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Diagnosis of bone metastasis in patients with non-small cell lung cancer by combined detection of CEA, CYFRA21-1, and ALP

Dijagnoza metastaza u kostima kod obolelih od nesitnoćelijskog karcinoma pluća kombinovanom detekcijom CEA, CYFRA21-1 i ALP

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Abstract

Background/Aim. Despite significant advances in the diagnosis and treatment of non-small cell lung cancer (NSCLC), the overall prognosis remains poor, especially when bone metastasis occurs with disease progression. The aim of this study was to examine the diagnostic value of combined detection of carcinoembryonic antigen (CEA), cytokeratin 19 fragment antigen 21-1 (CYFRA21-1), and alkaline phosphatase (ALP) for bone metastasis in NSCLC patients. **Methods.** In total, 148 patients with NSCLC were included in the study. They were selected from patients hospitalized for treatment between April 2020 and March 2022. Out of the total number of patients, 68 were assigned to the metastasis group and 80 to the non-metastasis group. Their blood samples were collected to measure CEA, CYFRA21-1, and ALP levels in the serum. Multivariate logistic regression analysis was conducted to determine the factors contributing to bone metastasis. Receiver operating characteristic (ROC) curves were plotted to analyze the diagnostic value. **Results.** Bone metastasis occurred in 68 (45.94%) patients. The baseline data exhibited no significant intergroup differences ($p > 0.05$). The metastasis group had significantly raised serum CEA, CYFRA21-1, and ALP levels compared to those of the non-metastasis group ($p < 0.05$). The increases in serum CEA [odds ratio (OR): 1.062, 95% confidence interval (CI): 1.031–1.094], CYFRA21-1 (OR: 1.155, 95% CI: 1.061–1.258), and ALP (OR: 1.027, 95% CI: 1.008–1.047) were risk factors for bone metastasis (OR > 1, $p < 0.05$). The areas under the ROC curves of CEA, CYFRA21-1, ALP, and their combination were all greater than 0.600, suggesting high diagnostic values. **Conclusion.** CEA, CYFRA21-1, and ALP levels in the serum can predict bone metastasis in NSCLC patients, and the predictive value of their combination is higher than that of any single indicator.

Key words:
biomarkers, tumor; bone neoplasms; carcinoma, non-small-cell lung; diagnosis; neoplasm metastasis; treatment outcome.

Apstrakt

Uvod/Cilj. Uprkos značajnom napretku u dijagnostici i lečenju nesitnoćelijskog karcinoma pluća (*non-small cell lung cancer* – NSCLC), ukupna prognoza ostaje loša, posebno kada se sa progresijom bolesti javljaju metastaze u kostima. Cilj rada bio je da se ispita dijagnostička vrednost kombinovane detekcije karcinoembrionalnog antigena (*carcinoembryonic antigen* – CEA), fragmenta citokeratina 19 (*cytokeratin 19 fragment antigen 21-1* – CYFRA21-1) i alkalne fosfataze (*alkaline phosphatase* – ALP) za predikciju metastaza u kostima kod obolelih od NSCLC. **Metode.** U studiju je ukupno bilo uključeno 148 obolelih od NSCLC. Oni su odabrani među bolesnicima koji su bili hospitalizovani radi lečenja između aprila 2020. i marta 2022. godine. Od ukupnog broja bolesnika, njih 68 svrstano je u grupu sa metastazama, a 80 u grupu bez metastaza. Prikupljeni su uzorci krvi bolesnika da bi se izmerili nivoi CEA, CYFRA21-1 i ALP u serumu. Multivarijantna logistička regresija korišćena je da bi se utvrdili faktori koji doprinose nastanku metastaza u kostima. Za analizu dijagnostičke vrednosti korišćene su *receiver operating characteristic* (ROC) krive. **Rezultati.** Metastaze kostiju javile su se kod 68 (45,94%) bolesnika. Osnovni podaci bolesnika nisu pokazali značajne razlike između grupa ($p > 0,05$). Grupa bolesnika sa metastazama imala je značajno povišene nivoe CEA, CYFRA21-1 i ALP u serumu, u poređenju sa grupom bez metastaza ($p < 0,05$). Povišeni nivoi CEA [*odds ratio* (OR): 1,062, 95% *confidence interval* (CI): 1,031–1,094], CYFRA21-1 (OR: 1,155, 95% CI: 1,061–1,258) i ALP (OR: 1,027, 95% CI: 1,008–1,047) u serumu bili su faktori rizika za metastaze kostiju (OR > 1, $p < 0,05$). Površine ispod ROC kriva za CEA, CYFRA21-1, ALP i njihove kombinacije bile su veće od 0,600, što ukazuje na visoke dijagnostičke vrednosti. **Zaključak.** Nivoi CEA, CYFRA21-1 i ALP u serumu mogu predvideti metastaze kostiju kod obolelih od NSCLC, pri čemu je prediktivna vrednost njihove kombinacije veća od bilo kog pojedinačnog markera.

