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ORIGINAL ARTICLE

Clinicopathological and prognostic significance of Nestin expression in patients with non-small cell lung cancer: a systematic review and meta-analysis

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Abstract Latest evidence indicates that Nestin expression may be associated with the high malignancy and poor prognosis of non-small cell lung cancer (NSCLC), but a relevant consensus has not been reached until now. Therefore, we conducted this meta-analysis to evaluate the clinicopathological and prognostic significance of Nestin expression in patients with NSCLC. We searched PubMed, EMBASE and the Web of Science for eligible full-text articles. Odds ratio (OR) and hazard ratio (HR) with 95 % confidence interval (95 % CI) severed as the summarized statistics. Q-test and I^2-statistic were applied to evaluate the heterogeneity, and sensitivity analysis was conducted for adjustments. Publication bias was detected by Begg's test and Egger's test. Finally, eight eligible articles with 834 NSCLC cases were included. Nestin expression was found to be significantly associated with the unfavorable outcomes of differentiation degree (OR: 2.47; 95 % CI 1.61–3.79; $P < 0.001$), lymphatic metastasis (OR: 2.45; 95 % CI 1.41–4.25; $P = 0.001$), TNM stage (OR: 1.73; 95 % CI 1.07–2.79; $P = 0.025$) and tumor size (OR: 2.68; 95 % CI 1.20–5.98; $P = 0.016$), but not associated with gender, age, smoking status and NSCLC subtypes. Nestin expression could significantly predict the lower overall survival of NSCLC (HR: 2.41; 95 % CI 1.72–3.38; $P < 0.001$). The prognostic value of Nestin remained statistically reliable in the subgroups stratified by statistical analysis, patients' origins and follow-up periods, but not significant in patients with squamous cell carcinoma. In conclusion, Nestin expression may be an independent predictor for the poor prognosis and clinicopathological characteristics of NSCLC. Further studies are necessary to validate our discoveries.

Keywords Nestin · Non-small cell lung cancer · Prognosis · Systematic review · Meta-analysis

Introduction

Lung cancer is the leading cause of malignancy-related deaths around the world, and non-small cell lung cancer (NSCLC) accounts for more than 85 % of all cases [1, 2]. The morbidity and mortality rates of both male and female NSCLC patients have rapidly increased during the last decade, especially in heavily smoking peoples. According to authoritative estimations, the overall 5-year survival rate of NSCLC patients approximates 15 %, indicating the present poor prognosis [3, 4]. However, the specific 5-year survival rate in patients with early stage NSCLC can be more than 80 % [5]. It has been commonly recognized that advanced stage, early metastasis and poor response to treatments can result in the poor prognosis of NSCLC. Regarding the current diagnostic and therapeutic regimens for NSCLC, identifying novel biomarkers efficiently predicting the clinicopathological and prognostic characteristics of NSCLC has been urgently required.

Recently, oncologists have increasingly focused on the biological functions of cancer stem cells (CSCs) in malignancy-related diseases [6]. As an extensively studied component of class VI intermediate filament protein (IFP), Nestin is mainly expressed in neural stem cells and has

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been demonstrated to be a putative marker for CSCs [7, 8]. New evidence from laboratorial researches has revealed a potentially crucial role of Nestin in regulating malignant cell proliferation, differentiation and survival [9, 10]. The latest clinical reports also indicate that Nestin expression may be associated with the increased risk of high malignancy and poor prognosis in many cancers, including NSCLC [11–21]. A recent evidence-based review has concluded that Nestin expression may be significantly correlated with the advanced stages and lymphatic metastasis of various cancers [22]. However, the integrated details for the prognosis and some other clinicopathological parameters of NSCLC are not systematically described in this study. The prognostic roles of Nestin in NSCLC and its relationship to the clinicopathological characteristics have not reached a consensus until now [11–18]. Some controversial results reported in previous studies have not yet been well explained.

Meta-analysis is regarded as a well-established statistical method quantitatively pooling the homogeneous evidences to formulate a global conclusion. By applying this evidence-based method to a large number of enrolled samples, the pooled data may help to clarify some pending issues [22–24]. Therefore, we conducted this systematic meta-analysis to evaluate the clinicopathological and prognostic significance of Nestin expression in patients with NSCLC.

Materials and methods

Protocol

No protocol had been previously published for this review. Systematic reviews and meta-analyses do not require necessary patients' consent or ethical approval. We conducted this systematic meta-analysis in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [25]. The PRISMA 2009 checklist is given in the *Online Resource 1*.

Search strategy

The literature retrieval of our meta-analysis ranged January 4, 2015–January 8, 2015. We searched three universal electronic databases, including PubMed, EMBASE (via Ovid interface) and the Web of Science (via the campus network of Sichuan University), to identify the eligible full-text papers published up to January 4, 2015. We combined several keywords with two Boolean operators ("AND" and "OR") to formulate the comprehensive search strings. The keywords are listed as follows: (I) "Nestin"; (II) "lung cancer," "lung carcinoma," "lung neoplasm" and "lung tumor"; (III) "pulmonary cancer," "pulmonary carcinoma," "pulmonary neoplasm" and "pulmonary tumor." Meanwhile, the reference lists in retrieved papers were also manually searched for additional studies with no duplication. The details of search strings in each database are listed in the *Online Resource 2*.

