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# Over-expression of S100B protein as a serum marker of brain metastasis in non-small cell lung cancer and its prognostic value

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ABSTRACT

Validated serum biomarkers for patients suffering from non-small cell lung cancer (NSCLC) brain metastasis are urgently needed for early diagnosis, treatment monitoring, and prognostic classification in daily clinical practice. Serum S100B was reported to be a marker of leaky blood-brain barrier (BBB), which was often caused by brain tumors. This study aimed to investigate the role of S100B in NSCLC brain metastasis. The results showed that serum S100B correlated significantly with NSCLC brain metastasis ($P < 0.001$). When evaluated by the ROC curve, at the cutoff point 13.83 pg/ml, the sensitivity and specificity were 94% and 93%, respectively (AUC = 0.938, $P < 0.001$). High level of serum S100B was significantly correlated with a higher number of brain metastases and significantly worse prognosis ($P < 0.05$). In addition, S100B was an independent prognostic factor ($P < 0.001$). In conclusion, serum S100B was a sensitive and specific marker for early detection of brain metastasis in NSCLC and could be used as a surveillance tool for prognosis evaluation.

## 1. Introduction

The brain metastasis is common in patients with non-small cell lung cancer (NSCLC), occurring in about 24%–50% of patients [1–3]. The incidence of brain metastasis has increased as a result of advances in imaging and improved systemic control [4]. Brain metastases often lead to deterioration in neurologic and neurocognitive function [1], and are associated with significant morbidity [5], including a high risk of spontaneous hemorrhage [6]. Median overall survival (OS) of untreated patients with NSCLC brain metastasis is less than 2 months, which can extend to 16 months with radiosurgery, chemotherapy, bevacizumab, and/or Tyrosine kinase inhibitors [7]. Current National Comprehensive Cancer Network guidelines recommend routine brain magnetic resonance imaging (MRI) screening to detect brain metastases only in patients with stage II to IV NSCLC, regardless of high risks, high expenses, and inconvenience in longitudinal follow-up [8]. Thus, looking for biomarkers able to accurately characterize brain metastasis is the principle warrant in NSCLC management [9].

Brain metastasis is always associated with impaired blood-brain barrier (BBB). Leaky BBB may lead to extravasation of cells and molecules from the brain metastases to the peripheral blood [10]. S100B, which is an organotropic and promigratory molecule [11,12], is glial-specific and expressed primarily by astrocytes in the brain. It can also be found in peripheral nervous system, especially in the Schwann cells, and outside the nervous system in adipocytes, chondrocytes, as well as melanocyte [13,14]. Nevertheless, the levels of serum S100B would not increase on account of extracranial damage. It can only be released into the peripheral blood when the BBB is disrupted by some factors, such as growth of brain metastases or neovascularization [10].

Recently, the levels of serum S100B have been reported to elevate in patients with brain metastases in lung cancer [15,16], however, the predictive and prognostic power of S100B in brain metastasis is still unclear. The limitation of these studies were single center studies with a small sample size and without non-cancerous controls. Further multi-center-controlled trails are needed to confirm the role of S100B in NSCLC brain metastasis. The aim of our study was to identify whether the concentrations and integrity index of S100B in serum can be used as a diagnostic and prognostic biomarker for NSCLC patients with brain metastasis.

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## 2. Materials and methods

### 2.1. Specimen preparation

An informed consent was signed by every patient according to the Declaration of Helsinki and institutional review protocols at the Affiliated Cancer Hospital of Zhengzhou University and the First Affiliated Hospital of Zhengzhou University. 100 patients with NSCLC brain metastasis, 50 patients with stage IV NSCLC but without brain metastasis and 50 patients with cerebrovascular diseases were enrolled in this prospective study (100 patients from the Affiliated Cancer Hospital of Zhengzhou University and the others from the First Affiliated Hospital of Zhengzhou University). To be included in this study, patients with NSCLC should be newly diagnosed without anti-cancer therapy and without neurological symptoms. All patients were screened by whole-body computed-tomography (CT) and brain MRI. Patients enrolled in accordance with the following criteria: age between 18 and 70 years, therapy-naive, Eastern Cooperative Oncology Group performance status (ECOG PS) 0–1 and adequate organ function. Patients were excluded if they had a previous or secondary malignancy. Patients with cerebrovascular diseases including ischemic and hemorrhagic stroke, were used as a control group. Blood samples were obtained in accordance with the protocol, which was at the timepoints of 0 (before therapy) and disease progression. All participants in this study should avoid strenuous exercises at least 3 days before blood collection. Blood samples were collected in test tubes without gelation inhibitor and centrifuged immediately at 3500 rpm for 15 min at 4℃, after which the liquid component without sediment was stored at -70℃ until assay.

