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Original Article

Clinical value of serum osteocalcin (N-MID) combined with thyroid transcription factor-1 for predicting bone metastasis of lung cancer

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Abstract: This study aimed to evaluate the clinical predictive value of combining N-terminal serum osteocalcin (N-MID) with thyroid transcription factor-1 (TTF-1) for bone metastasis in lung cancer. This retrospective analysis included 147 lung cancer patients admitted to the hospital between February 2020 and October 2024. Patients were divided into a bone metastasis group (n=83) and a non-bone metastasis group (n=64) based on the presence of bone metastases. Baseline clinical data and laboratory parameters, including TTF-1, N-MID, tumor abnormal protein (TAP), parathyroid hormone (PTH), vascular endothelial growth factor (VEGF), total N-terminal propeptide of type I collagen (tPINP), and β-C-terminal telopeptide of type I collagen (β-CTX) were collected. Univariate and multivariate logistic regression analyses were performed to identify independent risk factors for bone metastasis. Model performance was evaluated using receiver operating characteristic (ROC) curves, integrated discrimination improvement (IDI), category-based net reclassification improvement (cNRI), the Hosmer-Lemeshow goodness-of-fit test, and decision curve analysis (DCA). Age-stratified analysis and external validation were further conducted to ensure the generalizability of study. Univariate analysis showed that TTF-1 positivity rate and the levels of TAP, PTH, VEGF, tPINP, β-CTX, and N-MID were significantly higher in the bone metastasis group than in the non-bone metastasis group (all $P<0.05$). Multivariate logistic regression confirmed that TTF-1 (OR=2.217, 95% CI: 1.329-3.698, P<0.001) and N-MID (OR=2.519, 95% CI: 1.404-4.518, $P<0.001$) were independent risk factors for bone metastasis in lung cancer. After adding TTF-1 and N-MID to a baseline model that included PTH, VEGF, tPINP, and β-CTX, the area under the curve (AUC) increased from 0.752 to 0.837, with an IDI of 0.079 and a cNRI of 0.609 (both P<0.001). The Hosmer-Lemeshow test indicated good model fit, and external validation demonstrated good concordance between predicted probabilities and observed outcomes. Age-stratified analysis revealed that in elderly populations (70-79 years and 80-89 years), the combined detection of TTF-1 and N-MID yielded significantly higher AUC than either marker alone. Decision curve analysis confirmed that the combined detection provided a higher net benefit within clinically reasonable threshold ranges. In conclusion, TTF-1 and N-MID are independent risk factors for bone metastasis in lung cancer, and their combination significantly enhances the diagnostic performance of the predictive model, with particularly notable clinical value in elderly patients. These findings provide evidence-based references for early identification of bone metastasis and individualized clinical intervention in lung cancer.

Keywords: Lung cancer, bone metastasis of lung cancer, N-MID, thyroid transcription factor-1

Introduction

Lung cancer is a complex disease composing various histological and molecular types [1]. According to epidemiological data in China, the incidence and mortality rates of lung cancer rank first among all malignant tumors, with an annual incidence rate of approximately 53.6 per 100,000 population and a mortality rate of 45.6 per 100,000 per year [2]. Bone metastasis in lung cancer involves a complex regulatory network with multiple pathways. Within the bone microenvironment, the receptor activator for nuclear factor-κB ligand (RANKL)/receptor activator of nuclear factor-κB (RANK) signaling axis represents a critical pathway in lung cancer bone metastasis [3, 4]. Bone metastasis is a major cause of pain in cancer patients and

increases the risk of skeletal-related events (SREs) [5]. In addition to impairing patients' quality of life, it also increases medical costs and mortality risk. Domestic epidemiological studies have shown that the incidence of bone metastasis in lung cancer patients is approximately 15%-30%, and the median survival after bone metastasis is generally 8-12 months [6]. Therefore, early identification of bone metastasis in lung cancer patients is of great clinical significance for guiding individualized treatment strategies, improving prognosis, and prolonging survival.

Currently, the diagnosis of bone metastasis in lung cancer mainly relies on radionuclide bone scan (emission computed tomography, ECT) or positron emission tomography-computed tomography (PET-CT), combined with X-ray, CT, and magnetic resonance imaging (MRI) [7, 8]. However, these examinations involve radiation exposure and are costly, making them unsuitable for short-term dynamic monitoring. In recent years, serum markers for bone metastasis in lung cancer have attracted attention. Peripheral blood sampling is minimally invasive, convenient, cost-effective, and suitable for dynamic monitoring. Bone metabolism markers in blood, such as serum osteocalcin (N-MID), are closely related to the occurrence, progression, metastasis, and prognosis of bone metastatic tumors [9, 10]. Thyroid transcription factor-1 (TTF-1) is a protein marker widely used in medicine to assist in tumor typing, mostly for diagnosing lung cancer and thyroid cancer. Existing studies have found that TTF-1 expression is more common in lung adenocarcinoma patients with bone metastasis harboring epidermal growth factor receptor (EGFR) mutations [11]. Currently, there are few studies on the relationship between these molecular markers and bone metastasis of lung cancer. Therefore, this study aimed to investigate the relationship between N-MID and TTF-1 levels and bone metastasis in lung cancer patients, to clarify the potential value of N-MID and TTF-1 in early risk prediction of bone metastasis, and to provide references for early clinical identification and intervention of bone metastasis in lung cancer.

