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Research Article

Thyroglobulin Measurement in the Washout of Fine Needle Aspirates for the Diagnosis of Suspicious Cervical Lymph Nodes

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Abstract

Ultrasound-guided fine-needle aspiration cytology (FNAC) for suspicious cervical lymph nodes (CLN) is the gold standard technique for the identification of metastases from differentiated thyroid carcinomas. Thyroglobulin protein (Tgp) assay in the washout of needles employed for FNA biopsies (FNAB) has been reported to refine and support FNAC performances, especially in cases of inadequate sampling or cystic lymph nodes. In the present work, we evaluated the usefulness of routine measurement of Tgp in the FNAB washout of suspicious cervical lymph nodes (CLN), and its ability to increase the FNAC accuracy in the diagnosis of metastatic CLN.

A case study of 45 CLN with histological diagnosis from 36 patients was analyzed. Histology showed metastases from papillary thyroid carcinomas (PTC) in 31 CLN, from anaplastic thyroid cancer (ATC) in 3 CLN, from medullary thyroid cancer (MTC) in 4 CLN, and metastases from extrathyroidal malignancies in 5 CLN. Two CLN analyzed were found to be non-neoplastic.

The overall accuracy of FNAC was 82.9%, and that of Tgp was 91.1%, not statistically different. However, Tgp determination was found essential in 4 cases of metastatic CLN from DTC with inadequate cytology, and in 1 case in which the FNAC provided a false negative result.

We demonstrated that FNAC and Tgp assay show similar diagnostic accuracies, and that Tgp measurement may represent the only available information in case of inadequate lymph node sampling or cystic lymph nodes.

Abbreviations (if used)

FNAC - Ultrasound-Guided Fine-Needle Aspiration Cytology
Tgp - Thyroglobulin Protein
FNAB - Fine-Needle Aspiration Biopsies
CLN - Cervical Lymph Nodes
PTC - Papillary Thyroid Carcinomas
MTC - Medullary Thyroid Cancer
ATC - Anaplastic Thyroid Cancer
DTC - Differentiated Thyroid Carcinomas
PDTC - Poorly Differentiated Thyroid Carcinomas

Introduction

Thyroid cancer (TC) is the most common type of endocrine neoplasia, accounting for 3.1% of all new cancers and for 0.4% of cancer-related deaths worldwide in 2018 [1,2]. The majority of TC arise from the epithelial follicular cells, and are classified as differentiated thyroid carcinomas (DTC), comprising the papillary (PTC) and follicular (FTC) carcinomas, poorly differentiated (PDTC) and anaplastic thyroid carcinomas (ATC) [3]. Medullary thyroid carcinomas (MTC), originated from the parafollicular C cells, represent 5-10% of thyroid cancers with a 1-2% prevalence in thyroid nodules [3]. The annual incidence of TC has displayed an increasing trend in the last decades which, however, is not attributable to a real escalation in the onset of tumors, but rather to the improved detection of microcarcinomas in small non-palpable thyroid nodules [4]. The latter are very common, with a prevalence of 19% to 67% in the adult population, while malignant lesions occur only in about 5% of them [5,6]. Therefore, the first aim of clinical evaluation of thyroid nodules is to discriminate against their benign or malignant nature [5,6]. In this regard, fine-needle aspiration cytology (FNAC) represents the main diagnostic tool owing to its good diagnostic accuracy (84-95%), reproducibility and cost effectiveness [5-9]. Total thyroidectomy, with eventual dissection of the central and/or lateral compartment neck, is recommended for TC patients, followed in most cases by I^{131} ablation of the remnant [5,6,10,11].

Post-operative management of patients comprises periodic neck ultrasound, I^{131} whole-body scintigraphy, and serum Tg measurement after exogenous stimulation with recombinant human TSH 6-12 months after surgery [5,6]. DTC patients have a favorable prognosis, with 10-years-survival rate of roughly 90%. Nonetheless, approximately 10% to 15% of them exhibit persistent or recurrent TC, one third of whom face the loss of radioiodine avidity, poor response to conventional therapies and cancer-related death [5,6,10]. For such reasons, the identification of new prognostic molecular markers is highly required to improve the prognostic stratification of thyroid cancer patients [12-18].

