Lowered cutoff of lymph node fine-needle aspiration thyroglobulin in thyroid cancer patients with serum anti-thyroglobulin antibody

Kwanhoon Jo¹, Min-Hee Kim¹, Yejee Lim¹, So-Lyung Jung², Ja-Seong Bae³, Chan-Kwon Jung⁴, Moo-Il Kang¹, Bong-Yun Cha¹ and Dong-Jun Lim¹

Division of Endocrinology and Metabolism, Departments of ¹Internal Medicine, ²Radiology, ³Surgery and ⁴Hospital Pathology, College of Medicine, Seoul St Mary's Hospital, The Catholic University of Korea, #505 Banpo-Dong, Seocho-Gu, Seoul 137-701, Korea

Correspondence should be addressed to D-J Lim
Email
ldj6026@catholic.ac.kr

Abstract

Objective: Fine needle aspiration cytology (FNAC) and measurement of thyroglobulin (Tg) in needle washout (FNA-Tg) are recommended for the diagnosis of metastatic or recurrent lymph nodes (LNs) in differentiated thyroid cancer (DTC). However, the effect of serum Tg antibody (TgAb) on FNA-Tg levels still remains unclear in the preoperative setting. We analyze the interference of serum TgAb on FNA-Tg levels as proof of concept in the diagnostic advantage of serum TgAb combined with FNA-Tg.

Subjects and methods: A total of 370 suspicious cervical LNs from 273 patients with DTC were included. The primary tumor was confirmed as DTC on preoperative pathology in all patients. We performed FNA-Tg measurement and FNAC on suspicious LNs and evaluated the diagnostic performance of FNAC and FNA-Tg according to TgAb status. Final diagnoses were confirmed by histological examination of excised specimens or by follow-up ultrasonography for at least 6 months.

Results: Data from 273 subjects with suspicious 370 LNs were evaluated. Fifty-five LNs (14.9%) were from TgAb+ positive serum TgAb (TgAb+) patients. Serum Tg and FNA-Tg levels were significantly lower in patients with TgAb+ than in those with TgAb-negative (TgAb-). Final pathology confirmed 109 LNs (29.5%) as malignant. Diagnostic performance of FNA-Tg at the same cutoff level was lower in the TgAb+ than TgAb- group. FNA-Tg cutoff levels determined by ROC curve were lower in the TgAb+ group.

Conclusion: The results suggested that the cutoff value of FNA-Tg should be lowered in suspicious LN before thyroidectomy in thyroid cancer patients with TgAb.

European Journal of
Endocrinology
(2015) 173, 489–497

Introduction

Thyroid cancer incidence has increased over the last decade worldwide. Although differentiated thyroid cancer (DTC) shows good prognosis, 5% to 20% of patients treated with total thyroidectomy developed cervical lymph node (LN) metastases at the time of diagnosis or during postoperative follow-up (1, 2).

Fine needle aspiration cytology (FNAC) is recommended for diagnosis of metastatic LNs in thyroid cancer (3, 4). When

FNAC results are nondiagnostic or indeterminate, thyroglobulin (Tg) measurement in washout fluid of FNA (FNA-Tg) may improve the diagnostic performance of FNA. Many previous studies reported that FNA-Tg is more sensitive than FNAC, and a combination of FNAC and FNA-Tg can improve diagnostic accuracy for detecting metastasis (4, 5, 6, 7, 8, 9, 10, 11, 12, 13). Therefore, recent guidelines indicate FNAC with FNA-Tg for all suspicious LNs of thyroid cancer (14, 15, 16).

Positive serum Tg antibody (TgAb) is known to lower serum Tg (sTg) levels, and this may be even more important in DTC patients prior to thyroidectomy and/or radioactive iodine therapy in whom a higher rate of positive TgAb has been reported (17). Thus, the high rate of positive serum TgAb can lower sTg levels and influence FNA-Tg levels, which is possibly associated with altered diagnostic accuracy. Few prior studies indicate the potential interference of TgAb on FNA-Tg levels (5). However, the influence of TgAb on FNA-Tg remains unclear; furthermore, the status of the thyroid operation had not been considered in most of the earlier studies.

We aimed to determine the effects of serum TgAb on the cutoff value of FNA-Tg to detect metastatic lesions in suspicious LNs of preoperative DTC patients and to improve the diagnostic accuracy of FNA and FNA-Tg.

Subjects and methods

Patients

All three inclusion criteria should be satisfied before study enrollment: i) consecutive patients should have confirmed diagnosis of primary DTC, ii) ultrasound-guided FNAC were performed along with FNA-Tg measurement for suspicious LN, and iii) FNAC, FNA-Tg and TgAb measurement should be done before surgery.

Initially, 1050 LNs in 661 patients with suspicious LNs detected by neck ultrasound were targeted at a tertiary referral hospital between May 2011 and December 2013. Two hundred and seventy-three patients with 474 LNs who underwent lobectomy or total thyroidectomy before FNA were excluded. Additionally, 388 patients with 206 LNs were excluded due to no primary thyroid cancer ($n=2$), no follow-up or confirmation of LNs by pathology ($n=155$) and no data of preoperative sTg and TgAb ($n=49$) (Fig. 1). A total of 273 patients with 370 LNs were evaluated according to the presence or absence of TgAb. The institutional review board approved the study (KC14RISI0259).

