







# Risk factors for brain metastases from non-small-cell lung cancer

## A protocol for observational study

Jinlin He, MD<sup>a</sup> , Xiaolei Wang, MD<sup>b</sup> , Rensen Xiao, MD<sup>c</sup> , Wei Zuo, MD<sup>c</sup> , Wei Zhang, MD, PhD<sup>c</sup> , Huiming Yao, MD<sup>c,\*</sup> 

### Abstract

Brain metastasis is a common site of distant metastasis of non-small-cell lung cancer (NSCLC) that greatly reduces the prognosis of patients. In this study, we explored the correlation between different clinical factors and secondary brain metastases in NSCLC in an attempt to identify NSCLC patient populations at high risk of metastasis to the central nervous system.

We collected data for 350 NSCLC patients from the medical record system of the First Affiliated Hospital of Nanchang University from June 2015 to June 2019, and these patients had pathologically verified diagnoses. The correlations between age at the time of diagnosis, sex, histological type, calcium concentration, hemoglobin (HB), fibrinogen (Fbg), activated partial thromboplastin time (APTT), alkaline phosphatase (ALP), carcinoembryonic antigen (CEA), CA125, and CA199 levels and brain metastasis were analyzed. Multivariate logistic regression analysis was used to identify risk factors for NSCLC brain metastasis. A receiver operating characteristic (ROC) curve was used to calculate the cutoff, sensitivity, and specificity of the independent related factors.

Of the 350 patients, 57 were diagnosed with brain metastases. Univariate and multivariate logistic regression analysis indicated that lesion diameter, calcium concentration, and CEA level were independent risk factors correlated with brain metastasis ( $P < .05$ ). There were no significant differences in age, sex, type of histopathology, presence or absence of mediastinal lymph node metastasis, HB, Fbg, APTT, ALP, cancer antigen 125 (CA-125), or cancer antigen 199 (CA-199) levels between patients with brain metastases and patients without brain metastases ( $P > .05$ , respectively). ROC curves demonstrated that these factors had comparable accuracy in predicting brain metastasis (area under the curve [AUCs] were 0.620, 0.661, and 0.729, respectively). The cutoff values for lesion diameter, calcium, and CEA were 5.050 cm, 2.295 mmol/L, and 11.160 ng/mL, respectively. The sensitivities for prediction brain metastasis were 59.6%, 64.9%, and 73.3%, with specificities of 63.1%, 59.2%, and 70.3%, respectively.

According to our study, lesion diameter, calcium concentration, and CEA level are independent risk factors for brain metastases in NSCLC patients. Thus, we can strengthen the regular follow-up of NSCLC patients with tumor diameter  $> 5.050$  cm, calcium  $> 2.295$  mmol/L, CEA  $> 11.160$  ng/mL, and use these factors as a reference for preventive treatments.

**Abbreviations:**  $\beta$  = coefficient regression, ALP = alkaline phosphatase, APTT = activated partial thromboplastin time, AUC = area under the curve, CA-125 = cancer antigen 125, CA-199 = cancer antigen 199,  $Ca^{2+}$  = calcium, CEA = carcinoembryonic antigen, CIs = confidence intervals, CNS = central nervous system, EMT = epithelial-to-mesenchymal transition, Fbg = fibrinogen, HB = hemoglobin, NSCLC = non-small-cell lung cancer, OR = odds ratio, ROC = receiver operating characteristic.

**Keywords:** brain metastasis, calcium concentration, CEA, lesion diameter, non-small-cell lung cancer (NSCLC), risk factors

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JH and XW contributed equally to this article.

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All data generated or analyzed during the present study are included in this published article.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

<sup>a</sup> Department of Neurology Medicine, The First Affiliated Hospital of Nanchang University, Nanchang 330006, <sup>b</sup> Second Department of Cardiovascular Medicine, Jiangxi Provincial People's Hospital Affiliated to Nanchang University, Nanchang, <sup>c</sup> Department of Respiratory and Critical Care Medicine, The First Affiliated Hospital of Nanchang University, Nanchang 330006, Jiangxi, China.

