Evaluation of neutrophil-to-lymphocyte ratio and calcitonin concentration for predicting lymph node metastasis and distant metastasis in patients with medullary thyroid cancer

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Abstract. The aim of the present study was to investigate potential predictive factors of lymph node metastasis (LNM) and distant metastasis (DM) of medullary thyroid cancer (MTC). A total of 61 patients newly diagnosed with MTC at the General Hospital of the Chinese People's Liberation Army between January 2001 and January 2016 were enrolled and divided into two groups according to the results of preoperative examinations and surgical histopathology as follows: Group NM (without metastases), and group M (with metastases). Univariate logistic regression analysis demonstrated that sex, tumor size, preoperative peripheral blood neutrophil-to-lymphocyte ratio (NLR), and concentration of carcinoembryonic antigen (CEA) and calcitonin (Ctn), were significantly associated with LNM and DM. The multivariate analysis revealed that a Ctn concentration of >500 pg/ml [odds ratio (OR)=21.422; 95% confidence interval (CI): 2.611-175.731] and the NLR (OR=5.918; 95% CI: 1.147-30.541) were positively correlated with LNM and DM. The optimal cut-off value of the NLR for predicting LNM and DM obtained from receiver operating characteristic curve analysis was 1.784 (sensitivity 68.3% and specificity 80%), and the area under the curve was 0.717. In conclusion, the findings of the present study strongly suggest that inflammation and immune activation of MTC cells promote LNM and DM, and that higher values of NLR and Ctn concentration confer a high risk of metastasis.

Introduction

Medullary thyroid carcinoma (MTC) is a rare neuroendocrine tumor, which arises from parafollicular calcitonin-secreting cells (C-cells), and accounts for 3-10% of thyroid malignancies, although MTC is responsible for up to 13.4% of thyroid cancer-related deaths (1). MTC is of intermediate malignancy, characterized by partly invasive growth, and is more prone to generating lymph node metastasis (LNM) and distant metastasis (DM) compared with differentiated thyroid cancer, with ~10-15% of MTC patients having DM when first diagnosed. Unfortunately, the number of available studies on the predictors of metastasis in patients with MTC is limited.

Sex, age, tumor size, calcitonin (Ctn) and carcinoembryonic antigen (CEA) concentrations, among other factors, have been found to be associated with MTC (2-4). Ctn is a 32-amino acid residue peptide, encoded by a cognate gene located on the short arm of chromosome 11 in the C-cell. Ctn serves as a very sensitive and specific tumor marker for the diagnosis of MTC (5).

Neutrophils are the most abundant leukocyte population in the circulation and are the first cells recruited to the site of infection or inflammation (6). Neutrophils are common infiltrating cells in acute inflammation and a component of chronic inflammatory infiltrates. Research suggests that an inflammatory environment contributes to tumor angiogenesis, mutations, cell migration and metastatic progression through this particular environment that promotes the growth of malignant cells (7-9).

Numerous studies have reported that neutrophils play a critical role in tumor progression through the release of cytokines and angiogenic factors (10). Lymphocytes, which are key factors involved in the immune surveillance of tumor cells (11), kill cancer cells to suppress the progression of cancer. Furthermore, the development of several malignant tumors is closely correlated with an imbalance in the size of the populations of functional lymphocytes. A growing body of evidence highlights the role of lymphocytes as a central factor associated with the prognosis of patients with locally advanced cancer (12).

The systemic inflammatory response, a subject of intensive research, suppresses the activity of host immune cells through promoting microvascular regeneration, which facilitates the proliferation and differentiation of tumor cells, thus enhancing the invasion of tissues by tumor cells (13-15). The neutrophil-to-lymphocyte ratio (NLR) serves as an accurate and reliable index of systemic inflammation and, thus, serves as a systemic marker of inflammation (16). Furthermore, strong evidence indicates that a higher NLR is associated with the growth and migration of malignant cells.

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Numerous studies suggest that the initiation and progression of thyroid cancer is closely associated with inflammation (17). Moreover, neutrophils indicate the presence of inflammation, and lymphocytes reflect the function of the immune system. The NLR is a relevant factor of several malignancies, such as urothelial carcinoma (18), lung cancer (19), gastric cancer (20) and colorectal cancer (21). Unfortunately, the number of studies assessing the clinical factors that may help predict those MTC patients who have metastases is limited. The aim of the present study was to investigate the clinical data of 61 patients with MTC to identify predictors of metastasis.

