Prediction of central compartment lymph node metastasis in papillary thyroid microcarcinoma

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Summary

Objectives We aimed to determine the predictive factors for central compartment lymph node metastasis (LNM) in papillary thyroid microcarcinoma (PTMC).

Design and patients We undertook a retrospective study of 291 patients treated for PTMC. The following criteria were assessed to predict the presence of central compartment LNM: sex, age, tumour multifocality, tumour size, tumour bilaterality, extracapsular spread (ECS), lateral neck LNM, coexistence of chronic lymphocytic thyroiditis, BRAFV600E mutation and ultrasonography (US) features. Univariate and multivariate analyses were performed to identify clinicopathological characteristics and US findings in predicting central compartment LNM from PTMC.

Results The central compartment LNM affected 133 (45% of 291) patients. With use of univariate and multivariate analyses, male gender (OR 2.020; P = 0.039), tumour size (>5 mm) (OR 3.687; P = 0.015), ECS (OR 2.330; P = 0.044), lateral LNM (OR 15.075; P = 0.000) and BRAFV600E mutation (OR 2.464; P = 0.000) were independently correlated with central compartment LNM. Age, tumour multifocality, tumour bilaterality, coexistence of chronic lymphocytic thyroiditis and US characteristics were not significantly related to the presence of central compartment LNM. We have also developed a nomogram to predict the probability of central compartment LNM for an individual patient. The sensitivity was 71.9% and specificity was 70.3%, with an under the receiver operating characteristic (ROC) curve of 0.772.

Conclusions A prophylactic neck dissection of the central compartment should be considered particularly in PTMC patients with male gender, a >5 mm tumour size, ECS of the tumours, lateral LNM and positive BRAFV600E mutation.

Introduction

Papillary thyroid microcarcinoma (PTMC) is a papillary thyroid carcinoma (PTC) with a lesion measuring 10 mm or less in its greatest diameter according to the World Health Organization classification system for thyroid tumours. Central compartment LNM is found in 40–60% of patients with PTC.1 The rate of central LNM has been shown to increase as tumours progress in size, extrathyroidal extension and locally advanced disease.2

The standard care for involved lymph node is total thyroidectomy with ‘therapeutic’ cervical lymph node dissection. A more controversial topic is whether routine central lymph node dissection should be performed in the PTMC patients without evident LNM. One important reason is that prophylactic central lymph node dissection seemed to have little prognostic value.3–5 Therefore, investigation of the clinicopathologic factors associated with central compartment LNM is of clinical significance. The utility of pre-operative ultrasonography (US) to evaluate LNM in the central compartment is often limited. Recently, a novel parameter BRAFV600E mutation for pre-operative risk estimation could be extremely valuable in the management of PTMC.6

The purpose of this study was to determine the factors predictive of central compartment LNM, as well as to create a nomogram to estimate the probability of central compartment LNM in PTMC. The outcome of the current study could assist greatly in decision-making regarding further treatment.
Patients and method

Patients and data collection

Data were obtained through a retrospective review of a database in the hospital. Informed consent was obtained from each participant at the time of surgery. The study design was approved by local hospital ethics committee. The study population consisted of 291 patients with PTMC who underwent total thyroidectomy with prophylactic central compartment lymph node dissection, from June 2007 to December 2010. All patients underwent pre-operative examination by US and/or neck computed tomography to evaluate the size of the tumour and the presence of LNM. Potential patients who had central compartment LNM by pre-operative imaging were excluded from our study. Bilateral central lymph node dissection, which included dissection of the pretracheal, prelaryngeal and paratracheal lymph nodes, was performed in all patients. Lateral compartment dissection was selectively performed in patients diagnosed with LNM on pre-operative US-guided fine-needle aspiration. If lymph node suspicious for metastases was found at the time of the operation or on neck computed tomography and were not detected on staging US, lymph node sampling and frozen-section examinations were performed. If metastasis was confirmed, lateral compartments were dissected, including levels II, III, IV and anterior V. Of the 291 patients with PTMC, 56 (19%) were shown on pre-operative US to have findings suspicious for metastatic lymph node at the lateral compartment. Among them, 49 patients underwent US-guided fine-needle aspiration, and 31 patients (31/49, 63%) had results positive for malignancy, 15 patients (15/49, 31%) had results negative for malignancy, and the results in 3 patients (3/49, 6%) were nondiagnostic. For the remaining seven patients, who did not undergo pre-operative US-guided fine-needle aspiration, frozen biopsy at the time of the operation gave pathology results positive for malignancy in 5 patients. On final pathologic reports, 36 patients had lateral LNM. US was performed with Acuson Sequoia and 128XP sonographic scanners (Siemens Medical Solutions, Mountain View, CA, USA) equipped with commercially available 8- to 13-MHz linear probes. All patients were diagnosed with PTMC pre-operatively by fine-needle aspiration biopsy or intra-operatively on frozen section. Thyroid fine-needle aspiration biopsy was performed on the primary tumour in these patients, either in the outpatient clinic with ultrasound guidance or in the operating room before total thyroidectomy.

