scanner and all scans were performed by the same operator. Each lesion was evaluated by conventional ultrasound assessing whether it was a solitary or multinodular goiter. The location (right, left, both sides, isthmus) of the nodule, as well as its features (cystic, complex or solid), echogenicity (iso, hyper or hypoechoic), margins (regular or irregular), halo (complete or incomplete) and size were considered. The Doppler pattern (peripheral, central, peripheral and central or non-vascular) and the presence or absence of thyroiditis pattern were evaluated.

Elastography and Micropure

Each lesion was examined by MicroPure imaging, a microparticle visualization technique, as complementary testing, and the presence or absence of microparticles was determined using software integrated to the ultrasound machine. For elastographic testing, all 127 nodules were examined by the semiquantitative elastographic technique (strain elastography) using a Toshiba Apio 400 (Toshiba Medical Siemens, Tokyo) ultrasound scanner with a 5-12 MHz transducer. Lesions were in turn evaluated by conventional qualitative analysis, by semiquantitative and elasticity values, and the soft tissue deformation ratio was determined using measurement software included in the elastography module (fig. 1). To perform the necessary compression during the elastography of the thyroid, a linear high-frequency transducer was positioned in contact with the skin surface of the anterior neck, using gel as conductive material. The field of view included both the thyroid nodule and adjacent thyroid parenchyma, and for a better anatomic localization, the dual mode was selected; therefore the same structures were seen in grayscale images. Subsequently, several cycles of compression were applied via the transducer in the direction of the beam axis (fig. 2).

Figure 1 Grayscale axial image with the corresponding real-time elastogram of a thyroid nodule. The lesion appears blue, which is suggestive of a stiff lesion.
To assist the operator in optimizing the compression technique and to select representative elastograms for analysis, we used a system that gives compression techniques a numerical score and a graphic scale in real time (fig. 3).

**Fine needle aspiration methodology**

FNA was performed according to the following protocol:
1. The patient's informed consent was obtained;
2. A screening test for coagulation was previously performed;
3. For FNA, the patient was placed in a supine position with the neck slightly hyperextended. After the lesion was localized, the skin was cleansed with a 10% povidone-iodine solution and the transducer was placed with a sterile cover. Povidone-iodine solution was used as conductive material. Local anesthesia was applied only in large fluid-filled nodules, with the aim of completely aspirating the fluid with thicker needles. In these cases, 1-2 ml of lidocaine without epinephrine was injected into the subcutaneous tissue and thyroid capsule using a 25G needle.
Specimens were collected using fine needles (preferably 25G), attached to a 10-cc syringe. The sample was obtained by capillary action, after multiple cutting maneuvers up and down the lesion. Only one or two aspirations were performed during the procedure with all needle movements being continuously visualized in real time. Continuous aspiration during the entire procedure was reserved only for hard nodules from which specimens could not be obtained by capillary action. During the procedure, the transverse plane was generally used for better visualization of all neck structures. The use of color Doppler and power Doppler was of vital importance to avoid any blood vessels in the needle path and thus reduce blood sampling during puncture.

Patients were instructed not to swallow or speak during the procedure in order to avoid any movement of the gland. After the procedure, all patients remained in observation for 30 minutes and were instructed to visit the emergency room if neck swelling occurred.

In this series, no complications were detected. The collected material was placed on glass slides, smeared, and fixed in 95% ethyl alcohol. In some cases, the syringe was rinsed with normal saline solution to obtain any remaining cells for further testing. For cytology, two modalities were adopted: intraprocedural and delayed cytologic evaluation. Intraprocedural cytologic examination, with a pathologist present at the site of ultrasound, was performed in all cases. Once
a specimen was fully examined and proved to be quantitatively and qualitatively adequate, the interventionist physician was informed of its adequacy for delayed examination. Otherwise, repeat FNAs were performed (following the above-mentioned steps) until adequate material was obtained.

**Delayed cytologic examination**

The remaining smears were stained using the Papanicolaou technique and were sectioned and mounted in synthetic Canada balsam.

