Human Pathology (2016) **48**, 132–141

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Concordance study between one-step nucleic acid amplification and morphologic techniques to detect lymph node metastasis in papillary carcinoma of the thyroid

Sofía del Carmen MD[a,1], Sonia Gatius MD[b,1], Guzmán Franch-Arcas MD, PhD[a], José Antonio Baena MD[b], Oscar Gonzalez MD[c], Carlos Zafon MD[c], Dolors Cuevas MD[b], Joan Valls PhD[b], Angustias Pérez MD[a], Mercedes Martinez MD[a], Susana Ros MD[b], Carmen García Macías MD[a], Carmela Iglesias MD[c,2], Xavier Matías-Guiu MD, PhD[b,*,2], Enrique de Álava MD, PhD[a,,2]**

[a]*Hospital Universitario de Salamanca, Salamanca, 37007, Spain*
[b]*Hospital Universitari Arnau de Vilanova de Lleida, IRB Lleida, Lleida, 25198, Spain*
[c]*Hospital Vall d'Hebron, VHIR, Barcelona, 08035, Spain*

Received 3 June 2015; revised 2 September 2015; accepted 13 September 2015

Keywords:
Papillary carcinoma;
Thyroid;
Lymph node;
OSNA;
Metastasis

Summary Tumor resection in papillary thyroid carcinoma (PTC) is often accompanied by lymph node (LN) removal of the central and lateral cervical compartments. One-step nucleic acid amplification (OSNA) is a polymerase chain reaction–based technique that quantifies cytokeratin 19 (CK19) messenger RNA copies. Our aim is to assess the value of OSNA in detection of LN metastases in PTC, in comparison with imprints and microscopic analysis of formalin-fixed, paraffin-embedded (FFPE) tissue. A total of 387 LNs from 37 patients were studied. From each half LN, 2 imprints were taken and analyzed with hematoxylin and eosin (H&E) and CK19 immunostaining. One half of the LN was submitted to OSNA and one half to FFPE processing and H&E and CK19 staining. For concordance analysis, every single LN was considered as a case. A group of 11 cases with discordant results between OSNA and H&E/CK19 FFPE sections were subjected to additional FFPE serial sectioning and H&E and CK19 staining. We found a high degree of concordance between the assays used, with sensitivities ranging from 0.81 to 0.95, and specificities ranging from 0.87 and 0.98. OSNA allowed upstaging of patients from pN0 to pN1, in comparison with standard pathologic analysis. Identification of a metastatic LN with more than 15 000 CK19 messenger RNA copies predicted the presence of a second LN with macrometastasis (<5000 copies). In summary, the study shows that OSNA application in sentinel or suspicious LN may be helpful in assessing nodal status in PTC patients.

* Correspondence to: X. Matias-Guiu, Department of Pathology, Hospital Universitari Arnau de Vilanova, Institut de Recerca Biomedica de Lleida, Av Rovira Roure, 80, 25198, Lleida, Spain.
** Correspondence to: E. de Alava, Hospital Universitario Virgen del Rocío, IBiS Sevilla, Sevilla, Spain.
E-mail addresses: fjmatiasguiu.lleida.ics@gencat.cat (X. Matías-Guiu), enrique.alava.sspa@juntadeandalucia.es (E. de Álava).
[1] Contributed equally.
[2] Co-senior authors.

http://dx.doi.org/10.1016/j.humpath.2015.09.020
0046-8177/

1. Introduction

The prognostic impact of lymph node (LN) metastasis in papillary thyroid carcinoma (PTC) is controversial, particularly regarding microscopic metastasis. However, surgical treatment of PTC may include central compartment lymphadenectomy (CCL) [1]. CCL is considered to be prophylactic when no pathologic LNs are detected clinically and radiologically. CCL is also performed when a positive or suspicious cytologic diagnosis of metastatic PTC is obtained from fine-needle aspiration of a cervical LN, or when a suspicious LN is detected during surgery. CCL shows higher postsurgical adverse effects compared with simple thyroidectomy, including hypoparathyroidism or recurrent laryngeal nerve injury, particularly when bilateral CCL is performed [2,3].