Ključne reči:
tumorski markeri; kosti, neoplazme; pluća, nesitnoćelijski karcinom; dijagnoza; neoplazme, metastaze; lečenje, ishod.

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Introduction

Classified as lung cancer with the highest incidence rate, non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases [1]. Despite significant advances in the diagnosis and treatment of NSCLC in recent years, the overall prognosis remains poor, especially when bone metastasis occurs with disease progression, which not only remarkably reduces the quality of life but also seriously affects the survival rate of patients. Furthermore, bone metastasis increases pain and fracture risks, and it is likely to cause serious complications such as spinal cord compression and hypercalcemia [2]. Therefore, early diagnosis and effective monitoring of bone metastasis are essential for patients with NSCLC to perfect treatment options and improve prognosis. The frequently applied imaging techniques include magnetic resonance imaging (MRI), X-ray, positron emission tomography-computed tomography (PET-CT), and bone scan [3]. A bone scan can sensitively detect bone metastases by measuring the uptake of radioisotope-labeled compounds into the bones of the whole body. MRI can more clearly display the disease condition within the bone marrow.

Due to imaging limitations, the detection of serum tumor markers has become important for diagnosing and monitoring bone metastasis. For instance, carcinoembryonic antigen (CEA) is a glycoprotein secreted by tumor cells and some normal cells in the liver, pancreas, and gastrointestinal tract, which was widely studied and initially applied as a marker for colorectal cancer. The significant elevation of CEA has been observed not only in colorectal cancer but also in lung cancers, mammary gland, pancreas, and stomach [4]. Notably, elevated CEA levels in NSCLC patients have been associated with higher metastatic burden, including bone metastasis [5].

Cytokeratin 19 fragment antigen 21-1 (CYFRA21-1) plays a pivotal role as an important tumor marker besides CEA in clinical diagnosis and monitoring. In spite of a significant expression under squamous cell carcinoma, CYFRA21-1 also exhibits high diagnostic sensitivity and specificity in NSCLC patients, especially those with bone metastasis [6]. With unique biological characteristics, CYFRA21-1 is irreplaceable in the early diagnosis, treatment response assessment, and condition monitoring of NSCLC. It may outperform CEA in detecting bone metastatic involvement due to a higher correlation with tumor burden in the skeletal system [7].

Alkaline phosphatase (ALP), an enzyme closely related to bone metabolism, also presents an evidently raised level in NSCLC patients with bone metastasis, which can reflect the process of bone formation and destruction; hence, it possesses crucial reference value in the diagnosis of bone metastasis. The detection of ALP not only helps capture early signals of bone metastasis but also provides dynamic monitoring information during treatment [8]. Serum ALP level is significantly higher in NSCLC patients with bone metastasis compared to those without, suggesting its utility for early detection and monitoring [9].