Inclusion and exclusion criteria

The following inclusion and exclusion criteria were imposed to determine the included studies of our meta-analysis.

Inclusion criteria (I) the target disease is NSCLC; (II) the expression level of Nestin is analyzed independently, rather than in company with other biomarkers; (III) the demographics or statistics assessing the relationship between Nestin expression and clinicopathological characteristics of NSCLC are available; (IV) the statistics revealing the prognostic significance of Nestin expression in NSCLC, either from multivariate analysis or univariate analysis, are reported; (V) the survival events or Kaplan–Meier survival curves (K–M curves) with P value from log-rank test are published; (VI) the overall survival (OS) serves as the summarized endpoint.

Exclusion criteria (I) the following articles are immediately excluded: reviews, preclinical experiments, letters and conference abstracts; (II) the expression of Nestin is uncertain; (III) the continuous variables are not considered.

Quality assessment

Newcastle–Ottawa Scale (NOS) was used to evaluate the quality level of original non-randomized studies [26]. Three perspectives involving selection, comparability and exposure were considered for a semiquantitative estimation. The "star system" with a maximum of nine stars was employed as the assessment tool. After grading all of the included studies, we regarded 8–9 stars as a good quality, 6–7 stars as a fair quality and lower than six stars as a poor quality.

Data collection

We designed a Microsoft Excel sheet to collect the following information: (I) publication data including authors and publication years; (II) experimental data including study design, study period, patients' origins, investigating categories, experimental materials, detecting methods and sites, cutoff values, endpoints and follow-ups; (III) demographic data including enrolled samples, the number of patients with positive and negative expression of Nestin, the number of squamous cell carcinoma (SCC) and adenocarcinoma (AC) cases; (IV) statistical data

including summarized statistics with their sources, and statistical analysis methods (including multivariate analysis and univariate analysis).

Statistical analysis

To assess the relationship between Nestin expression and clinicopathological characteristics of NSCLC, odds ratio (OR) with 95 % confidence interval (CI) was determined as the appropriate summarized statistics. OR could be extrapolated by demographics or relevant statistics published in original articles. In addition, if relative risk (RR) or hazard ratio (HR) was reported, they could be directly incorporated into the meta-analysis [27].

To assess the prognostic value of Nestin expression in NSCLC, HR with 95 % CI served as the appropriate summarized statistics. HR is generally considered as the only statistic compatible for both censoring and time to events [28]. Incorporating the multivariate HR outcomes without the bias risks from other confounding factors was our first priority. Moreover, if RR was reported from multivariate analysis, it could be regarded as HR and included into quantitative synthesis [27]. If multivariate analysis was not performed in original studies, we extrapolated the HR with 95 % CI by reported demographics according to a practical method described by Tierney et al. [29] and then incorporated them into our meta-analysis. The relevant formulas are listed as follows:

$$O - E = \frac{\sqrt{\text{Total observed events} \times \text{Analyzed research} \times \text{Analyzed control}}}{(\text{Analyzed research} + \text{Analyzed control})} \times (Z \text{ score for } P \text{ value}/2)$$

$$V = \frac{\text{Total observed events} \times \text{Analyzed research} \times \text{Analyzed control}}{(\text{Analyzed research} + \text{Analyzed control})^2}$$

$$\text{HR} = \text{Exp}\left(-\frac{O - E}{V}\right)$$

where O − E is the log-rank observed minus expected events and V is the log-rank variance [29]. If necessary, we also extracted the survival details by Engauge Digitizer 4.1 (http://sourceforge.net) from the K–M curves published in original articles to measure the accuracy of estimated HRs.

Q-test and I^2-statistic served to evaluate the level of heterogeneity within our meta-analysis. Fine heterogeneity was defined as $I^2 < 50$ % and $P > 0.1$, and a standard fixed-effect model (Mantel–Haenszel method) would be supplied to integrate the ORs or HRs. Otherwise, if significant heterogeneity was revealed by $I^2 \geq 50$ % or $P \leq 0.1$, a random effect model (DerSimonian and Laird method) would be determined [30].

We performed an additional sensitivity analysis to further examine the robustness of our meta-analysis. We removed the study which might contributed to the significant heterogeneity and repeated a pooled analysis of the remaining studies for adjustments. The stability of our meta-analysis would be affirmed if there was no substantial variation between the adjusted summarized outcomes and primary summarized outcomes [31].

Begg's test and Egger's test were commonly used to detect the potential publication bias in our meta-analysis. The presence of publication bias was suggested by the symmetry of funnel plot conducted from Begg's test, in which log ORs or log HRs were plotted against their corresponding standard errors (SEs) [32]. The significant bias was also revealed when Egger's P value < 0.05.

Finally, we declared that all of above statistical analyses were accomplished by STATA 12.0 (STATA Corporation, College Station, TX).