Materials and methods

Study subjects

This retrospective cohort study included 147 lung cancer patients treated in Tongji University Affiliated Tianyou Hospital from February 2020 to October 2024. They were divided into a bone metastasis group (BM group, 83 cases) and a non-bone metastasis group (non-BM group, 64 cases). In the BM group, there were 59 males and 24 females, with a mean age of 75.34±7.51 years; in the non-BM group, there were 40 males and 24 females, with a mean age of 74.76±8.23 years. Another 68 lung cancer patients admitted from November 2024 to March 2026 were selected as an external validation set. Inclusion criteria for lung cancer patients with bone metastasis: (1) complete clinical data without missing key information; (2) confirmed diagnosis of lung cancer by cytological or histopathological examination [12]; (3) confirmed presence of bone metastatic lesions by ECT and CT; (4) no history of diseases affecting bone metabolism; (5) no use of medications that might interfere with bone metabolism within 3-6 months before enrollment. Inclusion criteria for non-bone metastasis patients: (1) complete clinical data; (2) confirmed diagnosis of lung cancer by cytological or histopathological examination; (3) no evidence of bone metastasis by ECT and CT. Exclusion criteria: (1) history of diseases affecting bone metabolism (e.g., hyperparathyroidism, chronic metabolic bone disease, severe osteoporosis); (2) use of medications that might interfere with bone metabolism within 3 months before enrollment (e.g., bisphosphonates, denosumab, glucocorticoids, sex hormones); (3) presence of other primary malignancies; (4) severe liver or kidney dysfunction; (5) incomplete clinical data with missing key information. All patients underwent whole-body bone ECT for screening. For abnormal lesions detected by ECT, CT and/or MRI were further performed to confirm the presence of bone destruction. The final diagnosis of bone metastasis was made independently by two radiologists with more than 10 years of experience, based on ECT, CT, and MRI findings, and in cases of disagreement, a consensus was reached through consultation with an experienced radiologist.

Data collection

Baseline data collection: Baseline data including age, sex, body temperature, respiratory rate, smoking history, alcohol consumption history, chronic disease history, presence of hyper-

tension, diabetes, coronary heart disease, and cerebrovascular disease, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), histological type (adenocarcinoma, squamous cell carcinoma, small cell lung cancer, others), and clinical stage (stage I-II, III, IV) were collected.

Measurement of laboratory parameters: All patients provided fasting venous blood samples in the morning (fasting ≥8 h). After serum separation, the samples were uniformly stored at -80°C. Relevant blood parameters were measured using an automatic biochemical analyzer and a hematology analyzer. Thyroid transcription factor-1 (TTF-1) was detected by immunohistochemistry using lung cancer tissue specimens. The clinical significance, normal reference ranges, and abnormal value interpretations for each indicator are as follows:

Serum osteocalcin (N-MID): A classic bone formation marker reflecting osteoblast activity. Normal reference range: 11-43 ng/mL for premenopausal women, with slightly higher values in postmenopausal women and men. Elevated levels indicate compensatory osteoblast activity and are commonly seen in tumor bone metastasis and osteoporosis.

Parathyroid hormone (PTH): A core hormone regulating calcium-phosphorus metabolism and bone remodeling. Normal reference range: 15-68 pg/mL. Elevated levels suggest calcium metabolism disorders, activate osteoclasts, accelerate bone destruction, and serve as a concomitant indicator of tumor bone metastasis.

Total procollagen type I N-terminal propeptide (tPINP): A specific bone formation marker. Normal reference range: 13.72-65.49 ng/mL. Increased levels indicate enhanced bone synthesis, which may be compensatory in tumor bone metastasis.

β-C-terminal telopeptide of type I collagen (β-CTX): A specific bone resorption marker. Normal reference range: 0.10-0.61 ng/mL. Elevated β-CTX reflects excessive osteoclast activation and aggravated bone destruction, making it a sensitive indicator for tumor bone metastasis.

Tumor abnormal protein (TAP): A tumor-associated marker. Normal reference range: <120 μm^2. Elevated levels suggest increased tumor burden and active tumor proliferation.

Vascular endothelial growth factor (VEGF): Regulates tumor angiogenesis. Normal reference range: <200 pg/mL. Elevated levels indicate active tumor angiogenesis and increased metastatic risk.

Routine blood parameters (absolute lymphocyte count [LYM], platelet [PLT], platelet-to-lymphocyte ratio [PLR], hemoglobin [HB]): These parameters reflect immune and hematopoietic status, indirectly assessing the overall condition of cancer patients.

TTF-1 (Thyroid transcription factor-1): Detected by immunohistochemical SP method on lung cancer tissue sections. Interpretation criteria: Positive if ≥10% of tumor cell nuclei show staining; negative if <10%. Clinical interpretation: TTF-1 is a specific pathological marker for lung adenocarcinoma and small cell lung cancer. Positivity suggests pulmonary origin of the tumor, and its expression status is associated with tumor invasion and metastatic potential. Variable type: This is a binary qualitative variable.

For variable grouping and assignment rules: For serum continuous variables including N-MID, PTH, VEGF, tPINP, and β-CTX, the median value of the test results from all study participants was used as the cut-off to divide patients into high-expression and low-expression groups. TTF-1, a binary qualitative immunohistochemical indicator, was classified as negative or positive and assigned a value of 0 (negative) or 1 (positive) for statistical analysis. All measurements were performed strictly in accordance with the standard operating procedures for automated biochemical analyzers and pathological testing equipment, as well as the instructions provided with the corresponding reagent kits.

All case data were obtained from the inpatient electronic medical records and imaging system of our hospital. Only anonymized and de-identified clinical data were used for retrospective analysis, and no additional interventions, blood sampling, or invasive procedures were performed on patients. All procedures followed the principles of the Declaration of Helsinki. The

study protocol was reviewed and approved by the Medical Ethics Committee of Tongji University Affiliated Tianyou Hospital (LIsc2024-1102, LIsc20260402). Given the retrospective design and the fact that all data were de-identified for privacy, the ethics committee waived the requirement for informed patient consent.