The electronic clinical and pathologic records collected included sex, age, tumour multifocality, tumour size, tumour bilaterality, extracapsular spread (ECS), lateral LNM, chronic lymphocytic thyroiditis, BRAFV600E mutation and central compartment lymph node involvement. The dichotomous variables were age at diagnosis (<45 y or ≥45 y), sex, multifocality (unifocal, multifocal), tumour diameter (>5 mm or ≤5 mm), tumour bilaterality (unilateral, bilateral), ECS, lateral LNM, chronic lymphocytic thyroiditis, BRAFV600E mutation. US features were recorded according to composition, echogenicity, calcifications, margin and shape. When multiple PTMC were found in the specimen, the largest tumour or the most suspicious dominant nodule was analysed.

Detection of the BRAFV600E mutation

BRAFV600E mutation was assayed as previously described. Briefly, DNA was extracted from the fine-needle aspiration biopsy samples with a QIAamp DNA Micro Kit (QIAGEN), according to the manufacturer’s protocol. We amplified the BRAF exon 15 by polymerase chain reaction (PCR) with the following primers: forward, 5'-TCTAATGTCTGGTCTTAGA-3'; reverse, 5'-GGCCTAAATTATGCTGG-3'. The amplicon size was 215 bp. The specificity and integrity of the PCR were confirmed by visualization of a single-band PCR product with the expected molecular weight on a 1.5% agarose gel. The samples were analysed on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems) to identify the mutation.

Statistical analysis

The statistical analysis was performed with SPSS (version 18.0). All significance tests were two sided. P < 0.05 was considered statistically significant. Univariate analysis with the χ2-test was used to analyse the statistical correlation between the factors and central compartment LNM. Multivariate logistic regression analysis was performed to identify the multivariate correlates of central compartment LNM. Results were presented as odds ratio (OR) with 95% confidence interval (CI) and P value. The nomogram for predicting central compartment LNM was developed based on the following factors – sex, tumour size, ECS, lateral LNM and BRAFV600E mutation. The predictive value of the model was measured using the area under the receiver operating characteristic (ROC) curve.

Results

Patient population

A total of 291 patients with PTMC were included in this study, among whom 243 were female and 48 were male. Patient age at time of initial treatment ranged from 21 to 76 years (mean age 44.7 years; SD 10.2 years).

Demographic variables

Table 1 shows the clinical and pathological characteristics of patients. Multifocal PTMC was present in 65 cases (22.3%) and bilateral PTMC in 36 (12.3%). Median tumour size was 5.91 mm (range 0.8–10 mm), with 155 tumours (53.3%) <5 mm and 136 tumours (46.7%) larger than 5 mm in diameter. ECS was found in 37 cases (12.7%). A total of 92 patients (31.6%) had chronic lymphocytic thyroiditis. BRAFV600E mutation was observed in 124 patients (42.6%). The central compartment LNM affected 133 (45.7%) of 291 patients. The mean number of removed central lymph nodes was 12.9 (range 8–20), of which 3.1 (range 1–9) had metastases. Lateral LNM was
detected in 36 patients (12.3%). The mean number of harvested lateral neck nodes was 14.8 (range 6–35), of which 2.0 (range 1–7) had metastases.