\* Correspondence: Huiming Yao, Department of Respiratory and Critical Care Medicine, The First Affiliated Hospital of Nanchang University, No. 17 Yongwaizheng Street, Donghu District, Nanchang 330006, China (e-mail: yaohuiming312@163.com).

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## 1. Introduction

The incidence of lung cancer is the highest among all tumors and cancers (mainly breast cancer, lung cancer, kidney cancer, etc), the cumulative incidence of brain metastases with lung cancer in 1 year and 5 years accounted for the first place, accounting for 14.8% and 16.3%, respectively.<sup>[1]</sup> In addition, non-small-cell lung cancer (NSCLC) accounts for most types of lung cancer. Therefore, NSCLC is the most common primary tumor in patients developing brain metastasis. Some studies have shown that the brain metastasis rate of NSCLC is approximately 10% to 20%<sup>[2–4]</sup> and that the brain metastasis rate is higher in patients with locally advanced non-small-cell lung cancer at approximately 30% to 50%.<sup>[3,5–7]</sup> The brain is the most common site for distant metastases of NSCLC, at the same time, simple brain metastases of NSCLC with no other metastases account for about 20% of all distant metastases of NSCLC.<sup>[5]</sup> Therefore, the most common site of distant metastasis in NSCLC patients is also the brain. Moreover, with the widespread use of craniocerebral magnetic resonance, those data are still increasing. Due to advances in the treatment of NSCLC, such as surgery combined with neoadjuvant therapy or multimodality treatment, the survival of patients has been extended, and the probability of brain metastasis in NSCLC has also increased.<sup>[2,3,5,7]</sup> However, once primary lung tumors invade the brain, there are very few targeted treatments, so brain metastasis is the main treatment obstacle for patients with lung cancer.

Severe neurological dysfunction often occurs in those patients because lung cancer patients can cause increased intracranial pressure during brain metastases, manifesting as headache, nausea and vomiting, and some patients can develop epilepsy, hemiplegia or mental symptoms, or even dead. These symptoms severely affect patients' quality of life. Because of the occurrence of brain metastases in patients with NSCLC, the median survival time has been greatly reduced.<sup>[4,8]</sup> Most of these central nervous system (CNS) recurrences occurred within 2 years of initial diagnosis.<sup>[2,5]</sup> Some studies indicate that the median survival time of NSCLC with brain metastases is approximately 6 to 30 months, and the survival time with early diagnosis is longer.<sup>[2,3,6,8]</sup> To improve the quality of life of patients and prolong the survival of patients, the most important method is to detect high-risk groups early, to shorten the follow-up time of these patients, and even to give preventive treatment. However, the efficacy and adverse effects of preventive treatment still need to be further proven in future research. The purpose of this study was to explore the risk factors for brain metastases in patients with NSCLC to determine the definition of high risk for NSCLC-related brain metastases.

## 2. Materials and methods

### 2.1. Patient selection

This research was a retrospective experiment. We searched the First Affiliated Hospital of Nanchang University (Nanchang, Jiangxi, China) Data Base and selected a series of consecutive patients with NSCLC confirmed by biopsy and pathology from June 2015 to June 2019. Exclusion criteria included incomplete chart information. In addition, we also analyzed cranial magnetic resonance imaging data of patients with NSCLC diagnosed with brain metastasis.

### 2.2. Data collection

We examined baseline demographic information and clinical variables, including patient age at diagnosis of the primary tumor, sex, lesion diameter, presence of mediastinal lymph node metastasis, histopathological type, laboratory test results when diagnosing the primary tumor, for example alkaline phosphatase (ALP) (140 U/L is considered the upper limit of the normal range), calcium (2.6 mmol/L is considered the upper limit of the normal range), coagulation markers (especially fibrinogen (Fbg) and activated partial thromboplastin time [APTT]) and hemoglobin (HB), common tumor markers (serum carcinoembryonic antigen [CEA], CA125 and CA199 values were determined in the same laboratory, and the normal ranges were 0–6.5 ng/mL, 0–35 U/mL, 0–27 U/mL, respectively). We collected data on the above factors in all patients and analyzed the correlation between these clinical parameters and brain metastases to determine risk factors for brain metastases in non-small-cell lung cancer.