Most of relapses appear within the central or lateral neck. When a mass in the thyroid bed and/or a lymphadenopathy are observed by ultrasound, FNAB is performed to diagnose a locally recurrent TC. Therefore, at present, FNAC is of crucial importance both in preoperative phase, to establish the surgical approach and prognostic stratification of TC patients, and in the follow-up to identify relapses [5,6,10,19-22]. An important limitation of this technique is that it depends totally on pathologist's experience for recognition of metastases from TC, metastases from extra-thyroidal neoplasms or non-tumoral diseases [23-25]. In addition, it is worth to note that inadequate cellularity prevents diagnosis in approximately 15% of cases [26-28]. Several studies suggested that measurement of thyroglobulin protein (Tgp) in the washout of the same needle used for FNAC could improve the diagnostic accuracy on CLN harboring metastases from DTC [29-46]. As a consequence, routine association of Tgp with FNAC in the diagnosis of CLN metastasis from DTC has been endorsed [29-46].

Here, we evaluated 45 CLN with histological report to assess the diagnostic value of Tgp, quantified in the washout of needles used for FNAC, in the identification of lymph nodal metastasis from thyroid tumors. The results showed that FNAC and Tgp have a similar diagnostic performance, and that Tgp could be informative in case of inadequate lymph node sampling.

Materials and Methods

Patients

From September 2004 to June 2016, FNAC and Tgp measurements were performed in parallel on suspicious CLN at the outpatients' clinic of Endocrinology and Thyroid Diseases of the Polyclinic Umberto I of Rome (Italy). Forty-five CLN obtained from 36 consecutive patients, for which histological report was available, were selected for the present study. Of these 32 CLN, corresponding to 26 patients, were already analyzed in a previous report [44]. The present study received the approval of the ethical committee of the Polyclinic Umberto I (protocol no. 2615), and all patients signed the consent form. Twelve males and 24 females (median age 44 yr, range 20-76 yr) were subjected to FNAB in one or multiple suspicious CLN. Among them, 10 patients had been formerly thyroidectomized for PTC, and hence they were suspected of relapse (see table 1). All patients with preoperative diagnosis of TC underwent unilateral neck dissection. The histological analysis evidenced that, of the 45 CLN analyzed, 31 had metastatic PTC, 4 had metastatic MTC, 3 had metastatic ATC, and 5 harbored metastases from extrathyroidal cancers (metastasis from non-Hodgkin lymphoma were contained in 3 CLN, metastasis from a rhino-pharyngeal carcinoma in 1 CLN, and metastasis from lung carcinoma in 1 CLN). Cervical lymph nodes 41 and 45 (patients 32 and 36) of table 1, with a non-neoplastic cytological diagnosis underwent surgical removal because of the presence of thyroid nodule with cytological diagnosis of PTC, that following surgery was confirmed by histology.

Table 1: *Patient's age and gender, cytological, molecular and histological diagnoses of suspicious metastatic cervical lymph nodes (CLN) included in the case study. RPC, rhino-pharyngeal carcinoma; LC, lung cancer. *Previously thyroidectomized patients.*