Statistical methods

SPSS version 22.0 software was used for data processing and analysis. Categorical data were described as numbers and percentages (n, %), and comparisons between groups were performed using the χ^2 test. Normally distributed continuous data were expressed as mean ± standard deviation (SD), and comparisons between two groups were performed using the independent-samples t-test. Variables with statistical significance in univariate analysis were included in multivariate binary logistic regression. TTF-1 was treated as a binary immunohistochemical variable (negative =0, positive =1). Serum continuous indicators including N-MID, PTH, VEGF, tPINP, and β-CTX were dichotomized using the median as the cutoff (≤ median =0, > median =1). Histological type and clinical stage were categorical variables with more than two categories; dummy variables were created for each variable and entered into the model. For histological type, non-adenocarcinoma was used as the reference; for clinical stage, stages I-II were used as the reference. Receiver operating characteristic (ROC) curves were used to evaluate the diagnostic performance of each indicator and the combined model, with calculation of the area under the curve (AUC), sensitivity, and specificity. The bootstrap method (with 1,000 resampling iterations) was used to calculate the integrated discrimination improvement (IDI) and categorical net reclassification improvement (cNRI) to quantify the incremental value of the model. The Hosmer-Lemeshow goodness-of-fit test was used to assess the calibration of the predictive model, with the degrees of freedom set to 8 by default; a test *P*-value >0.05 indicated good model calibration. Decision curve analysis (DCA) was performed to calculate the clinical net benefit at different threshold probabilities, and the bootstrap method was used to construct 95% confidence intervals, with results for key threshold points presented in tabular form. All statistical tests were two-sided, and a *P*-value <0.05 was considered statistically significant.

Results

Comparison of baseline data between the two groups

Among the 147 lung cancer patients, there were 83 cases (56.46%) in the BM group and 64 cases (43.54%) in the non-BM group. Comparison of baseline clinical data between the two groups showed that, in terms of demographic characteristics and general clinical indicators, there were no significant differences in sex, age, body temperature, heart rate, respiratory rate, smoking history, alcohol consumption history, chronic disease history (hypertension, diabetes, coronary heart disease, cerebrovascular disease), SBP, DBP, or MAP between the two groups (all $P>0.05$), indicating that the baseline data of the two groups were comparable.

Regarding histological type distribution, the difference between the two groups was statistically significant ($\chi^2=48.215$, $P<0.001$). In the BM group, adenocarcinoma was predominant, accounting for 64 cases (77.11%), significantly higher than 21 cases (32.81%) in the non-BM group; squamous cell carcinoma accounted for a higher proportion in the non-BM group (28 cases, 43.75%) compared to the BM group (13 cases, 15.66%); small cell lung cancer and other types accounted for a low proportion in both groups.

Regarding clinical stage distribution, the difference between the two groups was also statistically significant ($\chi^2=87.364$, $P<0.001$). In the BM group, stage IV patients accounted for the highest proportion, 57 cases (68.67%), significantly higher than 10 cases (15.63%) in the non-BM group; stage I-II patients accounted for a higher proportion in the non-BM group (31 cases, 48.44%) compared to the BM group (4 cases, 4.82%). These results suggest that histological type (adenocarcinoma) and clinical stage (stage IV) are closely associated with the occurrence of bone metastasis in lung cancer. Detailed data are presented in **Table 1**.

Comparison of laboratory parameters between the two groups

Comparison of laboratory parameters between the BM and non-BM groups showed that the BM group had significantly higher TTF-1 positivity rate and levels of TAP, PTH, N-MID, VEGF, tPINP, and β-CTX, with statistically significant differences ($P<0.05$; **Table 2**).

Table 1. Comparison of baseline data between the two groups of lung cancer patients [n (%), $\bar{x}\pm s$]

Variables	BM group (n=83)	Non-BM group (n=64)	χ^2/t	*P*
Sex (male/female)	59/24	40/24	1.211	0.271
Age (years)	75.34±7.51	74.76±8.23	0.483	0.630
Body temperature (°C)	36.93±0.85	37.02±0.79	0.656	0.513
Heart rate (beats/min)	95.30±21.25	97.58±25.87	0.586	0.558
Respiratory rate (breaths/min)	23.51±6.76	22.12±4.89	1.388	0.167
Smoking history [n (%)]	45 (54.22)	38 (59.38)	0.391	0.532
Alcohol consumption history [n (%)]	37 (44.58)	25 (37.50)	0.451	0.502
Chronic disease history [n (%)]	10 (12.05)	13 (20.31)	1.870	0.171
Hypertension [n (%)]	13 (15.66)	18 (28.12)	3.373	0.066
Diabetes [n (%)]	14 (16.87)	11 (17.18)	0.003	0.959
Coronary heart disease [n (%)]	20 (24.10)	15 (23.44)	0.009	0.926
Cerebrovascular disease [n (%)]	16 (19.28)	13 (20.31)	0.024	0.876
Systolic blood pressure (mmHg)	126.23±21.37	128.75±25.42	0.652	0.515
Diastolic blood pressure (mmHg)	70.72±15.19	71.28±15.54	0.219	0.827
Mean arterial pressure (mmHg)	91.87±17.42	92.36±18.27	0.166	0.869
Histological type			48.215	<0.001
Adenocarcinoma	64 (77.11)	21 (32.81)		
Squamous cell carcinoma	13 (15.66)	28 (43.75)		
Small cell lung cancer	5 (6.02)	10 (15.63)		
Others	1 (1.20)	5 (7.81)		
Clinical stage			87.364	<0.001
Stage I-II	4 (4.82)	31 (48.44)		
Stage III	22 (26.51)	23 (35.94)		
Stage IV	57 (68.67)	10 (15.63)		