Serum Tg, TgAb, FNAC and FNA-Tg measurement

Both serum and FNA washout Tg levels were measured using an immunoradiometric assay kit (CIS Bio International, Saclay, France), with a functional sensitivity of 0.7 ng/ml. Serum TgAb was measured by a competitive RIA kit (ZenTech, Angleur, Belgium). Positive TgAb was defined as serum TgAb of ≥ 70 IU/ml; negative TgAb as a serum TgAb of < 70 IU/ml.

Figure 1

Case selection.

Ultrasound-guided FNAC was performed by one of several radiologists with the use of a 23-gauge needle. While a liquid-based cytology (LBC) was used in the diagnosis of primary thyroid tumor (18), FNAC for suspicious LN was evaluated by the conventional method: the aspirates were immediately smeared on slides, fixed in 95% ethanol and processed by both hematoxylin and eosin (H&E) and Papanicolaou stain. The same needle and syringe were rinsed with 2 ml of normal saline, and the washout fluid was submitted for FNA-Tg measurement.

FNAC and final pathology

Cytology findings of the LNs reviewed by experienced pathologists were categorized as non-diagnostic, benign and metastatic papillary thyroid carcinoma (PTC; suspicious malignancy, malignancy) (5, 10, 19, 20, 21).

Final outcome was confirmed by post-operative pathology in malignant LNs. When LNs were suspicious for malignancy in ultrasonography but cytology results revealed benign LNs, LN dissection and histopathologic confirmation were performed if there were malignant LNs in the same compartment (7, 13). Otherwise, decreased or same-size LNs in follow-up ultrasound after 6–12 months were defined as benign in the final outcome (21).

Clinical Study	K Jo and others	Washout thyroglobulin and anti-thyroglobulin	173:4	491
----------------	-----------------	--	-------	-----

Table 1 Patient characteristics according to TgAb.

Characteristics	TgAb–	TgAb+	P value
Number of patients (%) ^a	218 (79.9)	55 (20.1)	
Age	47.1 ± 12.7	45.5 ± 11.0	0.369
Male sex (%)	47 (21.6)	5 (9.1)	0.036
Preoperative serum Tg (ng/ml)	13.6 (0.2–557.6)	7.2 (0.0–71.2)	0.040
Preoperative TgAb (IU/ml)	12.6 (0.1–69.5)	209.2 (73.0–8560.0)	0.000
Number of lymph nodes (%)	289 (78.1)	81 (21.9)	
FNA-Tg (ng/ml)	0.4 (0.0–1698.5)	0.9 (0.1–1121.2)	0.026

^aData are expressed as mean ± s.d. or median (range).

Statistical analysis

Data were expressed as mean ± s.d. or median (minimum–maximum value). We performed receiver operating characteristic (ROC) curve analysis to confirm the cutoff level of FNA-Tg. Diagnostic performances of FNA and FNA-Tg were evaluated with respect to sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. The χ^2 test or Fisher exact test was used to determine the differences between benign and metastatic LNs according to categorical variables. Statistical significance was determined when the *P* value was <0.05. All reported *P* values are two-sided. All analyses were conducted by SPSS Software (version 18.0).

Results

Patient and LN characteristics

Finally, 273 patients with 370 LNs were included in this study. The median patient age at the time of FNA was 45 years (range 21–81) and 221 (81%) were female

(Table 1). Of the 273 patients, 218 (79.9%) were in the TgAb negative (TgAb–) group and 55 (20.1%) in the TgAb positive (TgAb+) group. There was no significant difference in age but sex distribution between groups. However, FNA-Tg and preoperative serum Tg levels were significantly lower in the TgAb+ group vs TgAb– group (Table 1).

Two hundred and sixty-one (70.5%) of 370 LNs were benign (Table 2); 55 LNs were confirmed by surgery and subsequent postoperative pathology and 206 LNs by follow-up US. The other 109 LNs (29.5%) were confirmed as malignant on postoperative pathology (Table 2 and Fig. 2). According to the final outcome and TgAb status, we compared four LN groups (Table 2). In benign LNs, there were no significant differences in FNA-Tg and preoperative sTg between the TgAb+ and TgAb– groups; however, in malignant LNs, median sTg and FNA-Tg levels were significantly lower in patients with serum TgAb+ than in those with TgAb– (8.2 vs 29.7 ng/ml, *P*=0.000; 398.5 vs 881.3 ng/ml, *P*=0.012).

Diagnostic performance of FNAC

Among 109 metastatic LNs, FNAC resulted in a correct diagnosis in 92 cases but failed to diagnose 17 cases with a resultant 84.4% sensitivity and 99.6% specificity. Among the 17 misdiagnosed metastatic LNs, 15 had been reported as benign, one unsatisfactory and one instance of macrophages on FNAC. Among 261 benign LNs, 259 LNs were correctly diagnosed as benign in FNAC but one LN showed malignancy and another LN as unsatisfactory.

