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Suspicious Thyroid Fine Needle Aspiration Aspiration Biopsy: TSH as a Malignancy Marker?

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1. Introduction

Thyroid nodules are common, affecting from 5 to 15% of the population (Tunbridge et al., 1977; Vander et al., 1968). Thyroid cancer, on the other hand, is uncommon and represents only 5% of all nodules, with an incidence in the United States of 1/10,000 inhabitants (Davies & Welch, 2006). Among thyroid neoplasms, the differentiated carcinomas (DTC) are the most frequent, and are responsible for about 75% of the malignant nodules of this gland (Schlumberger & Pacini, 1997).

Despite the fact that the great majority of thyroid lesions are benign and the mortality rate due to thyroid cancer is low (Schlumberger & Pacini, 1997), the incidence of thyroid cancer is increasing at a rate of greater than 5% per year (Davies & Welch, 2006). Thus, it is important to identify the nodules which are malignant and require surgical treatment.

The method of choice for the diagnostic evaluation of thyroid nodules is fine needle aspiration biopsy (FNAB) (Bennedbaek et al., 1999; Bennedbaek & Hegedus, 2000; Cooper et al., 2006; Schlumberger, 1998). FNAB is both highly sensitive (65 – 98%) and highly specific (72 – 100%) (Gharib & Goellner, 1993; Mazzaferri, 1993; Sherman, 2003), and provides satisfactory diagnostic results in 80% of cases, with an increase of this percentage after a new FNAB (Gharib & Goellner, 1993). In cases of DTC, FNAB has been shown to be particularly useful in the diagnosis of papillary carcinoma (PC). However, in cases of follicular (FC) and Hürthle carcinoma (HC), as in the case of the follicular variant of PC (FVPC), FNAB is useful only as a screening test. In these cases FNAB indicates the corresponding cytological pattern (follicular or Hürthle), but is not able to differentiate benign tumors from the malignant tumors. In these cases, the patients undergo surgery for histological analysis and definitive diagnosis (Faquin & Baloch, 2010; Tuttle et al., 1998).

Faced with the uncertainty of the diagnostic evaluation of thyroid nodules, several clinical risk factors (Kimura et al., 2009; Tuttle et al., 1998), imaging tests (Wiest, 1998; Frates, 2006) and molecular markers (Melck & Yip, 2011) have been proposed as malignancy indicators. Recently, some studies have reported an association between increased serum levels of

thyroid stimulating hormone (TSH) and thyroid cancer (Boelaert et al., 2006; Gul et al., 2010; Jonklaas et al., 2008). However, this relationship has not yet been established for the more doubtful cases, such as those with an inconclusive cytological diagnosis for FC or HC.

The objective of this study was to evaluate whether the TSH serum levels can help to differentiate benign cases from the malignant cases in patients with an FNAB that shows a follicular or Hürthle pattern.

2. Material and methods

This retrospective study was approved by the Committee of Ethics in Research of Botucatu Medical School (FMB) of the Sao Paulo State University - UNESP (protocol number 3626-2010).

We analyzed the cytological reports from patients carrying thyroid nodules that were submitted for thyroid FNAB at the Clinics Hospital FMB–Unesp between the years of 2003 and 2008. Of these, 59 cases with suspicious or inconclusive cytological diagnosis for FC or HC were selected. We included those nodules that presented a follicular or Hürthle pattern with the following reported descriptions: "follicular lesion," "follicular tumor," "follicular neoplasia," "Hürthle follicular lesion," "Hürthle tumor" or "Hürthle neoplasia." The medical data of these patients were evaluated, and we found 31 cases that were submitted to surgery and with histological diagnosis. The effective study sample consisted of 28 women and three men, with an average age of 52.1 years.

The patients were divided into two groups according to the presence (group M) or absence (group WM) of a histological diagnosis of malignancy. Pre-operative TSH serum levels were compared between group M and group WM. The two groups were also compared in regards to gender, age, smoking history, previous exposure to radiation and free thyroxine (FT4) serum levels. These same comparisons were performed after the exclusion of the cases which presented hypo- or hyperthyroidism.