Patients and methods

Patients. A retrospective analysis of 61 patients newly diagnosed with MTC at the General Hospital of the Chinese People's Liberation Army (PLAGH) between January 2001 and January 2016 was performed. The patients were diagnosed according to the findings of the histopathological examination. The inclusion criteria were as follows: i) MTC was confirmed using thyroid tissue obtained by ultrasound-guided aspiration biopsy or surgery; ii) the patients were not administered chemotherapy, radiotherapy or hormone therapy; iii) routine blood tests were conducted within 3 days prior to surgery or within 1 week prior to biopsy; iv) serum Ctn and CEA assays were conducted within 3 days prior to surgery or within 1 week after diagnosis by biopsy; and v) the patients underwent preoperative ultrasonography of the thyroid gland and lymph nodes, as well as fluorodeoxyglucose (FDG)-positron emission tomography PET/computed tomography (CT). Patients with suspicious foci detected using FDG-PET/CT underwent CT or magnetic resonance imaging (MRI) of the neck, chest and abdomen, as well as emission-CT (ECT) of bone. The exclusion criteria were as follows: i) Infectious disease, ii) other malignancies and iii) inflammatory conditions within 1 month prior to the surgery or biopsy. The final study population comprised 61 patients (27 men and 34 women, aged 25-73 years), 59 of whom had sporadic and 2 hereditary MTC. All the patients in the present study signed written informed consent forms prior to admission, and they acknowledged that their clinical data would be used for clinical studies. The study protocol was granted ethical approval by the Ethics Committee of the General Hospital of the Chinese People's Liberation Army (Beijing, China).

Ultrasound-guided fine-needle aspiration. The fine-needle aspiration procedure was performed using a standard 21-gauge needle. Sampling typically targeted the solid component of the lesion. If there was more than one nodule, a sample was taken from the nodule with suspicious or atypical ultrasound characteristics.

Surgery. When MTC is clinically apparent (thyroid nodule and a positive fine-needle aspiration), measurements of serum Ctn, CEA and calcium concentrations, as well as ultrasound imaging of the neck should be performed. The surgeon should examine the results of thoracic and superior mediastinal CT/MRI scans if enlarged lymph nodes are identified, or if the preoperative serum Ctn concentration is >400 pg/ml.

The surgical approaches used at our hospital to treat patients with MTC were as follows: i) Total thyroidectomy (TT) with central lymph node dissection for unilateral intrathyroidal tumors sized ≤1 cm; preoperative serum Ctn ≤400 pg/ml; and absence of suspected central neck or lateral cervical lymph nodes metastases on ultrasound. ii) TT with dissection of the central lymph node compartment (level VI) and dissection of the involved lateral neck compartments (levels II-V) when any of these features are present: Tumor >1 cm, suspected lateral cervical lymph node metastases on ultrasound, and preoperative serum Ctn concentrations >400 pg/ml. iii) TT for patients with confined bilateral nodularity, dissection of the central lymph node compartment (level VI), and dissection of the bilateral lateral neck compartments (levels II-V). Patients' sex, age at diagnosis, histological characteristics of the tumors and neck lymph nodes (such as tumor size, focality and extrathyroidal extension) were recorded. All thyroid and lymph node specimens were evaluated by pathologists at the PLAGH.

Blood tests. Routine blood tests were performed within 3 days prior to surgery or within 1 week prior to biopsy. Samples were analyzed using a Sysmex XE-2100 hematology automated analyzer (Sysmex, Kobe, Japan) with the supplied reagents, red blood cell controls and calibrators (Sysmex, Kobe, Japan). The concentrations of serum Ctn and CEA were determined within 3 days prior to surgery or within 1 week after diagnostic biopsy. The analyzer employs a chemiluminescent immunoassay to measure the concentrations of Ctn and CEA. Ctn assessment was performed using an IMMUNITE1000 chemiluminescence analyzer (Siemens AG, Munich, Germany). The detection limits ranged from 2 to 2,000 pg/ml. The optimal Ctn cut-off value was 8.4 and 5.0 pg/ml for men and women, respectively. CEA measurement was performed using an ARCHITECT i4000 SR chemiluminescence analyzer (Abbott, Chicago, IL, USA). The detection limits of this method range from 0.5 to 1,500 ng/ml. The optimal cut-off value of CEA was 5.0 ng/ml.