### 2.3. Statistical analysis

Univariate and multivariate logistic regression analyses were used to explore the association of age at diagnosis, sex, tumor histology, lesion diameter, and mediastinal lymph node metastasis of brain metastases. Quantitative variables were reported as the means  $\pm$  standard deviation and compared with independent sample *t* test or univariate analysis. Qualitative variables were expressed as numbers and percentages and were assessed by the Chi-square test. The independent risk factors related to brain metastases were analyzed by a multivariate logistic regression analysis model. Adjusted odds ratios (ORs) were calculated with 95% confidence interval (CI). The sensitivity and specificity were calculated based upon optimal cutoff scores.  $P < .05$  was considered significant, and all statistical were performed by IBM SPSS Version 22 (SPSS Inc., Chicago, IL).

## 3. Results

### 3.1. Baseline characteristics of patients

A total of 350 patients with NSCLC were included in this study. A summary of all patients' demographics is presented in Table 1. The median age was 64 years (range: 54–74). The majority of the patients were men (267 cases, 76.29%), and most of the histopathologic types were adenocarcinoma (180 cases, 51.43%). The probability of primary lung lesion appearance was approximately equal in the right and the left lung.

### 3.2. The distribution of brain metastases in patients with NSCLC

The lesion sites of NSCLC brain metastases are detailed in Table 2. In this study, 57 NSCLC patients with brain metastases were diagnosed by cranial magnetic resonance or computed tomography. We analyzed the relevant characteristics of these patients, such as sex, primary site, and number of metastatic sites. We observed brain metastases are common in each lobe (frontal lobe 47.37%, temporal lobe 21.05%, occipital lobe 36.84%, parietal lobe 40.35%) and cerebellum (31.58%), while brain stem (7.02%) brain metastasis is the least. Only 1 brain metastasis site accounted for half of the NSCLC brain metastases patients (28 cases, 49.12%).

**Table 1**  
**Baseline characteristics of patients with NSCLC.**

Patient characteristics	Number of patients (%) (N = 350)
Age at diagnosis, mean ± SD, yr	63.93 ± 9.69
Gender	
Female	83 (23.71)
Male	267 (76.29)
Primary site	
Right	182 (52.00)
Left	156 (44.57)
Bilateral	12 (3.43)
Histopathological types	
Adenocarcinoma	180 (51.43)
Squamous cell carcinoma	155 (44.29)
Adenosquamous carcinoma	8 (2.28)
Large cell carcinoma	4 (1.14)
Other	3(0.86)

NSCLC = non-small-cell lung cancer.

**3.3. Correlation between diverse clinical factors and brain metastases**

Based on univariate analysis, we analyzed differences in biomarkers and clinically relevant indicators in patients with or without brain metastases. The variables analyzed were age at the time of diagnosis, sex, lesion diameter, histological type (adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma, large cell carcinoma, and other), calcium concentration, HB, Fbg, APTT, ALP, CEA, CA125, and CA199. The results showed that among the risk factors we studied, lesion diameter, calcium concentration, and CEA were related to brain metastatic cancer because these factors were significantly different between patients with brain metastatic cancer and patients without brain metastatic cancer (Table 3).

**3.4. Multivariate logistic regression models analysis the risk factors**

In addition, we performed a multivariate logistic regression model analysis to identify independent risk factors for brain metastases. The results showed that lesion diameter (OR 1.161,

**Table 2**  
**The distribution of brain metastases in patients with NSCLC.**

Patient characteristics	Number of patients (%) (N = 57)
Gender	
Female	14 (24.56)
Male	43 (75.44)
Primary site	
Frontal lobe	27(47.37)
Temporal lobe	12 (21.05)
Occipital lobe	21 (36.84)
Parietal lobe	23(40.35)
Brain stem	4(7.02)
Cerebellum	18(31.58)
Others	16(28.07)
Number of metastatic sites (n)	
One site	28(49.12)
Two sites	13(22.81)
Three and more sites	16(28.07)

NSCLC = non-small-cell lung cancer.

