ORIGINAL RESEARCH

Prediction of central lymph node metastasis in papillary thyroid microcarcinoma according to clinicopathologic factors and thyroid nodule sonographic features: a case-control study

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Background: Preoperative diagnosis of central lymph node metastasis (CLNM) poses to be a challenge in clinical node-negative papillary thyroid microcarcinoma (PTMC). This research work aims at investigating the association existing between BRAF mutation, clinicopathological factors, ultrasound characteristics, and CLNM, in addition to establishing a predictive model for CLNM in PTMC.

Materials and methods: The study included 673 PTMC patients, already undergone total thyroidectomy or lobectomy with prophylactic central lymph node dissection. The predictor factors were identified through univariate and multivariate analyses. The support vector machine was put to use to develop statistical models, which could predict CLNM on the basis of independent predictors.

Results: Tumor size (>5 mm), lower location, no well-defined margin, contact of >25% with the adjacent capsule, display of enlarged lymph nodes, and BRAF mutation were independent predictors of CLNM. Through the use of the predictive model, 79.6% of the patients were classified accurately, the sensitivity and specificity amounted to be 85.1% and 75.8%, respectively, and the positive predictive value and negative predictive value stood at 71.6% and 87.6%, respectively.

Conclusions: We established a predictive model in order to predict CLNM preoperatively in PTMC when preoperative diagnosis of CLNM was not clear.

Keywords: papillary thyroid microcarcinoma, central lymph node, predictive factor, support vector machine

Background

Thyroid cancer (TC) was the most frequently found endocrine malignancy.[1] As of now, the incidence of TC had been experiencing a growing trend throughout the past era, being faster in comparison with any other cancer type in the USA.[2,3] Among these patients, papillary thyroid cancer (PTC) appeared to be the most widespread histological TC.[3] The definition of the papillary thyroid microcarcinoma (PTMC) was made as a tumor with a maximum diameter of ≤1.0 cm,[4] and this proportion had increased over time because of the utilization of sonography in addition to ultrasound (US)-guided fine-needle aspiration biopsy (FNAB); up to 50% of newly diagnosed PTC were in the form of PTMC.[5,6] Even though most of the patients having PTMC possessed an indolent clinical course and positive prognosis,[7] yet they usually had lymph node metastasis when diagnosed.[8] Central lymph node metastasis (CLNM) had been taken into consideration being an element of risk regarding the recurrence,

distant metastasis, and reduced survival.[9–11] Lateral lymph node metastasis was normally via central compartment.[12] Therefore, it was crucial to find the elements of risk predicting CLNM in PTMC.

BRAF mutation was the most commonly found genetic alteration in TC, taking place in approximately 45% of sporadic PTCs in addition to being a major reason giving rise to atypical trigger of the MAPK pathway in human cancers.[13] The T1799A BRAF mutation gives birth to a V600E amino acid variation in the BRAF protein and occurs uniquely in PTC as well as some PTC-derived anaplastic TCs.[14] This mutation was associated with clinicopathological characteristics that relate to tumor progression and recurrence such as extrathyroidal invasion, lymph node metastasis, and advanced tumor stages.[15]

It was brought to light by the past reports on PTMC that aggressive characteristics and poor outcomes had association with US characteristics.[16–18] In this study, we investigated the association between BRAF mutation, clinicopathological factors, US characteristics, and CLNM in PTMC. Furthermore, we also developed a predictive model for the purpose of predicting preoperative CLNM in PTMC.

Materials and methods
Patients
The study included 673 PTMC patients who had undergone total thyroidectomy or lobectomy with prophylactic central lymph node dissection (pCLND) at the First Affiliated Hospital of Wenzhou Medical University from March 2016 to March 2017. All pathological diagnoses were made by three experienced pathologists. Preoperative US-guided FNAB was also carried out on the primary thyroid tumor in each patient suspected of malignancy. The PTMC patients included 147 males and 526 females. All patients had no history of neck surgery or neck irradiation. Retrospective reviews of electronic clinical and pathologic records were carried out to collect the clinicopathologic information. Approval of the research protocol was received from the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University.