Statistical analysis. Statistical analyses were performed using SPSS 21.0 software (IBM, Armonk, NY, USA). The Kolmogorov-Smirnov test was used to ensure that the data were normally distributed, and such data are presented as mean ± standard deviation, which were subjected to an independent samples t-test to evaluate the significance of differences between groups. Data not normally distributed are presented as median and quartiles, and they were evaluated using the Mann-Whitney test. Data are presented as frequency and rate. Chi-squared tests were used to analyze categorical variables. Receiver operating characteristic (ROC) curves and the area under the ROC curve (AUC) were used to determine the optimal cut-off values for the NLR to predict LNM and DM in MTC. Then, multivariate logistic regression analyses were used to identify factors independently associated with metastasis. P-values <0.05 were considered to indicate statistically significant differences.

Results

Identification of independent predictors of metastasis. Of the 61 patients with MTC, 41 were confirmed to have metastases (group M), and 20 were confirmed to be metastasis-free (group

Table I. Single-factor analysis results of lymphatic metastasis and/or distant metastasis of MTC.

Variables	N=61 (%)	Group M, n=41 (%)	Group NM, n=20 (%)	P-value
Sex (%)				0.034
Male	27 (100)	22 (81.5)	5 (18.5)	
Female	34 (100)	19 (55.9)	15 (44.1)	
Age (years)				0.689
<45	28 (100)	20 (71.4)	8 (28.6)	
≥45	33 (100)	22 (66.7)	11 (33.3)	
Maximum nodal axis (cm)				
≤2	32 (100)	18 (56.2)	14 (43.8)	
2-4	21 (100)	15 (71.4)	6 (28.6)	0.055
>4	8 (100)	8 (100)	0 (0)	0.034
Focality				0.098
Solitary	40 (100)	24 (60)	16 (40)	
Multifocal	21 (100)	17 (81)	4 (19)	
Ctn (pg/ml)				
<20	3 (100)	1 (33.3)	2 (66.7)	
20-50	5 (100)	1 (20)	4 (80)	0.2
50-200	7 (100)	2 (28.6)	5 (71.4)	0.006
200-500	4 (100)	2 (50)	2 (50)	0.000
>500	42 (100)	35 (83.3)	7 (16.7)	0.000
CEA (μg/l), median (range)	34.3 (0.6-731.4)	46.9 (4.2-731.4)	16.6 (0.66-136.3)	0.007
NLR, Median (range)	1.8 (0.69-5.53)	1.92 (0.76-5.53)	1.69 (0.69-2.76)	0.019

Significance was set at P<0.05. Group M, MTC with metastasis; group NM, MTC without metastasis; Ctn, calcitonin; CEA, carcinoembryonic antigen; NLR, neutrophil-to-lymphocyte ratio; MTC, medullary thyroid cancer.

Table II. Multivariate logistic analysis.

Variables	B	SE	Wald χ^2	OR	P-value	95% CI
Ctn (>500 pg/ml)	3.064	1.074	8.145	21.422	0.004	2.611-175.731
NLR	1.778	0.837	4.509	5.918	0.034	1.147-30.541

Significance was set at P<0.05. Ctn, calcitonin; NLR, neutrophil-to-lymphocyte ratio; OR, odds ratio; CI, confidence interval.

NM). Single-factor analysis revealed statistically significant differences between groups regarding sex, tumor size, Ctn and CEA concentrations and NLR (P<0.05). There was no significant difference between the groups according to age and solitary focal/multifocal thyroid carcinoma (P>0.05) (Table I). Multivariate logistic regression analysis revealed that Ctn concentrations >500 pg/ml and the NLR were independent predictors of metastasis (Table II).

ROC curve analysis and cut-off value of the NLR. ROC curve analysis indicated that the optimum NLR cut-off for lymphatic metastasis and/or distant metastasis was 1.784 (the sensitivity and specificity were 68.3 and 80%, respectively). The AUC value of the NLR was 0.717 (95% confidence interval: 0.583-0.851) (Fig. 1).