Patient number	Age (yr)	Gender	CLN number	Cytology	Tg protein (ng/FNAB)	Histology
1	20	F	**1**	Inadequate	6620	PTC
			2	Inadequate	7480	PTC
2	28	F	**3**	PTC	5652	PTC
3*	69	F	**4**	PTC	5293	PTC
4*	54	F	**5**	PTC	18612	PTC
5	46	F	**6**	PTC	1625	PTC
6	45	M	**7**	MTC	0.9	MTC
7*	28	F	**8**	Inadequate	37.5	PTC
8	35	M	**9**	PTC	26108	PTC
9	66	F	**10**	ATC	<0.15	ATC
10	52	F	**11**	PTC	37250	PTC
11	69	M	**12**	PTC	0.8	ATC
			13	PTC	2.82	ATC
12*	63	F	**14**	PTC	114	PTC
13*	34	F	**15**	PTC	288	PTC
14	30	F	**16**	PTC	1151	PTC
			17	PTC	3714	PTC
15	34	F	**18**	PTC	480	PTC
16	29	M	**19**	PTC	<0.15	MTC
			20	PTC	<0.15	MTC
17*	33	F	**21**	PTC	62908	PTC
18	31	F	**22**	PTC	72.3	PTC
			23	PTC	10349	PTC
19	25	M	**24**	PTC	384	PTC
20*	53	F	**25**	PTC	728	PTC
21	68	M	**26**	PTC	53	PTC
22	41	M	**27**	Epithelial cancer	<0.15	RPC
23	71	F	**28**	Lymphoma	10.9	Lymphoma
24	68	F	**29**	Lung cancer	<0.15	Lung cancer
25	38	M	**30**	PTC	3361	PTC
26*	28	F	**31**	Non-neoplastic	4395	PTC
			32	PTC	4388	PTC
27	43	F	**33**	PTC	125	PTC
28	58	F	**34**	PTC	1580	PTC
			35	PTC	562	PTC
29*	25	F	**36**	Inadequate	<0.15	PTC
30	56	F	**37**	Non-neoplastic	<0.15	Lymphoma
			38	Non-neoplastic	<0.15	Lymphoma
31	62	F	**39**	PTC	87.7	PTC
32*	51	M	**40**	PTC	7810	PTC
			41	Non-neoplastic	<0.15	Non-neoplastic
33	21	M	**42**	PTC	27359	PTC
34	25	F	**43**	PTC	9632	PTC
35	76	M	**44**	MTC	<0.15	MTC
36	62	M	**45**	Non-neoplastic	<0.15	Non-neoplastic

Ultrasonography and Fine-Needle Aspiration Biopsy (FNAB)

Ultrasonography (US) was carried out by means of the Aplio XV (Toshiba, Japan) system equipped with a linear transducer (PLT-805AT). The following US parameters were considered suggestive of tumor spreading to CLN: rounded shape, long axis/short axis ratio < 1.5, irregular echotexture, absence of echogenic hilum, sharp borders, presence of microcalcifications, liquefaction or coagulation necrosis, aberrant distribution of vessels. Patients selected for FNAB on the basis of US characters were recommended not to take anticoagulants during the 5 days before procedure.

Each CLN was stung for aspiration two or three times with a 22-gauge needle, performing a minimum of 2 and a maximum of 4 passes every time for adequate sampling. The aspirates were expelled onto one or more glass slides and prepared as previously described [44]. After that, the needle was rinsed in 1 ml of sterile phosphate buffered saline (PBS), which was centrifuged at 1200rpm for 5min. in order to eliminate cellular debris. Supernatants were then transferred in new tubes and frozen at -20°C until Tgp assay.

Cytological and Histological Analysis

Both slides and surgical samples were examined by three experienced pathologists (CDG, VA, ER and DB), which delivered consistent reports in all cases. All pathologists were unaware of Tgp results and, vice versa, the biologists who carried out the Tgp assay were uninformed of the pathologists' diagnoses.

Tgp and Tg Autoantibody Measurements

All the FNAB washouts were analyzed within 10 days from collection. Tgp and Tg autoantibody assays were accomplished with, respectively, the immune-luminometric assay Tg-PluS and the Anti-Tgn kit from

B.R.A.H.M.S. (Hennigsdorf, Germany). All samples were read in duplicate, and in some cases read back after dilution in PBS. Results were expressed as ng/FNAB, and Tgp values below 0.15ng/ml, i.e. the functional sensitivity of assay, were considered negative. All samples were negative for the presence of Tg autoantibodies.