Imaging analysis
The performance of US was made with the help of Acuson Sequoia and 128XP sonographic scanners (Siemens Medical Solutions, Mountain View, CA, USA) that were equipped with 8–13 MHz linear probes. The factors selected to analyze the risk of CLNM are shown in Table 1. Assessment of the tumor size was done by maximum diameter based on US.

The definition of Multifocal PTMC was provided as being above one lesion in a paraffin section, and the lesion with the maximum diameter or the most doubtful dominant nodule was brought under analysis. Shape was divided into regular or irregular. Classification of internal composition was made as either totally solid or largely solid (proportion of solid components ≥50%) cystic. Classification of margin was made as well defined and not well defined. On the basis of preoperative US images, classification of tumor position was made in four areas (upper, middle, lower, and isthmus). Contact of thyroid mass with the thyroid capsule as seen on US was recorded, and >25% contact with the adjacent capsule is the most accurate measurement for predicting extrathyroidal extension.[18]

DNA extraction and ARMS PCR
Two or three US-guided fine-needle aspiration using a 25-guage needle was carried out for the purpose of collecting material for both the cytological and the molecular analyses. The extraction of the DNA from the FNAB specimens was performed with the use of the DNA Extraction Kit (Amoy Diagnostics, Xiamen, China) in accordance with the guidelines of the manufacturer. Human BRAFV600E ARMS-PCR Kit (Amoy Diagnostics, Xiamen, China) based on Amplification Refractory Mutation System (ARMS) was used for the detection of BRAFV600E mutation (approved by China Food and Drug Administration [CFDA]). Every sample was subjected to an external control assay and a mutations assay in the same well. Each test included a positive control and a negative control. BRAFV600E mutation analysis was carried out on the ABI prism 7,500 series real-time polymerase chain reaction (PCR) instrument (Applied Biosystems, Carlsband, CA, USA). PCR procedure was as follows: Stage I: 5 minutes, 95°C for 1 cycle; Stage 2: 25 seconds, 95°C, 20 seconds, 64°C, 20 seconds, 72°C, for 15 cycles; Stage 3: 25 seconds, 93°C, 35 seconds, 60°C, 20 seconds, 72°C for 31 cycles (Data Collection).

Model development
The support vector machine (SVM) was put to use in respect of the development of statistical models that could predict CLNM based on BRAF mutation, clinicopathological factors, and US characteristics. We established the SVM model by utilizing Libsvm 3.20 for the prediction of CLNM with the modeling platform of MATLAB 2014a. The C-SVC together with radial basis function kernel was chosen. Moreover, the grid search approach was employed for debugging the model during the development of the SVM model. The grid c stood from –8 to 8, the grid c step amount to be 0.5, the grid g bound

Table 1 Baseline characteristics of papillary thyroid microcarcinoma patients stratified by the status of metastatic central lymph nodes