**Table 3**  
**Correlation between diverse clinical factors and brain metastases.**

Factors	Brain metastases	Non-brain metastases	P
Age at diagnosis, mean ± SD, yr	62.68 ± 9.93	64.17 ± 9.63	.288
Gender			.869
Female	14	69	
Male	43	224	
Lesions diameter (cm)	5.44 ± 1.96	4.72 ± 2.11	.019
Mediastinal lymph node metastasis			.296
Yes	39	179	
No	18	114	
Histopathological types			.617
Adenocarcinoma	32	148	
Squamous cell carcinoma	22	133	
Adenosquamous carcinoma	2	6	
Large cell carcinoma	0	4	
Other	1	2	
Calcium (mmol/L)	2.36 ± 0.21	2.25 ± 0.18	.001
Hb (g/L)	123.05 ± 16.30	124.35 ± 17.88	.610
Fbg (g/L)	4.32 ± 1.40	4.53 ± 1.44	.295
APTT (s)	28.42 ± 2.43	28.84 ± 3.19	.343
ALP (U/L)	96.84 ± 24.56	93.29 ± 39.43	.513
CEA (ng/mL)	46.09 ± 97.68	23.50 ± 59.00	.020
CA125 (U/mL)	88.31 ± 90.97	61.97 ± 119.34	.115
CA199 (U/mL)	33.35 ± 109.41	39.96 ± 111.21	.727

Normal ranges: Calcium 2.11–2.52 mmol/L, HB 115–150 g/L, Fbg 1.8–3.5 g/L, APTT 25–31.3s, ALP 50–135 U/L, CEA 0–6.5 ng/mL, CA125 0–35 U/L, CA199 0–27 U/L.

95% CI: 1.015–1.328,  $P = .029$ ), calcium (OR 3.014, 95% CI: 4.031–102.988,  $P < .001$ ), CEA (OR 0.004, 95% CI (1.001–1.007,  $P = .020$ ) is an independent risk factor for brain metastases in patients with NSCLC (Table 4).

**3.5. Values of risk factors in predicting brain metastases**

Finally, we analyzed the accuracy, specificity, and sensitivity of lesion diameter, calcium concentration and CEA level. Figures 1–3 show the receiver operating characteristic (ROC) curves of these independent risk factors for predicting the risk of brain metastasis. These factors had comparable accuracy in predicting brain metastasis from RCC (the area under the curve [AUCs] of those factors were 0.620, 0.661, and 0.729, respectively). The cutoff values of those factors were 5.050 cm, 2.295 mmol/L, and 11.160 ng/mL. CEA had a higher specificity (70.3%) and had a higher sensitivity (73.7%) among these 3 factors. Additionally, when analyzing the independent or combined prognostic power of these factors, the combination of lesion diameter with calcium and CEA levels had the highest specificity (lesion diameter + calcium: 76.40%; lesion diameter + CEA: 65.5%; CEA + calcium: 63.0%; lesion diameter + calcium + CEA: 78.4%) compared to 1 single factor (Table 5).

**Table 4**  
**Multivariate logistic regression model analysis of the risk factors of brain metastasis from NSCLC.**

Factors	β	OR	OR(95% CI)	P
Lesions diameter (cm)	0.149	1.161	1.015–1.328	.029
Calcium (mmol/L)	3.014	20.374	4.031–102.988	<.001
CEA (ng/mL)	0.004	1.004	1.001–1.007	.020

β = coefficient regression, CI = confidence interval, NSCLC = non-small-cell lung cancer, OR = odds ratio, normal ranges: calcium 2.11–2.52 mmol/L, CEA 0–6.5 ng/mL.

**Table 5****Cutoff value, sensitivity, and specificity of lesion diameter, calcium concentration, and CEA for diagnosing brain metastases.**

Factors	Cutoff value	Sensitivity (%)	Specificity (%)	AUC	95% CI	P
Lesions diameter (cm)	5.050	0.596	0.631	0.620	0.544–0.696	.004
Ca <sup>2+</sup> (mmol/L)	2.295	0.649	0.592	0.661	0.582–0.741	<.001
CEA (ng/mL)	11.160	0.737	0.703	0.729	0.667–0.791	<.001
Lesions diameter + Ca <sup>2+</sup>		0.526	0.764	0.677	0.601–0.753	<.001
Lesions diameter + CEA		0.667	0.655	0.653	0.580–0.727	<.001
Calcium + CEA		0.649	0.630	0.674	0.594–0.754	<.001
Lesions diameter + Ca <sup>2+</sup> + CEA		0.509	0.784	0.690	0.615–0.765	<.001

AUC=area under curve, Ca<sup>2+</sup>=calcium concentration, CI=confidence interval, normal ranges: calcium 2.11–2.52 mmol/L, CEA 0–6.5 ng/mL.

