

Diagnostic utility of thyroglobulin measurement in the fine needle aspirates from cervical lymph nodes: a case report

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SUMMARY: Diagnostic utility of thyroglobulin measurement in the fine needle aspirates from cervical lymph nodes: a case report.

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Fine needle aspiration cytology (FNAC) is the more accurate diagnostic method for cervical lymph node (CLN) metastasis from differentiated thyroid cancers (DTC). However, FNAC diagnosis of cystic CLN is, in most cases, uninformative due to inadequate cellularity. Recently, thyroglobulin (Tg) detection in FNAC needle washout fluid has been shown to improve the diagnostic accuracy of FNAC, and its routine association with cytology is recommended. We here describe the case of a 20 yr old girl complaining of the recent appearance of palpable non-painful laterocervical nodes in the neck. Ultrasound examination revealed the presence of 3 cystic CLNs and 2 mixed thyroid nodules,

with the larger one showing irregular margins. On the latter, and on 2 larger CLNs, FNAC was performed, and both Tg protein and mRNA were determined in the needle washout. The cytological analysis was not diagnostic for the two CLNs, while that of the thyroid nodule reported the presence of colloid and groups of thyrocytes with normal morphology. Both CLNs showed, however, high levels of Tg protein and were positive for Tg mRNA, suggestive of metastatic DTC. Based on these findings, the FNAC analysis was performed on the second smaller thyroid nodule suggesting (Tir4) the presence of PTC. The patient was then subjected to total thyroidectomy with lymph nodes resection of the central and homolateral compartments. The histological diagnosis confirmed the presence of a PTC in the small nodule and metastatic lymph nodes.

In conclusion, this case confirms that the cytological diagnosis of cystic lymph nodes is challenging, and that the measurement of Tg protein and/or mRNA in the needle washout may overcome this limitation.

KEY WORDS: Thyroid cancer - Lymph node - Metastasis - Diagnosis - Thyroglobulin - Fine-needle aspiration cytology - Follow-up.

Introduction

Thyroid cancer represents the most frequent endocrine neoplasia, being the fifth most common tumor in women in the United States (1-4). The majority of thyroid carcinomas arise from the epithelial follicular thyroid cells and are represented by differentiated carcinomas (DTC), subdivided into papillary (PTC) and follicular (FTC) histotypes, while 1-2% of them are undifferentiated highly aggressive anaplastic carcinomas (ATC) (3-5). About 3-4% of thyroid cancers derives from

the parafollicular C cells and is named medullary thyroid carcinoma (MTC) (3-6). The prognosis for thyroid cancer patients relies on clinical parameters, that is cancer type and stage of the disease at the time of diagnosis (2, 4, 7-11). The prognosis of DTC patients is favorable, with 10-year survival rate of about 90%, while that of ATC patients is very poor with 5-year survival below 5% (2, 4). The prognosis of MTC patients depends essentially on the state of the disease at diagnosis, with a 10-year survival rate of 90% when the disease is confined to the gland, of 70% when loco-regional metastases are present, and of 20% when distant metastases occur (2, 4).

Thyroid neoplasms manifest themselves as nodules and are very common, affecting 19% to 67% of the adult population (6, 7). Since only a minority of them (about 5%) harbors a malignant lesion the first aim of their clinical evaluation is to exclude malignancy (4, 12, 13). The diagnosis is based on patient's clinical parameters, ultrasonography (US), scintigraphy, and fine needle aspi-

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ration cytology (FNAC) (4, 12, 14-16). In addition to thyroid nodule, the accurate diagnosis of metastases to loco-regional lymph nodes is also crucial for the initial surgical approach as well as during patients' follow-up (17-19). To date, FNAC has represented the main diagnostic tool for cervical lymph node (CLN) metastasis (20-23). However, inadequate cellularity, often associated with cystic lymph nodes, prevents diagnosis in about 20% of samples (24, 25).

Over the last years, different reports have suggested that measurement of thyroglobulin (Tg) protein and/or mRNA in the FNAC needle washout fluid may increase the diagnostic performance of FNAC in identifying CLN metastases from DTC (26-34). In line with these expectations, we here describe the case of a 20 yr old girl suffering from lymph node metastatic PTC, demonstrating the clinical utility of Tg protein and mRNA detection in the fine needle washout.