CEA, CYFRA21-1, and ALP are each valuable in identifying some patients diagnosed with cancer, but single-marker testing usually fails to achieve sufficient diagnostic accuracy and sensitivity. In clinical practice, therefore, it is often necessary to combine the detection results of multiple markers to improve the overall diagnostic accuracy and reliability. However, the detection of a single marker cannot often meet clinical needs.

The aim of this study was to investigate the combined detection of CEA, CYFRA21-1, and ALP in serum to evaluate its diagnostic value and clinical application prospects for diagnosing bone metastasis in NSCLC patients. With this, we hope to achieve earlier and more accurate diagnosis, and provide patients with more timely and effective treatment strategies.

Methods

Subjects

A total of 148 NSCLC patients admitted to the Department of Laboratory Medicine, Xinchang Hospital of Traditional Chinese Medicine, Shaoxing, China, from April 2020 to March 2022 were selected for this study. Of them, according to the presence or absence of bone metastasis, 68 were assigned to the metastasis group and 80 to the non-metastasis group. Inclusion criteria were as follows: patients with NSCLC diagnosed pathologically [10], those with complete clinical data, and those who were informed of and consented to this study. Exclusion criteria included patients with primary bone tumors or osteoporosis.

The study was approved by the Ethics Committee of Xinchang Hospital of Traditional Chinese Medicine, China, (from April 20, 2020).

Detection methods and tools

To ensure detection accuracy and reliability, all procedures of sample collection and processing were performed under standard laboratory conditions using Cobas® 8000 modular analyzer (Roche, Basel, Switzerland) and AU5800 analyzer (Beckman Coulter, Brea, USA), as well as kits from Roche Diagnostics Shanghai Ltd. (Shanghai, China) and Beckman Coulter Laboratory Systems Suzhou Co., Ltd. (Suzhou, China). Briefly, fasting venous blood was collected in a volume of 5 mL and allowed to stand for 30 min to facilitate serum separation, followed by centrifugation at 3,000 revolutions *per* minute (rpm) for 10 min. Then, the acquired supernatant (serum) was utilized for determining the levels of serum CEA [normal range (NR): 0–5 ng/mL], CYFRA21-1 (NR: 0–3.3 μg/L), and ALP (NR: 45–125 U/L) using the abovementioned analyzers and kits.

Diagnostic criteria for bone metastasis

All patients underwent a whole-body bone scan to initially detect the presence or absence of signs of bone metas-

tasis. If the scan results suggested possible bone metastases, further evaluation by more than two physicians was required. CT, MRI, and other imaging examinations were also required to confirm the diagnosis results and accurately locate bone metastases.

Statistical analysis

Statistical analysis was performed using SPSS 26.0 software. The count data were presented with numbers or percentages, while measurement data were expressed as mean ± standard deviation. Intergroup comparisons were accomplished through the *t*-test and the Chi-square test. The value of $p < 0.05$ indicated a difference of statistical significance.

Results

Bone metastasis occurred in 68 (45.94%) out of the 148 patients.

The baseline data manifested no statistically significant intergroup differences ($p > 0.05$) (Table 1).

The metastasis group, compared to the non-metastasis group, presented significantly raised concentrations of serum CEA, CYFRA21-1, and ALP, and the differences were statistically significant ($p < 0.05$) (Table 2).

Multivariate logistic regression analysis was carried out with serum CEA, CYFRA21-1, and ALP as independent variables, and bone metastasis present or absent in NSCLC patients as a dependent variable (0 = metastasis, 1 = non-metastasis). The results revealed that the increases in serum CEA [odds ratio (OR): 1.062, 95% confidence interval (CI): 1.031–1.094], CYFRA21-1 (OR: 1.155, 95% CI: 1.061–1.258), and ALP (OR: 1.027, 95% CI: 1.008–1.047) were risk factors for bone metastasis (OR > 1, $p < 0.05$) (Table 3).

