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Accuracy of Fine Needle Aspiration Biopsy with and without the Use of Tumor Markers in Cytologically Indeterminate Thyroid Lesions

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A B S T R A C T

We investigated if the use of two tumor markers, galectin-3 and CD44v6, could improve diagnostic accuracy of thyroid fine needle aspiration biopsy (FNAB) in cytologically indeterminate lesions (CIL). 351 patients with CIL [cellular follicular lesion/suspicious follicular neoplasm/suspicious Hürthle cell neoplasm (CFL/sFN/sHCN), Hürthle cell neoplasm (HCN), and follicular neoplasm (FN)] and surgical follow-up were investigated. 251 patients had FNAB diagnoses made without help of tumor markers and the rest of 100 patients had FNAB diagnoses made with a known expression of tumor markers determined by the reverse transcription (RT)-PCR. Risk of malignancy in all 351 patients with CIL was 6.8%. In the group with FNAB made without RT-PCR, there were 140 CFL/sFN/sHCN with the risk of malignancy of 4.2%, 92 FN with the risk of malignancy of 13.0%, and 19 HCN with the risk of malignancy of 5.2%. In the group with FNAB made with RT-PCR, there were 49 CFL/sFN/sHCN with the risk of malignancy of 2.0%, 40 FN with the risk of malignancy of 7.5%, and 11 HCN with the risk of malignancy of 9.0%. In the group with at least one positive tumor marker (N=69), the risk of malignancy was 3.1% for CFL/sFN/sHCN, 11.1% for FN, and 10.0% for HCN. In the group with negative tumor markers (N=31) there were no malignancies. The use of tumor markers, galectin-3 and CD44v6, determined by RT-PCR improves only sensitivity of thyroid FNAB in CIL. In most patients with CIL, and negative both tumor markers, conservative approach is advisable.

***Key words**: thyroid disease, galectin-3, CD44v6 antigen, reverse transcriptase-polymerase reaction, biopsy, fine needle aspiration cytology*

Introduction

Fine-needle aspiration biopsy (FNAB) of the thyroid is a rapid, minimally invasive, accurate, and inexpensive procedure for evaluation of thyroid nodules[1]. The main goal of thyroid FNAB is to distinguish nodules that require surgery from those that do not, thereby decreasing the number of diagnostic surgical procedures[2–6]. However, FNAB of the thyroid is indeterminate for neoplasia in 5–29% of patients[7–9]. Surgical excision, with its attendant high cost and potential morbidity, usually is required to fully evaluate these lesions.

The most controversial decision is managing patients with cytologically indeterminate thyroid lesions. The diagnosis of follicular thyroid carcinomas is made only by identifying histopathologic infiltration into blood vessels or capsule, or by detection of distant metastasis. Cytologic diagnosis has its limitations because of the similar cytologic findings in aspirates from adenomatoid nodules, follicular adenomas, well-differentiated follicular carcinomas and papillary carcinomas of the follicular variant or with a prominent follicular component[4,10–14].

Reverse-transcription polymerase chain reaction (RT-PCR) has been used to search for the expression of potential thyroid carcinoma associated molecular markers within the cells obtained from FNAB of the thyroid[15,16]. Among them the most promising were two lectin-related molecules: the beta-galactoside-binding protein galectin-3 and CD44v6, an isoform of CD44, the cell-surface receptor for hyaluronic acid[17–21].

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We investigated whether the use of two tumor markers, galectin-3 and CD44v6 determinated by RT-PCR, could improve diagnostic accuracy of FNAB in cytologically indeterminate thyroid lesions.

Patients and Methods

We undertook a retrospective search of all patients who underwent a thyroid surgical procedure at University Hospital »Sestre milosrdnice«, Zagreb between March 1995 and April 2008 and had a preoperative FNAB diagnosis of cytologically indeterminate thyroid lesion. FNAB diagnoses included cellular follicular lesion/suspicious follicular neoplasm/suspicious Hürthle cell neoplasm (CFL/sFN/sHCN), Hürthle cell neoplasm (HCN), and follicular neoplasm (FN).