Case report

In December 2005 a 20 yr old girl was admitted to the Endocrinology Unit of the Umberto I General Hospital of Rome after complaining of the recent appearance of palpable, mobile and non-painful laterocervical nodes in the left side of the neck. The patient had normal thyroid function as revealed by normal TSH, FT₄ and FT₃ serum levels, with negative anti-TPO and anti-Tg antibodies. There was no familial history of thyroid cancer or previous exposure to either medical (radiation treatment to the head and neck region) or environmental (from fallout following nuclear power plant accidents) radiation.

US performed on the cervical area using the Aplio XV (Toshiba, Japan) system equipped with a linear transducer (PLT-805AT) revealed the presence of three enlarged cervical lymph nodes, two of which (lymph nodes A and B) are depicted in Figure 1. The latter showed a cystic appearance and diameter ratios comprised between 1.5 and 2.0. In addition, color-flow doppler analysis demonstrated in both an irregular vascularization (hilar and peripheral). Within the thyroid gland US evidenced two nodules (Figure 2): the first (nodule A of figure 2), mixed with regular margins and major diameter of 14.2 mm, was localized in the upper left lobe, while the second (nodule B of figure 2), mixed with irregular margins and major diameter of 22.1 mm, was localized in the inferior part of the left lobe.

Based on clinical and US examinations the patient was selected for FNAC and instructed not to take aspirin or any other anticoagulant in the 5 days prior to biopsy. The dominant thyroid nodule (nodule B of figure 2) and the two lymph nodes described in figure 1 were analyzed. To this end, a 25-gauge needle attached to 20 ml plastic syringe was used to aspirate nodes under US assistance.

All aspirates were smeared directly on glass slides which were either hair-dried or wet-fixed using the Bio-Fix (Bio-Optica, Milan, Italy). Hair-dried slides were stained with the May Grunwald-Giemsa solution, while the wet-fixed slides were stained with the Papanicolaou solution. The slides were then interpreted by a cytopathologist expert in thyroid cytology.

At the same time, the needle was washed with 1 ml of phosphate buffered saline by multiple pumping actions, and the suspension was centrifuged at 1200 rpm for 5 min. The supernatant was collected and analyzed for Tg protein by means of the immunoluminometric assay (ILMA) Tg-PluS (B.R.A.H.M.S., Hennigsdorf, Germany), whi-

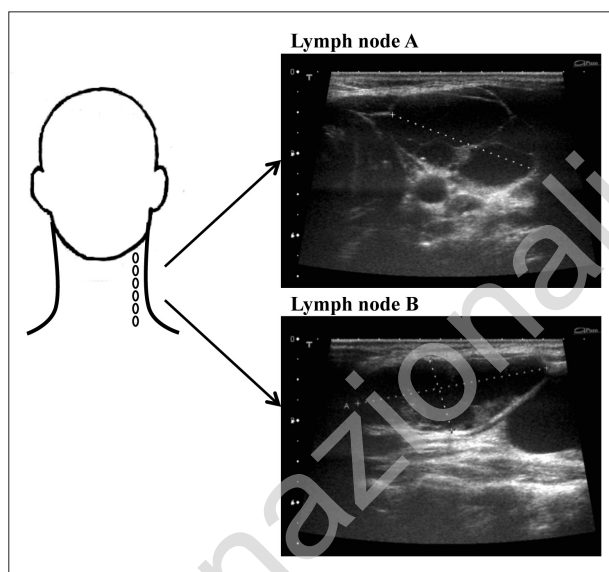


Fig. 1 - Ultrasonography of the neck area. Three enlarged lymph nodes were found, two of which (lymph nodes A and B) were selected for FNAC. Both these lymph nodes showed a cystic appearance and irregular vascularization (hilar and peripheral), with diameter ratios comprised between 1.5 and 2.0.