#### 4. Discussion

Due to the non-specific symptoms of brain metastasis, the detection of brain metastasis is late, which is not conducive to early treatment and makes the disease difficult to control. Therefore, brain metastasis is a poor prognostic factor of non-small-cell lung cancer. In order to find high-risk patients with brain metastasis, to intervene in these patients as soon as possible and achieve better treatment results, it is necessary to find the risk factors for brain metastasis in patients with non-small-cell lung cancer which is the goal of our research.

In our study, we divided patients with or without brain metastasis into 2 different groups. Based on the results of some previous prospective studies, we selected possible risk factors (such as age, sex, primary lesion diameter, mediastinum involvement lymph node metastasis, and pathological category), and after univariate and multivariate analysis, it was found that tumor diameter, calcium, and CEA were significantly related to brain metastases. Since our study is a study on the screening of risk factors for brain metastases in NSCLC, some common laboratory indicators have also been included in our parameters, even though previous studies have not confirmed that these indicators are related to brain metastasis.

Brain metastasis is a major treatment barrier for many patients with lung cancer and other malignancies, once tumors invade the brain, current treatment strategies rarely affect the survival rate. Therefore, the timely discovery of effective and valuable

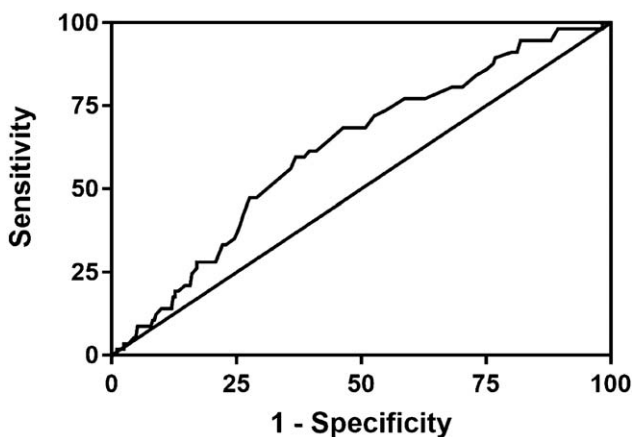
predictors is of great significance for timely intervention to prevent and delay brain metastases. Based on our research results, we compared the results of research related to our research direction.

Akinori Iwasaki et al<sup>[9]</sup> reported that the 3-year survival rate of patients with high CEA is 0%, and the 3-year survival rate of patients with normal CEA is 39.6%. So serum CEA has been confirmed as an independent risk factor for brain metastases by some researches.<sup>[10,11]</sup> Another study showed that CEA is expressed on cancer cells, particularly in non-squamous cell carcinomas and that elevated serum CEA levels can reliably reflect the metastatic trend throughout the body.<sup>[12]</sup> Therefore, serum CEA is an important predictor of brain metastasis. The relationship between elevated CEA levels and brain metastases may reflect tumor heterogeneity, but the exact mechanism is unclear. Further clinical trials and functional studies are needed to validate our findings.

Tumor diameter is another important predictor of brain metastasis. As we all know, tumor size is closely related to tumor staging, and the brain metastasis rate in the late stage of the tumor is indeed significantly higher than that in the early stage. Therefore, basically all studies on risk factors related to brain metastasis consider that tumor diameter is a predictor of a high risk of brain metastasis.<sup>[2–7,13]</sup>