351 patients with cytologically indeterminate lesions and surgical follow up were found. 251 patients had FNAB diagnoses made without help of tumor markers and the rest of 100 patients had FNAB diagnoses made with a known expression of tumor markers, galectin-3 and CD44v6, determined by the RT-PCR.

We investigated the risk of malignancy in both groups of patients (separately by the type of FNAB diagnosis), and then compare the risk of malignancy in patients with negative tumor markers, patients with at least one positive tumor marker and patients without RT-PCR results.

Aspirates were obtained by ultrasound guided FNAB and smeared for conventional cytology (MGG staining). One to three punctures per nodule were performed depending on the size of the nodule. In patients with RT-PCR analysis, the leftover material in the needle was used for RT-PCR analysis. RT-PCR analysis was performed as described by Samija et al.[22]. The value of marker analysis was considered positive if at least one marker, galectin-3 or CD44v6, was positive.

Statistical analysis was performed using Fisher's exact test. A p value less than 0.05 were considered significant.

Definition of the diagnostic categories for indeterminate thyroid lesions

CFL is best described as »probably neoplastic«. FNAB shows relatively abundant, slightly atypical, follicular cells and scant colloid.

FN is best described as »probably malignant«. It includes follicular adenoma and follicular carcinoma. It's characterized by irregular microfollicles with nuclear overlap and central, dense colloid, in association with cytologic nuclear atypia.

HCN includes Hürthle cell adenoma and Hürthle cell carcinoma. It's characterized by a single cell population of Hürthle cell in a background of minimal or absent colloid.

FNA reports of »sFN/sHCN« include cytomorphologic features of CFL and FN/HCN as well.

TABLE 1
CYTOLOGIC DIAGNOSIS *VERSUS* PATHOHISTOLOGICAL DIAGNOSES IN PATIENTS WITH FNAB DIAGNOSIS MADE WITHOUT RT-PCR

Cytologic diagnosis	PHD								Total	Risk of malignancy
	NG	HT	FA	HCA	PC	FC	HCC	MC		
CFL/FN?/HCN?	54	2	64	14	4	1	1	0	140	4.2%
FN	20	0	57	3	4	5	2	1	92	13.0%
HCN	7	0	3	8	1	0	0	0	19	5.2%
Total	81	2	124	25	9	6	3	1	251	7.6%

PHD – pathohistological diagnosis, NG – nodular goiter, HT – Hashimoto thyroiditis, FA – follicular adenoma, HCA – Hürthle cell adenoma, PC – papillary carcinoma, FC – follicular carcinoma, HCC – Hürthle cell carcinoma, MC – medullary carcinoma, CFL – cellular follicular lesion, FN – follicular neoplasm, HCN – Hürthle cell neoplasm

TABLE 2
CYTOLOGIC DIAGNOSIS *VERSUS* PATHOHISTOLOGICAL DIAGNOSES IN PATIENTS WITH FNAB DIAGNOSIS MADE WITH RT-PCR

Cytologic diagnosis	PHD								Total	Risk of malignancy
	NG	HT	FA	HCA	PC	FC	HCC	MC		
CFL/FN?/HCN?	21	1	23	3	1	0	0	0	49	2.0%
FN	14	0	20	3	1	1	0	1	40	7.5%
HCN	2	1	1	6	0	0	1	0	11	9.0%
Total	37	2	44	12	2	1	1	1	100	5.0%

PHD – pathohistological diagnosis, NG – nodular goiter, HT – Hashimoto thyroiditis, FA – follicular adenoma, HCA – Hürthle cell adenoma, PC – papillary carcinoma, FC – follicular carcinoma, HCC – Hürthle cell carcinoma, MC – medullary carcinoma, CFL – cellular follicular lesion, FN – follicular neoplasm, HCN – Hürthle cell neoplasm

TABLE 3
CYTOLOGIC DIAGNOSIS *VERSUS* PATHOHISTOLOGICAL DIAGNOSES IN PATIENTS WITH FNAB DIAGNOSIS AND EITHER GALECTIN-3 OR CD44V6 POSITIVE BY RT-PCR