The histological diagnosis of malignancy was based on the criteria set by the World Health Organization (WHO). Reports of PC, FC, HC and FVPC were considered to be malignant, and reports of follicular adenoma, Hürthle adenoma, colloid goiter and Hashimoto's thyroiditis were considered to be benign (DeLellis et al., 2004).

The serum levels of TSH and FT4 were obtained from the medical records. The average pre-operative hormone levels of each patient were determined by the average of three separate test results for these hormones, which were collected at different times up to one year prior to surgery. TSH and FT4 were measured by chemiluminescence, with a normal range of 0.8-1.9 ng/dL and 0.4-4.0 mUI/mL, respectively (DPC, Los Angeles, CA). Thyroid function was considered normal when TSH and FT4 were within normal reference ranges; hypothyroid, when TSH was elevated; and hyperthyroid, when TSH was suppressed.

2.1 Statistical analysis

The collected data were charted in a Microsoft Excel® worksheet (Microsoft Corporation, EUA) and submitted to statistical analysis through the computer program SPSS/Windows (version 10.0.7). To study the association between the qualitative variables, we used the Chi-square test. For the quantitative variables, we used the Student's T test. The significance level was of 5% (Zar, 1999).

3. Results

Of the 31 cases submitted to surgery, 14 showed malignancy upon histopathological analysis (group M). The malignancies included nine PCs, three FCs (one case with both PC and FC), one HC, one Hürthle tumor and one follicular tumor of uncertain malignant meaning. Thus, the concordance of suspicious or inconclusive FNAB for FC or HC with malignancy was 45.2% and the concordance with FC or HC was 12.9%. 17 patients (54.8%) had benign histological reports (group WM).

The M and WM groups did not differ significantly as to serum levels of TSH and FT4 ($p>0.05$). There were no significant differences in age or gender distribution ($p>0.05$). There were two smokers in the WM group and no smokers in the M group. In addition one patient in the WM group had previous exposure to radiation (Table 1).

Seven patients (22.6%) presented hypo- or hyperthyroidism and were under treatment with thyroid medication. These included two (28.6%) patients from the M group and five (71.4%) patients from the WM group. After excluding such cases, the M and WM groups still did not differ in regards to the analyzed parameters (Table 2).

Data	Group		P
	M	WM	
Female Gender n (%)**	12 (85.7)	16 (94.1)	0.43
Age (years)*	48.8 ± 12.5	54.8 ± 13.9	0.21
Smoking n (%)***	0 (0.0)	2 (100.0)	-
Previous exporsure to radiation ***	0 (0.0)	1 (100.0)	-
TSH (mUI/L)*	2.04 ± 1.74	3.08 ± 2.67	0.22
FT4 (ng/dL)*	1.56 ± 0.59	1.35 ± 0.19	0.17

* Average ± standard deviation (Student's T test); ** Chi-square test; ***not submitted to statistical analysis due to small sample number. M: presence of malignancy; WM: absence of malignancy; TSH: thyroid stimulating hormone; FT4: free thyroxine.

Table 1. General data from 31 patients, with fine needle aspiration biopsies (FNAB) with suspicious or inconclusive cytological diagnosis for FC or HC, submitted to thyroidectomy, according to the final histological diagnosis of presence (group M) or absence (group WM) of malignancy.

Data	Group		P
	M	WM	
Female Gender n (%)**	10 (83.3)	11 (91.7)	0.54
Age (years)*	50.4 ± 11.3	52.3 ± 15.8	0.74
Smoking n (%)***	0 (0.0)	2 (100.0)	-
TSH (mUI/L)*	1.79 ± 1.03	1.81 ± 1.16	0.96
FT4 (ng/dL)*	1.59 ± 0.64	1.33 ± 0.18	0.19

* Average ± standard deviation (Student's T test); ** Chi-square test; ***not submitted to statistical analysis due to small sample number. M: presence of malignancy; WM: absence of malignancy; TSH: thyroid stimulating hormone; FT4: free thyroxine.