### 2.2. Routine brain MRI scans

Before this study, routine brain MRI scans including T1-weighted sequences with and without gadolinium (Gd-DTPA), T2-weighted and fluid-attenuated inversion recovery (FLAIR) images, were used to detect the vascular changes or metastatic lesions.

### 2.3. Routine CT scans

Whole body CT scans including the chest, abdomen and pelvis were used for diagnosis, effect evaluation, and exclusion of other combined diseases.

### 2.4. Enzyme-linked immunosorbent assay (ELISA)

High levels of S100B have shown to trigger a specific autoimmune cascade characterized by elevated titers of anti-S100B IgGs in the peripheral blood [15]. However, there has been only one clinical study about the role of S100B and S100B antibody in NSCLC brain metastasis to date. As a result, both serum S100B protein and anti-S100B immunoglobulin G (IgG) were measured by ELISA in all participants in this study. S100B protein was measured via a commercially available ELISA Kit (S100B Human ELISA kit, Abnova, Germany) according to the procedure [17–20]. The details of S100B ELISA can be found in the supplemental online methods (http://www.abnova.com/). Serum anti-S100B IgG was tested according to the ELISA assay procedure in Choi's study [15].

### 2.5. Statistical analysis

All statistical analyses were conducted using Statistical Analysis Software SPSS 19.0 at a nominal significance level of 0.05. The differences of S100B and anti-S100B IgG between groups were measured by Analysis of variance (ANOVA). Sensitivity and specificity were used to evaluate the predictive value of S100B for brain metastasis in NSCLC patients, and the cutoff value was determined by time-dependent receiver operating characteristic (ROC) analyses. The optimal cutoff point was determined by maximizing the sum of sensitivity and specificity corresponding to the observation furthest separated from the diagonal on the ROC curve. The progression of the disease in NSCLC patients was determined according to the criteria of Response Evaluation Criteria in Solid Tumors (RECIST version 1.1). The follow-up time was from 1 st December 2012 until death or the study closing date of 1 st December 2017. The progression-free survival (PFS) and overall survival (OS) were estimated by Kaplan-Meier analysis. Outcome events were defined as the progression of brain metastases in NSCLC and the death. The difference of the survival curve between S100B low and high groups was tested using Log-rank test. Hazards Ratio (HR) and 95% Confidence Interval (CI) were estimated by Cox regression model adjusting for age, gender, EGFR, and the stage of tumor, which was used to evaluate the association between S100B and risk of brain metastasis. In addition, Pearson chi-square tests or Fisher exact tests were used to assess the correlation between S100B and clinical variables.

## 3. Results

### 3.1. Clinical characteristics of the participants

The clinical characteristics of the patients with NSCLC are shown in Table 1. At the closing time of our study, 7 patients with NSCLC (4.67%) were still alive. Among the 100 NSCLC patients with brain metastasis (BM + ), 39 (39%) patients had 0–2 brain metastases and 61 (61%) patients had greater than or equal to 3 brain metastases. Compared to the NSCLC patients without brain metastasis (BM-), characteristics including age, gender, histology, targetable genomic alterations (GAs, including EGFR, KRAS, HER2 mutations, ALK or MET translocations) and organs with metastasis were similar ($P > 0.05$).

### 3.2. Elevated serum S100B and brain metastasis

The average levels of serum S100B in NSCLC with brain metastasis, NSCLC without brain metastasis, and patients with cerebrovascular diseases (CV), were 59.32 ± 11.62 pg/mL, 6.74 ± 3.29 pg/mL and 6.64 ± 3.39 pg/mL, respectively. Serum S100B was significantly elevated in NSCLC patients with brain metastasis, compared to NSCLC patients without brain metastasis and patients with cerebrovascular diseases ($P < 0.001$). However, there were no significant differences between NSCLC patients without brain metastasis and patients with cerebrovascular diseases ($P = 0.95$) (Fig. 1a).