It has been suggested that CCL may be unnecessary in an important group of patients because only 20% to 50% of PTC patients show metastatic LN in central compartment. Reducing the number of unnecessary CCL would presumably avoid postsurgical adverse effects [2,4,5]. Recent guidelines of the American Thyroid Association [6] accept conservative surgery (thyroidectomy without CCL) in patients with small tumors (<4 cm), without extrathyroidal invasion and without clinical, radiologic, or intraoperative pathologic LN.

Prophylactic CCL is controversial; and there are pros and cons [2]. There are opinions that support prophylactic dissection, based on the fact that LN metastases do not influence patient outcome and that metastatic LN cannot be reliably identified during surgery. Meticulous CCL has a beneficial effect on the subsequent course [7,8]. Furthermore, CCL can be performed safe, and reoperation for central neck recurrence has greater morbidity [9]. On the other hand, the cons for prophylactic CCL include a higher risk of postsurgical hypoparathyroidism and increased risk of recurrent laryngeal nerve lesions [2].

Sentinel node biopsy of central compartment has a high positive predictive value. It would allow for selection of patients who would have real benefit of CCL and would avoid unnecessary surgery [5]. Unfortunately, conventional intraoperative analysis of metastatic LN has a 17% rate of false negatives, particularly important when LN contains micrometastasis or isolated tumor cells. LN analysis by means of one-step nucleic acid amplification (OSNA) assay is a procedure used for intraoperative staging of patients with breast cancer [10–13], based on real-time amplification and quantification of cytokeratin 19 (CK19) messenger RNA (mRNA) in LN samples. Because CK19 is also expressed in PTC [14,15], our aim is to determine the value of OSNA for intraoperative detection of LN metastasis of PTC in comparison with classical pathologic methods (intraoperative imprints and formalin-fixed, paraffin-embedded sections). Our study could provide interesting information regarding the possible application of OSNA to sentinel node biopsies of the central compartment or to a suspicious LN detected during surgery.

2. Materials and methods

2.1. Study design

The present is a prospective, observational, and multicentric study. Samples were obtained from Hospital Universitari Arnau de Vilanova de Lleida (HUAV), Hospital Universitario de Salamanca (HUS), and Hospital Universitari Vall d'Hebron de Barcelona (HUVH). A specific informed consent was obtained from each patient, and the study was approved by the ethical committees of the 3 centers (reference OSNACAT).

2.2. Inclusion criteria

Selected patients were older than 18 years. All of them had a confirmed pathologic diagnosis of PTC in the surgical specimen, with positive CK19 immunoreactivity in PTC cells in the primary tumor. Each of them was prospectively recruited by fulfilling 1 of the 2 following preoperative diagnostics:

1. Follicular lesion of uncertain malignancy with intraoperative pathologic LN
2. Suspicious or confirmed PTC in previous fine-needle aspiration

A series of patients with primary hyperparathyroidism was included as a control to evaluate the confusion effect if a parathyroid gland, which is CK19 positive, is processed as an LN using the OSNA method.

2.3. Patient samples and procedures

All primary tumors were classical variants of PTC, and each of them positive was for CK19 with a cytoplasmic pattern. A total of 387 LNs from 37 patients were included in the study (HUAV: 18 patients and 214 LNs; HUS: 8 patients and 95 LNs; HUVH: 11 patients and 78 LNs). Each LN had a diameter equal or greater than 3 mm (weight >20 mg). LNs were dissected from the central or laterocervical compartment. Each LN was weighted and bisectioned. One half was entirely submitted to OSNA, and the other half for pathologic processing by formalin fixation and paraffin embedding (FFPE), and preparation of a single 3-μm section, stained by hematoxylin and eosin (H&E) and one by CK19.

From each half LN, 2 imprints were taken. Imprints from the half LN analyzed using OSNA were called O imprints. Imprints from the half LN analyzed by FFPE were called P