Results

The selection of included studies

Complete procedure for the literature retrieval was displayed as a PRISMA diagram (Fig. 1). The primary retrieval identified a total of 260 citations by searching through three electronic databases, including 90 citations in PubMed, 60 citations in EMBASE and 110 citations in the Web of Science. After removing 144 duplicates, the remaining 116 publications received initial filtration by screening titles and abstracts. Then, 70 of them were directly excluded because of the irrelevant article styles, including 15 reviews, 42 experimental studies and 13 conference abstracts. The further filtration was based on reading through the full text of the remaining 46 studies. After excluding 37 irrelevant studies, nine articles were identified for possible eligibility of qualitative synthesis. For quantitative synthesis, we further excluded one of them because only continuous variables correlated with Nestin

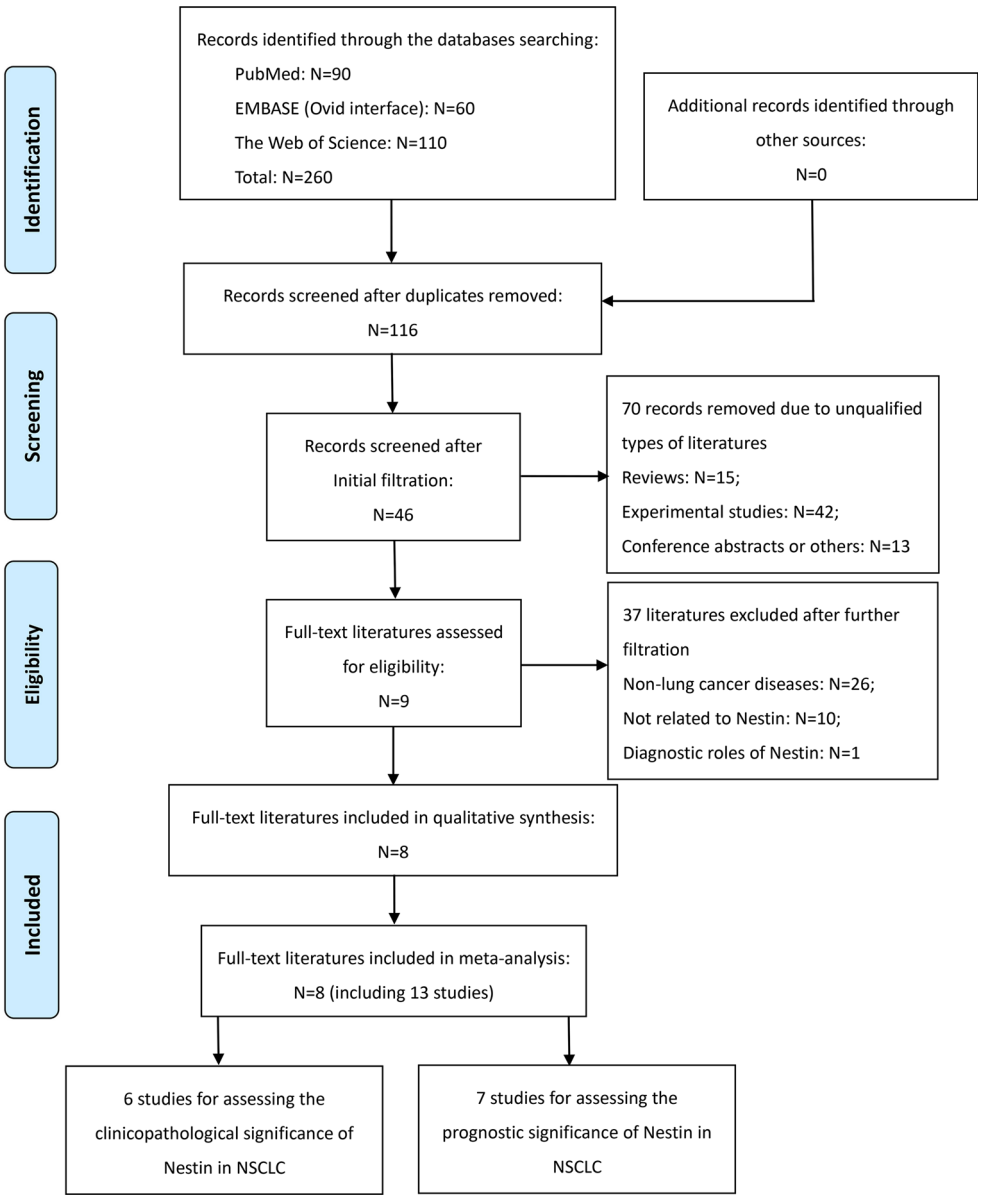

Fig. 1 PRISMA flow diagram of the literature retrieval. *NSCLC* non-small cell lung cancer, *PRISMA* preferred reporting items for systematic reviews and meta-analyses

expression in NSCLC were reported in the results [33]. Therefore, eight articles met all of the eligibility criteria and were finally included into our meta-analysis [11–18].

The quality level of included studies

After grading all of the included studies, the mean NOS score was 7.75 (ranged from 7 to 8), suggesting a generally good quality level of our meta-analysis. The complete details of estimations are listed in Table S1 (see *Online Resource 3*).