The Bethesda System for Reporting Thyroid Cytopathology, 2009 was the diagnostic method adopted. This system offers a six-category scheme and strict and specific cytopathologic criteria for unifying terminology, thus facilitating interdisciplinary communication among the different health-care professionals managing thyroid nodular disease. Hence, the Bethesda system for reporting thyroid cytopathology is divided into:

- Category 1: nondiagnostic or unsatisfactory (fig. 4).
- Category 2: benign (figs. 5-7). This category includes a benign follicular nodule (adenomatoid nodule, colloid nodule, etc), lymphocytic thyroiditis (Hashimoto thyroiditis) in the adequate context, (subacute) granulomatous thyroiditis and other diagnoses (such as acute thyroiditis or Riedel’s thyroiditis).
- Category 3: atypia or follicular lesion of undetermined significance (fig. 8). The degree of cellular atypia is higher than in specimens with clearly benign changes.
- Category 4: follicular neoplasm or suspicious for follicular neoplasm (specify if it is a Hurtle cell lesion) (fig. 9). This category applies to smears consisting mostly of abnormal arrangements of follicular cells, represented mainly by crowded grouping, microfollicles or both.
- Category 5: suspicious for papillary, follicular or medullary carcinoma, metastasis, lymphoma and other diagnoses. The specimen has some malignant features that allows diagnostic suspicion but are insufficient for confirmation.
- Category 6: malignant (fig. 10). This category includes cytomorphologic findings conclusive of malignancy: papillary, follicular, medullary or undifferentiated (anaplastic) carcinoma, metastasis, Hodgkin’s lymphoma and other diagnoses.

![Figure 8 Morular cellular structure with oxyphilic features, slight anisokaryosis and partial nuclear grooves. Bethesda III.](image)

![Figure 9 Monotonous pattern of epithelial cells in microfollicular architecture. Bethesda IV.](image)

![Figure 10. Neoplastic cells with nuclear grooves. Bethesda V-VI.](image)
Statistical analysis

The statistical analysis was performed using the IBM SPSS Statistics 20 software. Continuous variables were expressed as mean ± standard deviation (SD) and dichotomous variables were expressed as proportions. Of continuous variables was evaluated by the Kolmogorov-Smirnov test. Univariate inferential statistical analysis was performed using T test for 2 independent samples (parametric variables) and test of proportions (dichotomous variables). For multivariate analysis, multiple logistic regression analysis was the chosen method. Statistical significance was considered when $p \leq 0.05$ (two-tailed test). For evaluating sensitivity and specificity, the cutoff point was examined by ROC curve and then data were analyzed in a 2x2 contingency table.

Results

Finally 127 nodules were evaluated. Patients (82% female) had an average age of 59±16 years. Of the lesions analyzed, 120 were classified as Bethesda II (94%), with irregular margins (15%) and positive Micropure findings (13%), while those lesions classified as Bethesda V-VI had irregular margins (43%; $p: 0.05$) and positive MicroPure findings (57%; $p: 0.010$).

The mean strain ratio for Bethesda I-II nodules was 1.94±2.12 vs. 7.07±5.46 for Bethesda V-VI nodules ($p:0.048$). An elastography strain ratio $\leq 2$ (87 of 127 nodules) had a sensitivity of 85.7% and a specificity of 81.7% for predicting Bethesda associated with benign pathology, with a negative predictive value (NPV) of 99% and a positive predictive value of 15% (figs. 11-14).

Figure 11 (a) Gray scale US image showing a space-occupying solid lesion with liquid centre, net margins and complete halo of 27.1 mm in maximum diameter. (b) Power Doppler examination of the same lesion reveals predominantly peripheral vascularity. (c) Micropure imaging shows a lesion with no microcalcifications. (d) The Elastogram confirms the similarity between the color scale of the lesion and the surrounding healthy parenchyma. Strain ratio 0.72. Adenomatous nodule by cytology – Bethesda II.
Discussion

Elastography combined with conventional ultrasound is a new imaging modality that allows real time assessment of the structural organization of tissues, comparing the hardness of a lesion in respect to the surrounding normal parenchyma. Thus, tissue elasticity analysis provides information that, combined with data obtained from conventional and Doppler ultrasound may assist in the sonographic diagnosis of malignity.

Elastography was initially described by Ophir and further improved by Pesavento. By this technique, tissue is compressed and the resulting deformation of the tissue is reflected in an image. As a diagnostic imaging modality, this technique has been used for the evaluation of various organs (liver, breast, prostate, heart, soft tissue and blood vessels), adding structural information to the morphological data provided by conventional ultrasound. Every tissue in the body has typical mechanical properties and it behaves accordingly in response to the compression applied via the transducer, displaying a specific image on the scanner.

Elastographic imaging techniques are based on the hypothesis that healthy tissues deform more than lesions, and these differences can be quantified and differentiated in images. The elasticity values obtained for various conditions are closely related to the matrix of such diseases. Hence, we can infer that a malignant lesion will have a lower elasticity value than a benign lesion or normal tissue.