To explore the diagnostic value of serum biomarkers in relation to NSCLC histological subtypes, patients were stratified into adenocarcinoma and squamous cell carcinoma subgroups. For each subtype, the levels of CEA, CYFRA21-1, and ALP were compared between the metastasis and non-metastasis groups. In the squamous cell carcinoma subgroup, serum CYFRA21-1 level was significantly higher in patients with bone metastasis compared to those without metastasis ($p < 0.05$). No significant differences were observed in CEA or ALP levels between metastasis and non-metastasis groups ($p > 0.05$). In contrast, within the adenocarcinoma subgroup, CEA, CYFRA21-1, and ALP levels were all significantly elevated in the metastasis group ($p < 0.05$) (Table 4).

Table 1

Clinical data of patients

Parameters	Metastasis group (n = 68)	Non-metastasis group (n = 80)	χ^2/t	*p*
Age, years	65.32 ± 7.45	66.18 ± 7.12	0.716	0.474
Gender				
male	41 (60.29)	46 (57.50)	0.118	0.730
female	27 (39.71)	34 (42.50)		
Diabetes mellitus				
yes	26 (38.24)	32 (40.00)	0.048	0.826
no	42 (61.76)	48 (60.00)		
Hypertension				
yes	32 (47.06)	37 (46.25)	0.009	0.921
no	36 (52.94)	43 (53.75)		
Smoking				
yes	29 (42.65)	33 (41.25)	0.029	0.863
no	39 (57.35)	47 (58.75)		
Pathological type				
adenocarcinoma	43 (63.24)	49 (61.25)	0.061	0.804
squamous cell carcinoma	25 (36.76)	31 (38.75)		
Site of lesion				
left	28 (41.18)	32 (40.00)	0.021	0.884
right	40 (58.82)	48 (60.00)		

n – number.
All values are given as numbers (percentages) or mean ± standard deviation.

Table 2

CEA, CYFRA21-1, and ALP in the serum

Parameters	Metastasis group (n = 68)	Non-metastasis group (n = 80)	*t*	*p*
CEA (ng/mL)	12.43 ± 2.42	6.23 ± 1.53	18.903	0.001
CYFRA21-1 (μg/L)	6.49 ± 2.78	4.14 ± 0.21	7.540	0.001
ALP (U/L)	115.32 ± 21.56	106.24 ± 15.48	2.972	0.003

CEA – carcinoembryonic antigen; CYFRA21-1 – cytokeratin 19 fragment antigen 21-1; ALP – alkaline phosphatase; n – number.
All values are given as mean ± standard deviation.

Table 3

Associations of serum CEA, CYFRA21-1, and ALP with bone metastasis in NSCLC patients assessed by multivariate logistic regression analysis

Variables	*β*	SE	Wald	*p*	OR	95% CI
CEA	0.060	0.015	15.803	0.001	1.062	1.031–1.094
CYFRA21-1	0.144	0.044	10.980	0.001	1.155	1.061–1.258
ALP	0.027	0.010	7.881	0.005	1.027	1.008–1.047

NSCLC – non-small cell lung cancer; SE – standard error; OR – odds ratio; CI – confidence interval. For other abbreviations, see Table 2.

Table 4

Serum biomarker levels in patients stratified by histological subtype

Histological subtype	Metastasis group	Non-metastasis group	*t*	*p*
Squamous cell carcinoma				
CEA (ng/mL)	11.06 ± 2.29	6.03 ± 1.21	1.923	0.061
CYFRA21-1 (μg/L)	7.18 ± 2.81	5.31 ± 1.97	3.004	0.004
ALP (U/L)	112.40 ± 19.85	107.65 ± 14.29	1.142	0.258
Adenocarcinoma				
CEA (ng/mL)	13.11 ± 2.33	6.42 ± 1.61	16.267	< 0.001
CYFRA21-1 (μg/L)	6.06 ± 2.74	3.58 ± 0.87	6.471	< 0.001
ALP (U/L)	118.55 ± 21.71	104.33 ± 13.98	3.973	< 0.001

For abbreviations, see Table 2.