The basic characteristics of included studies

Baseline characteristics of eight eligible papers are summarized in Table 1. These articles actually reported 13 retrospective observational studies published between 2010 and 2014. Among them, six studies from six papers [11–13, 15–17] investigated the relationship between Nestin expression and major clinicopathological characteristics of NSCLC, and seven studies from seven papers [11, 13–18] evaluated the prognostic roles of Nestin expression in NSCLC. Sterlacci et al. [18] collected the clinical data of 371 NSCLC cases from Austria and performed a survival analysis for 106 evaluable SCC cases and 189 evaluable AC cases, respectively. However, only the survival data of some significant variables were validly reported in this study because of the multitude of evaluated biomarkers. A K–M curve comparing Nestin expression in 189 evaluable AC cases was finally published, but the relevant details of SCC cases were not shown. Moreover, various

Table 1 Baseline characteristics of the included studies

References	Patients' origins	Study design	Study period	Categories		NOS	No. samples			Stage	Histology		
				CP features	Prognosis		Total	PE	NE		SCC	AC	Others
Chen et al. [11]	China	ROS	2003–2004	✔	✔	8	71	35	36	I–IV	34	35	2
Chen et al. [12]	China	ROS	2003–2004	✔	✗	7	52	25	27	I–IV	25	26	1
Narita et al. [13]	Japan	ROS	1994–2001	✔	✔	8	95	45	50	I–III	47	48	–
Janikova et al. [14]	Czech	ROS	1996–2000	✗	✔	8	112	74	38	NI	82	39	–
Ryuge et al. [15]	Japan	ROS	2002–2004	✔	✔	8	171	27	144	I–III	31	131	9
Ryuge et al. [16]	Japan	ROS	1999–2009	✔	✔	8	30	8	22	I–III	–	–	30
Skarda et al. [17]	Czech	ROS	NI	✔	✔	7	114	40	74	I–IV	78	36	–
Sterlacci et al. [18]	Austria	ROS	1992–2004	✔	✔	8	189	57	132	I–IV	–	189	–

References	Material	Detecting method	Cutoff value	Positive site	Outcomes	Sources	Statistical analysis	Endpoints	Follow-up (months)
Chen et al. [11]	Paraffin-embedded tissue	IHC	50 % staining	Nuclei	OR, HR	DE, reported	U and M	OS	60
Chen et al. [12]	Paraffin-embedded tissue	IHC	50 % staining	Nuclei	OR	DE	U	–	–
Narita et al. [13]	Paraffin-embedded tissue	IHC	30 % staining	Cytoplasm	OR, HR	DE	U	OS	100
Janikova et al. [14]	Paraffin-embedded tissue	IHC	10 % staining	Nuclei	HR	DE	U	OS, DFS	144
Ryuge et al. [15]	Paraffin-embedded tissue	IHC	5 % staining	Cytoplasm	OR, HR	DE, reported	U and M	OS	52
Ryuge et al. [16]	Paraffin-embedded tissue	IHC	5 % staining	Cytoplasm	OR, HR	DE, reported	U	OS	124
Skarda et al. [17]	Paraffin-embedded tissue	IHC	*H*-score[a]	Cytoplasm	OR, HR	DE	U	OS, DFS	64
Sterlacci et al. [18]	Paraffin-embedded tissue	IHC	50 % staining	Cytoplasm	HR	DE	U and M	OS	180

[a] The *H*-score was used in Refs. [17] to determine the Nestin positivity (0—none, 1—weak, 2—moderate and 3—strong)

AC adenocarcinoma, *CP* clinicopathological, *DE* demographics extrapolated, *DFS* disease-free survival, *HR* hazard ratio, *IHC* immunohistochemistry, *M* multivariate, *NE* negative expression, *NI* no information, *OR* odds ratio, *OS* overall survival, *PE* positive expression, *ROS* retrospective observational study, *U* univariate

clinicopathological parameters of NSCLC were also involved but not comprehensively reported in this study [18]. Given such concerns, a total of 834 NSCLC patients were ultimately enrolled in the quantitative synthesis, including 504 cases with ACs, 297 cases with SCCs and 42 cases with other NSCLCs. More than half of the enrolled patients were from Asian countries (419/834, ratio = 50.2 %), including 296 patients from Japan [13, 15, 16] and 123 patients from China [11, 12]. The remaining 415 patients came from European countries (415/834, ratio = 49.8 %), including 226 patients from Czech [14, 17] and 189 AC cases from Austria [18]. All of the included studies commonly used immunohistochemistry (IHC) to detect Nestin expression in paraffin-embedded specimens [11–18]. However, the cutoff definitions and positive-stained sites varied largely across these studies (Table 1). Positive Nestin expression was identified in 311 patients, with the positive ratio of 37.3 % (311/834). Meanwhile, the maximum follow-up periods in these studies ranged from 52 to 180 months, and OS was defined as the major endpoint (Table 1).

The statistical characteristics of included studies

To assess the relationship between Nestin expression and clinicopathological characteristics of NSCLC, six included studies (from six articles) reported the demographic data, but none of them published any statistical result from multivariate analysis [11–13, 15–17]. Therefore, all of the estimated ORs were extrapolated by reported demographics, which were based on the univariate analysis (Table 1).