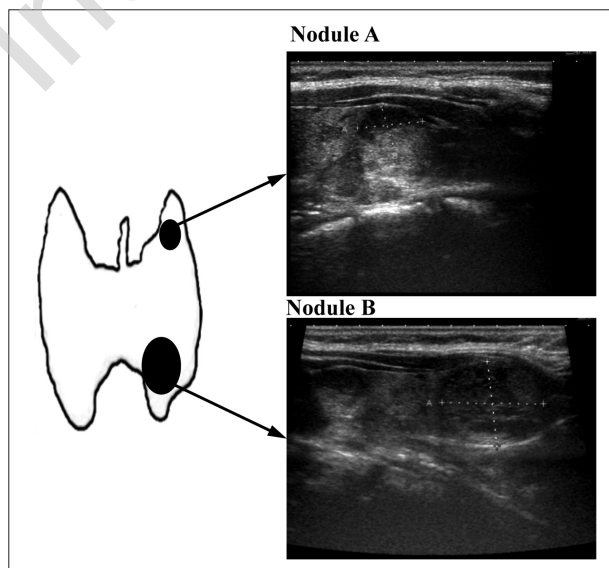


Fig. 2 - Ultrasonography of the thyroid gland. Arrows indicate the positions of two nodules: the nodule A is mixed with regular margins and major diameter of 14.2 mm, while the nodule B is mixed with irregular margins and major diameter of 22.1 mm.

le total RNA was extracted from the cell pellet and analyzed for Tg and calcitonin (CT) mRNA content as previously described (35, 36). The RT-PCR demonstrated the presence of Tg mRNA, but not CT mRNA, in the thyroid nodule (Figure 3). Similarly, the two lymph nodes analyzed were positive for Tg mRNA and negative for CT mRNA, suggesting the presence of metastases from DTC. These data were corroborated by the high level of Tg protein observed in both lymph nodes, that is 6620 ng/ml in lymph node A and 7480 ng/ml

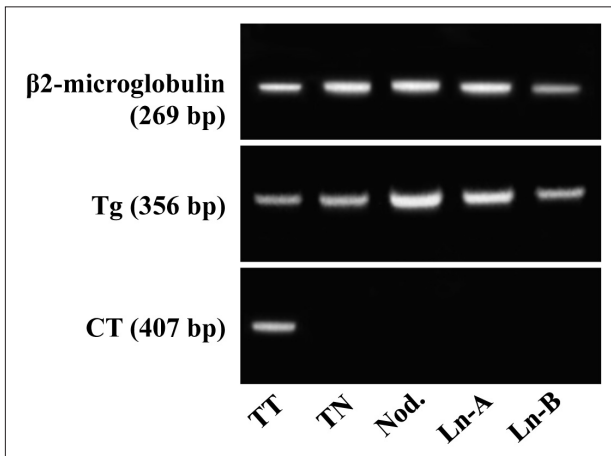


Fig. 3 - RT-PCR results obtained for the nodule B and the two lymph nodes. Positive controls are represented by the MTC derived cell line TT for calcitonin, normal human thyroid tissue (TN) for thyroglobulin, and b-actin as reference gene. Negative controls were performed by omitting the reverse transcriptase in parallel RT-PCR experiments (data not shown). The specificities of PCR products were checked by DNA sequencing. Tg, thyroglobulin; CT, calcitonin; Ln-A, lymph node A; Ln-B, lymph node B.

in lymph node B. However, the cytological diagnosis reported the presence of colloid and groups of thyrocytes with normal morphology in the thyroid nodule aspirated materials, while both lymph node FNACs were non diagnostic due to the presence of few lymphocytes and red blood cells.

Based on Tg mRNA and protein values found in the lymph nodes, it was decided to perform FNAC also on the second smaller thyroid nodule (nodule A), which suggested the presence of PTC. As a consequence, the patient was subjected to total thyroidectomy with lymphadenectomy of the central and homolateral cervical lymph node compartments. Histological analysis of the removed tissues (Figure 4) confirmed the presence of PTC in the apical portion of the left lobe, and a benign nodule in the inferior pole of the left lobe. In addition, three lymph nodes of the central compartment and three of the laterocervical compartment (the latter showed in figure 4) were metastatic.

