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Fine Needle Aspiration Biopsy of Follicular Thyroid Tumors

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A B S T R A C T

US-guided fine needle aspiration cytology is currently the best diagnostic tool for thyroid nodules. The aim of this research was to make a detailed and objective determination of the morphological characteristics of cells in cytological smears in an attempt to distinguish benign from malignant follicular tumors. The research included 62 patients with cytologically diagnosed follicular or oncocytic tumors, and 15 patients with nodular hyperplasia. Echographic findings were divided into three groups: isoechogenic, hypoechogenic and hyperechogenic nodules. We analyzed the cellularity of the smear, cohesion between follicular cells, acinar formations, bare nuclei, characteristics of the nucleus and the cytoplasm, and the presence of colloid. The statistical analysis of cytological parameters has indicated that none of the cytological parameters alone is discriminating enough between non-tumor and tumor changes, or benign and malignant follicular thyroid nodules. The analysis of age, sex, nodule size and ultrasound findings has not shown the correlation between any of these parameters with the malignant or benign follicular tumors. The cytological analysis of the smears for patients with follicular tumors, in combination with clinical data and other diagnostic methods, contributes to more precise diagnostics, but is not sufficient for the differentiation between benign and malignant follicular tumors.

Key words: *FNAB, thyroid nodule, follicular thyroid neoplasm*

Introduction

According to the 2004 WHO histological classification of thyroid tumors, they occur as carcinoma, adenomas and related tumors, and other thyroid tumors.

Adenomas are the most frequent of thyroid tumors. Echographically, like nodular hyperplasia, they look as isoechogenic and hypoechogenic nodes. A follicular carcinoma can be defined as an invasive neoplasm of follicular cells, without typical nuclear characteristics of the papillary carcinoma. It can be minimally invasive, with a limited capsular and/or blood vessel invasion, or overtly invasive, when the infiltration into the surrounding tissue and/or blood vessels is significant[1–5]. Oncocytic tumors (Hürthle cell tumors) are defined as neoplasms originating from the follicular epithelium, built entirely or predominantly (>75%) from oncocytic (Hürthle) cells[6]. Whereas for most thyroid tumors there are clear cytological criteria, the boundaries between a well differentiated follicular carcinoma, follicular adenoma, and nodular hyperplasia are not well defined cytologically.

The cytological pictures of a follicular adenoma and a well differentiated follicular carcinoma are often identical: high cellularity, thyreocites in the shape of a rosette, uniform size nuclei and macronucleosis, bare nuclei, nucleoli, not well differentiated, vacuolised light cytoplasm, which is often missing, with only little or no colloid. In patients with not well differentiated follicular carcinoma, the malignancy criteria are more apparent: higher cellularity, more expressed anisomacronucleosis, three-dimensional thyreocite clusters, reduced tendency for creating micro-follicles and many bare nuclei[7–12]. The cytological smear of patients with the histologically verified nodular hyperplasia often matches the cytological picture of a follicular tumor: in cellular smears we often find clusters of medium-sized follicular cells in the shape of

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honey combs, without morphological abnormalities, microfollicles and rosettes. We can also see pycnotic nuclei and oncocytic cells, as well as colloid[3,13].

The aim of this research was to make a detailed and objective determination of the morphological characteristics of cells in cytological smears of pathohistologically verified follicular tumors.

Materials and Methods

The research included 62 patients with cytologically diagnosed follicular or oncocytic tumors, histologically verified as follicular or oncocytic adenoma or carcinoma, and 15 patients with histologically diagnosed nodular hyperplasia.

Ultrasound examinations were conducted on all patients using the SHIMASONIC SDL-310 Diagnostic Ultrasound device, with the 7.5 MHz linear ultrasound probe, and SONOLINE Adara with the 8.5 MHz linear probe. Echographic findings were divided into three groups: isoechogenic, hypoechogenic and hyperechogenic nodules.

According to the WHO pT classification[14], the nodules were divided into three groups: pT1 (≤10 mm), pT2 (>10, ≤40 mm), and pT3+[a] (pT3 (>40 mm)+pT4 (any pT with the thyroid capsule invasion).