Characteristics	The status of metastatic central lymph nodes		P-value
	Positive	**Negative**	
Total	281 (41.8%)	392 (58.2%)	
Tumor size (mm)	7.11±1.90	6.44±1.81	<0.001
≤5 (n, %)	68 (24.2%)	123 (31.4%)	0.042
>5 (n, %)	213 (75.8%)	269 (68.6%)	
Age at diagnosis (years)	46.37±10.44	47.58±10.44	0.14
<45 (n, %)	121 (43.1%)	152 (38.8%)	0.264
≥45 (n, %)	160 (56.9%)	240 (61.2%)	
Gender			0.13
Male (n, %)	82 (29.2%)	94 (24.0%)	
Female (n, %)	199 (70.8%)	298 (76.0%)	
BRAF mutation			<0.001
Yes (n, %)	243 (86.5%)	294 (75.0%)	
No (n, %)	38 (13.5%)	98 (25.0%)	
Tumor location			<0.001
Lower (n, %)	78 (27.8%)	61 (15.6%)	
Upper/middle/isthmus (n, %)	203 (72.2%)	331 (84.4%)	
Margin			0.002
Well defined (n, %)	167 (59.4%)	278 (70.9%)	
Not well defined (n, %)	114 (40.6%)	114 (29.1%)	
Contact of >25% with the adjacent capsule			<0.001
Yes (n, %)	159 (56.6%)	55 (15.2%)	
No (n, %)	122 (43.4%)	337 (84.8%)	
Display of enlarged lymph nodes			<0.001
Present (n, %)	157 (55.9%)	127 (32.4%)	
Absent (n, %)	124 (44.1%)	265 (67.6%)	
No. of nodules			0.117
Single (n, %)	221 (78.6%)	327 (83.4%)	
Multiple (n, %)	60 (21.4%)	65 (16.6%)	
Hashimoto's thyroiditis			0.262
Yes (n, %)	57 (20.3%)	95 (24.2%)	
No (n, %)	224 (79.7%)	297 (75.8%)	
Calcification			0.001
Present (n, %)	175 (62.2%)	194 (49.5%)	
Absent (n, %)	106 (37.8%)	198 (50.5%)	
Shape			0.659
Regular (n, %)	239 (85.1%)	337 (86.0%)	
Irregular (n, %)	42 (14.9%)	55 (14.0%)	
Intact capsule			0.480
Yes (n, %)	152 (54.1%)	223 (56.9%)	
No (n, %)	129 (45.9%)	169 (43.1%)	
Composition			0.793
Solid (n, %)	274 (97.5%)	384 (98.0%)	
Cystic or mixed (n, %)	7 (2.5%)	8 (2.0%)	
Vascularization			0.506
Present (n, %)	14 (5.0%)	25 (6.4%)	
Absent (n, %)	267 (95.0%)	367 (93.6%)	

value came out to be –8 to 8, and the grid g step valued at 0.5. The fold number of cross-validation came out to be 5.

$$\text{Plabel} = \text{sgn}\left(\sum_{i=0}^{n} w_i \exp(-\text{gamma}|(x_i-x)|^2 + b)\right)$$

The "sgn" function is to maintain the positive or negative of the parameter. The meaning of "plabel" is to get the numeric symbols. As we divided patients into two groups, positive value means central lymph node metastasis positive and negative value means central lymph node metastasis negative.

Statistical analysis

We created two groups of the patients in accordance with the status of metastatic central lymph node: CLNM positive and

CLNM negative. The application of independent two-sample *t*-test was made in order to carry out the comparison of data on normal distribution. Categorical variables were expressed as percentage and were compared with chi-squared test or Fisher's exact test, as appropriate. Variables with *P*<0.05 in the univariate analysis were progressed to a multivariate analysis using forward stepwise selection. The findings were brought forth as odds ratio (OR) having 95% CI as well as *P*-value. All *P*-values were two sided. Moreover, the value <0.05 was taken into consideration as statistically significant. Statistical analysis was performed with SPSS software version 22.0 (SPSS, Chicago, IL, USA).

Ethics approval and informed consent

The research protocol used in this study was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University. All of the patients provided written informed consent.

Results

Relationship of central lymph node metastasis of PTMC with clinicopathologic factors and US features

We carried out the investigation of the relationship of CLNM with clinicopathologic factors and US features through univariate analysis (Table 1). The results showed that larger tumor size (*P*<0.001), BRAF mutation (*P*<0.001), lower location (*P*<0.001), presence of calcification (*P*<0.001), no well-defined margin (*P*=0.002), contact of >25% with the adjacent capsule (*P*<0.001), and display of enlarged lymph nodes (*P*<0.001) have considerable association with CLNM.