**Univariate analysis**

Table 2 demonstrates the relationship between predictive factors and central compartment LNM. Five factors were significantly related to central compartment LNM: male gender ($P = 0.010$), multifocality ($P = 0.019$), tumour size (>5 mm) ($P = 0.005$), ECS ($P = 0.000$), lateral LNM ($P = 0.000$) and BRAFV600E mutation ($P = 0.000$). Age, tumour bilaterality, chronic lymphocytic thyroiditis were not significantly related to the presence of central compartment LNM. None of the US features were found to be statistically significant in predicting central LNM in PTMC.

**Multivariate analysis**

A multivariate analysis was performed to determine whether these parameters were independently correlated with central compartment LNM. Male gender (OR 2.020; $P = 0.039$), tumour size (>5 mm) (OR 3.687; $P = 0.015$), ESC (OR 2.330; $P = 0.044$), lateral LNM (OR 15.075; $P = 0.000$) and BRAFV600E mutation (OR 2.464; $P = 0.000$) turned out to be independently predictive for central compartment LNM (Table 3).
under the ROC curve of 0 for the population was accurate and discriminative, with an area of central compartment LNM was defined as a value 0 based on the prognostic model score, a cut point for prediction (mm).

The role of routine central lymph node dissection in the treatment for PTMC remains debated. It is generally agreed that therapeutic neck dissection should be performed to remove macroscopic lymph node metastases because this treatment reduces the chance of PTC persistence/recurrence. However, there are no randomized controlled trials to support the concept that routine prophylactic central lymph node dissection impacts the recurrence or survival rates of PTMC. In addition, whether prophylactic central lymph node dissection may increase the risk of complications such as hypocalcaemia and recurrent laryngeal nerve palsy remains controversial. These controversies are, to a large extent, due to the imprecision in the risk estimation of thyroid cancer aggressiveness based on the clinical and testing information available pre-operatively. The utility of pre-operative US to evaluate LNM in the central compartment is often limited. Thus, it would be important to investigate the pre-operative predictive factors of central LNM when developing an individualized-based treatment plan.

The predictive factors for central compartment LNM in patients with PTMC were not well defined. However, it is generally accepted that prognosis depends on sex, tumour multifocality, capsular invasion and tumour size. This study included nine clinicopathological parameters and US features as potential predictors of central compartment LNM. As a result, male gender, tumour size, ECS, lateral LNM and BRAFV600E mutation were found to be independent predictors of central compartment LNM.

The cut-off age of 45 years is widely used as a clinical marker for prognosis. Among patients with PTC, an inverse correlation is usually seen between age and prognosis. The current study showed that age was not predictive of central compartment LNM. Previous studies had also reported that age was not associated with LNM in patients with PTMC.

Male gender has previously been suggested as important indicator for prophylactic lymph node dissection. In a series of studies, male gender was associated with higher rate of LNM. In this study, male gender (OR 2.020; P = 0.039) was independently predictive of central compartment LNM, which agreed with previous reports.

PTMC is often multifocal (15.5–40% in surgical series and over 80% in systematic autopsy studies). Some studies showed that multifocality was associated with tumour recurrence. This study showed significant relationship between tumour multifocality and central compartment LNM in univariate analysis, but multifocality was not an independent predictor of central compartment LNM in multivariate analysis (P = 0.444). Lee SH et al. have also found no correlation between tumour multifocality and central compartment LNM in a retrospective study of 52 patients. The presence of multiple foci may not indicate increased aggressiveness. It appears reasonable that tumour multifocality represents multiple numbers of the tumour that have individual characteristics of their own.

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The tumour size has been repeatedly confirmed as an independent predictor of both pathologic and clinical outcomes. The LNM is known to increase with tumour size. Lim et al.\cite{10} reported that tumour size (>5 mm) was a significant predictive factor of LNM in PTMC. Machens et al.\cite{16} and Roti et al.\cite{9} also had demonstrated that PTMC of >5 mm were more associated with poor prognostic factors compared with those of <5 mm. The results of this study confirmed that tumour size (>5 mm) was significantly correlated with central compartment LNM. Therefore, tumour size can also assist in the decision to perform surgery.