The material for cytological analysis was obtained using US-guided fine needle aspiration. Cytological smears were stained according to the standard Pappenheim method (May-Grünwald-Giems), and analyzed under a light microscope. For the purpose of this research, all cytological findings were revised and analyzed again. The incidence of particular morphological characteristics was determined using the semi-quantitative analysis of the aspirated material[8,15–19].

»Cellularity of the specimen« refers to the presence of epithelium in the cytological smear, and it is determined according to the number of clusters and cells per cluster. [1 – low (less than 6 groups with 5–10 cells), 2 – moderate (6–10 groups with 10–15 cells), 3 – abundant (more than 10 groups with 10–15 cells)]. »Cohesion between follicular cells«: 1-low (<50% thyreocytes in clusters), 2-moderate and high (>50% thyreocytes in clusters). The »prevalence of acinar formations« relative to the total number of clusters in the smear was also determined: [0 – no acinus, 1 (<25% in the cytological smear), 2 (25–75% in the cytological smear), 3 (>75% in the cytological smear)]. »Macronucleosis« included nuclear size greater than twice the size of an erythrocyte (0 – not expressed, 1 – expressed). »Nucleoli« needn't be visible – absent (0) or present (1). »Bare nuclei«: 0 – not found in the smear, 1 – <20% relative to the total number of cells, 2 – 20–50% relative to the total number of cells, 3 – >50% relative to the total number of cells. »Cytoplasm« may have marginal vacuoles or be absent (1), or not well differentiated, gently basophilic (2). »Presence of colloid«: 0 – absent, 1 – present.

The statistical analysis was conducted using the SPSS 9.0, and included the analysis of categorical variables (2x2 and RxC contingency tables), as well as the analysis of correlation and variance.

Results

Descriptive analysis

The study included the tissue samples of 66 female and 11 male patients, between 17 and 72 years old. There were no major age differences or age-based tendencies in certain pathohistological groups, and neither were there any differences in age between female and male patients [52 (44–63) *vs*. 41 (30–56) yrs., F:M, median (interquartile range), p=0.061, Table 1].

The distribution of nodules according to size (concerning the pT category) did not show a significant difference in nodule size for different pathohistological categories. There were also no differences between female and male patients [diameter: 28 (18–35) *vs*. 30 (12–35) mm, F:M, nodule median, p=0.808. In conditions of limited statistical strength, the size of the nodule did not correlate with the age of patients (Table 2).

Among the analyzed histological categories there were no significant differences in ultrasound characteristics of nodules, both concerning dimensions (Table 2), and concerning the echogenicity of nodules (Table 3).

Analysis of cytological findings

Cellularity of the specimen

The pathohistological categories covered in this analysis do not differ in cellularity of cytological smears. Furthermore, follicular adenomas and carcinoma, when grouped according to their malignant potential, did not differ in cellularity of cytological smears (p=0.108, Freeman-Halton exact test, Table 4).

TABLE 1
DEMOGRAPHIC FEATURES OF PARTICIPANTS

Phenotype	Sex	Age (years)	
		Median (IQR)*	Range (min-max)
Follicular adenoma	men (n=2) women (n=14)	54 (45–60)	24–72
Follicular carcinoma	men (n=2) women (n=16)	48 (38–52)	29–72
Oncocytic adenoma	men (n=1) women (n=14)	63 (42–69)	30–76
Oncocytic carcinoma	men (n=4) women (n=9)	51 (43–64)	41–76
Nodular hyperplasia	men (n=2) women (n=13)	51 (40–60)	19–72

[a]Kruskall-Wallis χ^2-test, χ^2=4.667, df=4, p=0.323; Levene test of variable homogenization $F_{(4,72)}$=0.544, p=0.704

TABLE 2
DISTRIBUTION OF NODULE DIAMETERS IN ANALYZED HISTOLOGICAL SAMPLES

Histological diagnosis	N	Diameter (mm)		Range (mm)
		$\overline{X}$±SD	Median (IQR)	
Follicular adenoma	16	30±14.9	27 (20–39)	13–65
Follicular carcinoma	18	33±15.5	33 (22–36)	11–80
Oncocytic adenoma	15	24±11.3	20 (15–35)	7–42
Oncocytic carcinoma	13	30±14.7	30 (20–40)	8–63
Nodular hyperplasia	15	22±11.4	22 (10–30)	10–45