**Table 1**
Clinical characteristics of NSCLC tissues.

| Characteristic | BM + N (%) | BM- N (%) | *P* |
|---|---|---|---|
| **Age** | | | 0.62 |
| ≥60 | 21(21%) | 11(22%) | |
| < 60 | 79(79%) | 39(78%) | |
| **Gender** | | | 0.58 |
| Female | 68(68%) | 40(80%) | |
| Male | 32(32%) | 10(20%) | |
| **Histology** | | | 0.80 |
| Adenocarcinoma | 68(68%) | 35(70%) | |
| Squamous carcinoma | 32(32%) | 15(30%) | |
| **Genomic alterations (GAs)** | | | 0.80 |
| GA + | 30(30%) | 14(28%) | |
| GA- | 70(70%) | 36(72%) | |
| **Organs with metastasis** | | | |
| Liver | 38(38%) | 21(42%) | 0.56 |
| Bone | 43(43%) | 29(58%) | 0.52 |

GA + patients include patients with targetable genomic alterations, such as EGFR, KRAS, HER2 mutations, ALK or MET translocations.

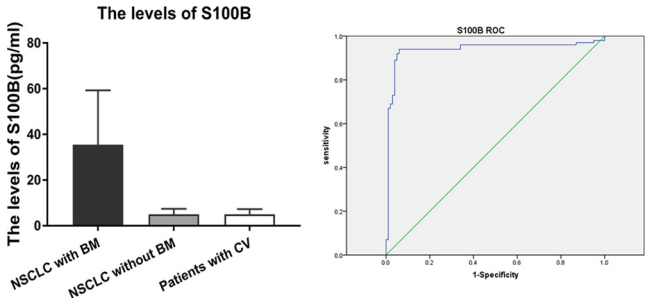


**Fig. 1.** a.The average levels of serum S100B. Serum S100B was significantly elevated in NSCLC patients with brain metastasis (BM), compared to NSCLC patients without BM and patients with cerebrovascular diseases (CV) ($p < 0.001$). b. ROC analysis curve of S100B. A ROC analysis curve of S100B for obviously discriminating between NSCLC patients with BM (blue line) and those without BM (including NSCLC patients and patients with CV) (green line as the control). AUC ($p < 0.001$), area under the curve is 0.938 (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

### 3.3. Serum S100B antibody and brain metastasis

The average levels of serum S-100 antibody in NSCLC with BM, NSCLC without BM, patients with CV, were 2.70 ± 1.71 pg/ml, 2.72 ± 2 pg/ml, and 3.29 ± 1.86 pg/ml, respectively. Serum S100B antibody was increased in patients with CV, but the difference did not reach statistical significance ($p > 0.05$). S100B antibody was not a serum marker of brain metastasis in NSCLC.

### 3.4. The cutoff value of S100B was determined by ROC analyses

A ROC curve was generated to evaluate the predictive value of S100B in NSCLC brain metastasis. As shown in Table 2 and Fig. 1b, when the level of S100B was 13.83 pg/ml, the sensitivity and specificity reached their peak value, were 94% and 93%, respectively (AUC = 0.938, $P < 0.001$).

### 3.5. S100B and prognosis of NSCLC patients with brain metastasis

Among the patients with brain metastasis, 97 patients (97%) had deceased until the time of analysis. The median PFS and OS were 10.08 months and 12.49 months, respectively. The 5-year survival probabilities were 3% in the NSCLC patients with brain metastasis. According to the median level of S100B, all patients of NSCLC with brain metastasis were divided into low and high expression groups. 50 cases were assigned to the low-S100B level group, while the other 50 cases were assigned to the high-S100B level group. The median PFS in low-S100B level group and that in high level group were 15.69 months (95%CI 13.75–17.65) Vs 4.56 months (95%CI 3.95–5.19, $P < 0.001$), respectively (Fig. 2a). The median OS in low-S100B level group and that in high level group were 18.75 months (95%CI 16.58–20.01) Vs 6.23 months (95%CI 5.60–6.88, $P < 0.001$), respectively (Fig. 2b). Kaplan-Meier survival analysis and log-rank test demonstrated that the S100B-high level group had significantly shorter OS as well as PFS than the S100B-low group (Fig. 2a and b).

