

Serum thyroglobulin elevation after needle aspiration of the lymph nodes: the predictive value for detecting metastasis in papillary thyroid cancer patients – a pilot study

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Abstract

Ultrasonography (USG)-guided fine needle aspiration (FNA) is widely used for diagnosis of lymph node (LN) metastasis in papillary thyroid cancer (PTC). However, FNA cytology sometimes shows inconclusive results. Recently, the measurement of thyroglobulin (Tg) in FNA washout fluid (aspirate-Tg) has been widely adopted, but there are some difficulties in the preparation of the sample and standardization of the procedure. Here, we examined serum Tg after FNA as a new predictive marker for LN metastasis of PTC. We performed USG-guided FNA cytology and examined aspirate-Tg in PTC patients showing suspicious metastatic LNs during follow-up. We measured baseline serum thyroid stimulating hormone (TSH), Tg, and Tg antibody levels before FNA, and serum Tg level within an hour after FNA. We defined aspirate-Tg level above 0.9 ng/mL as positive, and a 30% increase in serum Tg level after FNA compared to the baseline as elevation of serum Tg. Twenty-two patients were included in our study. Nine patients (40.9%) showed elevation of Tg level after FNA, and the mean value of Tg elevation was 24.8 ± 48.0 ng/mL. Among these 9 patients, 8 were diagnosed with PTC and 1 patient showed cellular atypia on cytopathology. All these patients showed positive aspirate-Tg. Thirteen patients (59.1%) did not show elevation of Tg level after FNA. Among these patients, 2 had PTC, 2 had cellular atypia, and 9 yielded negative results for malignancy on cytopathology. Elevation of serum Tg level after FNA might have a diagnostic role for predicting LN metastasis of PTC.

Abbreviations: Aspirate-Tg = thyroglobulin in fine needle aspiration washout fluid, FNA = fine needle aspiration, HIFU = high intensity focused ultrasound, LN = lymph node, PTC = papillary thyroid cancer (PTC), Tg = thyroglobulin, Tg-Ab = thyroglobulin-antibody, USG = ultrasonography.

Keywords: fine needle aspiration, lymph node, metastasis, thyroglobulin, thyroid cancer

1. Introduction

Papillary thyroid cancer (PTC) is the most common endocrine cancer, and the prevalence of PTC has risen in the last few decades. Even though PTC patients have excellent survival, more than 60% of PTC patients harbor cervical lymph node (LN)

metastasis.^[1] Moreover, up to 30% of PTC patients experience recurrence or persistent metastasis of the cervical LNs.^[2]

Ultrasonography (USG) is a crucial tool for detecting LN metastasis in PTC patients.^[3] USG-guided fine needle aspiration (FNA) has been widely used for the diagnosis of LN metastasis. However, FNA shows a 6% to 8% false-negative rate and a 5% to 10% inadequacy rate.^[4] Since Pacini et al first introduced the measurement of thyroglobulin (Tg) in FNA washout fluid (aspirate-Tg),^[5] aspirate-Tg has been widely used and has shown promising results for improved diagnostic sensitivity of metastatic thyroid cancer.^[6] However, there are some difficulties in preparation of the sample and standardization of the procedure.^[7]

Serum Tg in patients who undergo total thyroidectomy followed by radioiodine ablation is considered an excellent specific tumor marker for detecting recurrent or persistent thyroid cancer. Tg is a large glycoprotein with a molecular weight of 660 kDa, which is produced in both benign thyroid follicular cells and malignant cells, including PTC cells.^[8] There have been some reports that cancer markers in the serum can be increased after procedures or chemotherapy to cancerous tissues.^[9,10] Therefore, we hypothesized that serum Tg after FNA might be affected by the FNA procedure, and we measured serum Tg after FNA of suspicious metastatic LNs in PTC patients who underwent total thyroidectomy and radioiodine ablation.

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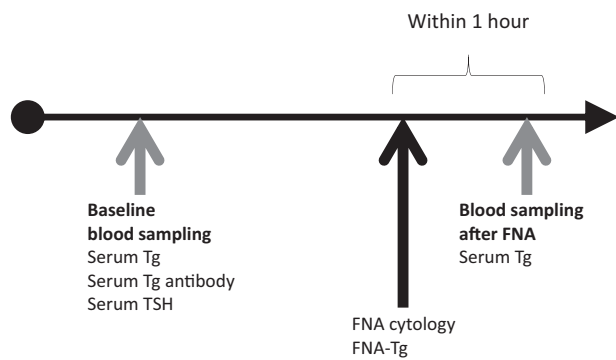


Figure 1. Sequence of thyroglobulin (Tg), Tg-antibody (Tg-Ab), and thyroid stimulating hormone (TSH) level measurement with regard to fine needle aspiration (FNA).