Multivariate logistic regression analysis of TTF-1, N-MID and risk of bone metastasis

To investigate the effects of bone metabolism and tumor-associated markers on TTF-1 and N-MID expression, each of these two indicators was use as a binary dependent variable for multivariate logistic regression analysis. N-MID was divided into high-expression group (assigned =1) and low-expression group (assigned =0) based on the median value. TTF-1 was classified as positive (assigned =1) or negative (assigned =0) according to immunohistochemical results. TAP, PTH, VEGF, tPINP, and β-CTX were included as independent variables, and separate multivariate logistic regression models were constructed to analyze the independent associations of these indicators with the expression status of TTF-1 and N-MID.

Multivariate logistic regression analysis showed that in the model with TTF-1 as the dependent variable (**Table 3**), PTH (OR=2.321, 95% CI: 1.297-4.156, *P*=0.005), tPINP (OR=2.044, 95% CI: 1.174-3.588, *P*=0.011), and β-CTX (OR=2.524, 95% CI: 1.376-4.632, *P*=0.003) were independent risk factors for high TTF-1 expression; In contrast, TAP and VEGF showed no significant association with TTF-1 expression (both *P*>0.05). Similar results were observed in the model with N-MID as the dependent variable (**Table 4**): PTH (OR=2.140, 95% CI: 1.205-3.798, *P*=0.009), tPINP (OR=1.978, 95% CI: 1.149-3.406, *P*=0.013), and β-CTX (OR=2.408, 95% CI: 1.327-4.365, *P*=0.004) were also identified as independent risk factors for high N-MID expression; TAP and VEGF showed no independent association with N-MID expression (both *P*>0.05).

Multivariate logistic regression analysis of factors influencing bone metastasis in lung cancer patients

To comprehensively evaluate the independent effects of various factors on bone metastasis outcome, a multivariate logistic regression analysis was performed with "presence or absence of bone metastasis" as the dependent variable, including all variables that showed sig-

Table 2. Comparison of laboratory parameters between the two groups [*n* (%), $\bar{x}\pm s$]

Variables	BM group (n=83)	Non-BM group (n=64)	χ^2/t	*P*
LYM (×10^9/L)	1.37±0.33	1.35±0.28	0.389	0.698
PLT (×10^9/L)	207.48±40.73	201.14±45.29	0.891	0.374
PLR	165.32±30.03	158.79±37.26	1.177	0.241
TTF-1 positivity [n (%)]	53 (63.86)	5 (7.81)	47.512	<0.001
Hb (g/L)	132.33±17.24	130.67±18.36	0.563	0.575
TAP (μm^2)	126.35±26.18	111.84±19.75	3.696	<0.001
PTH (pg/mL)	102.46±32.73	85.62±28.46	3.271	0.001
N-MID (ng/mL)	19.16±3.42	15.73±3.85	5.707	<0.001
VEGF (pg/mL)	262.54±56.38	219.47±48.62	4.871	<0.001
tPINP (ng/mL)	78.62±23.85	66.43±17.92	3.412	0.001
β-CTX (ng/mL)	0.87±0.29	0.42±0.17	11.033	<0.001

Note: TTF-1 was assessed using the qualitative criteria (positive/negative) recommended by the kit. TTF-1: thyroid transcription factor-1. TTF-1 was a qualitative immunohistochemical indicator, and was assigned as negative =0 and positive =1 for statistical analysis. Serum biomarkers were dichotomized into high- and low-expression groups based on the overall median. LYM: lymphocyte count; PLT: platelet count; PLR: platelet-to-lymphocyte ratio; Hb: hemoglobin; TAP: tumor abnormal protein; PTH: parathyroid hormone; N-MID: N-terminal serum osteocalcin; VEGF: vascular endothelial growth factor; tPINP: total N-terminal propeptide of type I collagen; β-CTX: β-C-terminal telopeptide of type I collagen.

Table 3. Multivariate logistic regression analysis of TTF-1 and risk of bone metastasis

Variable	*β*	*SE*	*Wald* χ^2	*OR* (95% *CI*)	*P*
TAP (μm^2)	0.295	0.182	2.621	1.343 (0.938-1.922)	0.105
PTH (pg/mL)	0.842	0.298	7.985	2.321 (1.297-4.156)	0.005
VEGF (pg/mL)	0.236	0.219	1.162	1.266 (0.823-1.947)	0.281
tPINP (ng/mL)	0.715	0.283	6.403	2.044 (1.174-3.558)	0.011
β-CTX (ng/mL)	0.926	0.309	8.947	2.524 (1.376-4.632)	0.003

Table 4. Multivariate logistic regression analysis of N-MID and risk of bone metastasis

Variable	*β*	*SE*	*Wald* χ^2	*OR* (95% *CI*)	*P*
TAP (μm^2)	0.278	0.189	2.164	1.320 (0.912-1.909)	0.141
PTH (pg/mL)	0.761	0.292	6.802	2.140 (1.205-3.798)	0.009
VEGF (pg/mL)	0.192	0.215	0.797	1.212 (0.791-1.857)	0.372
tPINP (ng/mL)	0.682	0.275	6.153	1.978 (1.149-3.406)	0.013
β-CTX (ng/mL)	0.879	0.303	8.472	2.408 (1.327-4.365)	0.004

nificant differences between the two groups in **Tables 1** and **2** (including histological type, clinical stage, TAP, PTH, VEGF, tPINP, β-CTX. N-MID, TTF-1), to construct a prediction model.