SD – standard deviation, IQR – interquartile range
*Kruskall-Wallis χ^2=6.952, df=4, p=0.138

TABLE 3
ULTRASOUND FINDINGS ACCORDING TO THE HISTOLOGICAL DIAGNOSES

Histological diagnosis	Ultrasound finings	
	hypoechogenic	isoechogenic
Follicular adenoma (n=16)	11	5
Follicular carcinoma (n=18)	14	4
Oncocytic adenoma (n=15)	12	3
Oncocytic carcinoma (n=13)	9	4
Nodular hyperplasia (n=15)	5	10

p=0.059, Fisher-Freeman-Halton exact test, 10^4 Monte Carlo simulation

Cohesion between follicular cells

The cohesion of follicular adenomas and carcinoma differed significantly, i.e. there was a significant loss of cohesiveness in follicular carcinoma smears. When grouped according to the malignant potential, adenomas and carcinoma differed significantly in cell cohesion (p=0.0016). However, in terms of diagnostic value, sensitivity (42%), specificity (87%), positive (PPV) (76%) and negative predicative values (NPV) (60%) of low cohesion were insufficient and unreliable for the discrimination between malignant and benign tumors.

The prevalence of acinar formations

There was no significant difference in the number of acini between follicular adenomas and carcinoma (p=0.727, Freeman-Halton exact test, Table 4). It was, however, significant for follicular and oncocytic tumors (regardless of their malignant potential). Similarly, oncocytic adenomas and carcinoma did not differ statistically concerning the number of acini. There was no difference in the number of acinary formations between oncocytic tumors (neither adenomas nor carcinoma) and nodular hyperplasia, whereas this difference was significant for nodular hyperplasia and benign and malignant follicular tumors.

The analysis of »bare nuclei« in the cytological smear showed the significant difference only between follicular carcinoma and nodular hyperplasia ($p=5.5 \times 10^{-4}$, Table 4). As far as the diagnostic value, the sensitivity (45%), specificity (81%), PPV (70%) and NPV (60%) of the increasing number of bare nuclei in the smear were insufficient and unreliable for discriminating between adenomas and carcinoma.

Characteristics of the nucleus

Neither macronucleosis (despite significant differences) nor the presence of nucleoli in omnibus testing showed statistically significant differences between pathohistological categories (Table 4).

Cytoplasm

There was no significant difference in the characteristics of the cytoplasm between follicular adenomas and carcinoma, as well as between oncocytic adenoma and carcinoma, whereas the follicular and oncocytic tumors (regardless of their malignant potential) differ significantly (Table 4). In terms of diagnostic value, the characteristics of the cytoplasm in differentiating between adenomas and carcinoma were not sufficient and relevant

TABLE 4
SEMIQANTITATIVE CYTOLOGICAL ANALYSIS OF THE SMEARS

PHD	Cellularity			Cohesivity			Acinar formations				Bare nuclei				Macronucleosis		Nucleoli		Cytoplasm		Colloid	
	1	2	3	1	2	3	0	1	2	3	0	1	2	3	0	1	0	1	0	1	0	1
F Ad (N=16)	0	10	16	3	7	6	0	3	9	4	6	5	5	0	11	5	12	4	14	2	12	4
F Ca (N=18)	3	5	10	9	9	0	0	6	9	3	0	6	9	3	6	12	11	7	16	2	17	1
O Ad (N=15)	1	7	7	1	10	4	12	3	0	0	5	9	1	0	4	11	10	5	2	13	15	0
O Ca (N=13)	1	4	8	4	8	1	9	3	1	0	3	8	2	0	3	10	8	5	5	8	13	0
NH (N=15)	1	9	5	2	9	4	10	4	1	0	6	8	1	0	10	5	13	2	5	10	10	5

F Ad – follicular adenoma, F Ca – follicular carcinoma, O Ad – oncocytic adenoma, O Ca – oncocytic carcinoma, NH – nodular hyperplasia

[sensitivity (45%), specificity (81%), PPV (70%) and NPV (60%)].