FNA-Tg and final outcome

We compared sensitivity, specificity, PPV, NPV and diagnostic accuracy of FNA-Tg levels of LN according to the functional sensitivity of Tg (0.7 ng/ml) (3, 22) and the value determined by ROC curve (6.0 ng/ml), with those of FNAC (Table 4). In addition, FNA-Tg/sTg ratio was also

Table 2 Lymph node characteristics according to final outcome and TgAb.

Final outcome	Benign		Malignant		P value			
	TbAb– ^a	TbAb+ ^b	TbAb– ^c	TbAb+ ^d	a vs b	c vs d	a vs c	b vs d
Number of lymph nodes	200	61	89	20				
FNA-Tg (ng/ml)	0.2 (0.0–1253.7)	0.3 (0.1–833.5)	881.3 (0.2–1698.5)	398.5 (0.3–1121.2)	0.930	0.012	0.000	0.000
Preoperative serum Tg (ng/ml)	11.5 (0.2–366.2)	6.8 (0.0–71.2)	29.7 (0.4–557.6)	8.2 (0.1–71.2)	0.127	0.000	0.004	0.767
Preoperative TgAb (IU/ml)	14.2 (0.1–66.9)	218.9 (75.9–8560.0)	12.5 (0.3–69.5)	515.8 (73–6483.1)	0.001	0.013	0.004	0.625

Figure 2

A 72-year-old female presented with thyroid nodule and a suspicious lymph node in the left side of the neck. She underwent fine-needle aspiration cytology (FNAC) with wash-out thyroglobulin (FNA-Tg) for lymph node. Her FNA-Tg, serum Tg, and serum Tg antibody were 809.0 ng/ml, 18.5 ng/ml and 19.1 IU/ml respectively. (A) FNAC of cervical lymph node shows cellular clusters with characteristic nuclear features of papillary thyroid carcinoma (Papanicolaou stain, $\times 400$). (B) On pathologic examination of resected specimen, metastatic papillary carcinoma is seen within the lymph node (H&E stain, $\times 12$). A full colour version of this figure is available at <http://dx.doi.org/10.1530/EJE-15-0344>.

used as a cut-off value to diminish the influence of sTg in preoperative patients (19, 23) (Table 4)

FNAC alone showed the lowest sensitivity (88.4%) but the highest specificity (99.6%). With FNA-Tg 0.7 added to FNAC, sensitivity was the highest (99.1%) but PPV was the lowest (63.9%). The combination of FNA-Tg 6.0 and FNAC

showed the best diagnostic accuracy, with 97.3% sensitivity, 94.6% specificity, 88.3% PPV, 98.8% NPV and 95.4% accuracy. The diagnostic value of FNA-Tg/sTg ratio was inferior to that of FNA-Tg alone.

All combinations of FNA-Tg and FNAC showed higher sensitivity and NPV than FNAC or FNA-Tg alone.

Optimal cutoff values of FNA-Tg in determining malignant LNs according to TgAb status

Diagnostic performance of FNAC, all FNA-Tg levels and their combinations were lower in the TgAb+ group than TgAb- group. When applying ROC curve cutoff of the entire group (6.0 ng/ml), sensitivity and PPV were reduced by > 10% in the TgAb+ group, as compared to the TgAb- group (Table 5). Comparing FNA-Tg alone with FNAC combined with FNA-Tg at each cutoff value of FNA-Tg, there was no significant improvement in diagnostic performance found in the TgAb- group, but sensitivity was greatly improved (85–95%) in the TgAb+ group. Sensitivity and NPV improved when applying a lower cutoff value of FNA-Tg (6.0 ng/ml to 2.5 ng/ml) combined with FNAC in the TgAb+ group. On the contrary, TgAb- group showed better diagnostic performance when applying a higher cutoff of FNA-Tg (6.0 ng/ml to 8.5 ng/ml) (Table 5).

Presentation of LNs with discordant results

FNAC results and postoperative pathology were not in agreement in 19 LNs of the 19 patients: 15 LNs that were negative for malignancy in FNAC were malignant on postoperative pathology (Table 6). Four patients out of 19 discordant cases showed positive TgAb (21.1%). Among 15 malignant LNs, only three LNs showed FNA-Tg of <6 ng/ml (cases 1–3). Case 2 with positive TgAb (173.7 IU/ml) had 2.8 ng/ml of FNA-Tg, which could have been detected by the cutoff value (2.5 ng/ml) with ROC analysis in the TgAb+ group.

Table 3 Correlation of FNAC results with postoperative pathology in suspicious lymph node.

FNAC results	Postoperative pathology		Total
	Benign	Malignancy	
Benign	259	15	274
Malignant	1	92	93
Unsatisfactory	1	1	2
Other	0	1	1
Total	261	109	370

Clinical Study	K Jo and others	Washout thyroglobulin and anti-thyroglobulin	173:4	493
-----------------------	------------------------	---	--------------	------------

Table 4 Comparison between diagnostic performances of FNAC, FNA-Tg and FNA-Tg/sTg ratio.