Discussion

To the best of our knowledge, metastatic MTC is incurable; therefore, the clinician must select patients that are likely to benefit from therapy, balancing the often slow rate of tumor progression associated with good quality of life against the limited efficacy and potential toxicities of local and systemic therapies (22). There are no known predictive factors for LNM and DM of MTC. Ctn produced by thyroid C-cells is the most sensitive diagnostic marker of MTC. The NLR is a simple index of a systemic inflammatory response, and an elevated NLR may reflect an imbalanced inflammatory state that facilitates tumor growth (23).

In the present study, among the 41 patients with metastasis, 40 (97.56%, 40/41) had Ctn concentrations >500 pg/ml or an

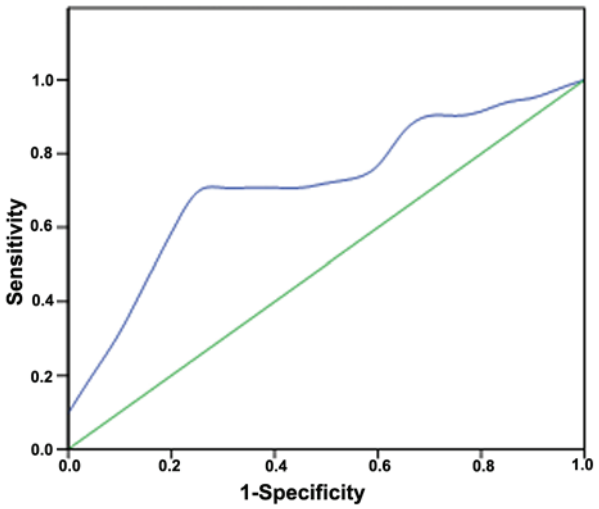

Figure 1. The ROC curve for NLR level of lymphatic metastasis or and/or distant metastasis. The AUC value for NLR is 0.717 (P<0.05). NLR, neutrophil-to-lymphocyte ratio; ROC, receiver operating characteristic; AUC, area under the ROC curve.

NLR >1.784. Therefore, Ctn concentration and NLR may help predict metastasis. These findings provide compelling evidence that the serum Ctn concentration and the NLR may be used as a primary screen of patients with MTC to detect LNM and DM. Routine implementation of these measurements may facilitate earlier detection of LNM and DM, allowing earlier medical intervention.

MTC is an aggressive tumor, prone to hematogenous and lymphatic metastasis (24). The prognosis of patients with MTC differs between those with differentiated thyroid carcinoma or anaplastic thyroid carcinoma. The tumor metastasizes early to paratracheal and lateral cervical lymph nodes, and distant metastases may develop in the liver, lungs and bones. Given its propensity to spread to lymph nodes and distant organs, MTC is often difficult to cure if not detected early. When lymph node involvement or distant metastases are present at diagnosis, the prognosis is usually poor, even when the primary tumor is quite small (25-27). Therefore, the treatment of such patients differs from that of patients without metastasis, and accurate assessment of a patient's metastatic status is crucial for prescribing optimal individualized treatment that will increase the survival rate.

Ctn, which is produced by thyroid C-cells, is the most sensitive marker available for the diagnosis of MTC. Moreover, the concentration of serum Ctn represents an accurate and sensitive marker for preoperative diagnosis and post-surgical follow-up. For example, Ctn concentrations >100 pg/ml have a positive predictive value (PPV) of 100% for MTC (27). The PPV associated with Ctn concentrations ≥50 and <100 pg/ml vs. Ctn concentrations ≥20 and <50 pg/ml is 25 and 8.3%, respectively. The preoperative basal serum Ctn concentration helps assess the extent of lymph node metastasis (22). By contrast, CEA is not a specific biomarker of MTC, although serum CEA concentrations are useful for evaluating disease progression in patients with clinically evident MTC. Furthermore, the preoperative serum CEA concentration is correlated with the number of metastatic lymph nodes, and combining the Ctn and CEA data increases the rate of successful diagnosis.

