Fine Needle Aspiration Biopsy of Follicular Thyroid Tumors

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ABSTRACT

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 significant1–5. Oncocytic tumors (Hürthle cell tumors) are defined as neoplasms originating from the follicular epithelium, built entirely or predominantly (>75%) from oncocytic (Hürthle) cells6. 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, thyreocytes 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 thyreocyte clusters, reduced tendency for creating micro-follicles and many bare nuclei7–12. 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
honey combs, without morphological abnormalities, microfollicles and rosettes. We can also see pycnotic nuclei and oncocytic cells, as well as colloid\textsuperscript{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\textsuperscript{14}, the nodules were divided into three groups: pT1 (≤10 mm), pT2 (>10, ≤40 mm), and pT3+\textsuperscript{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 adenomas or carcinomas was determined using the semi-quantitative analysis of the aspirated material\textsuperscript{3,13,15–19}.

«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» refers to the total number of clusters in the smear. 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>
<thead>
<tr>
<th>TABLE 1</th>
<th>DEMOGRAPHIC FEATURES OF PARTICIPANTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotype</td>
<td>Sex</td>
</tr>
<tr>
<td>Follicular adenoma</td>
<td>men (n=2)</td>
</tr>
<tr>
<td>Follicular carcinoma</td>
<td>men (n=2)</td>
</tr>
<tr>
<td>Oncocytic adenoma</td>
<td>men (n=1)</td>
</tr>
<tr>
<td>Oncocytic carcinoma</td>
<td>men (n=4)</td>
</tr>
<tr>
<td>Nodular hyperplasia</td>
<td>men (n=2)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Kruskall-Wallis $\chi^2$-test, $\chi^2$=4.667, df=4, $p=0.323$; Levene test of variable homogenization $F_{(4,72)}=0.844$, $p=0.704$
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 predictive 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 acini between follicular and 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 × 10⁻⁴, Table 4). As far as the diagnostic value, the sensitivity (45%), specificity (81%), positive (PPV) (76%) and negative predictive values (NPV) (60%) of 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.

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### TABLE 2
DISTRIBUTION OF NODULE DIAMETERS IN ANALYZED HISTOLOGICAL SAMPLES

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>N</th>
<th>Diameter (mm)</th>
<th>Range (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular adenoma</td>
<td>16</td>
<td>30±14.9</td>
<td>27 (20–39)</td>
</tr>
<tr>
<td>Follicular carcinoma</td>
<td>18</td>
<td>33±15.5</td>
<td>33 (22–36)</td>
</tr>
<tr>
<td>Oncocytic adenoma</td>
<td>15</td>
<td>24±11.3</td>
<td>20 (15–35)</td>
</tr>
<tr>
<td>Oncocytic carcinoma</td>
<td>13</td>
<td>30±14.7</td>
<td>30 (20–40)</td>
</tr>
<tr>
<td>Nodular hyperplasia</td>
<td>15</td>
<td>22±11.4</td>
<td>22 (10–30)</td>
</tr>
</tbody>
</table>

SD – standard deviation, IQR – interquartile range

*Kruskall-Wallis χ²=6.952, df=4, p=0.138

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### TABLE 3
ULTRASOUND FINDINGS ACCORDING TO THE HISTOLOGICAL DIAGNOSES

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>Ultrasound finings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hypoechogenic</td>
</tr>
<tr>
<td>Follicular adenoma (n=16)</td>
<td>11</td>
</tr>
<tr>
<td>Follicular carcinoma (n=18)</td>
<td>14</td>
</tr>
<tr>
<td>Oncocytic adenoma (n=15)</td>
<td>12</td>
</tr>
<tr>
<td>Oncocytic carcinoma (n=13)</td>
<td>9</td>
</tr>
<tr>
<td>Nodular hyperplasia (n=15)</td>
<td>5</td>
</tr>
</tbody>
</table>

p=0.059, Fisher-Freeman-Halton exact test, 10⁴ Monte Carlo simulation

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### TABLE 4
SEMIQUANTITATIVE CYTOLOGICAL ANALYSIS OF THE SMEARS

<table>
<thead>
<tr>
<th>PHD</th>
<th>Cellularity</th>
<th>Cohesivity</th>
<th>Acinar formations</th>
<th>Bare nuclei</th>
<th>Macronucleosis</th>
<th>Nucleoli</th>
<th>Cytoplasm</th>
<th>Colloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>F Ad (N=16)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F Ca (N=18)</td>
<td>3</td>
<td>5</td>
<td>10</td>
<td>9</td>
<td>0</td>
<td>6</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>O Ad (N=15)</td>
<td>1</td>
<td>7</td>
<td>7</td>
<td>1</td>
<td>10</td>
<td>4</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>O Ca (N=13)</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>8</td>
<td>1</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>NH (N=15)</td>
<td>1</td>
<td>9</td>
<td>5</td>
<td>2</td>
<td>9</td>
<td>4</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

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

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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-dif-
fentiated follicular carcinoma, follicular adenomas and nodule hyperplasia is cytologically not well defined. A precise discrimination is not possible even on the basis of a clinical examination, scintigraphy, or ultrasound exami-
nation. 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 find-
ing clinically reliable tumor markers that would make the differentiation possible and reduce the number of un-
necessary surgeries.

The analysis of age and sex of patients showed no statistically significant differences in the occurrence of par-
ticular histological categories. We did not find a correla-
tion between nodule size and non-tumor and tumor for-
mations, 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 bio-
ological behaviour of those tumors. Deveci et al.26 did not prove the correlation between nodule size and its benign or malignant characteristics, but they did stress the in-
creased risk of malignant tumors in patients younger than 40. Baloch et al.21 also studied the correlation be-
tween a histological diagnosis, age, sex, and nodule size. They found the increased risk for the development of car-
cinoma 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 pa-
ients over 40, but again without a statistically signifi-
cant difference22–24.

Echographic characteristics of nodules showed no sig-
nificant differences regarding particular pathohistolo-
cal categories.

The following conclusions can be made from the se-
miquantitative analysis of cytological smears: neither of the cytological parameters alone was sufficient as a dis-
aginating factor for differentiating between non-tu-
mor formations and tumors; between follicular adeno-
mas and carcinoma and between oncocytic adenomas and carcinoma. The analysis of particular cytological pa-
rameters in cytological smears of follicular and oncocytic cell tumors, as well as nodular hyperplasia in this re-
search, is in line with the data found in literature. Ac-

cording to the results of similar studies, neither of the pa-
rameters alone was sufficiently discriminating for non-tumor nodules, or benign vs. malignant follicular tumors25–28.

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 dif-
fentiation between benign and malignant follicular tu-
mors.
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 onkokitnih tumora te odrediti vrijednost pojedinih citoloških parametara u diferencijaciji benignih i malignih tumora. U istraživanje je uključeno 62 ispitanika s citološkom dijagnozom folikularnog ili onkokitnog tumora te 15 ispitanika s čvorom 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.