Table 5 shows the regression results without the inclusion of TTF-1 and N-MID. The results showed that TAP was not independently associated with bone metastasis risk (OR=1.112, *P*=0.073). PTH (OR=1.672, *P*=0.022), VEGF (OR=1.530, *P*=0.038), tPINP (OR=2.070, *P*<0.001), β-CTX (OR=2.136, *P*<0.001), adenocarcinoma (OR=6.599, *P*<0.001), and stage IV (OR=12.654, *P*<0.001) were independent risk factors for bone metastasis in lung cancer patients.

Table 6 presents the results of the multivariate logistic regression analysis after further incorporation of TTF-1 and N-MID. The results showed that TAP was not independently associated with the risk of bone metastasis (*P*>0.05); VEGF was statistically significant in the baseline model (*P*=0.038) but became non-significant after the inclusion of TTF-1 and N-MID (*P*=0.131), suggesting that its predictive efficacy may be partially replaced by TTF-1 and N-MID. PTH (OR=1.923, *P*=0.017), tPINP (OR=1.880, *P*=0.003), and β-CTX (OR=2.384, *P*<0.001) remained independent risk factors. After adjustment, adenocarcinoma and stage IV still maintained independent associations, but their OR values decreased compared with those in **Table 5** (from 6.599 to 3.459 for adenocarcinoma, and from 12.654 to 6.482 for stage IV, both *P*<0.05). More importantly, after full adjustment for histological type and clinical stage, TTF-1 (OR=2.217, P<0.001) and N-MID (OR=2.519, P<0.001) were still confirmed as independent risk factors for bone metastasis in lung cancer, indicating that their predictive value is independent of tumor type and stage.

Contribution of TTF-1 and N-MID to the prediction model for bone metastasis

After adding TTF-1 and N-MID to the prediction model for bone metastasis (Model 1, which included PTH, VEGF, tPINP, and β-CTX), Model 2

Table 5. Multivariate logistic regression analysis of factors influencing bone metastasis in lung cancer patients (without TTF-1 and N-MID)

Variable	*β*	*SE*	*Wald* χ^2	*OR* (95% *CI*)	*P*
TAP (μm^2)	0.106	0.208	0.932	1.112 (0.735-1.681)	0.073
PTH (pg/mL)	0.514	0.235	4.751	1.672 (1.051-2.659)	0.022
VEGF (pg/mL)	0.425	0.211	4.065	1.530 (1.008-2.323)	0.038
tPINP (ng/mL)	0.728	0.325	5.018	2.070 (1.089-3.932)	<0.001
β-CTX (ng/mL)	0.759	0.352	4.655	2.136 (1.065-4.285)	<0.001
Adenocarcinoma (vs non-adenocarcinoma)	1.887	0.418	20.342	6.599 (2.914-14.945)	<0.001
Stage IV (vs Stage I-III)	2.538	0.485	27.415	12.654 (4.876-32.852)	<0.001

Table 6. Multivariate logistic regression analysis of factors influencing bone metastasis in lung cancer patients (with TTF-1 and N-MID)

Variable	*β*	*SE*	*Wald* χ^2	*OR* (95% *CI*)	*P*
TAP (μm^2)	0.228	0.227	1.012	1.256 (0.802-1.967)	0.057
PTH (pg/mL)	0.654	0.251	6.795	1.923 (1.173-3.154)	0.017
VEGF (pg/mL)	0.217	0.262	0.685	1.242 (0.740-2.085)	0.131
tPINP (ng/mL)	0.631	0.229	7.582	1.880 (1.196-2.953)	0.003
β-CTX (ng/mL)	0.869	0.308	7.956	2.384 (1.298-4.376)	0.001
Adenocarcinoma (vs non-adenocarcinoma)	1.241	0.396	9.824	3.459 (1.586-7.532)	0.002
Stage IV (vs Stage I-III)	1.869	0.450	17.236	6.482 (2.675-15.714)	<0.001
TTF-1	0.796	0.260	9.351	2.217 (1.329-3.698)	<0.001
N-MID	0.924	0.297	9.658	2.519 (1.404-4.518)	<0.001

Table 7. Comparison of efficacy of prediction models for bone metastasis in lung cancer patients

Parameter	AUC-ROC (95% *CI*)	[b]*P* value	IDI (95% *CI*)	[b]*P* value	cNRI (95% *CI*)	[b]*P*
Bone metastasis in lung cancer patients						
Model 1[a]	0.752 (0.683-0.821)					
Model 2[c]	0.837 (0.791-0.883)	0.045	0.079 (0.024-0.134)	<0.005	0.609 (0.472-0.746)	<0.001

Note: [a]Model 1 includes PTH, VEGF, tPINP, and β-CTX; [b]P value for comparison between Model 2 and Model 1; [c]Model 2 includes PTH, VEGF, tPINP, β-CTX, TTF-1, and N-MID.

Table 8. The Hosmer-Lemeshow test for prediction models of bone metastasis in lung cancer patients

Variable	χ^2	*df*	*P*
Model 1[a]	7.692	8	0.675
Model 2[b]	6.158	8	0.871

Note: [a]Model 1 includes PTH, VEGF, tPINP, and β-CTX; [b]Model 2 includes PTH, VEGF, tPINP, β-CTX, TTF-1, and N-MID.

was expanded to include PTH, VEGF, tPINP, β-CTX, TTF-1, and N-MID. The AUC of Model 1 for predicting bone metastasis was 0.752 (95% CI: 0.683-0.821). After the addition of TTF-1 and N-MID, the AUC significantly increased to 0.837 (95% CI: 0.791-0.883) (P< 0.001). To evaluate the incremental value of TTF-1 and N-MID, the IDI and cNRI were calculated. Compared with Model 1, the addition of TTF-1 and N-MID significantly improved the IDI (0.079, 95% CI: 0.024-0.134) and cNRI (0.609, 95% CI: 0.472-0.746), with statistically significant differences (P<0.001). See **Table 7**.