Statistical Analysis

Performances of FNAC and Tgp tests were compared by the one-tail Fisher exact test and refereed as statistically significant when the pertaining p value was < 0.05. Statistical analyses were made using the Stata software, version 8.0 (College Station, Texas, Stata Corporation, 2003).

Results

As reported in table 1, FNAC turned out to be inadequate in 4 (8.9%) out of the 45 CLN samples analyzed. The FNAC diagnosis was correctly provided in 34 cases out of 41 CLN, with an overall accuracy of 82.9%. In the remaining 7 cases, cytology erroneously identified 4 metastatic PTC that histology reported as 2 metastatic MTC (table 1, CLN n. 19 and 20) and 2 metastatic ATC (table 1, CLN n. 12 and 13); of 3 cytological diagnoses of non-neoplastic CLN, 1 was found to be a metastatic PTC (table 1, CLN n. 31), and

the other 2 were found to be lymphomas at histology (table 1, CLN n. 37 and 38). These 7 FNAC diagnoses were considered as false negative.

In agreement with other studies, we decided to adopt a Tgp cut-off of 1ng/FNAB, as it offered a good discrimination of tumors limiting false negative results at the same time [44, 46-48]. In these conditions, Tgp provided correct diagnoses in 41 CLN (31 true positive and 10 true negative), false negative results in 3 CLN, comprising 2 metastatic ATC (table 1, CLN n. 9 and 11), and one false positive in a CLN affected by lymphoma (table 1, CLN n. 28). Specificity, sensitivity, positive (PPV) and negative (NPV) predictive values, and accuracy are reported for FNAC and Tgp in table 2. It may be appreciated as Tgp showed a statistically significant higher NPV, while no differences were recorded among the two diagnostic approaches for sensitivity, specificity, PPV and overall accuracy. The comparison between these parameters of FNAC and Tgp was performed by analyzing either PTC and ATC taken together or the PTC cases alone (see table 3). In both situations, no statistically significant differences were observed among the different diagnostic results.

Table 2: *Diagnostic performances of fine-needle aspiration cytology (FNAC) and thyroglobulin protein (Tgp) assay for the diagnosis of all suspicious metastatic cervical lymph nodes (CLN) (n=45). PPV, positive predictive value; NPV, negative predictive value. In round brackets the number of cases. In square brackets the 95% confidence interval.*

	FNAC	Tgp	p
Sensitivity	82.1 (32/39) [66.5-92.5]	91.2 (31/34) [76.3-98.1]	0.216
Specificity	100 (2/2) [15.8-100]	90.9 (10/11) [58.7-99.8]	0.846
PPV	100 (32/32) [89.1-100]	96.9 (31/32) [83.8-99.9]	0.516
NPV	22.2 (2/9) [2.81-100]	76.9 (10/13) [46.2-95.0]	0.017
Accuracy	82.9 (34/41) [67.9-92.8]	91.1 (41/45) [78.8-97.5]	0.209

Table 3: *Diagnostic performances of fine-needle aspiration cytology (FNAC) and thyroglobulin protein (Tgp) assay for the diagnosis of suspicious metastatic cervical lymph nodes (CLN) of epithelial thyroid cancer patients (PTC+ATC) (n=36) or only PTC (n=33). PPV, positive predictive value; NPV, negative predictive value. In round brackets the number of cases. In square brackets the 95% confidence interval.*