Discussion

The accurate diagnosis of loco-regional lymph node metastasis is of primary importance for the initial surgical approach as well as for prognostic stratification and follow-up of thyroid cancer patients (17-19). FNAC represents the main diagnostic tool for the diagnosis of metastatic CLN from DTC, but the diagnosis may be challenging as lymph nodes could harbor metastases from a multiplicity of extra-thyroidal malignancies or be affected by several non-tumoral diseases (21-23). As a consequence, the accuracy of the technique, which was reported to vary from 73% to 94%, is highly dependent on the experience and ability of the cytopathologist (28-34). Different clinical reports demonstrated that Tg measurement in fine-needle washout may significantly improve the accuracy of FNAC in the evaluation of metastatic lymph

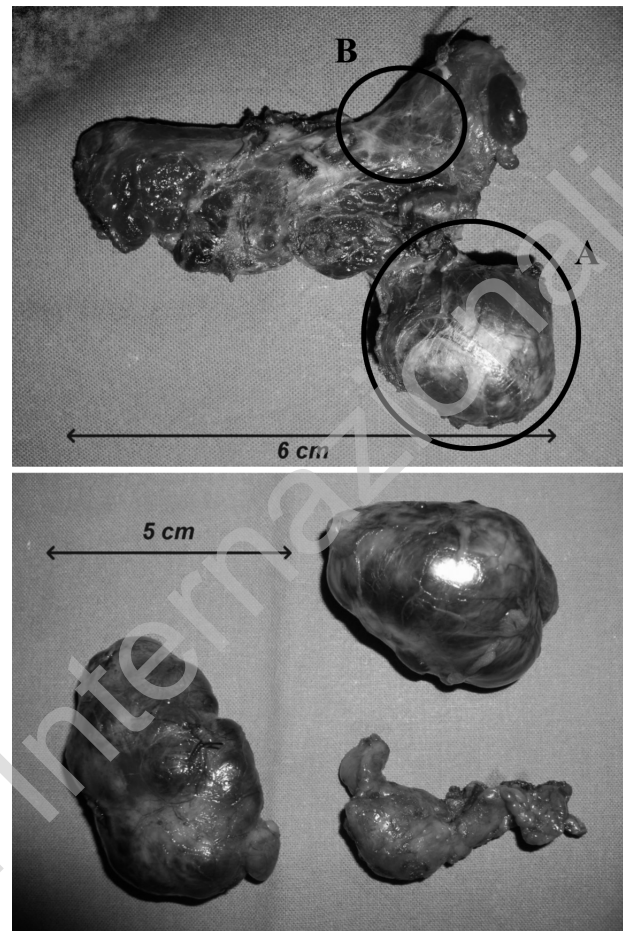


Fig. 4 - Image of the thyroid gland and the three metastatic cervical lymph nodes after resection. Rings indicate the positions of nodules A and B within the gland.

nodes from DTC, and hence the routine association of Tg measurement with FNAC in the preoperative diagnosis of suspicious CLN is currently recommended (18, 19, 28-34). In this context, we here report our experience on a case of a 20 yr old girl suffering from metastatic PTC, for which Tg protein and mRNA detection in the needle washout of CLN provided the accurate diagnosis, making up for uninformative FNAC due to cystic CLN. In addition, although the dominant nodule initially chosen for FNAC was benign, the information obtained from the molecular diagnosis redirected our attention to the second thyroid nodule of the patient, whose malignancy was shown following FNAC.

It has to be mentioned, however, that in our experience, based on a case study of 33 CLN, we found that FNAC and Tg protein or mRNA determination in the needle washout showed a 90% overall agreement, and that the molecular diagnosis became clinically useful only in cases in which FNAC provides uninformative dia-

gnosis, as in the present case report, or inconsistent diagnosis with regard to patient's biochemical and/or clinical parameters (unpublished observations). Based on such evidence, in our opinion FNAC remains the gold standard technique for the diagnosis of suspicious CLN due to its ability to efficiently diagnose metastases not only from thyroid cancers, but also from other primary tumors.