The Hosmer-Lemeshow goodness-of-fit test was used to assess the calibration of the two prediction models. For Model 1, the test result was χ^2=7.692, P=0.675, indicating good fit; for Model 2, χ^2=6.158, P=0.871, indicating ideal fit (**Table 8**).

Comparison of diagnostic performance of TTF-1 and N-MID for bone metastasis across different age groups

Age-stratified analysis (according to geriatric age classification criteria: 60-69 years, 70-79 years, and 80-89 years) revealed the following

Table 9. Comparison of diagnostic ability of TTF-1 and N-MID for bone metastasis in different age groups

Age group	Indicators	Sensitivity	Specificity	Accuracy	AUC
60-69 years	TTF-1	0.68	0.86	0.85	0.72
	N-MID	0.65	0.84	0.82	0.73
	TTF-1+N-MID	0.64	0.72	0.80	0.75
70-79 years	TTF-1	0.51	0.83	0.81	0.62
	N-MID	0.57	0.77	0.84	0.69
	TTF-1+N-MID	0.85	0.81	0.83	0.85
80-89 years	TTF-1	0.54	0.86	0.79	0.62
	N-MID	0.67	0.85	0.83	0.68
	TTF-1+N-MID	0.78	0.82	0.81	0.81

findings: in the 60-69 years cohort, the sensitivity of TTF-1 or N-MID alone was higher than that of combined detection; conversely, in the 70-79 and 80-89 years cohorts, the sensitivity of combined detection was significantly higher than that of either single indicator. Regarding specificity, in all age groups, the specificity of TTF-1 or N-MID alone was better than that of combined detection. In terms of diagnostic efficacy, in the 60-69 years group, the AUC of combined detection was not significantly different from that of TTF-1 or N-MID alone ($P>0.05$); however, in the 70-79 and 80-89 years groups, the AUC of combined detection was significantly higher than that of either single indicator ($P<0.05$), indicating that combined detection has superior overall diagnostic performance in the elderly population. See **Table 9** and **Figure 1**.

External validation

An independent validation set was established in this study to perform external validation of the model, with a focus on assessing the stability of model probability calibration. As shown by the calibration curve (**Figure 2**), the distribution of predicted probabilities across risk strata was in good agreement with the observed probabilities. The curve closely followed the ideal 45° diagonal line, with no obvious overestimation or underestimation. The Hosmer-Lemeshow goodness-of-fit test yielded a *P*-value >0.05, indicating good model calibration. These results suggest that the model maintains stable predictive performance and clinical generalizability in an independent population.

Decision curve analysis of TTF-1 and N-MID for predicting bone metastasis

Decision curve analysis, with net benefit on the Y-axis and high-risk threshold on the X-axis, showed that the N-MID model yielded negative net benefit within certain threshold ranges, whereas the other models exhibited positive clinical net benefit across the clinical threshold range, indicating favorable clinical value. Furthermore, net benefit increased as the threshold probability decreased. See **Table 10**.

Further analysis demonstrated that across most clinically reasonable threshold ranges, the combined detection of TTF-1 and N-MID provided a higher overall net benefit for predicting bone metastasis than either biomarker alone. This finding suggests that the combined detection strategy has greater practical value in decision-making. See **Figure 3**.

Comparison of N-MID as a prognostic indicator

Serum N-MID levels were measured at 3 months and 6 months of treatment in patients with and without bone metastasis, and the differences between the two groups were compared. At 3 months, the N-MID levels differed significantly between the two groups ($P<0.05$). At 6 months, although the mean value in the bone metastasis group was slightly higher than that in the non-bone metastasis group, the difference was not statistically significant ($P>0.05$). See **Table 11**.

Discussion

The most common metastatic sites in lung cancer patients include the nervous system, bone, liver, respiratory system, and adrenal glands. Bone metastasis in lung cancer is a major challenge. Survey data show that the long-term prognosis of patients with bone metastasis is poor; moreover, bone metastasis causes physical pain and fractures, greatly reducing patients' quality of life [13, 14]. In the process of bone metastasis, the "vicious cycle" theory is widely accepted [15], i.e., tumor cells secrete

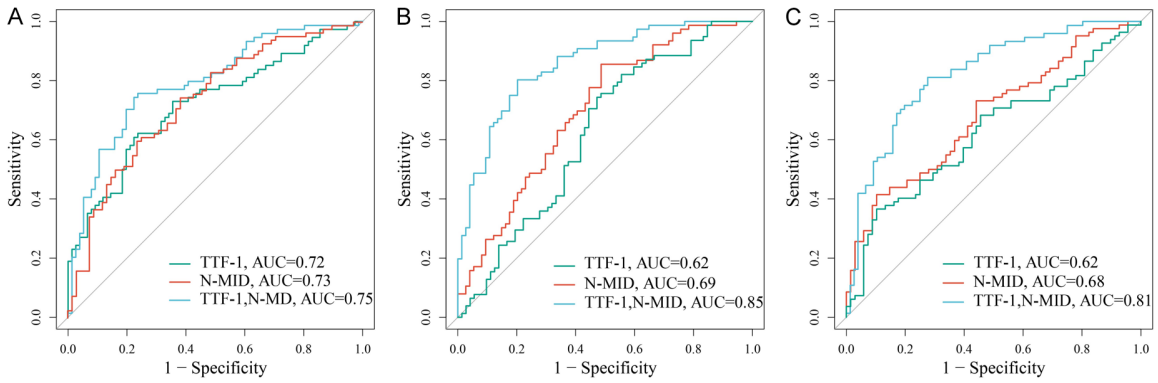

Figure 1. ROC curves for different age groups. Note: A: 60-69 years; B: 70-79 years; C: 80-89 years. TTF-1: thyroid transcription factor-1; N-MID: N-terminal osteocalcin.