	FNAC	Tgp	p
PTC + ATC			
Sensitivity	90 (27/30) [73.5-97.9]	91.2 (31/34) [76.3-98.1]	0.522
Specificity	100 (2/2) [15.8-100]	100 (2/2) [15.8-100]	0.758
PPV	100 (27/27) [87.2-100]	100 (31/31) [88.8-100]	0.574
NPV	40 (2/5) [5.27-85.3]	40 (2/5) [5.27-85.3]	0.720
Accuracy	90.6 (29/32) [75.0-98.0]	91.7 (33/36) [77.5-98.2]	0.557
Only PTC			
Sensitivity	96.3 (26/27) [81.0-99.9]	96.8 (30/31) [83.3-99.9]	0.569
Specificity	100 (2/2) [15.8-100]	100 (2/2) [15.8-100]	0.757
PPV	100 (26/26) [86.8-100]	100 (30/30) [88.4-100]	0.575
NPV	66.7 (2/3) [9.4-99.2]	66.7 (2/3) [9.4-99.2]	0.738
Accuracy	96.6 (28/29) [82.2-99.9]	97.0 (32/33) [84.2-99.9]	0.567

Discussions

In the present study, we showed that US-guided FNAC and Tgp assay had the same overall accuracy in the diagnosis of cervical lymph nodes (CLN) suspicious of malignancy. Although dependent on the experience and ability of the cytopathologists, FNAC was widely documented to be a reliable method for identifying CLN metastases, not only derived from thyroid cancers but also from other primary malignancies, as well as in recognizing non-tumoral diseases affecting CLN. Since the first observation, reported by Pacini and colleagues in 1992 (32), that Tgp determination in fine-needle washout could improve the accuracy of FNAC in the diagnosis of DTC metastatic CLN, several studies have confirmed its clinical utility, and its routine association with FNAC in the preoperative diagnosis of suspicious CLN has been recommended [11, 29-47].

Our experience on 45 CLN with available histological report encompassed cases of CLN harboring metastases from PTC, MTC, ATC, and non-thyroidal primary tumors. Considering the 36 CLN with metastases from PTC and ATC as a unique group, FNAC exhibited 90.6% accuracy, a datum in line with those reported from other studies [32,33,37,40,44,46]. In addition, Tgp evaluation on the same group offered a diagnostic performance comparable to that of FNAC. As expected, Tgp levels were very low in the three CLN harboring metastases from ATC, owing to the partial or total loss of expression of thyroid specific genes typically occurring in this cancer. In fact, in one of these lymph nodes the Tgp was still detectable and correctly indicated metastasis from thyroid carcinoma (CLN n. 13). Conversely, the absence or the low level (below the cut-off value of 1 ng) of Tgp in the other 2 lymph nodes were false negative results (CLN n. 10 and 12). The greatest diagnostic usefulness of Tgp was found on lymph nodes with inadequate FNAC (see CLN n. 1, 2 and 8), for which Tgp was able to provide the correct diagnosis. Similarly, in one case with cytological diagnosis reporting the absence of neoplastic features (CLN n. 31) Tgp correctly identified the metastatic nature of the CLN. False negative or non-diagnostic results characterize about 20% of FNAC diagnoses, and are frequently observed in presence of cystic or highly vascular lymph nodes [46]. It is in these conditions that Tgp determination could be of particular clinical utility, solving a major limit of the canonical FNAC due to the low cellularity of the collected samples. Actually, in order to reduce the cost bound to routine Tgp determination, we previously suggested that washout samples should be always collected, but analyzed for Tgp content only in case of uninformative or inconsistent FNAC diagnosis with respect to patient's clinical parameters [44]. More recently, Zhang and colleagues adopted a standard procedure for the diagnosis of CLN in patients with previous or concurrent diagnosis of PTC [48]. They suggest to evaluate FNA cytology first, and to analyze Tgp content only in those CLN samples not diagnosed as metastatic PTC [48].

Conclusions

FNAC represents the standard modality of evaluation of lymph-nodal metastases originated either from thyroid cancers or from other primary tumors. In the specific case of DTC detection, it is possible to combine this technique with Tg protein measurement without the need for further sampling. The results of the present study confirm previous observations about the diagnostic utility of Tgp assay in the FNA washout of CLN suspicious of metastatic thyroid cancer, particularly in the case of inadequate lymph node sampling.

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