All values are given as mean ± standard deviation.

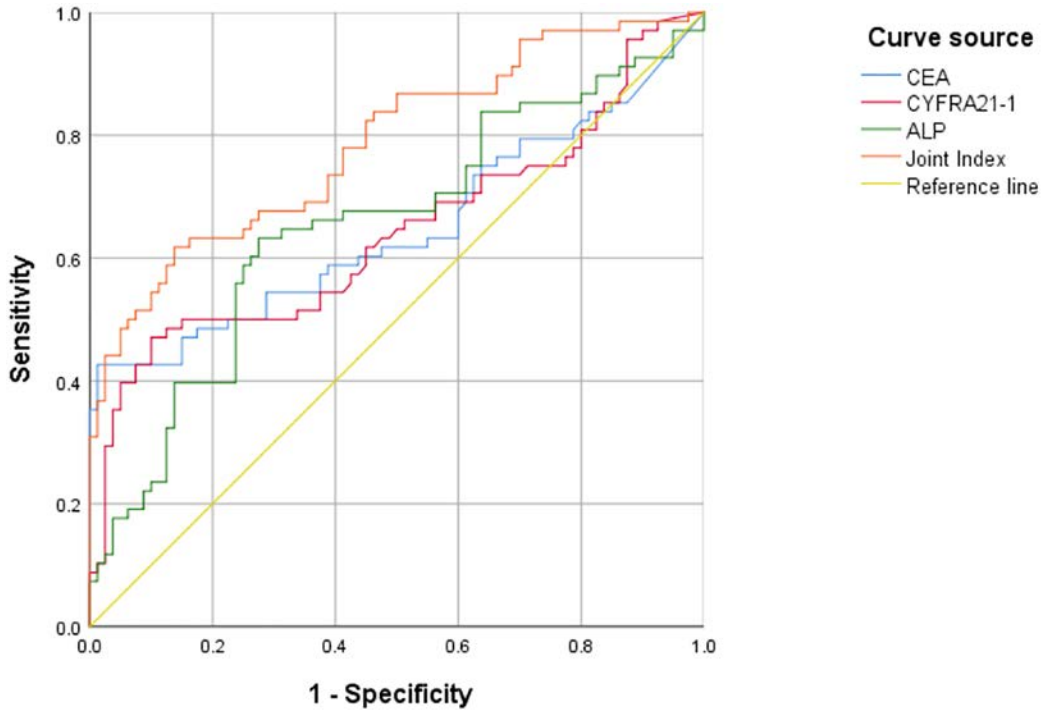

Fig. 1 – ROC curves of CEA, CYFRA21-1, and ALP in the serum and their combination for diagnosing bone metastasis in NSCLC patients. The yellow reference line represents the 45-degree line, which indicates no predictive ability. Factors with ROC curves that are farther away from this reference line have better predictive performance. ROC – receiver operating characteristic. For other abbreviations, see Tables 2 and 3.

Serum CEA, CYFRA21-1, ALP, and a combination of the three were set as test variables, and bone metastasis present or absent in NSCLC patients was determined as a state variable (0 = metastasis, 1 = non-metastasis) to generate receiver operating characteristic (ROC) curves. The results showed that CEA, CYFRA21-1, ALP, and their combination all had an area under the curve (AUC) greater than 0.600, suggesting that these indicators possess high diagnostic value for bone metastasis in NSCLC patients (Figure 1, Table 5).

Table 5

Significance of combined detection of CEA, CYFRA21-1, and ALP for diagnosing bone metastasis in NSCLC patients

Factor	AUC	95% CI	Specificity	Sensitivity	Youden index	Cut-off value
CEA	0.648	0.553–0.742	0.712	0.515	0.227	9.5 ng/mL
CYFRA21-1	0.641	0.548–0.735	0.562	0.574	0.136	5.3 μg/L
ALP	0.654	0.564–0.744	0.900	0.221	0.121	110.0 U/L
Combination	0.787	0.719–0.861	0.537	0.868	0.405	-

AUC – area under the curve; CI – confidence interval.
For other abbreviations, see Tables 2 and 3.