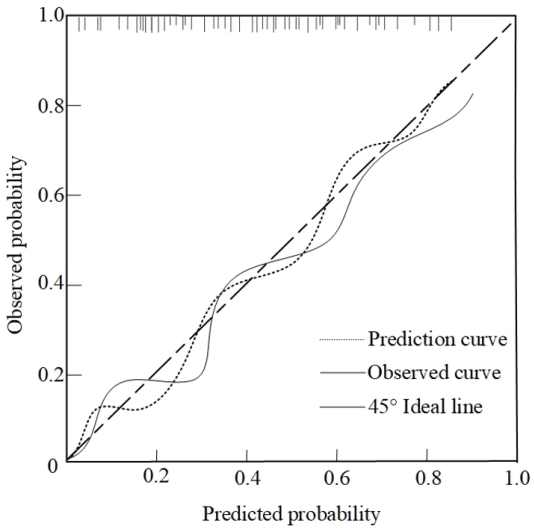

Figure 2. External validation calibration curve.

cytokines that activate osteoclasts and accelerate bone resorption and destruction, while growth factors released from the bone matrix in turn promote tumor cell proliferation, invasion, and colonization, creating a cascade amplification effect. According to this theory, osteoclasts are often regarded as "bone digesters", and secondary effects of bone resorption, including providing physical space for tumor growth and releasing tumor-promoting factors from the bone matrix-promote tumor colonization in bone [16, 17]. Although research on bone metastasis of lung cancer has a long history, existing prediction methods still have limitations (e.g., ECT and PET-CT involve high radiation doses and are expensive, and these are inappropriate for short-term dynamic follow-up and repeated screening of bone metastasis in lung cancer patients; conventional CT has lower radiation and cost but insufficient sensitivity for detecting early microscopic bone metastases). Therefore, this study combined N-ID and TTF-1 to construct a non-invasive, convenient, and repeatable predictive model for bone metastasis in lung cancer, aiming to address the shortcomings of imaging examinations in short-term dynamic screening.

Thyroid transcription factor-1 (TTF-1) promotes tumor cell proliferation and survival by regulating various downstream target genes, and its expression level is positively correlated with disease progression. Consequently, lung cancer patients with bone metastasis tend to have higher TTF-1 expression [18]. Osteocalcin (N-MID) is a bone metabolism marker that directly reflects the degree of bone destruction caused by metastasis. In lung cancer bone metastasis, tumor cells activate osteoclasts, leading to bone resorption, while the body enhances bone formation through compensatory mechanisms, promoting the release of N-MID into the blood, thereby increasing serum N-MID levels [19, 20].

In this study, serum levels of TTF-1 and N-MID at admission were significantly higher in lung cancer patients with bone metastasis than in those without bone metastasis. Furthermore, elevated TTF-1 and N-MID levels were significantly positively correlated with elevated levels of lung cancer bone metastasis risk indicators (PTH, tPINP, β-CTX). PTH, tPINP, and β-CTX are common markers reflecting bone metastasis in lung cancer, and their levels change significantly when bone metastasis occurs. PTH, secreted by tumor cells, activates downstream

Table 10. Net benefit of TTF-1, N-MID, and the combined model on decision curve analysis

Threshold probability (Pt)	TTF-1 model net benefit (95% CI)	N-MID model net benefit (95% CI)	Combined model (TTF-1+N-MID) net benefit (95% CI)
0.1	0.27 (0.24-0.30)	0.28 (0.25-0.31)	0.29 (0.26-0.32)
0.2	0.22 (0.19-0.25)	0.24 (0.21-0.27)	0.25 (0.22-0.28)
0.3	0.18 (0.15-0.21)	0.20 (0.17-0.23)	0.23 (0.20-0.26)
0.4	0.15 (0.12-0.18)	0.15 (0.12-0.18)	0.20 (0.17-0.23)
0.5	0.13 (0.10-0.16)	0.13 (0.10-0.16)	0.16 (0.13-0.19)
0.6	0.10 (0.07-0.13)	0.09 (0.06-0.12)	0.13 (0.10-0.16)
0.7	0.07 (0.04-0.10)	0.06 (0.03-0.09)	0.10 (0.07-0.13)
0.8	0.04 (0.01-0.07)	0.03 (0.00-0.06)	0.05 (0.02-0.08)

Note: Decision curve analysis was performed using the bootstrap resampling method to calculate the net benefit and 95% confidence intervals for each model at various threshold probabilities. Threshold probabilities ranging from 0.1 to 0.8 represent the clinically relevant decision interval.