To assess the prognostic value of Nestin expression in patients with NSCLC, only two studies [11, 15] reported the HR with 95 % CI conducted from multivariate analysis,

which adequately eliminated the bias risks from other confounders. Sterlacci et al. [18] performed both univariate analysis and multivariate analysis to determine the prognostic significance of Nestin expression in AC, but did not report any statistic derived from multivariate analysis. One study reported by Ryuge et al. [16] published the univariate HR with 95 % CI and the remaining studies [13, 14, 17] just provided the K–M curves with corresponding log-rank *P* value. Therefore, the majority of estimated HRs were extrapolated by survival demographics with log-rank *P* value published in the included studies, which were originated from the univariate analysis [13, 14, 16–18].

Association between Nestin expression and clinicopathological characteristics of NSCLC

Three clinical parameters of NSCLC considered for assessments included gender, age and smoking status. Five pathological parameters of NSCLC considered for assessments included differentiation, lymphatic metastasis, TNM stage, histological subtypes and tumor size.

On the one hand, the pooled ORs with low heterogeneity indicated that Nestin expression was significantly associated with some unfavorable pathological features, including differentiation degree (OR: 2.47; 95 % CI 1.61–3.79; $P < 0.001$) (Table 2; Fig. 2a), lymphatic metastasis (OR: 2.45; 95 % CI 1.41–4.25; $P = 0.001$; Table 2; Fig. 2b), TNM stage (OR: 1.73; 95 % CI 1.07–2.79; $P = 0.025$; Table 2; Fig. 2c) and tumor size (OR: 2.68; 95 % CI 1.20–5.98; $P = 0.016$; Table 2; Fig. 2e). But no evidence revealing any significant association between Nestin expression and histological subtypes of NSCLC was observed among the included studies (OR: 1.87; 95 % CI 0.46–7.60; $P = 0.383$; Table 2; Fig. 2d), with high level of heterogeneity ($I^2 = 91.1$ %, $P < 0.001$).

On the other hand, the pooled ORs revealed no significant relationship between Nestin expression and the clinical variables of NSCLC, including gender (OR: 0.92; 95 % CI 0.59–1.44; $P = 0.726$; Table 2; Fig. 3a), age (OR: 1.49; 95 % CI 0.86–2.60; $P = 0.156$; Table 2; Fig. 3b) and smoking status (OR: 1.33; 95 % CI 0.75–2.36; $P = 0.328$; Table 2; Fig. 3c).

Association between Nestin expression and prognosis of NSCLC

In overall analysis, the summarized HR integrating the appropriate data from seven included studies [11, 13–18] was 2.41 (95 % CI 1.72–3.38; $P < 0.001$; Table 3; Fig. 4), suggesting that positive Nestin expression could significantly predict the lower OS in patients with NSCLC, without any heterogeneity ($I^2 = 0.0$ %, $P = 0.736$).

To further investigate the prognostic roles of Nestin in detail, we classified all cases into several subgroups according to the statistical analysis, patients' origins, histological subtypes and follow-up periods, and performed a subgroup analysis.

In the subgroups stratified by statistical analysis, two included studies reported the HR outcomes from multivariate analysis [11, 15]. The univariate HRs could be

Table 2 Meta-analysis of the association between Nestin expression and clinicopathological characteristics of NSCLC

Characteristics	*N*	No. samples			Heterogeneity (I^2, *P*)	Model	OR (95 %CI)	*P* value	Publication bias		Conclusion
		Total	PE	NE					Egger (*P*)	Begg (*P*)	
Gender (male vs. female)	6	533	180	353	14.8 %, 0.319	Fixed	0.92 (0.59–1.44)	0.726	0.124	0.133	Not significant
Age (≥65 vs. <65 years)	3	296	80	216	0.0 %, 0.889	Fixed	1.49 (0.86–2.60)	0.156	0.517	1.0	Not significant
Smoking (Yes vs. No)	4	324	95	229	41.9 %, 0.160	Fixed	1.33 (0.75–2.36)	0.328	0.940	0.734	Not significant
Differentiation (G3 vs. G1 and G2)	5	503	172	331	39.5 %, 0.158	Fixed	2.47 (1.61–3.79)	<0.001	0.083	0.086	Significant
Lymphatic metastasis (Yes vs. No)	4	348	105	243	49.4 %, 0.115	Fixed	2.45 (1.41–4.25)	0.001	0.057	0.089	Significant
TNM stage (III/IV vs. I/II)	5	419	140	279	0.0 %, 0.498	Fixed	1.73 (1.07–2.79)	0.025	0.323	0.462	Significant
Histology (AC vs. SCC)	5	503	172	331	91.1 %, <0.001	Random	1.87 (0.46–7.60)	0.383	0.234	0.462	Not significant
Tumor size (>3 vs. ≤3 cm)	2	125	53	72	0.0 %, 0.593	Fixed	2.68 (1.20–5.98)	0.016	NI	1.0	Significant