Tumour bilaterality in PTC is traditionally considered indication for total or near-total thyroidectomy, but few data specifically examining the prognostic implications of bilateral disease have been reported. Wang et al.\cite{17} found that patients with bilateral PTC were more likely to have larger tumour size, extrathyroidal invasion, LNM and more advanced stage compared with unilateral PTC patients. Kim et al.\cite{18} also demonstrated central LNM in patients with PTMC was significantly associated with bilaterality. However, in this study, there were no statistical correlations between tumour bilaterality and central compartment LNM in PTMC.

ECS is thought to have predictive value for central compartment LNM. In our series, tumoral infiltration of the thyroid capsule was not rare (12.7%), in agreement with previous studies reporting a 9-9–26.8% rate.\cite{19,20} It has been demonstrated that ECS was independently predictive of subclinical central LNM. Our results indicated that ECS was an independent risk factor for central compartment LNM (OR 2.330; P = 0.044). This will prompt us to consider neck dissection of the central compartment in PTMC patients with this histological feature.

Cervical metastasis occurs firstly to the lymph nodes in the central compartment and subsequently to those in the lateral neck.\cite{21} The skip metastasis to the lateral compartment of the neck in the absence of central disease is uncommon.\cite{22} In current series, lateral LNM was present in 36 patients (12.3%) and underwent additional therapeutic neck dissection. A total of 34 of the 36 patients with positive lateral LNM had positive lymph node in the central compartment. With use of univariate and multivariate analyses, we have confirmed that lateral LNM was an independent risk factor for central compartment LNM (OR 15.075; P = 0.000). However, it is impossible to identify the rate of lateral LNM of all the cases because we did not undertake lateral neck dissection for all the enrolled patients.

The coexistence of chronic lymphocytic thyroiditis with PTC has been reported to range from 10% to 58%.\cite{23} The association of chronic lymphocytic thyroiditis and aggressive pathologic features of PTMC has been debated. There have been few reports of studies on the effects of coexisting chronic lymphocytic thyroiditis with PTC on the LNM. Loh et al.\cite{24} reported that patients with PTC and chronic lymphocytic thyroiditis had a good prognosis, because the frequency of extrathyroid extension, nodal metastasis and distant metastasis was low. Kim et al.\cite{25} reported a negative association between the coexistence of chronic lymphocytic thyroiditis and central compartment LNM. In this study, 92 patients (31.6%) had chronic lymphocytic thyroiditis, of which 39.1% was found with central compartment LNM, the result was similar to Kim’s report (LNM in chronic lymphocytic thyroiditis was identified in 52.5%). In the chronic lymphocytic thyroiditis group, central compartment LNM had a lower frequency compared with the control group (39.1% vs 48.7%), but we found no significant difference in the frequency of LNM (P = 0.125).

Recent evidences have focused on the BRAFV600E mutation as a novel prognostic marker that may be useful in stratifying the risk of LNM. The frequency of BRAFV600E mutation was reported as approximately 45% in PTC.\cite{26} The relationship between the BRAFV600E mutation and clinicopathologic features is still controversial. Some studies have established a strong association of BRAFV600E mutation with aggressive clinicopathologic characteristics of primary PTC, including extrathyroidal extension, LNM, histological subtypes with a poor prognosis and advanced disease stages, as well as disease persistence/recurrence.\cite{27,28} Xing et al.\cite{9} reported BRAFV600E mutation in fine-needle aspiration biopsy specimens was found in 38% of patients with PTC, significantly associated with LNM. In contrast, some studies failed to find a significant association between the BRAFV600E mutation and high-risk clinicopathologic characteristics. Kim et al.\cite{30} reported BRAFV600E mutation was not significantly associated with cervical LNM. We examined the BRAFV600E mutation status on pre-operative fine-needle aspiration biopsy specimens from 291 patients with PTMC. BRAFV600E mutation was found in 124 (42.6%) of 291 patients with PTMC. BRAFV600E mutation positivity was associated with central compartment LNM (P = 0.000) and remained an independent predictor of central compartment LNM on multiple logistic regression analysis (OR 2.464; P = 0.000). The present study demonstrated the predictive value of BRAFV600E mutation on fine-needle aspiration biopsy specimens for PTMC. The predictive role of BRAFV600E mutation was also studied by Rossi et al.\cite{31}, who used liquid-based cytology for the detection of BRAFV600E mutation on fine-needle aspiration cytology in the patients with PTMC. A significant association between BRAFV600E mutation and lymph node involvement was observed. Moreover, Nikiforov et al.\cite{32} found that a panel molecular analysis of mutations including BRAFV600E from thyroid fine-needle aspiration samples of patients with cytologically indeterminate thyroid nodules has significant diagnostic value and can be helpful for more effective clinical management of these patients. Thus, the BRAFV600E mutation analysis performed on cytologic material from fine-needle aspiration has significant diagnostic and predictive role and may help to identify a group of patients with a greater risk of LNM who should undergo more extensive surgery and prophylactic central lymph node dissection.