**Table 2**
Sensitivity and specificity of S100B to detect brain metastasis.

| S100B(pg/ml) | Sensitivity (%) | Specificity (%) |
|---|---|---|
| 1.18 | 100 | 0 |
| 3.11 | 97 | 7 |
| 5.12 | 96 | 43 |
| 7.12 | 96 | 61 |
| 9.48 | 94 | 69 |
| 11.00 | 94 | 75 |
| 12.00 | 94 | 84 |
| 12.89 | 94 | 90 |
| 13.06 | 94 | 91 |
| 13.36 | 94 | 92 |
| 13.83 | 94 | 93 |

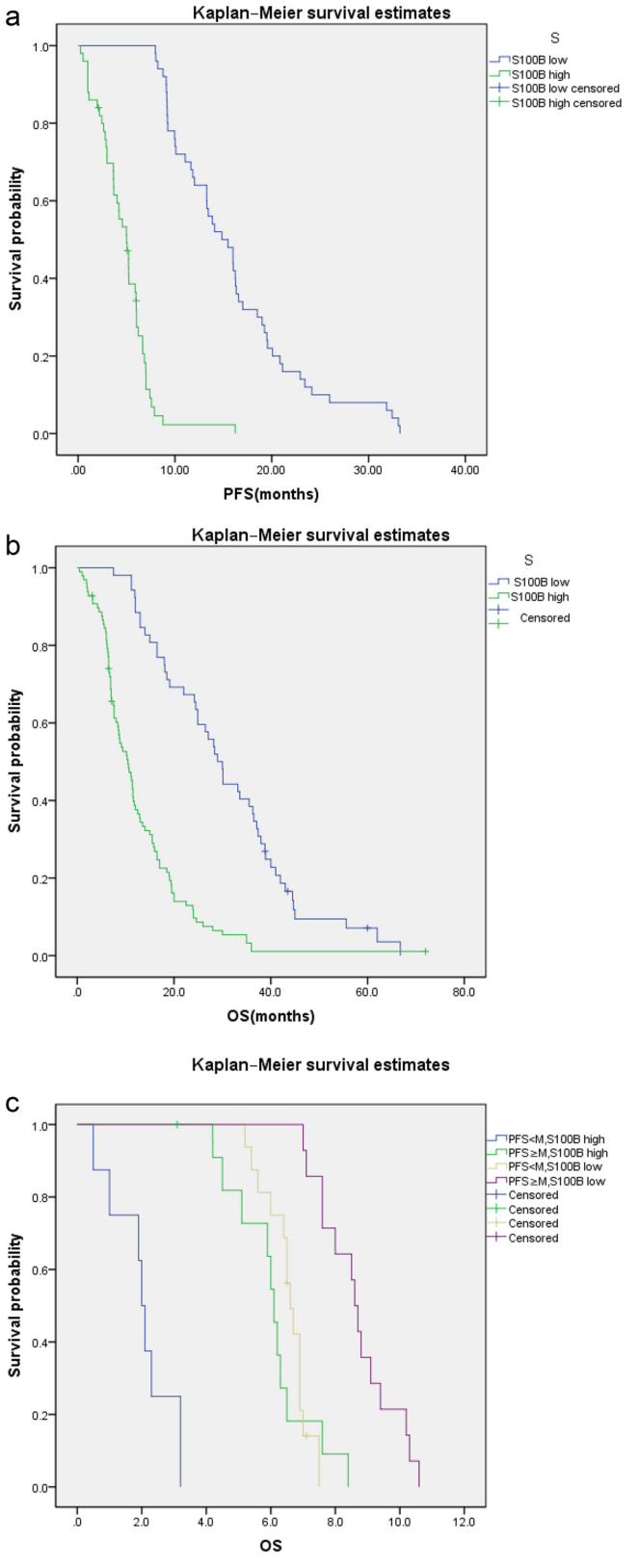


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**Fig. 2.** aThe PFS of patients with low-S100B and high-S100B. The PFS in low-S100B level group and that in high level group were 15.69 months Vs 4.56 months, respectively. Kaplan-Meier survival analysis and log-rank test demonstrated that the S100B-high level group had significantly shorter PFS than the S100B-low group ($p < 0.001$). b The OS of patients with low-S100B and high-S100B. The OS in low-S100B level group and that in high level group were 18.75 months Vs 6.23 months, respectively. Kaplan-Meier survival analysis and log-rank test demonstrated that the S100B-high level group had significantly shorter OS than the S100B-low group ($p < 0.001$). c Kaplan-Meier curves for OS with the influence of S100B levels and PFS. The median OS of PFS < M and high-S100B group was the shortest ($p < 0.001$), compared to other groups, and the median OS of PFS ≥ M and low-S100B groups was the longest ($p < 0.001$). The OS of PFS < M and low-level S100B group seemed to be slightly longer than that of PFS ≥ M and high-level S100B, but there was no significant difference between them($p = 0.068$).