Table 2. General data from 24 patients, without hypo- or hyperthyroidism, with fine needle aspiration biopsies (FNAB) with suspicious or inconclusive cytological diagnosis for FC or HC, submitted to thyroidectomy, according to the final histological diagnosis of presence (group M) or absence (group WM) of malignancy.

4. Discussion

Considering that in DTC the follicular cell physiologic characteristic of TSH responsiveness is preserved (Biondi et al., 2005; Carayon et al., 1980; Ichikawa et al., 1976), it is presumable that a greater stimulation provided by this hormone might be a contributing factor to the tumor genesis. In fact, some studies have reported an association between increased TSH serum levels and thyroid malignancy. Boelaert et al. have observed that the malignancy risk of a thyroid nodule rises along with the TSH serum levels, indicating that these levels are an independent prognostic factor for malignancy and may be used in conjunction with FNAB in detecting such tumors (Boelaert et al., 2006). Gul et al. have reported that low serum levels of FT4, associated with high levels of TSH (still within the normal patterns), are associated with a greater probability of thyroid cancer, independent of gender and goiter type (Gul et al., 2010). This study also suggests that hormone levels may be used in conjunction with FNAB in diagnosing thyroid cancer, as do gender, age and goiter type (Boelaert et al., 2006; Kumar et al., 1999; Tuttle et al., 1998).

Although FNAB is the method of choice for the evaluation of thyroid nodules, the technique presents limitations in the investigation of lesions of follicular or Hürthle patterns (Faquin & Baloch, 2010). Thus, the TSH serum levels might be used as a malignancy marker in such lesions. However, few studies have evaluated the relationship between thyroid malignancy and levels of this hormone specifically in follicular or Hürthle lesions (Tuttle et al., 1998). In the present study we have evaluated such relationship, and observed no association between TSH serum levels and malignancy in follicular or Hürthle lesions: the average serum levels of TSH and FT4 were not significantly different between the benign and the malignant cases.

One possible reason for such discordance in comparison with the majority of previous studies may be that, in this study, only the cases with suspicious or inconclusive aspiration for FC or HC were analyzed. Others have evaluated the presence of malignancy by studying DTC in general, including PC (Gul et al., 2010; Haymart et al., 2008; Jonklaas et al., 2008), or several types of carcinomas, including those independent from TSH (Boelaert et al., 2006; Polyzos et al., 2008). When evaluating only the FNABs with a diagnosis of follicular neoplasia, Tuttle et al. also did not find any differences in the results of thyroid function tests between benign and malignant cases (Tuttle et al., 1998).

Another reason for the distinct findings in this study may be that we have evaluated only the cases with histological confirmation of the diagnosis. Others have included non-thyroidectomized patients, who had "diagnostic confirmation" only through the evolutionary evaluation during a two year follow-up period (Boelaert et al., 2006; Polyzos et al., 2008; Fiore et al., 2009), a time that might be considered insufficient in the DTC cases.

Another divergence between this study and others (Gul et al., 2010; Jonklaas et al., 2008) is that this study excluded the cases with cytological diagnosis of malignancy and those without recent TSH level measurements. At first, the cases with radiation exposure were not excluded. However, only one patient had been submitted to previous external radiotherapy, presenting a final histological diagnosis of benignity. Although those with thyroid dysfunction were also not excluded at first, when these patients were withdrawn from the analysis, there was still no statistically significant difference between the hormone levels in benign and malignant cases.