AC adenocarcinoma, *CI* confidence interval, *NE* negative expression, *NI* no information, *NSCLC* non-small cell lung cancer, *OR* odds ratio, *PE* positive expression, *SCC* squamous cell carcinoma

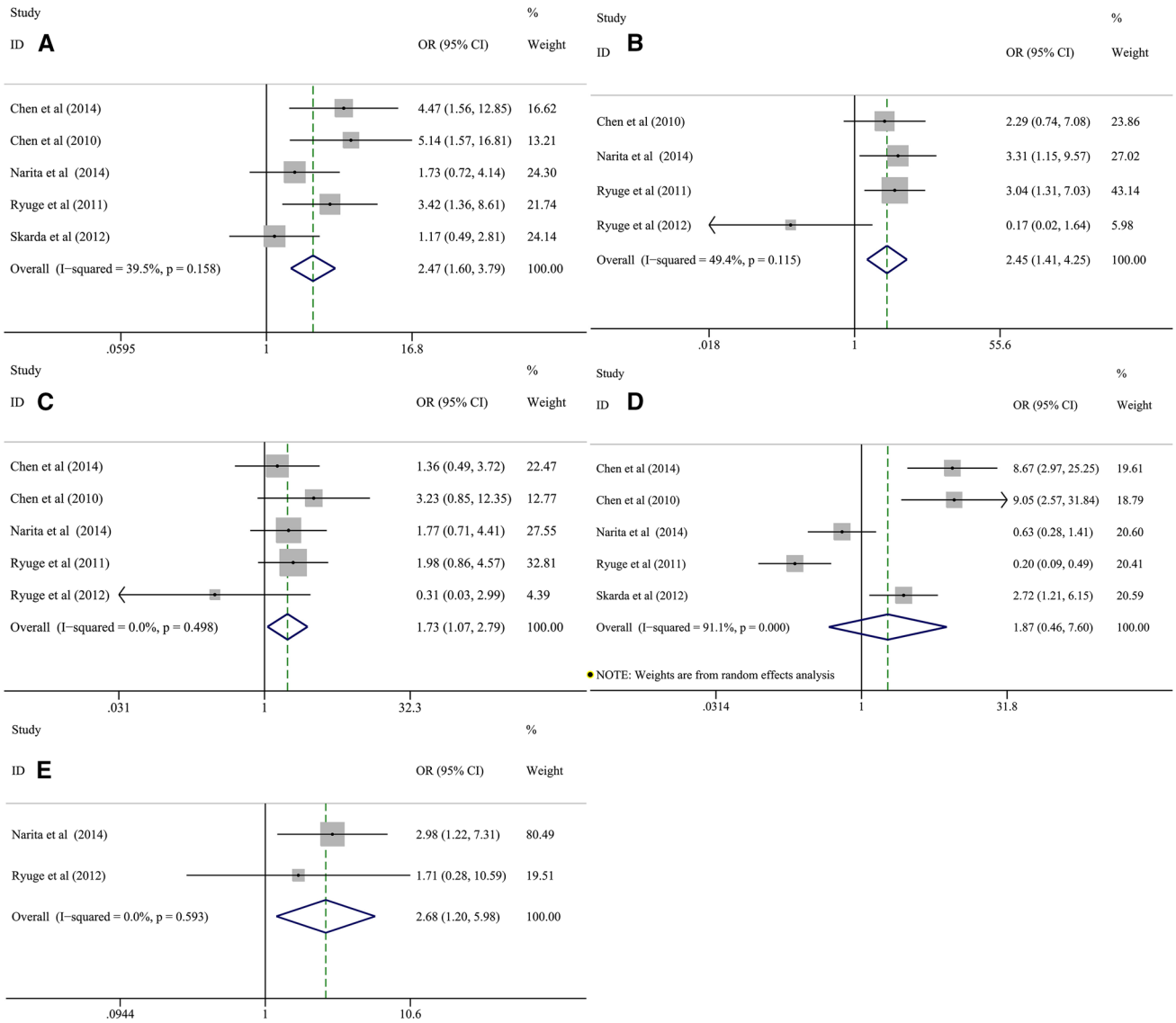

Fig. 2 Association between Nestin expression and pathological characteristics including **a** differentiation degree, **b** lymphatic metastasis, **c** TNM stage, **d** histological subtypes and **e** tumor size of NSCLC. *CI* confidence interval, *NSCLC* non-small cell lung cancer, *OR* odds ratio

extrapolated by demographics in four studies [13, 14, 17, 18] and directly extracted from the reported results in one study [16]. A significant relationship between Nestin expression and lower OS of NSCLC was revealed in both multivariate analysis group (HR: 2.74; 95 % CI 1.54–4.85; $P = 0.001$) and univariate analysis group (HR: 2.25; 95 % CI 1.49–3.42; $P < 0.001$), without any heterogeneity (Table 3; Fig. 5a).

In the subgroups stratified by origins of patients, the pooled HR was 2.98 (95 % CI 1.87–4.76; $P < 0.001$) in Asian populations enrolled from four included studies [11, 13, 15, 16]. The pooled HR was 1.91 (95 % CI 1.17–3.11; $P = 0.009$) for non-Asian group from the other three studies [14, 17, 18]. Both of the two summarized outcomes indicated the significant relationship between Nestin expression and poor prognosis of NSCLC, without ethnic differences (Table 3; Fig. 5b).