Diagnostic performance	Sensitivity	Specificity	PPV	NPV	Accuracy
FNAC	84.4	99.6	98.9	93.9	95.1
FNA-Tg 0.7 ^a	98.2	77.0	64.1	99.0	83.2
FNA-Tg 6.0 ^b	95.4	95.0	88.9	98.0	95.1
FNA-Tg/sTg 1	88.1	93.9	85.7	95.0	92.2
FNA-Tg/sTg 0.4 ^b	92.7	88.9	77.7	96.7	90.0
FNAC + FNA-Tg 0.7 ^a	99.1	76.6	63.9	99.5	83.2
FNAC + FNA-Tg 6.0 ^b	97.3	94.6	88.3	98.8	95.4
FNAC + FNA-Tg/sTg 1	92.7	93.5	85.6	96.8	93.2
FNAC + FNA-Tg/sTg 0.4 ^b	96.3	88.5	77.8	98.3	90.8

^aFunctional sensitivity of thyroglobulin assay.^bCutoff value according to ROC.

Another two non-diagnostic LNs were metastatic in the final report (cases 16 and 17) with FNA-Tg levels of >6 ng/ml, without TgAb. Case 18, initially misdiagnosed as papillary carcinoma by FNAC, was finally diagnose as parathyroid hyperplasia (Table 6).

Discussion

Although many clinical studies composed of total thyroidectomy patients evaluated optimal cutoff values of FNA-Tg levels in detecting metastatic LNs, there are no reliable studies on cutoff values of FNA-Tg levels in preoperative patients. Several studies were conducted on preoperative patients (7, 13, 23, 24, 25) but the small number of cases precluded any strong conclusion and the TgAb status was not considered. Prior studies also showed

the effect of TgAb status on FNA-Tg levels but did not consider the operative status (5, 10, 26).

In this study, preoperative patients who were diagnosed with PTC and had LNs with metastatic feature in US underwent FNAC and Tg estimation in washout fluid of FNAC. Our study confirmed that positive serum TgAb lowered FNA-Tg levels and influenced the diagnostic accuracy of FNA-Tg in suspicious LNs of preoperative patients.

All preoperative patients had normal thyroid tissues, which indicated an increased proportion of TgAb-positive patients, as compared to thyroidectomy patients with or without radioiodine remnant ablation (27). As increased serum TgAb levels lower sTg levels (28), we hypothesized that increased serum TgAb levels in thyroid cancer patients have lower FNA-Tg levels and subsequently

Table 5 Comparison between diagnostic performances of FNA-Tg, FNA-Tg/sTg ratio according to TgAb status.

Diagnostic performance	Sensitivity	Specificity	PPV	NPV	Accuracy
TgAb –					
FNAC	85.4	99.5	98.7	93.9	95.2
FNA-Tg 0.7 ^a	98.9	81.0	69.8	99.4	86.5
FNA-Tg 6.0 ^b	97.8	96	91.6	99.0	96.5
FNA-Tg 8.5 ^c	96.6	96.5	92.5	98.5	96.5
FNAC + FNA-Tg 0.7 ^a	98.9	80.5	69.3	99.4	86.2
FNAC + FNA-Tg 6.0 ^b	97.8	95.5	90.6	99.0	96.2
FNAC + FNA-Tg 8.5 ^c	96.6	96.0	91.5	98.5	96.2
TgAb +					
FNAC	80.0	100.0	100.0	93.9	95.1
FNA-Tg 0.7 ^a	95.0	63.9	46.3	97.5	71.6
FNA-Tg 6.0 ^b	85	91.8	77.3	94.9	90.1
FNA-Tg 2.5 ^d	95	83.6	65.5	98.1	86.4
FNAC + FNA-Tg 0.7 ^a	100.0	63.9	47.6	100.0	72.8
FNAC + FNA-Tg 6.0 ^b	95.0	91.8	79.2	98.3	92.6
FNAC + FNA-Tg 2.5 ^d	100.0	83.6	66.7	100.0	87.7

^aFunctional sensitivity of thyroglobulin assay.^bCutoff value according to ROC regardless of TgAb status.^cCutoff value according to ROC in the absence of TgAb.^dCutoff value according to ROC in the presence of TgAb.

Clinical Study	K Jo and others	Washout thyroglobulin and anti-thyroglobulin	173:4	494
----------------	-----------------	--	-------	-----

Table 6 Clinical characteristics of lymph nodes with discordant results between preoperative FNAC and postoperative pathology.