Discussion

As a complex and severe disease, lung carcinoma poses a great challenge to public health worldwide. According to statistics, pulmonary carcinoma emerges as a major contributor to carcinoma-associated death globally, with millions of deaths every year. From the perspective of the medical field, pulmonary carcinoma is classified primarily into two types (small cell lung carcinoma and NSCLC) according to its pathological characteristics, with NSCLC as the most ubiquitous type [11], accounting for nearly 85%. Bone metastasis is a ubiquitous and serious complication of NSCLC, which usually occurs at the progressive stage when cancer cells metastasize from the primary site to the bone. Bones are rich in blood supply and have an excellent bone marrow microenvironment; therefore, cancer cells can easily colonize and continue to grow in the bones [12]. Bone metastasis frequently occurs in the spine, pelvis, ribs, and long bones of the extremities of NSCLC patients, causing a variety of clinical problems ranging from hypercalcemia, pathological fractures, pain, to bone-spinal cord compression. The symptoms of bone metastasis are highly similar to those of primary bone disease, which complicates early diagnosis and accurate identification. Patients usually report nonspecific bone pain first, which may be intermittent or persistent, and which may gradually aggravate at night. As the disease progresses, the risk of pathological fractures will increase, especially at the bone under heavy load. When invading the spine, cancer cells are more likely to cause neurological dysfunction due to spinal cord compression, including paresthesia, dyskinesia, or even paralysis [13]. Imaging diagnosis using MRI, X-ray, CT, and bone scan plays an important role in detecting bone metastasis. X-rays can detect large-scale bone damage, but they are less sensitive to early small-scale lesions. Bone scans can detect bone lesions at multiple sites of the body, but are less specific [14]. Therefore, other ways of accurately identifying and diagnosing bone metastasis need to be considered in NSCLC patients.

Inflammation and immune response are key players in the occurrence and development of tumors, suggesting that the detection of indicators related to inflammation and immune response may contribute to earlier or more accurate diagnosis of bone metastasis [15]. In addition, biochemical markers of bone metabolism and tumor markers have been widely applied in the diagnosis and monitoring of diversified tumors, which provide key information about bone health as well as the tumor and its activity. The concentration of tumor markers can reflect the presence and progression of cancer, and these biomolecules are significantly important for cancer in terms of early diagnosis, therapeutic effect assessment, and relapse monitoring [16]. CEA and CYFRA21-1 are common tumor markers associated with lung cancer. CEA is a protein produced during embryonic development, and its level is normally very low in the blood of adults. Still, it can be significantly elevated in several carcinoma types (e.g., mammary adenocarcinoma, colorectal cancer, and pulmonary carcinoma) [17]. Therefore, CEA is considered a broad-spectrum tumor marker, and its level detected by immunochemistry can reflect the presence and progression of some tumors. Monitoring CEA helps assess efficacy and evaluate the prognosis of NSCLC [18]. In this study, there was a significant correlation between CEA and the presence or absence of bone metastasis ($p < 0.05$), and CEA was found to be a risk factor independently affecting bone metastasis, that is, the higher the CEA level was, the higher the bone metastasis potential in NSCLC patients would be (AUC = 0.648). However, CEA is a nonspecific cancer marker, and its elevation may also exist in some benign diseases and other non-cancerous conditions. Therefore, the detection of CEA alone is insufficient for cancer diagnosis, and it is generally necessary to combine it with other indicators for comprehensive evaluation. Being a soluble cytokeratin 19 fragment, CYFRA21-1 usually presents a high level, indicating a severe condition or active tumor, which is of essential reference value for clinicians in developing individualized treatment plans. Since it is widely applied for lung cancer, CYFRA21-1 has become one of the crucial diagnostic tools in clinical practice. CYFRA21-1 has also been discovered with critical effects on lung cancer regarding metastasis and prognosis [19]. In this study, the blood CYFRA21-1 level rose significantly in NSCLC subjects manifesting bone metastasis, which was determined through multivariate logistic regression analysis as a factor independently influencing bone metastasis (AUC = 0.641) ($p < 0.05$). Nevertheless, CYFRA21-1 is not a specific marker, and its elevated level may also be associated with other diseases like breast cancer, bladder carcinoma, and colorectal carcinoma [20]. Therefore, CYFRA21-1 is less specific and sensitive in diagnosing bone metastasis. In contrast, bone metabolic biochemical markers, possibly including blood calcium and phosphatase, can more directly and specifically reflect bone metabolic activities, which are often used to evaluate bone health status and diagnose bone diseases [21]. ALP, the most frequently employed biochemical indicator of bone metabolism, is a kind of enzyme system widely present in tissues of the human body, which is