Conclusion

Thyroglobulin protein and/or mRNA determination in fine needle washout is a clinically useful method to improve the diagnostic accuracy of fine needle aspiration cytology of cystic cervical lymph nodes suspected to harbor a metastasis from differentiated thyroid cancers.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010;127:2893-917.
2. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer Statistics, 2009. *Ca Cancer J Clin* 2009;59:225-49.
3. Trimboli P, Ulisse S, Graziano FM, Marzullo A, Ruggieri M, Calvanese A, Piccirilli F, Cavaliere R, Fumarola A, D'Armiento M. Trend in thyroid carcinoma size, age at diagnosis and histology in a retrospective study of 500 cases diagnosed over 20 years. *Thyroid* 2006;16:1151-5.
4. Sherman SI. Thyroid carcinoma. *Lancet* 2003;36:501-11.
5. American Thyroid Association Guidelines Task Force, Kloos RT, Eng C, Evans DB, Francis GL, Gagel RF, Gharib H, Moley JF, Pacini F, Ringel MD, Schlumberger M, Wells SA Jr. Medullary thyroid cancer: management guidelines of the American Thyroid Association. *Thyroid* 2009;19:565-612.
6. Sorrenti S, Gaitoli E, Catania A, D'Andrea V, Di Matteo FM, Nardi M, Prinzi N, Nardi F, Ascoli V, Baldini E, Ulisse S, De Antoni E. Surgical strategies in patients with medullary thyroid carcinoma. *Clin Ter* 2012;163:e303-6.
7. Xing M. BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications. *Endocr Rev* 2007;28:742-62.
8. Handkiewicz-Junak D, Czarniecka A, Jarzab B. Molecular prognostic markers in papillary thyroid cancer: current status and future directions. *Mol Cell Endocrinol* 2010;322:8-28.
9. Ulisse S, Baldini E, Sorrenti S, Barollo S, Gnessi L, Catania A, Pellizzo MR, Nardi F, Mian C, De Antoni E, D'Armiento M, Frati L. High expression of the urokinase plasminogen activator and its cognate 1 receptor associates with advanced stages and reduced disease-free interval in papillary thyroid carcinoma. *J Clin Endocrinol Metab* 2011;96:504-8.
10. Ulisse S, Baldini E, Sorrenti S, Barollo S, Prinzi N, Catania A, Nesca A, Gnessi L, Pellizzo MR, Mian C, De Vito C, Calvanese A, Palermo S, Persechino S, De Antoni E, D'Armiento M. In papillary thyroid carcinoma BRAFV600E is associated with increased expression of the urokinase plasminogen activator and its cognate receptor, but not with disease-free interval. *Clin Endocrinol* 2012. In press, doi: 10.1111/j.1365-2265.2012.04465.x.
11. Baldini E, Sorrenti S, D'Armiento E, Di Matteo FM, Catania A, Ulisse S. The urokinase plasminogen activating system in thyroid cancer: clinical implications. *G Chir* 2012;33(10):305-310.
12. Roman SA. Endocrine tumors: evaluation of the thyroid nodule. *Curr Opin Oncol* 2003;15:66-70.
13. Welker MJ, Orlov D. Thyroid nodules. *Am Fam Phys* 2003;67:559-66.
14. Sorrenti S, Trimboli P, Catania A, Ulisse S, De Antoni E, D'Armiento M. Comparison of malignancy rate in thyroid nodules with cytology of indeterminate follicular or indeterminate Hürthle cell neoplasm. *Thyroid* 2009;19:355-60.
15. Trimboli P, Ulisse S, D'Alò M, Solari F, Fumarola A, Ruggieri M, De Antoni E, Catania A, Sorrenti S, Nardi F, D'Armiento M. Analysis of clinical, ultrasound and colour flow-doppler characteristics in predicting malignancy in follicular thyroid neoplasms. *Clin Endocrinol* 2008;69:342-4.
16. Trimboli P, Condorelli E, Catania A, Sorrenti S. Clinical and ultrasound parameters in the approach to thyroid nodules cytologically classified as indeterminate neoplasm. *Diagn Cytopathol* 2009;37:783-5.
17. American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer, Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, Sherman SI, Steward DL, Tuttle RM. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid* 2009;19:1167-214.
18. Schlumberger M, Berg G, Cohen O, Duntas L, Jamar F, Jarzab B, Limbert E, Lind P, Pacini F, Reiners C, Sánchez Franco F, Toft A, Wiersinga WM. Follow-up of low-risk patients with differentiated thyroid carcinoma: a European perspective. *Eur J Endocrinol* 2004;150: 105-12.
19. Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, Wiersinga W; European Thyroid Cancer Taskforce. European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. *Eur J Endocrinol* 2006;154:787-803.
20. Cervin JR, Silverman JF, Loggie BW, Geisinger KR. Virchow's node revisited. Analysis with clinicopathologic correlation of 152 fine-needle aspiration biopsies of supraclavicular lymph nodes. *Arch Pathol Lab Med* 1995;8:727-30.
21. Stack BC Jr, Ferris RL, Goldenberg D, Haymart M, Shaha A, Sheth S, Sosa JA, Tufano RP; American Thyroid Association Surgical Affairs Committee. American thyroid association consensus review and statement regarding the anatomy, terminology, and rationale for lateral neck dissection in differentiated thyroid cancer. *Thyroid* 2012;22:501-8.
22. Florentine BD, Staymates B, Rabadi M, Barstis J, Black A; Cancer Committee of the Henry Mayo Newhall Memorial Hospital. The reliability of fine-needle aspiration biopsy as the initial diagnostic procedure for palpable masses: a 4-year experience of 730 patients from a community hospital-based outpatient aspiration biopsy clinic. *Cancer* 2006;107:406-16.
23. Cignarelli M, Triggiani V, Ciampolillo A, Ambrosi A, Giorgino F, Liso V, Giorgino R. High frequency of incidental diagnosis of extrathyroidal neoplastic diseases at the fine-needle aspiration