The median PFS of patients with NSCLC brain metastasis was 10.08months (M = 10.08months). Based on the median levels of S100B, the patients with brain metastasis were divided into four subgroups with respect to their median PFS time: PFS < M, S100B high; PFS ≥ M, S100B high; PFS < M, S100B low; PFS ≥ M, S100B low. As shown in Fig. 2c, in the PFS < M group, the median OS of the group with high-level S100B was significantly shorter than that of the group with low-level S100B, in other words, 3.03 months versus 7.54 months, respectively ($P < 0.001$). In the PFS ≥ M group, the median OS of the group with high-level S100B was significantly shorter than that of the group with low-level S100B, in other words, 7.07 months versus 10.68 months, respectively ($P < 0.05$). In the S100B high-level groups, the median OS of the group with PFS < M was significantly shorter than that of the PFS ≥ M group, in other words, 3.03 months versus 7.07 months, respectively ($P < 0.001$). The OS of PFS ≥ M and high-level S100B group seemed to be slightly shorter than that of PFS < M and low-level S100B, but there was no significant difference between them ($P = 0.068$) (Fig. 2c).

### 3.6. Prognostic significance of S100B in NSCLC with brain metastasis

The high levels of serum S100B were associated with NSCLC brain metastasis. Compared with low-S100B patients with brain metastasis, the death risk of NSCLC patients with high S100B increased for 1.16 times (HR 1.16, 95%CI 1.01–1.33, $P = 0.03$) after adjusting for age, GAs, sex and tumor stage. S100B was an independent prognostic factor (Table3).

### 3.7. The correlation between S100B and clinical variables

According to the median level of S100B, all patients with NSCLC were divided into low and high expression groups. The relationship of the high-S100B percent with various general clinicopathologic characteristics of NSCLC is shown in Table 4. There were no significant correlations between S100B and histologic type of NSCLC, status of targetable GAs, T classification of tumor stage, lymph node involvement, liver metastasis and bone involvement, whereas high level of serum S100B was significantly correlated with brain metastasis and a higher number of brain metastases ($P < 0.001$).

**Table 3**
Levels of S100B and death risk of brain metastasis in NSCLC patients.

| Clinicopathological characteristics | HR | 95%CI | P-value |
|---|---|---|---|
| S100B | 1.16 | 1.01-1.33 | 0.03 |
| Age | 0.95 | 0.80-1.12 | 0.56 |
| GAs | 1.46 | 0.66-3.24 | 0.35 |
| Sex | 0.75 | 0.42-3.14 | 0.97 |
| Stage | 2.54 | 0.01-4.86 | 0.95 |

**Table 4**
Relationships between levels of serum S100B and clinical variables.

| Characteristics | Low S100B N (%) | High S100B N (%) | *P* |
|---|---|---|---|
| **T classification** | | | 0.74 |
| T1, T2 | 25(36.8%) | 43(63.2%) | |
| T3, T4 | 28(34.1%) | 54(65.9%) | |
| **Lymph node metastasis** | | | 0.30 |
| Negative | 26(40%) | 39(60%) | |
| Positive | 27(31.8%) | 58(68.2%) | |
| **Organs with metastasis** | | | |
| Liver | 21(35.6%) | 38(64.4%) | 0.96 |
| Bone | 29(40.3%) | 43(59.7%) | 0.22 |
| Brain | 6(6%) | 94(94%) | < 0.001 |
| **Genomic alterations** | | | 0.59 |
| Negative | 36(34.0%) | 70(66.0%) | |
| Positive | 17(38.6%) | 27(61.4%) | |
| **Histology** | | | 0.61 |
| Adenocarcinoma | 35(34%) | 68(66%) | |
| Squamous carcinoma | 18(38.3%) | 29(61.7%) | |
| **Numbers of brain metastases** | | | < 0.001 |
| 0 | 47(94%) | 3(6%) | |
| 1-2 | 0(0) | 39(100%) | |
| ≥3 | 6(9.8) | 55(90.2) | |