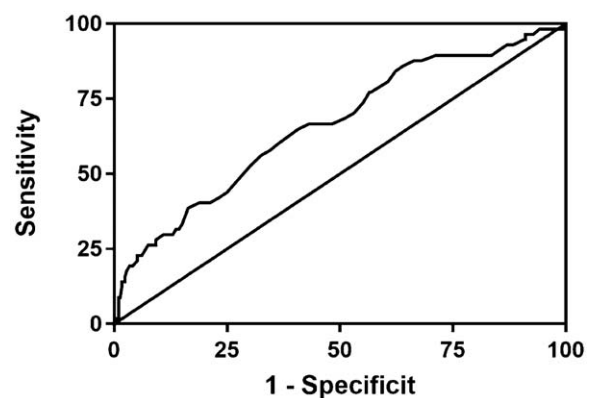
The last important predictor we analyzed was serum calcium concentration, which is rare in other reports of brain metastasis-related risk factors. In the univariate analysis and multivariate analysis of calcium in this study, calcium was significantly

#### ROC curve of tumor diameter



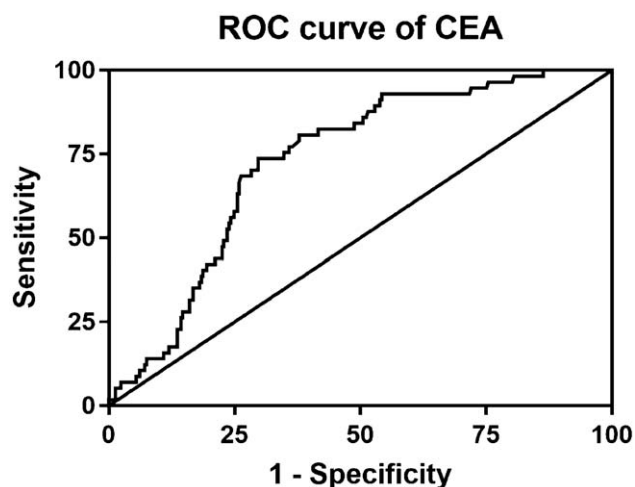
**Figure 1.** Receiver operating characteristic (ROC) curve demonstrated sensitivities and specificities of the lesion diameter for predicting the risk of brain metastasis.

#### ROC curve of blood calcium concentration



**Figure 2.** Receiver operating characteristic (ROC) curve demonstrated sensitivities and specificities of the concentrations of calcium for predicting the risk of brain metastasis.





**Figure 3.** Receiver operating characteristic (ROC) curve demonstrated sensitivities and specificities of the CEA level for predicting the risk of brain metastasis.

associated with a high risk of developing brain metastases ( $P = .001$ ,  $P < .001$ , respectively). Calcium ( $\text{Ca}^{2+}$ ) is a secondary messenger involved in a variety of cellular processes, which also means that calcium ions play an important role in tumor biology. It has been reported that  $\text{Ca}^{2+}$  channels are involved in the regulation of epithelial-to-mesenchymal transition (EMT), a process that is essential for cancer metastasis and also contributes to drug resistance, and the removal of calcium signals may be an approach to the treatment of metastatic cancer.<sup>[14]</sup> Due to the diverse pathophysiological mechanisms of calcium ions, the role of calcium channels in cancer development and progression remains largely unclear, although many studies have investigated this. A study has shown that voltage-gated calcium channels can be found in a variety of cancers, including lung and brain tumors. It may play a critical role in cancer biology and was proposed as a potential therapeutic target for these specific cancer subtypes.<sup>[15]</sup> Another study suggests that phosphatidyserine (PS) that normally exist in the inner surface of the cell membrane can become biomarkers of many cancers, including brain metastases, due to the exposure of cell carcinogenesis to the cell membrane through  $\text{Ca}^{+}$  influx.<sup>[16]</sup>

Some of our data also conflict with previous related research. For instance, many reports suggest that the younger the age of onset, the higher the probability of brain metastases, which may be related to the longer survival of young patients.<sup>[3-5,13,17]</sup> However, because our study did not analyze the interval between secondary brain metastases in NSCLC patients, the study results showed that there was no significant correlation between the age of onset and brain metastases. Similar to our results, a study has shown that the tumor pathological type is not an important factor in predicting brain metastasis,<sup>[13]</sup> and in some studies non-squamous cell carcinoma, especially adenocarcinoma, is associated with an increased risk of brain metastasis.<sup>[3,4,7,11,17]</sup> Overall, we found no significant correlation between age at onset, tumor pathological type, and mediastinal lymph node metastasis in association with brain metastases.