2. Materials and methods

2.1. Patients

We retrospectively enrolled PTC patients seen at our institution between October 2016 and July 2018 who had suspicious metastatic LN features on USG during follow-up after total thyroidectomy followed by radioiodine ablation. We performed an annual or bi-annual recurrence examination using serum Tg, Tg-antibody (Tg-Ab), TSH, and USG. Before USG-guided FNA of suspicious metastatic LNs, baseline serum Tg, Tg-Ab, and TSH levels were measured. Immediately after FNA, aspirate-Tg (wash-out of the syringe) level was measured, and serum Tg level was measured within an hour after FNA (Fig. 1). Institutional review board approval was obtained for this retrospective study, and the need for informed consent was waived.

2.2. USG-guided FNA

USG-guided FNA for suspicious LNs was performed by an experienced radiologist. Aspiration samples were dispersed onto slides and fixed with 95% ethanol. The same needle and syringe

were rinsed with 1 mL of distilled water, and the washout was immediately submitted for Tg measurement (aspirate-Tg). These procedures were performed under TSH suppression status.

2.3. Tg and Tg-Ab measurement

Commercial radioimmunoassay kits were used to measure serum Tg (THYRO, thyroglobulin IRMA; Cisbio Bioassays, Codolet, France) and Tg-Ab (B·R·A·H·M·S anti-Tg_n RIA, B·R·A·H·M·S GmbH, Henningsdorf, Germany) levels. The Tg assay had a functional sensitivity of 0.7 ng/mL. We defined aspirate-Tg level above 0.9 ng/mL as positive result, based on a previous meta-analysis.^[6] Elevation of Tg level after FNA was defined as a 30% increase in serum Tg level compared to baseline serum Tg level. Tg-Ab level higher than 140 U/mL was considered positive result, based on previous studies.^[11,12]

3. Statistical analysis

All statistical analyses were performed using open-source statistical software packages (R version 3.5.0, <http://www.R-project.org>). Continuous variables were expressed as mean ± standard deviation, and *P* values less than .05 were considered significant.

4. Results

Twenty-two PTC patients were included in our study. Five patients were men, and 17 patients were women. The mean age of the patients was 54.4 ± 18.0 years. All the patients underwent at least one time of radioiodine therapy (1.55 ± 0.86 times), and the cumulative dose was 217.7 ± 120.5 mCi. Cytopathologically, 9 patients yielded negative results for malignancy, 3 patients were diagnosed with cellular atypia, and 10 patients were diagnosed with PTC (Table 1). One patient of cellular atypia and 6 patients of PTC underwent surgical excision at our institution and confirmed as PTC. These patients showed decreasing pattern of serum Tg after surgical excision.

Table 1
Patients' laboratory and cytology findings.

No.	Baseline serum Tg (ng/mL)	Baseline serum Tg-Ab (U/mL)	Aspirate-Tg (ng/mL)	Serum Tg after FNA (ng/mL)	Cytopathology
1	3.64	19.03	380.1	8.75	PTC
2	8.28	16.03	3469	12.45	Cellular atypia
3	1.5	35.22	11,103	11.02	PTC
4	<0.24	55.2	16,429	33.16	PTC
5	<0.24	5474.4	27,621	3.98	PTC
6	2.2	34.1	28921	13.46	PTC
7	3.93	33.01	>40,000	7.06	PTC
8	30.03	40.16	42,311	180.2	PTC
9	0.25	21.9	67,295	3.23	PTC
10	<0.24	10.74	37.15	<0.24	Cellular atypia
11	<0.24	21.52	83.34	<0.24	Cellular atypia
12	4.4	12.78	86.66	2.01	Negative
13	<0.24	30.3	933.5	<0.24	PTC
14	0.93	40.76	983.0	0.79	PTC
15	<0.24	23.31	<0.24	<0.24	Negative
16	<0.24	47.14	<0.24	<0.24	Negative
17	<0.24	21.83	<0.24	<0.24	Negative
18	<0.24	13.42	<0.24	<0.24	Negative
19	3.57	41.85	<0.24	1.06	Negative
20	3.67	15.25	<0.24	1.86	Negative
21	4.11	44.32	<0.24	3.89	Negative
22	0.36	23.04	<0.24	0.56	Negative

Aspirate-Tg = washout Tg of syringe used in FNA, FNA = fine needle aspiration, Negative = negative for malignancy, PTC = papillary thyroid cancer, Tg = thyroglobulin, Tg-Ab = Tg-antibody.

Table 2**Relationships between elevation of thyroglobulin level after fine needle aspiration and cytopathologic result.**

Cytopathology	Elevation of serum Tg after FNA	
	Positive (N=9)	Negative (N=13)
Papillary thyroid cancer	8	2
Cellular atypia	0	2
Negative for malignancy	1	9

FNA = fine needle aspiration, Tg = thyroglobulin.

Nine patients (40.9%) showed elevation of Tg level after FNA, and the mean serum Tg level after FNA was 24.8 ± 48.0 ng/mL. Among these 9 patients, 8 were cytologically diagnosed with PTC, and the cytology of 1 patient showed cellular atypia. All 9 patients showed positive aspirate-Tg. Eight patients showed more than 1000 ng/mL of aspirate-Tg, and 1 patient had aspirate-Tg levels of 380.1 ng/mL.