mainly synthesized in the liver and bone tissues and catalyzes the hydrolysis reaction of phosphate compounds. The ALP level rises significantly when bone formation increases or bone resorption decreases, and its close correlation with NSCLC prognosis has been confirmed [22]. In this study, the sensitivity, specificity, and AUC of ALP for diagnosing bone metastasis in NSCLC patients were 22.1%, 90.0%, and 0.654, respectively, which could be used for combined de-tection.

ALP level can be an early marker for bone metastasis in NSCLC patients, and an abnormal ALP level may indicate a high potential for bone metastasis, especially in patients with less elevated CEA and CYFRA21-1 levels. These two indicators possess high predictive value for bone metastasis in NSCLC [23]. As a marker of bone destruction and formation, ALP exhibits unique diagnostic value for bone metastasis. As a result, the combined detection of CEA, CYFRA21-1, and ALP may provide a more accurate and effective clinical basis for early diagnosis of NSCLC patients with bone metastasis. In this study, CEA, CYFRA21-1, and ALP levels in the serum of NSCLC patients were detected and analyzed. Compared with patients without bone metastasis, those suffering from bone metastasis demonstrated increased content of these three markers. Specifically, elevated CEA usually indicated an increased tumor size or distant metastasis, elevated CYFRA21-1 further indicated enhanced activity and spread capacity of tumor cells, and elevated ALP directly reflected abnormal bone metabolism. The results of ROC curve analysis showed that a sensitivity of 86.8% and an AUC of 0.787 were obtained for the combined detection. Both values were higher than those of any marker alone, suggesting that this combined detection offers greater clinical value for diagnosing bone metastasis in NSCLC patients.

Nevertheless, this study has limitations. Firstly, the retrospective design may introduce selection bias, potentially limiting the generalizability of the results. Secondly, this study did not analyze the relationship between serum biomarker levels and the extent or anatomical distribution of bone metastases (e.g., solitary vs. multiple lesions; spinal vs. pelvic or appendicular sites) due to incomplete or inconsistent imaging records in the retrospective dataset. These stratifications should be carefully explored in future prospective studies with standardized imaging and staging protocols.

Conclusion

The detection integrating CEA, CYFRA21-1, and ALP is an effective means of diagnosing NSCLC patients for bone metastasis early, which can efficiently reduce missed diagnoses and misdiagnoses in bone metastasis screening. It not only expands the application of biomarkers in tumor diagnosis but also provides clinicians with more comprehensive and accurate information in NSCLC treatment and management. With the application and popularization of this detection strategy, the early screening results of NSCLC may be significantly improved, thereby prolonging both disease-free survival and overall survival, as well as raising the quality of life. As more in-depth research is conducted and high-precision detection technology develops in the future, testing incorporating CEA, CYFRA21-1, and ALP will have broader prospects in tumor diagnosis and treatment.

Conflict of interest

The authors declare no conflict of interest.

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