In the subgroups stratified by histological subtypes of NSCLC, the survival details of NSCLC histology were not available in four studies [12, 14, 15, 17]. Narita et al. [13] performed a survival analysis in 48 AC cases and 47 SCC cases, respectively. Only one study conducted by Ryuge et al. [16] evaluated the prognostic roles of Nestin expression in 30 cases with large cell neuroendocrine carcinoma (LCNEC). Therefore, by pooling two studies for AC, the summarized HR was 2.71 (95 % CI 1.40–5.24; $P = 0.003$), indicating that Nestin expression was significantly associated with the poor OS of AC (Table 3; Fig. 5b). The significant prognostic value of Nestin expression in LCNEC was also revealed by the reported

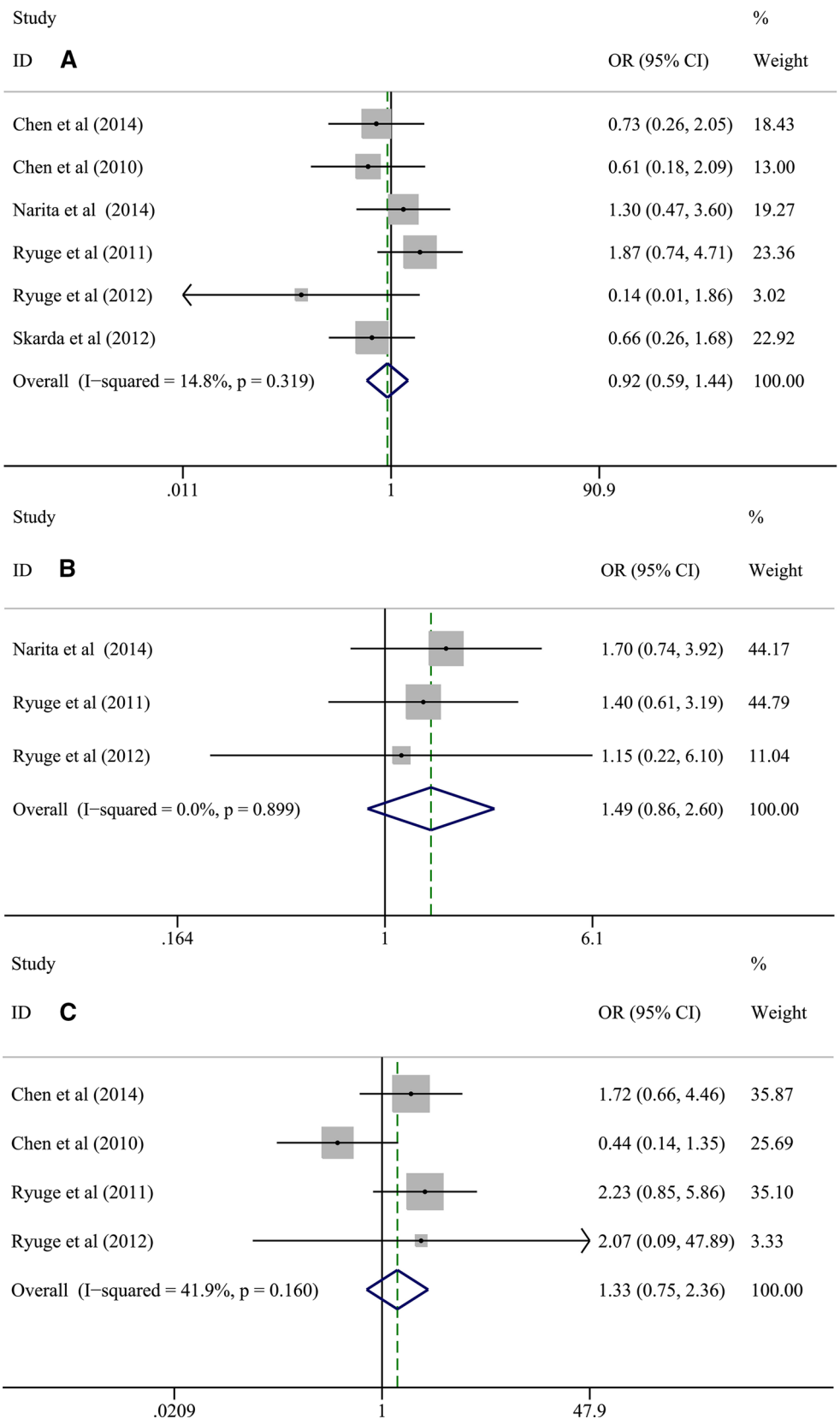

Fig. 3 Association between Nestin expression and clinical characteristics including **a** gender, **b** age and **c** smoking status in patients with NSCLC. *CI* confidence interval, *NSCLC* non-small cell lung cancer, *OR* odds ratio

Table 3 Prognostic significance of Nestin expression for OS in patients with NSCLC