Cytologic diagnosis	PHD								Total	Risk of malignancy
	NG	HT	FA	HCA	PC	FC	HCC	MC		
CFL/FN?/HCN?	13	1	15	2	1	0	0	0	32	3.1%
FN	9	0	14	1	1	1	0	1	27	11.1%
HCN	1	1	1	6	0	0	1	0	10	10.0%
Total	23	2	30	9	2	1	1	1	69	7.2%

PHD – pathohistological diagnosis, NG – nodular goiter, HT – Hashimoto thyroiditis, FA – follicular adenoma, HCA – Hürthle cell adenoma, PC – papillary carcinoma, FC – follicular carcinoma, HCC – Hürthle cell carcinoma, MC – medullary carcinoma, CFL – cellular follicular lesion, FN – follicular neoplasm, HCN – Hürthle cell neoplasm

TABLE 4
CYTOLOGIC DIAGNOSIS *VERSUS* PATHOHISTOLOGICAL DIAGNOSES IN PATIENTS WITH FNAB DIAGNOSIS AND BOTH GALECTIN-3 AND CD44V6 NEGATIVE BY RT-PCR

Cytologic diagnosis	PHD								Total	Risk of malignancy
	NG	HT	FA	HCA	PC	FC	HCC	MC		
CFL/FN?/HCN?	8	0	8	1	0	0	0	0	17	0 %
FN	5	0	6	2	0	0	0	0	13	0 %
HCN	1	0	0	0	0	0	0	0	1	0 %
Total	14	0	14	3	0	0	0	0	31	0 %

PHD – pathohistological diagnosis, NG – nodular goiter, HT – Hashimoto thyroiditis, FA – follicular adenoma, HCA – Hürthle cell adenoma, PC – papillary carcinoma, FC – follicular carcinoma, HCC – Hürthle cell carcinoma, MC – medullary carcinoma, CFL – cellular follicular lesion, FN – follicular neoplasm, HCN – Hürthle cell neoplasm

Results

Overall risk of malignancy in all 351 patients with cytologically indeterminate lesions was 6.8%. In the group of patients with FNAC diagnosis made without RT-PCR, there were 140 diagnoses of CFL/sFN/sHCN with the risk of malignancy of 4.2%, 92 diagnoses of FN with the risk of malignancy of 13.0%, and 19 diagnoses of HCN with the risk of malignancy of 5.2% (Table 1). In the group of patients with FNAC diagnosis made with RT-PCR, there were 49 diagnoses of CFL/sFN/sHCN with the risk of malignancy of 2.0%, 40 diagnoses of FN with the risk of malignancy of 7.5%, and 11 diagnoses of HCN with the risk of malignancy of 9.0% (Table 2). The risks of malignancy in these two groups didn't differ significantly ($p>0.05$). In the group of patients with at least one positive tumor marker (N=69), the risk of malignancy was 3.1% for diagnoses of CFL/sFN/sHCN, 11.1% for diagnoses of FN, and 10.0% for diagnoses of HCN (Table 3). The risk of malignancy in this group of patients (7.2%) didn't differ significantly ($p>0.05$) from the risk of malignancy in the previous two groups of patients (7.6 and 5.0, respectively). In the group of patients with negative both tumor markers (N=31) there were no malignancies (Table 4). Although there was a strong tendency ($p=0.1241$), we found no statistically significant ($p>0.05$) difference between the group of patients with negative both tumor markers and the group of patients with at least one positive tumor marker, according to the risk of malignancy.

Sensitivity and specificity of investigated tumors markers, galectin-3 and CD44v6, when the RT-PCR was considered positive for malignancy if at least one tumor marker is positive, was 100% and 60%, respectively.

Discussion and Conclusion

The ultimate goal in the preoperative evaluation of thyroid nodules is to accurately designate lesions as benign or malignant, and thereby allow malignant lesions to be surgically excised. Clinical features can help to predict an increase or decrease in the likelihood of cancer in a nodule, but these parameters have limited utility[23,24]. Standard cytological evaluation of thyroid nodules, even under the guidance of a highly trained cytopathologist, cannot definitively distinguish benign from malignant thyroid lesions in all cases. Thyroid cancer markers, such as CD44v6 and galectin-3 used in this study, are therefore in a focus of interest as potential tools to enhance the preoperative evaluation of thyroid nodules.