## 4. Discussion

Brain metastasis is one of the most important causes of morbidity and mortality in NSCLC. The identification of brain metastases in these patients has important implications for determining their treatment and prognosis [21]. BBB, which can prevent the therapeutic drugs into the brain, is a shelter for cancer cells, and thus makes the therapy of brain metastases a huge challenge. Astrocytic proteins such as S100B are synthesized in the brain and released from the brain to the capillaries nearby, but it is thought that they extravasate into the plasma only when the BBB is breached [22–26]. Many neurologic lesions and disorders are related to BBB disruption, such as primary and metastatic brain tumors, cerebrovascular diseases, and infection [27]. Contrast-enhancement MRI is an invaluable tool for detecting lesions that have a disrupted BBB. However, there are many controversies about the role of surveillance MRI in asymptomatic patients, because of high costs associated with longitudinal follow-up, difficulty in discriminating tiny lesions and long-term psychological impacts on patients [28,29]. More and more doctors and patients opt for serum markers of brain metastasis. Our study was prompted by the desire to discover unique patterns of serum proteins from NSCLC patients with brain metastasis, and to find a sufficiently sensitive or specific biomarker to warrant widespread clinical use as an alternative or supplement to MRI.

In humans, increasing preclinical studies have proved that S100B could promote cancer cell proliferation, metastasis and angiogenesis [30–35]. S100B is primarily synthesized in the brain by the end feet process of the astrocytes and releases quickly from the brain to the peripheral blood, when the BBB is impaired [36–40]. Choi reported that serum S100B combined with S100B antibody were able to detect brain metastases from lung cancer with high sensitivity and reasonable specificity in the patients from the USA [15]. However, our study demonstrated that levels of serum S100B antibody were not elevated in Chinese patients with brain metastasis, and serum S100B antibody was not a specific tumor marker. The probable reason might be that S100B antibody was unstable in the serum, or the ethnic differences.

Our present study was a multicenter-controlled trail to investigate the role of S100B in Chinese patients with NSCLC brain metastasis. NSCLC patients without brain metastasis and patients with cerebrovascular diseases were set up as control groups, which made the results much more convincing. Some studies reported that the levels of serum S100B could slightly increase in the course of playing football and other high cardiovascular output activities [39], thus strong

activities were prohibited within 3 days before blood-collection in this prospective study. CT scans of whole body were used to exclude other interference factors. Serum S100B was significantly elevated in NSCLC patients with brain metastasis, compared to NSCLC patients without brain metastasis and patients with cerebrovascular diseases, however, there were no significant differences between NSCLC patients without brain metastasis and patients with cerebrovascular diseases ($P > 0.05$). These results implied that S100B was not only a marker of BBB integrity, but also a specific tumor marker of NSCLC brain metastasis. When evaluated by the ROC curve, at the cutoff point 13.83 pg/ml, the maximizing the sum of sensitivity and specificity was the optimal level to detect brain metastasis in NSCLC. Serum S100B is an attractive biomarker for clinical use to detect brain metastasis in NSCLC at early stage. Impaired BBB or growth of brain metastases may be the reason of elevated serum S100B.

Early identification of brain metastasis by serum S100B before neurologic symptoms or tumor progression, provides patients with a wider set of treatment options and a better prognosis. High-risk patients of brain metastasis with high levels of S100B should be closely followed by surveillance MRI, even if at early cancer stages. Serum S100B, as an inexpensive, non-radiologic, and non-invasive tool is a candidate biomarker for NSCLC brain metastasis with high sensitivity and specificity in Chinese patients.

The median PFS and OS of the NSCLC patients with brain metastasis were 10.08 months and 12.49 months, respectively. These findings were similar with previous studies. High levels of serum S100B were associated with a significantly worse prognosis in this study. Nowadays, staging and PFS are used for prognosis evaluation in NSCLC patients with brain metastasis. From this study, combination of serum S100B and PFS could more accurately evaluate the prognosis of NSCLC patients with brain metastasis. S100B was an independent prognostic factor for NSCLC patients with brain metastasis.

S100B was not correlated with histological types of NSCLC, status of targetable GAs, primary tumor size, lymph node involvement, and distant bone or liver metastasis.

S100B is a biomarker of brain metastasis. High level of serum S100B could indicate brain metastasis or a higher number of brain metastases.

In summary, serum S100B was a sensitive and specific biomarker for early detection of brain metastasis in NSCLC and could assist clinicians in stratifying the high-risk patients. High levels of S100B were associated with poor prognosis in NSCLC patients with brain metastasis, and it was an independent prognostic factor. Serum S100B could be used as a surveillance tool for prognosis evaluation.

## Conflicts of interest

The authors declare no conflicts of interest.

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