Thirteen patients (59.1%) did not show elevated Tg after FNA. Among these patients, 2 were diagnosed with PTC, 2 patients showed cellular atypia, and 9 patients yielded negative results for malignancy on the cytopathologic assessment (Table 2). Five of 13 patients had positive aspirate-Tg result, and all the patients with negative aspirate-Tg results and without elevation of Tg levels after FNA yielded negative results for malignancy on the cytopathologic assessment.

Interestingly, all the patients with aspirate-Tg level higher than 1000 ng/mL showed elevation of serum Tg after FNA. In addition, only 1 patient (No. 5) had positive Tg-Ab result, but there was elevation of Tg level after FNA, and the aspirate-Tg level was 27,621 ng/mL.

5. Discussion

In this study, we observed that patients with positive aspirate-Tg results and elevation of Tg levels after FNA had the highest incidence of metastatic LNs. In contrast, patients with negative aspirate-Tg results and without elevation of Tg levels after FNA yielded negative results for malignancy on cytopathologic assessment. These results suggest that aspirate-Tg and elevation of serum Tg after FNA have a powerful role in predicting LN metastasis in PTC patients.

FNA is widely used for confirmation or exclusion of LN metastasis, but small LNs are difficult or impossible to aspirate, and enlarged LNs might show complex cytologic features.^[4] In these cases, measurements of aspirate-Tg and serum Tg after FNA may be quite useful for establishing future diagnostic or therapeutic plans for these patients.

Even though measuring aspirate-Tg significantly improves the sensitivity of FNA, it has major challenges for laboratories due to several factors that might alter the results, such as the lack of methodological standards, inadequate functional sensitivity, and variability in the specificity of commercially available antibody kits.^[6] These challenges make it difficult to define appropriate cut-off values for aspirate-Tg.^[13]

We assume that damage to the metastatic LN during FNA might result in elevation of serum Tg level. Even if FNA of metastatic LNs results in an inadequate cytologic sample, the FNA procedure can destroy the metastatic LN and release Tg into the blood, which results in elevation of serum Tg level after FNA. The current study demonstrated that elevation of serum Tg level after FNA might be a predictor of LN metastasis of PTC. Patients

with cytologic confirmed metastasis might undergo the standard treatment plan, such as, surgery or local therapy. However, some patients without cytological confirmation of metastasis might reveal elevation of serum Tg after FNA. In these cases, the serum Tg elevation could be one of markers which we need to consider presence of metastasis in the lymph node. Therefore, patients with serum Tg elevation after FNA and without cytologic confirmation might need surgical excision of suspected lymph node. However, surgical removal of LN should be done if LNs are highly suspicious for malignancy regardless of Tg change after FNA.

Interestingly, all the patients with a high aspirate-Tg levels (>1000 ng/mL) showed elevation of serum Tg levels after FNA. The authors think that LNs with a high aspirate-Tg level might release more Tg than those with a low aspirate-Tg level. As mean Tg half-life in the blood is 65.2 hours,^[14] serum Tg could be elevated for 3 to 4 weeks after FNA. Therefore, serum Tg level later than 1 hour after FNA might also have similar significance, although it requires additional studies.

As Tg levels can be affected by the presence of Tg-Ab,^[11,15] Tg-Ab interference was taken into consideration when measuring aspirate-Tg levels. Boi et al showed no significant interaction between Tg-Ab and aspirate-Tg.^[16] In our study, only 1 patient showed positive Tg-Ab result, and this patient also showed positive aspirate-Tg result. Even though baseline serum Tg seems to be suppressed by Tg-Ab, we could observe elevation of serum Tg after FNA. However, elevation of Tg level after FNA seems to be decreased compared with that in patients yielding negative results for Tg-Ab.

Serum tumor markers are known to be elevated after other tumor destructive procedures, such as chemotherapy and surgery.^[9,10] Radiofrequency ablation and ethanol injection are also administered to thyroid cancer patients,^[17,18] and a clinical trial for low-risk PTC using high intensity focused ultrasound (HIFU) is ongoing (NCT03327636). Elevation of serum Tg level after these non-surgical approaches can be a predictor of successful treatment. For deep-seated suspicious metastatic lesions where FNA is risky or not possible, elevation of serum Tg level after low or very low doses (diagnostic doses) of HIFU to the lesion can be used for diagnosis of the suspicious lesion.

There are several limitations in the current study. Although only 1 experienced radiologist performed all the procedures, the FNA procedure could vary according to the location and size of suspicious metastatic LNs. The relatively small number of patients and retrospective design of this study are the other limitations. A larger study including more patients is warranted to verify the diagnostic value of elevating Tg after FNA.

6. Conclusion

Elevation of serum Tg level after FNA might have a diagnostic role for predicting LN metastasis of PTC.

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