The use of multivariate logistic regression was made for the purpose of determining whether these elements were independently associated with CLNM (Table 2). The results showed that larger tumor (OR 1.164; *P*=0.005; 95% CI 1.048–1.294), lower location (OR 1.912; *P*=0.006; 95% CI 1.206–3.034), no well-defined margin (OR 1.695; *P*=0.010;

95% CI 1.136–2.530), contact of >25% with the adjacent capsule (OR 13.833; *P*<0.001; 95% CI 8.696–22.007), display of enlarged lymph nodes (OR 5.865; *P*<0.001; 95% CI 3.786–9.087), and BRAF mutation (OR 2.290; *P*=0.001; 95% CI 1.404–3.734) were independently predictive of CLNM.

The predictive model

An aggregate of six selected factors was put into use to predict CLNM in 673 patients. The details of patients are shown in Table 1. We established a model for predicting CLNM using SVM statistical model that combines the six factors. Five hundred thirty-seven of 673 patients (79.6%) were correctly classified and the details are shown in Table 3. The sensitivity and specificity for this model amounted to 85.1% and 75.8%, respectively, and the positive predictive value and negative predictive value stood at 71.6% and 87.6%, respectively (Table 4). The model had a satisfied predictive value when compared with other single predictors.

Discussion

In the current era, the prevalence of PTMC has experienced a rapid growth across the globe through preoperative sonography and US-guided FNAB. Despite the fact that most of the patients having PTMC had an indolent clinical course together with positive prognosis, the incidence of CLNM has been termed as an element of risk with respect to poor prognosis.[9–11] pCLND is brought forth as a suggestion in respect of the decrease of recurrence and metastasis; however, aggressive PTMC treatments can lead to negative results, for instance, temporary or permanent laryngeal nerve injury causing damage to the voice and temporary or permanent hypoparathyroidism.[19,20] Some studies reported that aggressive treatments were not linked to the improved outcome as regards reduced recurrence or prolonged survival.[9] That is why monitoring could be chosen for the patients with PTMC without unfavorable features.[7] Although the prognosis of most PTMC appears to be positive, a small part of the patients having PTMC has a more aggressive course of disease.[21] However, the decision of surgery is dependent on the opinion

Table 2 Multivariate analysis between the clinicopathologic factors and CLNM

Characteristic	OR	95% CI	*P*-value
Tumor size (mm)	1.164	1.048–1.294	0.005
Tumor location	1.912	1.206–3.034	0.006
Margin	1.695	1.136–2.530	0.010
Display of enlarged lymph nodes	5.865	3.786–9.087	<0.001
BRAF mutation	2.290	1.404–3.734	0.001
Contact of >25% with the adjacent capsule	13.833	8.696–22.007	<0.001

Abbreviations: CLNM, central lymph node metastasis; OR, odds ratio.

Table 3 The predictive model and pathological diagnosis of CLNM

Pathological diagnosis	Predictive model		
	Positive	Negative	Total
Positive	239	42	281
Negative	95	297	392
Total	334	339	673

Abbreviation: CLNM, central lymph node metastasis.

Table 4 The predictive value for the model and independent predictors

Independent predictors	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Size	75.8	31.9	79.8	64.8
Location	27.8	84.4	56.1	62.0
Margin	40.6	70.9	50.0	62.5
Display of enlarged lymph nodes	55.9	67.6	55.3	68.1
BRAF mutation	86.5	25.0	45.3	72.1
Contact of >25% with the adjacent capsule	56.6	86.0	74.3	73.4
The model	85.1	75.8	71.6	87.6

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

of the doctor or the patients. In this study, we set an aim to dig deep into the preoperative predicting elements for CLNM, together with allowing the surgeons and endocrinologists to formulate a more comprehensive operative plan.

In respect of this research work, the occurrence rate of CLNM in PTMC came out to be 41.8% that displayed harmony with the previous reports.[11,22] It was illustrated by the univariate analysis that larger tumor size, BRAF mutation, lower location, the presence of calcification, no well-defined margin, contact of >25% with the adjacent capsule, and display of enlarged lymph nodes were considerably linked to the status of CLNM. Multivariate logistic regression confirmed that larger tumor size, BRAF mutation, lower location, no well-defined margin, contact of >25% with the adjacent capsule, and display of enlarged lymph nodes were independently predictive of CLNM.