- biopsy of laterocervical lymph nodes in patients with thyroid nodules. *Thyroid* 2001;11:65-71.
24. Ustün M, Risberg B, Davidson B, Berner A. Cystic change in metastatic lymph nodes: A common diagnostic pitfall in fine-needle aspiration cytology. *Diagn Cytopathol* 2002;27:387-92.
 25. Kessler A, Rappaport Y, Blank A, Marmor S, Weiss J, Graif M. Cystic appearance of cervical lymph nodes is characteristic of metastatic papillary thyroid carcinoma. *J Clin Ultrasound* 2003;1:21-5.
 26. Cignarelli M, Ambrosi A, Marino A, Lamacchia O, Campo M, Picca G, Giorgino F. Diagnostic utility of thyroglobulin detection in fine-needle aspiration of cervical cystic metastatic lymph nodes from papillary thyroid cancer with negative cytology. *Thyroid* 2003;13:1163-7.
 27. 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. *J Clin Endocrinol Metab* 2007;92:4278-81.
 28. Kim MJ, Kim EK, Kim BM, Kwak JY, Lee EJ, Park CS, Cheong WY, Nam KH. Thyroglobulin measurement in fine-needle aspirate washout: the criteria for neck node dissection for patients with thyroid cancer. *Clin Endocrinol* 2009;70:145-51.
 29. Pacini F, Fugazzola L, Lippi F, Ceccarelli C, Centoni R, Miccoli P, Elisei R, Pinchera A. Detection of thyroglobulin in fine needle aspirates of nonthyroidal neck masses: a clue to the diagnosis of metastatic differentiated thyroid cancer. *J Clin Endocrinol Metab* 1992;74:1401-4.
 30. 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;14:959-63.
 31. Cunha N, Rodrigues F, Curado F, Ilhéu O, Cruz C, Naidenov P, Rascão MJ, Ganho J, Gomes I, Pereira H, Real O, Figueiredo P, Campos B, Valido F. Thyroglobulin detection in fine-needle aspirates of cervical lymph node: a technique for the diagnosis of metastatic differentiated thyroid cancer. *Eur J Endocrinol* 2007;157:101-7.
 32. 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;9:105-11.
 33. 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 Med J* 2012;53:126-31.
 34. Kim DW, Jeon SJ, Kim CG. Usefulness of thyroglobulin measurement in needle-washout of fine-needle aspiration biopsy for the diagnosis of cervical lymph node metastases from papillary thyroid cancer before thyroidectomy. *Endocrinol* 2012; 42:399-403.
 35. Morgenthaler NG, Froehlich J, Rendl J, Willnich M, Alonso C, Bergmann A, Reiners C. Technical evaluation of a new immunoradiometric and a new immunoluminometric assay for thyroglobulin. *Clin Chem* 2002;48:1077-83.
 36. Cianfarani F, Baldini E, Cavalli A, Marchioni E, Lembo L, Teson M, Persechino S, Zambruno G, Ulisse S, Odorisio T, D'Armi M. TSH receptor and thyroid specific genes expression in human skin. *J Invest Dermatol* 2010;130:93-101.