LN#s	Gender	Age	FNAC	Postoperative pathology	FNA-Tg	TgAb	Serum Tg	Cervical lymph node level
1	F	53	Negative for malignancy	Papillary carcinoma	0.2	1.1	14.4	L4
2	F	45	Negative for malignancy	Papillary carcinoma	2.8	173.7	23.4	R2
3	F	49	Negative for malignancy, many small and large polymorphic lymphoid cells	Papillary carcinoma	4.8	39.3	39.7	R4
4	F	35	Negative for malignancy	Papillary carcinoma	6.6	11.4	2.3	R4
5	F	60	Negative for malignancy	Papillary carcinoma	7.9	1552.2	9.1	R3
6	F	44	Negative for malignancy	Papillary carcinoma	9.3	24.4	11.8	L4
7	F	36	Negative for malignancy	Papillary carcinoma	26.0	8.6	514.4	L3
8	F	49	Negative for malignancy	Papillary carcinoma	27.1	191.1	36.9	R6
9	F	36	Negative for malignancy	Papillary carcinoma	29.4	664.2	71.2	R3
10	F	49	Negative for malignancy	Papillary carcinoma	36.2	13.0	28.9	L4
11	F	39	Negative for malignancy. Mainly blood and some inflammatory cells	Papillary carcinoma	890.8	22.0	15.2	R4
12	F	47	Negative for malignancy	Papillary carcinoma	1037.5	15.3	28.4	R2
13	M	63	Negative for malignancy	Papillary carcinoma	1195.5	13.0	5.7	R3
14	F	60	Negative for malignancy	Papillary carcinoma	1195.6	9.2	156.3	R4
15	M	43	Negative for malignancy	Papillary carcinoma	1295.7	6.8	49.6	L4
16	M	34	Non-diagnostic specimen; only fibrovascular and muscular tissue	Papillary carcinoma	413.8	12.1	25.4	L4
17	M	60	Other; many hemosiderin-laden macrophages	Papillary carcinoma	800.3	1.4	122.9	L4
18	F	40	Papillary carcinoma	Parathyroid hyperplasia	0.3	10.7	14.7	R6
19	F	38	Unsatisfactory; very low cellularity	Negative for malignancy	0.0	6.4	6.1	R4

L, left; R, right; number, cervical lymph node area (2, 3, 4, 6).

lower diagnostic accuracy of FNA-Tg in patients with suspicious LNs. Baskin *et al.* (26) first suggested that the measurement of FNA-Tg from LN was unlikely to be affected by the presence of TgAb in the peripheral blood, possibly because the intracellular Tg is not exposed to circulating TgAb, from the two patients who showed FNA-Tg levels of 24.9 and 67.5 ng/ml respectively but positive TgAb and undetectable sTg. Boi *et al.* (5) also concluded that the diagnostic performance of FNA-Tg seems to be not substantially affected by TgAb, although TgAb in FNA washout may lower FNA-Tg in a few selected cases. The rationale of no interference by TgAb is as follows: In metastatic LNs, FNA-Tg levels are much higher than TgAb levels that exist in the washout fluid. It can overcome interference by TgAb due to saturated TgAb-binding sites (5). Diluted TgAb in washout fluid (most likely being 1:50 or higher) (25) result in absence or scarcity of TgAb within the LNs.

However, one recent study by Jeon *et al.* (21) demonstrated that high serum TgAb levels could lower FNA-Tg levels, from 25 suspicious LNs in 14 patients who underwent total thyroidectomy and radioiodine remnant ablation. They showed a diagnosis based on FNA-Tg in metastatic LNs might have a lower sensitivity and NPV in

the TgAb-positive patients (19, 26). Consistent with their results, our study also showed a lower diagnostic accuracy of FNA-Tg, whether combined with FNAC or not, in the TgAb-positive patients (Table 5). However, a high proportion of patients (55/273, 20.1%) with high TgAb levels existed in our preoperative LN case series, whereas only 15 patients out of 207 (7.2%), in Jeon *et al.* (21), showed high TgAb levels and were evaluated by FNA-Tg for suspicious LNs over follow-up after total thyroidectomy. These findings suggest that TgAb has greater effect on FNA-Tg level of preoperative patients and justify TgAb measurement when planning to perform FNA-Tg evaluation before surgery.

We measured FNA-Tg levels of 370 LNs from preoperative patients according to the presence of TgAb. As FNA-Tg levels *per se* are too low in uninvolved benign LNs, the effect of TgAb positivity on FNA-Tg levels was negligible. On the other hand, the FNA-Tg levels from malignant LNs were significantly high, indicating that serum TgAb positivity might affect FNA-Tg as well as sTg. Usually, women showed a higher rate of TgAb positivity than men. This could be explained by the higher prevalence of autoimmune thyroiditis in women than men (29).

Our results indicated that FNA-Tg had a high NPV, a characteristic of a good diagnostic tool. Diagnostic performance of the combination of FNAC and FNA-Tg levels was better than the single use of FNAC or FNA-Tg (Table 4). When we used an FNA-Tg cutoff value of 6.0 ng/ml for preoperative patients, the TgAb– group showed the best diagnostic performance, but lower sensitivity and NPV were shown in the TgAb+ group. In comparison, lowering the FNA-Tg cutoff value led to increased sensitivity and NPV in the TgAb+ group (Table 5). This finding supported the hypothesis that the cutoff level of FNA-Tg in TgAb+ group should be lower than in the TgAb– group. Interestingly, combining FNAC with FNA-Tg showed the same sensitivity but lower specificity in the TgAb– group, because of one false-positive FNAC result.