In our study, cytological specimens from US-guided fine-needle aspiration were used to detect BRAF mutation and the occurrence of the BRAF mutation at 79.8% (537/673), which was higher than generally reported values of approximately 45%–50%.[23,24] One possible explanation suggests that the technique of ARMS-PCR had a higher sensitivity and specificity.[25] Another reason was that elevated consumption of iodine poses to be a sizeable element of risk with respect to the incidence of BRAF mutation.[26] Taking into consideration the eastern coastal regions in China, where the study was conducted, the population's diet is rich in iodine.

With the increase in size, lymph node metastasis increased in PTMC.[27] Several studies revealed that larger tumor size was an independent predictor of CLNM.[28,29] Kim et al[28] discovered that tumor size >5 mm in PTMC was sizably associated with the CLNM. Our research work brought to light the fact that tumor size >5 mm was independently predictive of CLNM in PTMC.

Some studies had reported that the incidence of CLNM was related to the location of PTMC.[11] Lower location in the thyroid lobe suggested an elevated risk factor of CLNM.[30] In the same way with previous studies, we discovered the fact that the tumor positioned in the lower pole of the thyroid was an independent factor associated with CLNM.

The thyroid nodule with no well-defined margin is more inclined to be malignant tumor. It was reported that no well-defined margin suggested a considerable US feature of aggressive PTMC.[17] Furthermore, we also discovered that no well-defined margin suggested an independent predictor of CLNM in PTMC.

Contact with >25% of the capsule is the most accurate measurement for predicting extrathyroidal extension.[18] It was reported by the past studies that extrathyroidal extension was independently predictive of CLNM in patients having PTMC.[27,31] In our study, we discovered the fact that thyroid nodules in contact with >25% of the capsule were an independent factor in respect of CLNM.

Enlarged lymph nodes are usually associated with lymph node metastasis and recur in the regional lymph nodes.[32] In our study, display of enlarged lymph nodes suggested an independent predictor of CLNM in accordance with the univariate and multivariate analyses.

The incidence of recurrence, distant metastasis, and reduced survival[9–11] increased with the existence of CLNM in PTMC. pCLND still continued to be a point that needs discussion. The CLND could be carried out intraoperatively if the CLNM could be diagnosed preoperatively. It had been reported by numerous studies that BRAF mutation,[13–15] overexpression of cyclin D1, underexpression of p27[33] together with other PTC-specific mutations, are likely to be linked with the aggressiveness of thyroid carcinoma. Moreover, the BRAF mutation was the most frequent somatic oncogene in PTC.[34] Owing to the fact that preoperative sonography for the detection of CLNM possessed low sensitivity, we employed a combination of BRAF mutation, clinicopathologic features, and US findings for the purpose of enhancing the diagnostic accuracy. Enlightened by a US scoring system in predicting CLNM,[35] we developed a model based on six factors. This model for the detection of CLNM had a sensitivity of 85.1%, a specificity of 75.8%, a positive predictive value of 71.6%, and a negative predictive value of 87.6%. Moreover, the model is expected to extend help to the surgeons when it comes to deciding the surgical strategy with respect to each patient in PTMC.

There were numerous constraints encountered by the current research work. Firstly, in some patients having multifocal nodules, we carried out analysis of the most suspicious or the largest one only, when the information about other nodules was not available. Secondly, the model has characterized a reference set, which needs more patients to examine the characteristics predicting CLN metastatic disease in a test set. Thirdly, there is a need for an extended follow-up time for the purpose of evaluating the survival rate as well as surgical complication. In summary, we discovered six independent factors, tumor size (>5 mm), lower location, no well-defined margin, contact of >25% with the adjacent capsule, display of enlarged lymph nodes, and BRAF mutation, being the independent predictors of CLNM. Thereafter, we established a predictive model with the use of a simple approach in order to predict CLNM preoperatively in PTMC as preoperative diagnosis of CLNM was not quite clear enough.

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Disclosure

The authors report no conflicts of interest in this work.

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