Several studies have reported pre-operative US findings of tumour margin (ill-defined), and presence of calcification were independent predictive factors for lateral LNM.\cite{24} However, few have studied the relationship between central compartment LNM and pre-operative US findings. Some studies showed US features including composition, echogenicity, calcifications, margin and shape were not associated with central compartment LNM.\cite{18} Also in this study, no significant association was seen.
among the US features of PTMC with central compartment LNM.

There were many reports about predictive factors of central compartment LNM, but none of them has incorporated all risk factors for prediction of central compartment LNM. In this study, nomogram has been developed to incorporate sex, tumour size, ECS, BRAFV600E mutation and lateral LNM from statistical analysis to further predict central compartment LNM. The nomogram had high predictive performance. When clinicians suspect central compartment LNM, they input the patient’s sex (male = 1, female = 0), tumour size (mm), ECS findings (1 if positive finding and 0 for negative), the presence or absence of lateral LNM (1 for present and 0 for not), BRAFV600E mutation (1 for positive, 0 for negative) into the equation. The computer will then calculate the final total score. Finally, they will get the estimated likelihood of central compartment LNM for the patient. Clinicians can tailor each patient’s follow-up treatment accordingly. To our knowledge, the present nomogram is the first that has been developed specifically to predict the central compartment LNM in patients with PTMC. However, the sensitivity of the equation was 71.9%, while the specificity was 70.3% for prediction which was not very high. The following possibilities may influence the nomogram’s accuracy. Firstly, lateral neck dissection was not performed in all patients, and there might be positive lateral lymph nodes which were not taken into consideration. Secondly, the cases were not large enough for performing subgroup analysis like numbers of involved lymph nodes per compartment. More importantly, the pre-operative novel and minimally invasive biomarkers, such as serum microRNA, should be incorporated into analysis. In recent report, several serum microRNAs in PTC have been shown to be associated with LNM.35

However, the current study has limitations. This study was conducted with a cross-sectional design. Thus, a cause-and-effect relationship between the risk and variables could not be determined. Furthermore, the nomogram has not been prospectively validated in a large independent data set. Much more work has to been carried out to perform the tool in prospective, multicentre, high-volume study. Herein, we focused on the search for risk factors predicting central LNM, tumour recurrence and survival after central compartment dissection was not studied. Further investigation with a long follow-up period is necessary to determine whether the studied parameters are associated with prognosis. Despite these limitations, our study is based on the variables obtained from patients within the same race and in a local environment. Thus, we believe it will be useful in the design of further studies.

In conclusion, the results of this study identified five statistically significant independent predictive factors for central compartment LNM in PTMC: male gender, ECS, a tumour size more than 5 mm, lateral LNM and BRAFV600E mutation. The nomogram, which combined modern molecular detection of BRAFV600E mutation with optimal clinicopathologic findings to estimate the risk of central compartment LNM, provides a sound basis for the selection of appropriate patients for entry into neck dissection of the central compartment. Further validation and follow-up studies will ultimately provide additional guidance to the clinician and patient.

Disclosure statement

The authors have nothing to disclose.

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