The analysis of the presence of *colloid* in the cytological smear showed no difference between particular histological categories (Table 4).

Discussion and Conclusion

Even though the ultrasound testing made it possible to discover and localize thyroid nodules, it cannot determine the type of lesion and its origin. Whereas there are clear cytological criteria for the differentiation between most thyroid tumors, the borderline between a well-differentiated follicular carcinoma, follicular adenomas and nodular hyperplasia is cytologically not well defined. A precise discrimination is not possible even on the basis of a clinical examination, scintigraphy, or ultrasound examination. Therefore, all patients with cytological diagnosis of a follicular tumor undergo a surgery. As only 5–10% of such tumors are malignant, there is a clear need for finding clinically reliable tumor markers that would make the differentiation possible and reduce the number of unnecessary surgeries.

The analysis of age and sex of patients showed no statistically significant differences in the occurrence of particular histological categories. We did not find a correlation between *nodule size* and non-tumor and tumor formations, or benign and malignant tumors. We also did not find the correlation between nodule size and patient age/sex. Several authors have tried to combine different clinical and anthropometric indicators to foresee the biological behaviour of those tumors. Deveci et al.[20] did not prove the correlation between nodule size and its benign or malignant characteristics, but they did stress the increased risk of malignant tumors in patients younger than 40. Baloch et al.[21] also studied the correlation between a histological diagnosis, age, sex, and nodule size. They found the increased risk for the development of carcinoma in male patients older than 40 with nodules larger than 3 cm. Other authors have also indicated an increased risk for the development of cancer in male patients over 40, but again without a statistically significant difference[22–24].

Echographic characteristics of nodules showed no significant differences regarding particular pathohistological categories.

The following conclusions can be made from the semiquantitative analysis of cytological smears: neither of the cytological parameters alone was sufficient as a discriminating factor for differentiating between non-tumor formations and tumors; between follicular adenomas and carcinoma and between oncocytic adenomas and carcinoma. The analysis of particular cytological parameters in cytological smears of follicular and oncocytic cell tumors, as well as nodular hyperplasia in this research, is in line with the data found in literature. According to the results of similar studies, neither of the parameters alone was sufficiently discriminating for non-tumor nodules, or benign vs. malignant follicular tumors[7,25–28].

The conclusion therefore reinforces the conclusions of earlier studies: the cytological analysis of the smears for patients with follicular tumors, in combination with clinical data and other diagnostic methods, contributes to more precise diagnostics, but is not sufficient for the differentiation between benign and malignant follicular tumors.

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CITODIJAGNOSTIKA FOLIKULARNIH TUMORA ŠTITNJAČE

S A Ž E T A K

Citološka punkcija pod kontrolom ultrazvuka je nedovoljno osjetljiva i specifična za razlikovanje benignih i malignih folikularnih tumora. Cilj istraživanja je bio utvrditi citomorfološke karakteristike stanica punktata histološki verificiranih folikularnih i onkocitnih tumora te odrediti vrijednost pojedinih citoloških parametara u diferencijaciji benignih i malignih tumora. U istraživanje je uključeno 62 ispitanika s citološkom dijagnozom folikularnog ili onkocitnog tumora te 15 ispitanika s čvorastom hiperplazijom. Ehografski, čvorovi su bili izoehogeni i hipoehogeni, a prema veličini su podijeljeni u skladu sa pT klasifikacijom SZO. Semikvantitativno je analizirana celularnost uzorka, kohezija među stanicama, morfologija nakupina, gole jezgre, karakteristike jezgre i citoplazme te koloid. Statistički, ni jedan od citoloških parametara sam za sebe nije dovoljan diskriminirajući faktor između netumorskih i tumorskih promjena, kao ni između benignih i malignih folikularnih tumora štitnjače. Analizom dobi, spola, veličine čvora ni UZV nalaza nije dokazana povezanost bilo kojeg od ovih parametara s malignim ili benignim folikularnim tumorom. Citološka analiza punkata u kombinaciji sa kliničkim podacima i drugim dijagnostičkim metodama doprinosi preciznijoj dijagnostici, ali sama za sebe nije dostatna za diferencijaciju benignih i malignih folikularnih tumora.