It has been suggested that sTg level should be considered with FNA-Tg by sTg to FNA-Tg ratio (23, 30, 31). This approach could potentially overcome interference by sTg with FNA-Tg, especially in preoperative patients, and therefore needs validation. In our study, a serum-to-FNA-Tg ratio of 1.0 had an 88.1% sensitivity and 93.9% specificity, which showed inferiority to FNA-Tg alone or FNA-Tg combined with FNAC (Table 4).

Generally, FNAC, FNA-Tg levels and their combination had lower diagnostic performance in TgAb+ group compared to the TgAb– group. These results indicated that a positive TgAb status has a low diagnostic rate at any cutoff level (Table 5). According to the current guidelines, there is no recommendation to measure serum TgAb levels preoperatively (16). However, TgAb should be measured in preoperative thyroid cancer patients with planned FNAC or FNA-Tg assays for suspicious LNs. Our results suggested a lowering of cutoff value of FNA-Tg levels when TgAb is present in patient serum.

For evaluation of FNAC on LN, we used the conventional smear (CS) method rather than LBC. There were several studies that showed better diagnostic performance in LBC than in CS in evaluating thyroid nodule (18, 32). However, there were rarely studies showing the usefulness of LBC in FNAC of the LN. One study showed that the diagnostic efficacy of FNAC of the LN is identical between the CS and LBC (33). In another study by Tripathy *et al.* (34) that evaluated 40 LNs (20 reactive hyperplasia, 12 granulomatous lymphadenitis, three lymphoma and five metastatic carcinoma), Reed–Sternberg cells were more easily diagnosed on LBC smears due to mono-layering. From a few prior studies, we could see the potential of LBC in FNA-Tg diagnosis. In the near future, comparative study should be included between LBC and the CS method for the improvement of diagnostic efficacy in LN FNAC.

The strength of this study was that all 370 LNs in 273 patients were preoperative cases that had never been assessed in detail up to that point. However, we did not measure the levels of TgAb in the FNA washout fluid (FNA-TgAb). This makes it impossible to know the exact impact of FNA-TgAb on FNA-Tg levels, even though FNA-TgAb is unlikely to have an influence on FNA-Tg levels, according to several reports (35, 36): first, the high Tg levels in metastatic LN (FNA-Tg) overcomes the blocking capacity of FNA-TgAb. Secondly, the FNA-TgAb is too low to interfere. Another limitation was that all suspicious LNs evaluated by FNAC and FNA-Tg were not pathologically confirmed. The LNs considered to be benign by FNAC were not the target of surgery, hence their final outcomes were dependent on follow-up US in 6–12 months.

Conclusion

Our data indicated that serum TgAb should be measured along with FNAC and FNA-Tg on suspicious LNs in preoperative patients, due to the different cutoff values of FNA-Tg based on serum TgAb status. We propose that the cutoff value of FNA-Tg should be lowered before thyroidectomy in thyroid cancer patients with positive serum TgAb.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

References

- Schlumberger MJ. Medical progress – papillary and follicular thyroid carcinoma. *New England Journal of Medicine* 1998 **338** 297–306. (doi:10.1056/NEJM199801293380506)
- Johnson NA & Tublin ME. Postoperative surveillance of differentiated thyroid carcinoma: rationale, techniques and controversies. *Radiology* 2008 **249** 429–444. (doi:10.1148/radiol.2492071313)
- Cunha N, Rodrigues F, Curado F, Ilheu O, Cruz C, Naidenov P, Rascao MJ, Ganho J, Gomes I, Pereira H *et al.* Thyroglobulin detection in fine-needle aspirates of cervical lymph nodes: a technique for the diagnosis of metastatic differentiated thyroid cancer. *European Journal of Endocrinology* 2007 **157** 101–107. (doi:10.1530/EJE-07-0088)
- Kwak JY. Indications for fine needle aspiration in thyroid nodules. *Endocrinology and Metabolism* 2013 **28** 81–85. (doi:10.3803/EnM.2013.28.2.81)
- Boi F, Baghino G, Atzeni F, Lai ML, Faa G & Mariotti S. The diagnostic value for differentiated thyroid carcinoma metastases of thyroglobulin