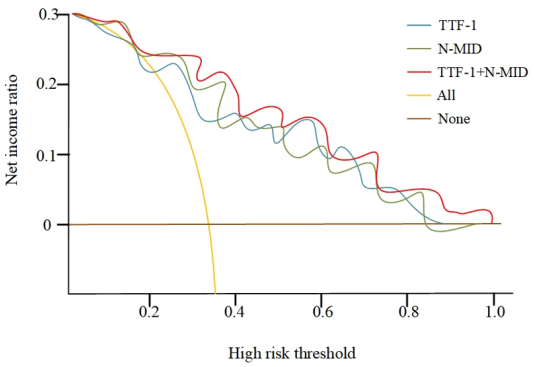

Figure 3. Decision curve for TTF-1 and N-MID in predicting bone metastasis in lung cancer patients.

pathways, indicating bone metastasis has been initiated [21]; β-CTX is a sensitive indicator of the degree of bone destruction and dissolution caused by cancer cells; tPINP is a marker of bone formation, and its elevation in the setting of bone metastasis represents a "compensatory destructive repair" response [1]. Therefore, elevated levels of PTH, tPINP, and β-CTX are generally markers of bone metastasis. Elevated TTF-1 and N-MID not only reflect further disease progression in lung cancer patients but are also closely associated with the bone metastasis process; thus, TTF-1 and N-MID may become predictive indicators for bone metastasis in lung cancer patients.

To systematically evaluate the clinical utility of TTF-1 and N-MID, this study performed multidimensional validation. First, multivariate logistic regression confirmed that both TTF-1 and N-MID are independent risk factors for bone metastasis of lung cancer, and including them in the model significantly improved predictive performance. Stratified analysis showed that in the elderly cohorts aged 70-79 years and 80-89 years, the AUC of combined TTF-1 and N-MID detection was significantly superior to that of either marker alone, indicating that the combined strategy has better diagnostic performance in the elderly population. Notably, in the 70-79 and 80-89 age groups, the sensitivity of combined detection was higher than that of individual markers, whereas in the 60-69 age group, the sensitivity of combined detection was slightly lower than that of individual markers. This age-stratified difference has reasonable clinical and pathophysiological explanations. In lung cancer patients aged 60-69 years, tumor aggressiveness is relatively lower, and the burden of potential micrometastasis and occult bone lesions is lower; single bone turnover and tissue markers already provide good screening performance. When the parallel combined diagnostic mode is applied, sensitivity may show a slight decrease due to individual biological heterogeneity and diagnostic threshold fluctuations. In contrast, in patients aged ≥70 years, who often have underlying bone metabolic disorders and reduced compensatory capacity, the risk of malignant tumor phenotypes and occult bone metastasis is higher. Single markers are prone to missed diagnosis, whereas combined detection effectively improves detection efficiency through marker complementarity. Differences in tumor biological characteristics, baseline bone metabolic status, and occult metastatic burden across age groups are the main reasons for the lack of complete consistency in stratified diagnostic trends. The stratified results of this study are consistent with the clinical applica-

Table 11. Comparison of N-MID levels between the two groups at different time points

indicators	Time	BM group (n=83)	Non-BM group (n=64)	t	*P*
N-MID	At 3 months of treatment	16.52±3.18	15.31±3.66	2.141	0.034
N-MID	At 6 months of treatment	15.48±2.95	14.92±3.51	1.050	0.295

Note: BM, bone metastasis.

tion patterns of bone metastasis markers in lung cancer patients, and the overall conclusion maintains good internal consistency. In an independent external validation set, the Hosmer-Lemeshow test yielded a *P*-value >0.05, and the calibration curve closely followed the ideal diagonal line, indicating that the predicted probabilities were highly consistent with the observed probabilities, with no overfitting or calibration drift. Finally, to address the limitation of traditional ROC analysis focusing only on discriminative ability, this study employed DCA to assess the clinical utility of the model. The results showed that across a range of clinically reasonable threshold probabilities, the net benefit of combined TTF-1 and N-MID detection was higher than that of either marker alone. The combined prediction model constructed in this study demonstrates good discrimination and calibration. Based on the optimal cut-off values of the model, a simple clinical risk scoring system can be established. By integrating TTF-1 expression status and serum N-MID levels, lung cancer patients can be stratified into low-, moderate-, and high-risk groups for bone metastasis. For newly diagnosed lung cancer patients, routine testing of TTF-1 and N-MID indicators can be used to assess risk by applying the prediction model. Low-risk patients may undergo routine follow-up; moderate-risk patients should have shortened follow-up intervals and regular re-examination of bone turnover markers, and high-risk patients should undergo ECT/CT imaging screening as early as possible, thereby achieving early warning, stratified management, and precise intervention for bone metastasis. This study moves from single-biomarker analysis to the construction of a combined prediction model and further proposes a clinical screening pathway, thereby bridging the gap from basic indicator testing to clinical decision-making guidance, and providing a practical and referable assessment tool for the early identification and individualized management of bone metastasis in lung cancer.

Although this study has yielded clinically meaningful findings, several limitations should be acknowledged. This study relied solely on clinical case analyses to investigate the association of N-MID and TTF-1 with bone metastasis in lung cancer. We did not construct cellular or animal models to conduct functional experiments, and therefore we are unable to elucidate the molecular pathways through which these two markers jointly mediate bone metastasis in lung cancer; in vivo and in vitro mechanistic validation is still lacking. In addition, this was a retrospective, single-center study with a relatively concentrated sample source, which may introduce some selection bias and affect the robustness of the results. Although an independent external validation set was used for preliminary validation of the prediction model, the external validation samples still came from the same institution. Therefore, the generalizability of the model to different regions, ethnicities, and medical settings requires further validation through multicenter, large-sample, prospective cohort studies. In the future, a series of experiments will be required to validate the mechanistic role of N-MID and TTF-1 in lung cancer bone metastasis.

In summary, this study found that serum TTF-1 and N-MID levels at admission in lung cancer patients are significantly associated with the risk of bone metastasis. Combined detection of the two markers shows high clinical value in predicting bone metastasis and exhibits a favorable net benefit in decision curve analysis, suggesting its potential application prospects in clinical practice.

Disclosure of conflict of interest

None.

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