Ultrasound in Med. & Biol., Vol. 00, No. 00, pp. 1–11, 2018

0301-5629/$ - see front matter

https://doi.org/10.1016/j.ultrasmedbio.2018.06.020

● *Original Contribution*

CONVENTIONAL ULTRASOUND, IMMUNOHISTOCHEMICAL FACTORS AND BRAF^{V600E} MUTATION IN PREDICTING CENTRAL CERVICAL LYMPH NODE METASTASIS OF PAPILLARY THYROID CARCINOMA

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(*Received* 29 *January* 2018; *revised* 28 *May* 2018; *in final from* 29 *June* 2018)

Abstract—The study was aimed at evaluating the correlation between central cervical lymph node metastasis (CLNM) in papillary thyroid carcinoma (PTC) patients and ultrasound (US) features, immunohistochemical factors and BRAF^{V600E} mutation. A total of 225 consecutive patients (225 PTCs) who had undergone surgery were included. All PTCs were pre-operatively analysed by US with respect to size, components, echogenicity, shape, margins, microcalcification, multiple cancers or not, internal vascularity and capsule contact or involvement. The presence of four immunohistochemical factors, including cytokeratin 19, human bone marrow endothelial cell 1, galectin 13 and thyroid peroxidase, and BRAF^{V600E} mutation was also evaluated. Univariate and multivariate analyses were performed to identify the risk factors for central CLNM, and a risk model was established. Pathologically, 44% (99/225) of the PTCs had central CLNMs. Multivariate analysis revealed that size ≤10 mm, microcalcification, internal vascularity, capsule contact or involvement and BRAF^{V600E} mutation were independent risk factors for central CLNM. The risk score for central CLNM was calculated as follows: risk score = 1.5 × (if lesion size ≤10 mm) + 1.9 × (if microcalcification) + 0.8 × (if internal flow) + 3.0 × (if capsule contact or involvement) + 1.5 × (if BRAF^{V600E} mutation). The rating result was divided into six stages, and the relevant risk rates of central CLNM were 0% (0/1), 0% (0/22), 7.4% (4/54), 48.6% (34/70), 71.2% (42/59) and 100% (19/19), respectively. In conclusion, PTC ≤10 mm, microcalcification, internal vascularity, capsule contact or involvement and BRAF^{V600E} mutation are risk factors for central CLNM. The risk model may be useful in treatment planning and management of patients with PTCs. (E-mail: xuhuixiong@126.com)

Key Words: **Papillary thyroid carcinoma, CK19, Human bone marrow endothelial cell-1, Galectin-13, Thyroid peroxidase, BRAF^{V600E} mutation, Central cervical lymph node metastases.**

INTRODUCTION

Papillary thyroid carcinoma (PTC) is one of the most common endocrine tumours, and its incidence has rapidly increased in recent years, with rates of 7.8% and 7.2% in men and women, respectively (Colonna et al. 2015). Although PTC is an indolent disease with satisfactory prognosis (Baek et al. 2010; Conzo et al. 2012; Jemal et al. 2005), cervical lymph node metastasis (CLNM) is often encountered and is present in approximately 20% to 80% of PTC patients (Chow et al. 2003; Kouvaraki et al. 2003; Roh et al. 2007). Among these metastases, central CLNM is common and may appear even when the PTC is small or non-invasive (Hay et al. 1992). Tumour cells typically metastasise to central CLNMs followed by lateral CLNMs (Hughes and Doherty 2011).

High-frequency ultrasound (US) is important in the diagnosis of CLNM. Round shape, hyper-echogenicity, cystic change, calcification and peripheral vascularisation

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are considered to be suspicious features of CLNM on US (Park et al. 2009). However, because of false-negative US features, approximately 90% of CLNMs might not be found pre-operatively (Cooper et al. 2009). The sensitivity of US for central CLNM was <50%, whereas it was approximately 70% to 80% for lateral CLNM (Choi et al. 2010; Khokhar et al. 2015). The prognosis of patients with CLNM might be poor, because CLNM is associated with increased local recurrence (Baek et al. 2010; Lundgren et al. 2006; White et al. 2007). According to the guidelines from the American Thyroid Association (ATA) and the European Thyroid Association (ETA), therapeutic central compartment node dissection (CCND) is recommended for patients at high risk for thyroid cancer, while prophylactic CCND is recommended for low-risk patients (Cooper et al. 2009; Pacini et al. 2006). However, prophylactic CCND does not decrease the frequency of local recurrence; instead, it increases complications such as low parathyroid function and recurrent nerve injury (Carling et al. 2010; Zetoune et al. 2010). Thus, it is essential to identify the risk factors for central CLNM and establish a risk model to stratify PTC patients, which is helpful in evaluating the prognosis and designing the treatment strategy. For instance, prophylactic CCND is not necessary for those with low risk of central CLNM, whereas it is necessary for those with high risk of central CLNM.