- (Tg) measurement in washout fluid from fine-needle aspiration biopsy of neck lymph nodes is maintained in the presence of circulating anti-Tg antibodies. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 1364–1369. (doi:10.1210/jc.2005-1705)
- 6 Baloch ZW, Barroeta JE, Walsh J, Gupta PK, Livolsi VA, Langer JE & Mandel SJ. Utility of thyroglobulin measurement in fine-needle aspiration biopsy specimens of lymph nodes in the diagnosis of recurrent thyroid carcinoma. *CytoJournal* 2008 **5** 1. (doi:10.1186/1742-6413-5-1)
 - 7 Sohn YM, Kim MJ, Kim EK & Kwak JY. Diagnostic performance of thyroglobulin value in indeterminate range in fine needle aspiration washout fluid from lymph nodes of thyroid cancer. *Yonsei Medical Journal* 2012 **53** 126–131. (doi:10.3349/ymj.2012.53.1.126)
 - 8 Mikosinski S, Pomorski L, Oszkowska L, Makarewicz J, Adamczewski Z, Sporny S & Lewinski A. The diagnostic value of thyroglobulin concentration in fine-needle aspiration of the cervical lymph nodes in patients with differentiated thyroid cancer. *Endokrynologia Polska* 2006 **57** 392–395.
 - 9 Jung JY, Shin JH, Han BK & Ko EY. Optimized cutoff value and indication for washout thyroglobulin level according to ultrasound findings in patients with well-differentiated thyroid cancer. *AJNR. American Journal of Neuroradiology* 2013 **34** 2349–2353. (doi:10.3174/ajnr.A3687)
 - 10 Frasoldati A, Toschi E, Zini M, Flora M, Caroggio A, Dotti C & Valcavi R. Role of thyroglobulin measurement in fine-needle aspiration biopsies of cervical lymph nodes in patients with differentiated thyroid cancer. *Thyroid* 1999 **9** 105–111. (doi:10.1089/thy.1999.9.105)
 - 11 Grani G & Fumarola A. Thyroglobulin in lymph node fine-needle aspiration wash-out: a systematic review and meta-analysis of diagnostic accuracy. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** 1970–1982. (doi: 10.1210/jc.2014-1098)
 - 12 Torres MR, Nobrega Neto SH, Rosas RJ, Martins AL, Ramos AL & da Cruz TR. Thyroglobulin in the washout fluid of lymph-node biopsy: what is its role in the follow-up of differentiated thyroid carcinoma? *Thyroid* 2014 **24** 7–18. (doi:10.1089/thy.2013.0244)
 - 13 Moon JH, Kim YI, Lim JA, Choi HS, Cho SW, Kim KW, Park HJ, Paeng JC, Park YJ, Yi KH *et al.* Thyroglobulin in washout fluid from lymph node fine-needle aspiration biopsy in papillary thyroid cancer: large-scale validation of the cutoff value to determine malignancy and evaluation of discrepant results. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 1061–1068. (doi:10.1210/jc.2012-3291)
 - 14 Leenhardt L, Erdogan MF, Hegedus L, Mandel SJ, Paschke R, Rago T & Russ G. European thyroid association guidelines for cervical ultrasound scan and ultrasound-guided techniques in the postoperative management of patients with thyroid cancer. *European Thyroid Journal* 2013 **2** 147–159. (doi:10.1159/000354537)
 - 15 Yi KH, Park YJ, Koong S-S, Kim J-H, Na DG, Ryu J-S, Park SY, Park IA, Baek C-H, Shong YK *et al.* Revised Korean Thyroid Association management Guidelines for patients with thyroid nodules and thyroid cancer. *Endocrinology and Metabolism* 2010 **25** 270–297. (doi:10.3803/EnM.2010.25.4.270)
 - 16 Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M *et al.* Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid* 2009 **19** 1167–1214. (doi:10.1089/thy.2009.0110)
 - 17 Kim ES, Lim DJ, Baek KH, Lee JM, Kim MK, Kwon HS, Song KH, Kang MI, Cha BY, Lee KW *et al.* Thyroglobulin antibody is associated with increased cancer risk in thyroid nodules. *Thyroid* 2010 **20** 885–891. (doi:10.1089/thy.2009.0384)
 - 18 Jung CK, Lee A, Jung ES, Choi YJ, Jung SL & Lee KY. Split sample comparison of a liquid-based method and conventional smears in thyroid fine needle aspiration. *Acta Cytologica* 2008 **52** 313–319. (doi:10.1159/000325513)
 - 19 Kim MJ, Kim EK, Kim BM, Kwak JY, Lee EJ, Park CS, Cheong WY & Nam KH. Thyroglobulin measurement in fine-needle aspirate washouts: the criteria for neck node dissection for patients with thyroid cancer. *Clinical Endocrinology* 2009 **70** 145–151. (doi:10.1111/j.1365-2265.2008.03297.x)
 - 20 Salmaslioglu A, Erbil Y, Citlak G, Ersoz F, Sari S, Olmez A, Tunaci M, Yilmazbayhan D, Colak N & Ozarmagan S. Diagnostic value of thyroglobulin measurement in fine-needle aspiration biopsy for detecting metastatic lymph nodes in patients with papillary thyroid carcinoma. *Langenbeck's Archives of Surgery* 2011 **396** 77–81. (doi:10.1007/s00423-010-0723-1)
 - 21 Jeon MJ, Park JW, Han JM, Yim JH, Song DE, Gong G, Kim TY, Baek JH, Lee JH, Shong YK *et al.* Serum antithyroglobulin antibodies interfere with thyroglobulin detection in fine-needle aspirates of metastatic neck nodes in papillary thyroid carcinoma. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 153–160. (doi:10.1210/jc.2012-2369)
 - 22 Snozek CL, Chambers EP, Reading CC, Sebo TJ, Sistrunk JW, Singh RJ & Grebe SK. Serum thyroglobulin, high-resolution ultrasound, and lymph node thyroglobulin in diagnosis of differentiated thyroid carcinoma nodal metastases. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 4278–4281. (doi:10.1210/jc.2007-1075)
 - 23 Chung J, Kim EK, Lim H, Son EJ, Yoon JH, Youk JH, Kim JA, Moon HJ & Kwak JY. Optimal indication of thyroglobulin measurement in fine-needle aspiration for detecting lateral metastatic lymph nodes in patients with papillary thyroid carcinoma. *Head & Neck* 2014 **36** 795–801. (doi:10.1002/hed.23371)
 - 24 Kim DW, Jeon SJ & Kim CG. Usefulness of thyroglobulin measurement in needle washouts of fine-needle aspiration biopsy for the diagnosis of cervical lymph node metastases from papillary thyroid cancer before thyroidectomy. *Endocrine* 2012 **42** 399–403. (doi:10.1007/s12020-012-9636-9)
 - 25 Sigstad E, Heilo A, Paus E, Holgersen K, Groholt KK, Jorgensen LH, Bogsrud TV, Berner A & Bjoro T. The usefulness of detecting thyroglobulin in fine-needle aspirates from patients with neck lesions using a sensitive thyroglobulin assay. *Diagnostic Cytopathology* 2007 **35** 761–767. (doi:10.1002/dc.20726)
 - 26 Baskin HJ. Detection of recurrent papillary thyroid carcinoma by thyroglobulin assessment in the needle washout after fine-needle aspiration of suspicious lymph nodes. *Thyroid* 2004 **14** 959–963. (doi:10.1089/thy.2004.14.959)
 - 27 Spencer CA, Takeuchi M, Kazarosyan M, Wang CC, Guttler RB, Singer PA, Fatemi S, LoPresti JS & Nicoloff JT. Serum thyroglobulin autoantibodies: prevalence, influence on serum thyroglobulin measurement, and prognostic significance in patients with differentiated thyroid carcinoma. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1121–1127.
 - 28 Spencer CA. Challenges of serum thyroglobulin (Tg) measurement in the presence of Tg autoantibodies. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 3702–3704. (doi:10.1210/jc.2004-0986)
 - 29 Office on Women's Health USDoHaHS. "Hashimoto's disease fact sheet". <http://www.womenshealth.gov/publications/our-publications/fact-sheet/hashimoto-disease.html> 2012.
 - 30 Jeon SJ, Kim E, Park JS, Son KR, Baek JH, Kim YS, Park do J, Cho BY & Na DG. Diagnostic benefit of thyroglobulin measurement in fine-needle aspiration for diagnosing metastatic cervical lymph nodes from papillary thyroid cancer: correlations with US features. *Korean Journal of Radiology* 2009 **10** 106–111. (doi:10.3348/kjr.2009.10.2.106)
 - 31 Urano T, Miyauchi A, Shimizu K, Tomoda C, Takamura Y, Ito Y, Miya A, Kobayashi K, Matsuzuka F, Amino N *et al.* Usefulness of thyroglobulin measurement in fine-needle aspiration biopsy specimens for diagnosing cervical lymph node metastasis in patients with papillary thyroid cancer. *World Journal of Surgery* 2005 **29** 483–485. (doi:10.1007/s00268-004-7701-0)
 - 32 Chang H, Lee E, Lee H, Choi J, Kim A & Kim BH. Comparison of diagnostic values of thyroid aspiration samples using liquid-based