Kazaure *et al* (1) advocate that the size of the primary tumor and extrathyroid extension is independently associated with the prognosis of MTC, whereas Dequanter and Lothaire (3) consider age to be an important prognostic factor. Furthermore, the basal mortality rate of MTC reveals that survival is independent of age (28). Inflammation is implicated in the initiation and progression of thyroid cancer. For example, the molecular inflammatory process plays a central role in the malignant progression of transformed thyroid cells (29,30). The NLR is a simple index of the systemic inflammatory response and serves as a prognostic indicator in certain cancers. Liu *et al* (23) suggested that an elevated NLR may serve as a marker of an imbalanced inflammatory state that facilitates tumor growth. Moreover, the NLR serves an independent prognostic predictor of patients with cancer (31-33).

The present study retrospectively analyzed 61 patients with newly diagnosed MTC. Univariate logistic regression analysis revealed that sex, tumor size, preoperative peripheral blood NLR and serum concentrations of CEA and Ctn were associated with LNM and DM. Multivariate analysis revealed that Ctn concentrations >500 pg/ml and NLR are independent predictors of metastasis. The Ctn concentrations in men were markedly higher compared with those in women, which may be explained by the abundance of thyroid C-cells in men (34). Furthermore, Ctn concentrations are inversely correlated with those of CEA and with tumor size (35,36).

The optimal cut-off value of the NLR for predicting metastasis obtained from ROC analysis was 1.784 (sensitivity 68.3% and specificity 80%, AUC 0.717). Furthermore, patients with an NLR >1.784 had a significantly higher rate of LNM and DM compared with patients with an NLR ≤1.784. Among the 41 patients with metastasis, 40 (97.56%) had Ctn concentrations >500 pg/ml or an NLR >1.784. Previous studies demonstrated that Ctn concentrations are associated with tumor load (37). Of the 42 patients with Ctn concentrations >500 pg/ml in the present study, 35 had LNM or/and DM. By contrast, other studies reported that high Ctn concentrations are consistent with LNM and higher TNM stage (38). In the present study, among the 32 patients with NLR >1.784, 28 had LNM or/and DM, supporting the conclusion that a high NLR is associated with metastasis.

There is a strong association between inflammation and MTC. For example, activated neutrophils may directly and indirectly stimulate tumor growth. Lymphocytes participate in the immune process, construct immune barriers, and kill tumor cells. An elevated NLR may indicate an excessive but ineffective immune response to the tumor load, or serve as a marker of an imbalanced inflammatory state, which facilitates tumor growth.

Whole-body imaging of all patients with MTC is costly and labor-intensive. Serum Ctn assays and NLR determination, which are cost-effective and universally available, may be used as a primary screen for MTC patients to detect LNM and DM. Furthermore, high-risk patients should undergo imaging examinations to facilitate early detection of metastasis, earlier diagnosis, and earlier medical intervention.

There were certain limitations to this study. First, this was a retrospective single-center study. Second, the

limited sample size of MTC patients did not permit us to perform more intensive analyses. Finally, only the NLR in the prediction of LNM and DM in MTC patients was analyzed, whereas no information was reported on other immunological parameters. A multicenter community-based prospective study, a study with a higher number of cases, and a more sophisticated study including eosinophils, basophils or IgG levels and other immunological parameters, are required in the future in order to investigate a more useful and precise method for predicting LNM and MD in patients with MTC.

In summary, the findings of the present study demonstrated that the serum Ctn concentration and the NLR helped predict the presence of LNM and DM in patients with MTC. Therefore, physicians should pay more attention to patients with Ctn concentrations >500 pg/ml or an NLR >1.784, and then perform imaging examinations to detect metastases as early as possible. These interventions are critically important for efforts to implement individualized treatment that improves the quality of life and prolongs survival.

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Availability of data and materials

All the data collected and analyzed in the study are available from the corresponding author on reasonable request.

Authors' contributions

All the authors have read and approved the final version of this manuscript. WT and NX designed the present study. NX, YJ and YW performed the experiments. All authors participated in the writing of the manuscript.

Ethics approval and consent to participate

The study protocol was granted ethical approval by the Ethics Committee of the General Hospital of the Chinese People's Liberation Army (Beijing, China). All the patients in the present study signed written informed consent forms prior to admission, and they acknowledged that their clinical data would been used for clinical studies.

Patient consent to publication

Not applicable.

Competing interests

The authors declare that they have no competing interests to disclose.

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