Figure 12 (a) Gray scale US image showing a space-occupying lesion with septation and fluid content, 9.3 mm in maximum diameter. (b) Power Doppler examination of the same lesion reveals peripheral vascularity. (c) Micropure imaging shows a lesion without microcalcifications. (d) The elastogram confirms the similarity in the color scale between the lesion and the surrounding healthy parenchyma. Strain ratio 0.19. Colloid nodule – Bethesda II.
There are two well-differentiated types of elastography: semi-quantitative (strain) elastography and quantitative (shear-wave) elastography. The former obtains information about the elasticity of different tissues by means of the deformation that occurs as a result of the compression applied by the transducer or other methods (such as the patient’s breathing movements or the high-intensity ultrasound waves). This information is displayed in a color map and the strain ratio is obtained by comparing the percentage of tissue deformation of the lesion and of the surrounding normal tissue. Quantitative elastography measures the propagation speed of ultrasound waves known as transverse waves or shear waves, which are characterized by being perpendicular to conventional US waves and having a speed that is proportional to tissue elasticity. The systems using this method express elasticity values in units of velocity or directly in kilopascals (kPa). This type of elastography requires excitation with ultrasound waves of a higher intensity than the ones used in 2D or Doppler ultrasound. The advantage of shear-wave elastography over strain elastography is the reduction of inter- and intra-observer variability (i.e. improved reproducibility). However, some recent reports indicate that, while quantitative elastography has the highest specificity, its sensitivity is lower than that of the semi-quantitative modality14,15.

A vast majority of the nodules undergoing FNA in our series (94%) were benign, in agreement with the literature. The addition of this method allows for a better selection of nodules for FNA. Thus, a nodule of regular margins with nega-

Figure 13 (a) Gray scale US image showing a solid space-occupying lesion with non-defined margins, 18.9 mm in maximum diameter. (b) Power Doppler examination of the same lesion reveals a high degree of central and peripheral vascularity. (c) Micropure imaging shows a lesion without microcalcifications. (d) The elastogram confirms the similarity in the color scale between the lesion and the surrounding healthy parenchyma. Strain ratio 1.26. Lymphocytic thyroiditis – Bethesda II.
tive findings at Micropure imaging and strain ratio < 2 might undergo follow-up, while a nodule with irregular margins, positive findings at Micropure imaging and strain ratio > 2 should undergo FNA, independently of its size, to rule out malignancy.

Even if we could not obtain a strain ratio above which the diagnosis of malignancy would be accurate (because of the small number of cases of malignant disease available to date), we did find a statistically significant difference between benign and malignant disease. Even more, the strain ratio < 2 showed acceptable sensitivity and specificity for predicting Bethesda associated with benign pathology, making it possible to establish this cutoff point.

For the statistical analysis of this study, nodules classified as Bethesda III and IV were excluded because of the impossibility of performing a comparative study. However, we consider that it is precisely in these categories, gray areas of thyroid pathology, that this methodology could be very useful for decision-making. These cases will be analyzed as soon as a larger number is available.

As regards the limitations of elastography, we may first mention operator dependence, but in our series we have also found that in solid-cystic nodules, compression is applied over the solid portion, which displaces the surrounding fluid, and thus the strain ratio obtained is higher than the real one. Furthermore, the presence of calcifications in benign nodules results in higher values that may be interpreted as malignant findings; nodules located in areas posterior or adjacent to the

Figure 14. (a) Gray scale US image showing a solid space-occupying lesion with complete halo, 9 mm in maximum diameter. (b) Power Doppler examination of the same lesion reveals central and peripheral vascularity. (c) Micropure imaging shows a lesion without central microcalcifications. (d) The elastogram confirms a difference in the color scale between the lesion (blue) and the surrounding normal tissue (green). Strain ratio 6. Papillary carcinoma – Bethesda V-VI.
trachea were difficult to compress, and the strain ratios were not reproducible in different measurements; in thyroiditis and multinodular goiter, the thyroid parenchyma is stiffer, which impedes obtaining reliable elastographic values. Finally, large thyroid nodules with minimal surrounding healthy parenchyma are not suitable for elastography because their stiffness cannot be compared against that of normal tissue. Attempts are currently being made to determine the definitive role of elastography within the diagnostic algorithm for thyroid nodules. For the time being, the elastographic technique is useful to define the mechanical features of nodular lesions, and provides further data to determine whether a lesion is malignant or benign. Thus, it is also useful for optimizing the pre-selection of patients for FNA.

Conclusions

In line with the proposed objectives, in our experience the elastography strain ratio allowed ruling out malignant nodules with a strain ratio ≤ 2 and a NPV of 99%, improving the selection of patients for FNA. The increase in the elastography strain ratio was associated with a higher probability of malignant thyroid pathology, although no cutoff value could be established because of the low number of cases with Bethesda V-VI nodules. The various pathologies included in the Bethesda II category could not be classified by means of the elastography strain ratio.

Conflicts of interest

The author declares no conflicts of interest.

References