In regards to the remaining clinical aspects examined in this study, there was also divergence between the present study and previous reports mentioned. We did not find any significant differences regarding age and gender that might predict nodular malignancy. In fact, the influence of such aspects is controversial and, similar to TSH evaluation, many of the previous studies did not restrict their evaluation only to cases with suspicion of FC or HC. Boelaert et al. showed an association of the male sex or the age extremes with a greater malignancy risk (Boelaert et al., 2006). Haymart et al. has also reported an association of greater risk of malignancy with the male sex and younger age groups, although they found no association with older age (Haymart et al., 2008). On the other hand, Gul et al. associated age of greater than 60 years to a greater risk of malignancy, but did not find an association with gender (Gul et al., 2010). Considering the lesions of follicular pattern, Raber et al., when evaluating cases with diagnostic FNAB for follicular neoplasia, also did not associate the age extremes or the male gender to a greater risk of malignancy (Raber et al., 2000). Tuttle et al. did not find an association between age and the occurrence of malignant tumors, although they did associate the male gender to a greater risk of malignancy (Tuttle et al., 1998). Schlinkert et al. also studied cases with diagnostic FNAB for follicular neoplasia and associated younger ages, but not older ages, with a greater risk of malignancy (Schlinkert et al., 1997). However, other authors have found an association between older ages and greater probability of malignant tumor (Cooper et al., 2006; Tuttle et al., 1998). Thus, there is a great divergence even among findings of studies that are restricted to the cytological diagnosis of follicular neoplasia. Moreover, the criteria for surgery submission were different in each study, which complicates the comparison between studies.

Another characteristic examined in this study was smoking history, which also could not be associated with a greater risk of malignancy. This finding is in agreement with other case-control studies (Kreiger & Parkes, 2000; Mack et al., 2003). In contrast to these studies, Sokic et al. found an association between smoking and a greater risk of thyroid cancer. However, the Sokic study was carried out in a population of hospitalized patients (Sokic et al., 1994), for whom tobacco exposure was modified due to the hospital condition.

We must highlight that the present study presents important limitations. One of the most relevant limitations is the small number of cases evaluated (31 patients). The retrospective nature of the study contributed to that small number by presenting problems such as irregular follow-up, non-submission to thyroidectomy and therefore absence of a histopathological report, and the absence of TSH measurements close in time to the surgical procedure. However, independent of the limitations of this and similar studies, it is a fact that the reports investigating nodular malignancy criteria in follicular tumors cases are not in unanimous agreement.

Therefore, there is still much controversy surrounding the pre-operative diagnosis of thyroid nodules with FNAB compatible with a follicular or Hürthle pattern. Recently, the *National Cancer Institute Thyroid Fine-needle Aspiration State of the Science Conference* (Bethesda, Maryland, USA) attempted to minimize such divergences by reclassifying many of these lesions as benign, follicular lesions of uncertain meaning or follicular neoplasia, presenting a malignancy risk lower than 1%, between 5 and 10% and between 20 and 30%, respectively (Baloch et al., 2008). However, many services have not yet adhered to this new cytological classification and, even when this classification is used, there is still a significant percentage of thyroid nodules for which diagnostic doubt will only be clarified after surgical approach.

5. Conclusion

In conclusion, in this study the serum levels of TSH and FT4, in addition to gender, age and smoking habits, were not useful in differentiating FC or HC from benign lesions of similar cytological patterns. Considering the controversy surrounding this area and the absence of significant evidence, there is a need for more studies examining the correlation between the cytological diagnoses in cases of follicular tumors with the pre-operative TSH serum levels. Future studies should have an adequate study design and a greater study population, in order to improve the diagnosis of these lesions.

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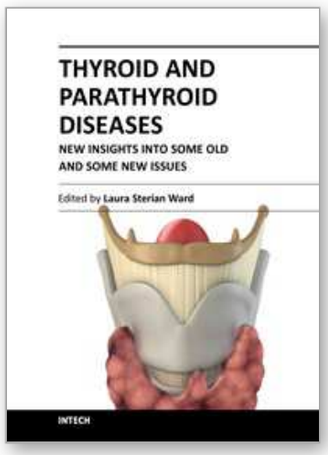
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Thyroid and Parathyroid Diseases - New Insights into Some Old and Some New Issues

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This book was designed to meet the requirements of all who wish to acquire profound knowledge of basic, clinical, psychiatric and laboratory concepts as well as surgical techniques regarding thyroid and parathyroid glands. It was divided into three main sections: 1. Evaluating the Thyroid Gland and its Diseases includes basic and clinical information on the most novel and quivering issues in the area. 2. Psychiatric Disturbances Associated to Thyroid Diseases addresses common psychiatric disturbances commonly encountered in the clinical practice. 3. Treatment of Thyroid and Parathyroid Diseases discusses the management of thyroid and parathyroid diseases including new technologies.

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