It is worth mentioning that we retrospectively analyzed 57 patients with brain metastases, which was confirmed by magnetic resonance imaging or computed tomography at the time of onset. The location of brain metastasis was analyzed, and it was found

that the probability of lesion in each lobe of the brain is roughly equal, but the probability of transfer to the brain stem is significantly lower, which improves the feasibility of local treatment. Meanwhile, it has been reported that the lack of cerebellar metastasis is an independent factor with a good prognosis.<sup>[18]</sup> Therefore, although NSCLC with brain metastasis is considered stage IV, some patients have better local control, so their prognosis is more favorable than that of others. For NSCLC patients with brain metastases, based on our results combined with the results of Akinori Iwasaki<sup>[9]</sup> and Rita Engenhardt,<sup>[19]</sup> we can choose those patients with stable vital signs, negative lymph nodes, normal calcium, CEA levels, small primary tumor lesions and only a single brain metastasis and try to remove lung and brain lesions, and whole-brain radiation therapy (WBRT). In addition, the current cutting-edge research shows that the combination of drugs targeting gene mutations in signal transduction pathways and drugs regulating tumor immunity are effective and less toxic treatments for patients with advanced BMs, and have broad prospects.<sup>[20,21]</sup>

There have been studies showing that prophylactic whole-brain radiation therapy can prolong the survival of patients at high risk of brain metastases,<sup>[10,13]</sup> but this treatment is often accompanied by neurotoxic effects,<sup>[17]</sup> so it is particularly important to choose those at high risk. Previous studies have shown that scoring the expression levels of 3 genes, CDH2 (N-cadherin), KIF1, and FALZ, can be a good predictor of brain metastases in lung cancer.<sup>[22]</sup> Moreover, we believe that even when genetic testing becomes widely available. Because of the simplicity and accessibility, the predictors in our study can be combined with genetic testing to better predict the risk of brain metastasis. If patients with a high risk of brain metastases can be reliably identified, this will help future research on preventive cranial irradiation (PCI) or other interventions. Our results did not have high specificity and sensitivity, and more reliable methods are needed to determine which patients are most likely to develop brain metastases. With the development of science and technology and the popularization of genetic analysis, we may be able to use gene sequencing to more accurately predict lung cancer patients at high risk of brain metastases.

In conclusion, the actuarial risk of brain metastases was 16.3% in this large group of patients with NSCLC. Although some epidemiological and clinical factors have been shown to be independently associated with an increased risk of central nervous system recurrence, a better understanding of the pathogenesis of brain metastases is needed to prospectively identify the highest risk subgroups. If this population can be identified, treatment rates for NSCLC patients will increase. Because of the current lack of effective treatment options for patients with brain metastases, with the improvement of local and systemic disease control, it is becoming increasingly important to reduce the risk of brain metastases in early and locally advanced diseases. Our study is a single-center retrospective study, and many variables cannot be controlled. The results we obtained need to be verified by large-sample prospective studies.

#### Author contributions

**Conceptualization:** Jinlin He, Rensen Xiao, Huiming Yao.

**Data curation:** Rensen Xiao, Huiming Yao.

**Formal analysis:** Jinlin He, Huiming Yao.

**Funding acquisition:** Wei Zhang, Huiming Yao.

**Investigation:** Jinlin He, Rensen Xiao, Huiming Yao.

**Methodology:** Huiming Yao.

**Project administration:** Rensen Xiao, Wei Zhang, Huiming Yao.

**Resources:** Rensen Xiao, Huiming Yao.

**Software:** Huiming Yao.

**Supervision:** Wei Zhang, Huiming Yao.

**Validation:** Huiming Yao.

**Visualization:** Wei Zuo, Wei Zhang, Huiming Yao.

**Writing – original draft:** Jinlin He, Xiaolei Wang, Wei Zuo, Wei Zhang, Huiming Yao.

**Writing – review & editing:** Jinlin He, Xiaolei Wang, Huiming Yao.

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