High-frequency US plays an important role in the detection and diagnosis of PTC (Haugen et al. 2016; Jiang et al. 2016; Kwak et al. 2011; Sakorafas 2010). However, as mentioned above, US alone offers insufficient diagnostic evidence for predicting central CLNM in PTC (Frates et al. 2005; Gharib et al. 2010; Liu et al. 2015).

On the other hand, it has been found that many immunohistochemical markers are correlated with PTC, such as cytokeratin 19 (CK 19), human bone marrow endothelial cell 1 (HBME-1), galectin 13 and thyroid peroxidase (TPO) (Cheung et al. 2001; Dencic et al. 2015; Rossi et al. 2006). However, the relation between the immunohistochemical markers and central CLNM in PTC is unclear. Furthermore, in some previous studies, immunohistochemical markers, especially in combination, were helpful in differentiating benign from malignant thyroid nodules, but their prediction of central CLNM in PTC and prognosis was not robust (Arcolia et al. 2017; Chen et al. 2016a, 2016b; Cheung et al. 2001; Dencic et al. 2015).

Recently, BRAF mutation has gained increasing attention in the pre-operative diagnosis and prognosis stratification for PTC patients; it is the most common alteration in PTC patients and has a prevalence of approximately 29% to 83% (Nikiforov 2011; Tang and Lee 2010). Most BRAF mutations in PTC are point mutations with a thymidine-to-adenosine transversion at nucleotide 1799 (1799T > A) in exon 15 and they result in a valine-to-glutamate substitution at residue 600 ($BRAF^{V600E}$), which leads to abnormal activation of the MARK pathway (Xing et al. 2004). Many studies have reported the correlation between $BRAF^{V600E}$ mutation and clinical characteristics, US features and poor prognosis of PTC such as central CLNM (Chen et al. 2016a; Lin et al. 2010; Zheng et al. 2012, 2013). However, the conclusions were inconsistent (Kwak et al. 2009; Lee et al. 2016; Moon et al. 2012); thus, further studies are warranted.

Although some previous studies have assessed the relevance between central CLNM and US features, as well as between central CLNM and molecular makers (Roh et al. 2007; Xu et al. 2016; Zhao et al. 2017), few studies have evaluated the predictive value of the combination of US features, immunohistochemical factors and $BRAF^{V600E}$ mutation. Based on these previous studies, we hypothesised that some US features, immunohistochemical factors and $BRAF^{V600E}$ mutation might be risk factors for CLNM in PTC patients. To confirm the hypothesis, we carried out this study to identify possible risk factors for predicting central CLNM in PTC patients and established a risk model to stratify the patients.

METHODS

Patients and data collection

The study was approved by the ethics committee of the University Hospital, and informed consent from the patients was waived because of the retrospective nature of the study. Between December 2015 and November 2016, a total of 257 consecutive patients (257 thyroid cancers) who had undergone surgery were included. All patients had undergone pre-operative US examination, post-operative immunohistochemical examination and post-operative BRAF mutation tests. The immunohistochemical factors included CK19, HBME-1, galectin-13 and TPO. The inclusion criteria were as follows: (i) The US imaging data must be retrievable; (ii) the US examination had to have been performed within the month before surgery; and (iii) PTC with or without central CLNMs had to have been confirmed by pathology after surgery. Twenty-four patients were excluded because the US imaging data were incomplete. Another 8 patients were excluded because they had different kinds of thyroid cancers, including 4 poorly differentiated squamous cell cancers and 4 follicular cancers. In patients with multiple PTCs, only the largest was selected. Thus, 225 patients (52 men and 173 women, mean age: 46.2 ± 12.2 y, range: 21−71 y) with 225 PTCs were included in the final analysis cohort.