Clinical Study	K Jo and others	Washout thyroglobulin and anti-thyroglobulin	173:4	497
----------------	-----------------	--	-------	-----

- preparation and conventional smear: one-year experience in a single institution. *APMIS* 2013 **121** 139–145. (doi:10.1111/j.1600-0463.2012.02944.x)
- 33 Garbar C, Rimmelink M & Mascaux C. Fine needle aspiration cytology of lymph node: experience of 2 university hospitals with conventional smears and liquid-based cytology. *Acta Cytologica* 2008 **52** 418–423. (doi:10.1159/000325546)
- 34 Tripathy K, Misra A & Ghosh JK. Efficacy of liquid-based cytology versus conventional smears in FNA samples. *Journal of Cytology* 2015 **32** 17–20. (doi:10.4103/0970-9371.155225)
- 35 Spencer C & Fatemi S. Thyroglobulin antibody (TgAb) methods – strengths, pitfalls and clinical utility for monitoring TgAb-positive patients with differentiated thyroid cancer. *Best Practice & Research. Clinical Endocrinology & Metabolism* 2013 **27** 701–712. (doi:10.1016/j.beem.2013.07.003)
- 36 Verburg FA, Luster M, Cupini C, Chiovato L, Duntas L, Elisei R, Feldt-Rasmussen U, Rimmele H, Seregni E, Smit JW *et al.* Implications of thyroglobulin antibody positivity in patients with differentiated thyroid cancer: a clinical position statement. *Thyroid* 2013 **23** 1211–1225. (doi:10.1089/thy.2012.0606)

Received 30 March 2015

Revised version received 2 July 2015

Accepted 22 July 2015