In our study, overall risk of malignancy in 351 patients with cytologically indeterminate lesions was 6.8%. In other publications, the possibility of malignancy in this cytologic category ranged from 0 to 43%, with a median of 3% and a mean of 12%[9].

In our study, sensitivity and specificity of galectin-3 and CD44v6 analyzed by RT-PCR for distinguishing benign from malignant lesions among cytologically indeterminate thyroid lesions was 100% and 60%, respectively. It is similar to the results of Maruta et al.[25] using the immunostaining method, who found sensitivity and specificity of either galectin-3 or CD44v6 of 97% and 52%, respectively, for distinguishing follicular carcinoma among FNAB diagnoses of follicular tumors. The lower specificity could be explained by the presence of macrophages and Hürthle cells in some samples. It has been shown that macrophages and Hürthle cells could be responsible for positive results of galectin-3 and CD44v6 in benign thyroid lesions[26].

The risk of malignancy in the group of patients with FNAB diagnosis made without RT-PCR (7.6%) and the risk of malignancy in the group of patients with FNAC diagnosis made with RT-PCR (5.0%) didn't differ significantly, presumably because patients were referred to surgical removal according solely to FNAB diagnosis.

Patients with at least one positive tumor marker and FNAB diagnoses of CFL/sFN/sHCN had the low risk of malignancy (3.1%) and could be monitored by close follow-up. The risk of malignancy (11.1%) for patients with at least one positive tumor marker and FNAB diagnoses of FN and the risk of malignancy (10.0%) for FNAB diagnoses of HCN were significantly higher and require surgical treatment.

In the group of patients with cytologically indeterminate lesions and both tumor markers negative, there were no malignancies and these patients could be monitored in follow-up evaluations.

In conclusion, results of this study demonstrate that RT-PCR improve diagnostic accuracy of FNAB avoiding unnecessary surgeries for many patients with cytologically indeterminate lesions.

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POUZDANOST CITOLOŠKE PUNKCIJE SA I BEZ UPOTREBE TUMORSKIH BILJEGA KOD CITOLOŠKI NEODREĐENIH PROMJENA ŠTITNJAČE

SAŽETAK

Istraživali smo da li upotreba dvaju tumorskih biljega, galektina-3 i CD44v6, može poboljšati pouzdanost citološke dijagnoze (CD) štitnjače kod citološki neodređenih nalaza (CNN) u pogledu malignosti. Analiziran je 351 pacijent s CNN [celularna folikularna promjena/suspektan folikularni tumor/suspektan Hürthleov tumor (CFP, sFT, sHT), Hürthleov tumor (HT) i folikularni tumor (FT)] kojemu je promjena kirurški odstranjena. Kod 251 pacijenta CD je postavljena bez upotrebe tumorskih biljega dok je kod ostalih 100 pacijenata dijagnoza postavljena uz poznatu vrijed-

nost tumorskih biljega određenih uz pomoć RT-PCR. Rizik malignosti kod svih pacijenata s CNN bio je 6,8%. U skupini s CD postavljenom bez RT-PCR, bilo je 140 CFP/sFT/sHT uz rizik malignosti od 4,2%, 92 FT uz rizik malignosti od 13,0%, te 19 HT uz rizik malignosti od 5,2%. U skupini s CD postavljenom sa RT-PCR, bilo je 49 CFP/sFT/sHT uz rizik malignosti od 2,0%, 40 FT uz rizik malignosti od 7,5%, te 11 HT uz rizik malignosti od 9,0%. U skupini sa barem jednim pozitivnim biljegom (N=69), rizik malignosti bio je 3,1% za CFP/sFT/sHT, 11,1% za FT, te 10,0% za HT. U skupini sa negativna oba tumorska biljega (N=31) nije bilo malignih tumora. Može se zaključiti da upotreba tumorskih biljega, galektina-3 i CD44v6, poboljšava samo senzitivnost CD štitnjače kod CNN. Kod većine pacijenata sa CNN i negativna oba tumorska biljega može se preporučiti konzervativni pristup.