Outcomes	*N*	No. samples			Heterogeneity (I^2, *P*)	Model	HR (95 % CI)	*P* value	Conclusion
		Total	PE	NE					
Overall	7	782	286	496	0.0 %, 0.736	Fixed	2.41 (1.72–3.38)	<0.001	Significant
Statistical analysis									
Multivariate analysis	2	242	62	180	0.0 %, 0.672	Fixed	2.74 (1.54–4.85)	0.001	Significant
Univariate analysis	5	540	224	316	0.0 %, 0.562	Fixed	2.25 (1.49–3.42)	<0.001	Significant
Origins of patients									
Asian countries	4	367	115	252	0.0 %, 0.806	Fixed	2.99 (1.87–4.76)	<0.001	Significant
Non-Asian countries	3	415	171	244	0.0 %, 0.582	Fixed	1.91 (1.17–3.11)	0.009	Significant
Histological subtypes[a]									
AC	2	237	77	160	23.1 %, 0.254	Fixed	2.71 (1.40–5.24)	0.003	Significant
SCC	1	47	25	22	–	–	1.34 (0.14–12.95)	0.80	Not significant
LCNEC	1	30	8	22	–	–	3.40 (1.18–9.77)	0.023	Significant
Follow-up periods									
≥100 months	4	426	184	242	0.0 %, 0.419	Fixed	2.24 (1.41–3.54)	0.001	Significant
<100 months	3	356	102	254	0.0 %, 0.882	Fixed	2.63 (1.60–4.32)	<0.001	Significant

[a] Nestin expression in each histological subtype of NSCLC was not reported in Refs. [12, 14, 15, 17]

AC adenocarcinoma, *CI* confidence interval, *HR* hazard ratio, *LCNEC* large cell neuroendocrine carcinoma, *NE* negative expression, *NSCLC* non-small cell lung cancer, *OS* overall survival, *PE* positive expression, *SCC* squamous cell carcinoma

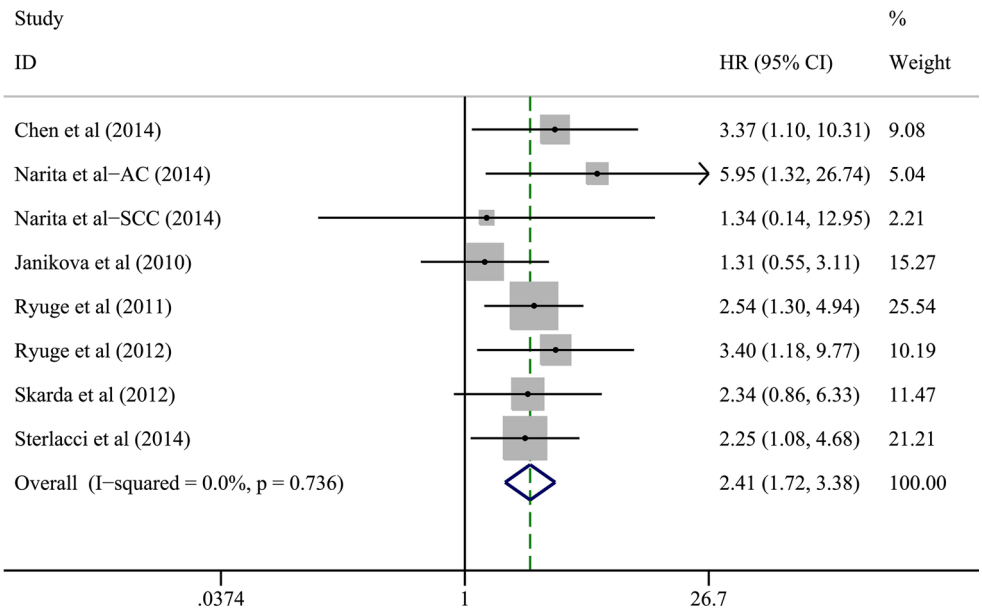

Fig. 4 Overall analysis for the prognostic value of Nestin expression in patients with NSCLC. *AC* adenocarcinoma, *CI* confidence interval, *HR* hazard ratio, *NSCLC* non-small cell lung cancer, *SCC* squamous cell carcinoma

HR results (HR: 3.40; 95 % CI 1.18–9.77; $P = 0.023$) [16]. However, as what Narita et al. [13] reported, no significant relationship was observed between Nestin expression and the prognosis of SCC (HR: 1.34; 95 % CI 0.14–12.95; $P = 0.80$; Table 3; Fig. 5c).

In the subgroups stratified by follow-up periods, the HR outcomes integrating three included studies with a maximum follow-up period shorter than 100 months [11, 15, 17] suggested that Nestin expression was significantly associated with the poor short-term OS of NSCLC (HR: 2.63; 95 % CI 1.60–4.32; $P < 0.001$). Similarly, the pooled HR of the remaining four studies [13, 14, 16, 18] with a maximum follow-up period longer than 100 months was 2.24 (95 % CI 1.41–3.54; $P = 0.001$), indicating that Nestin expression could also significantly predict the poor long-term OS of NSCLC (Table 3; Fig. 5d).

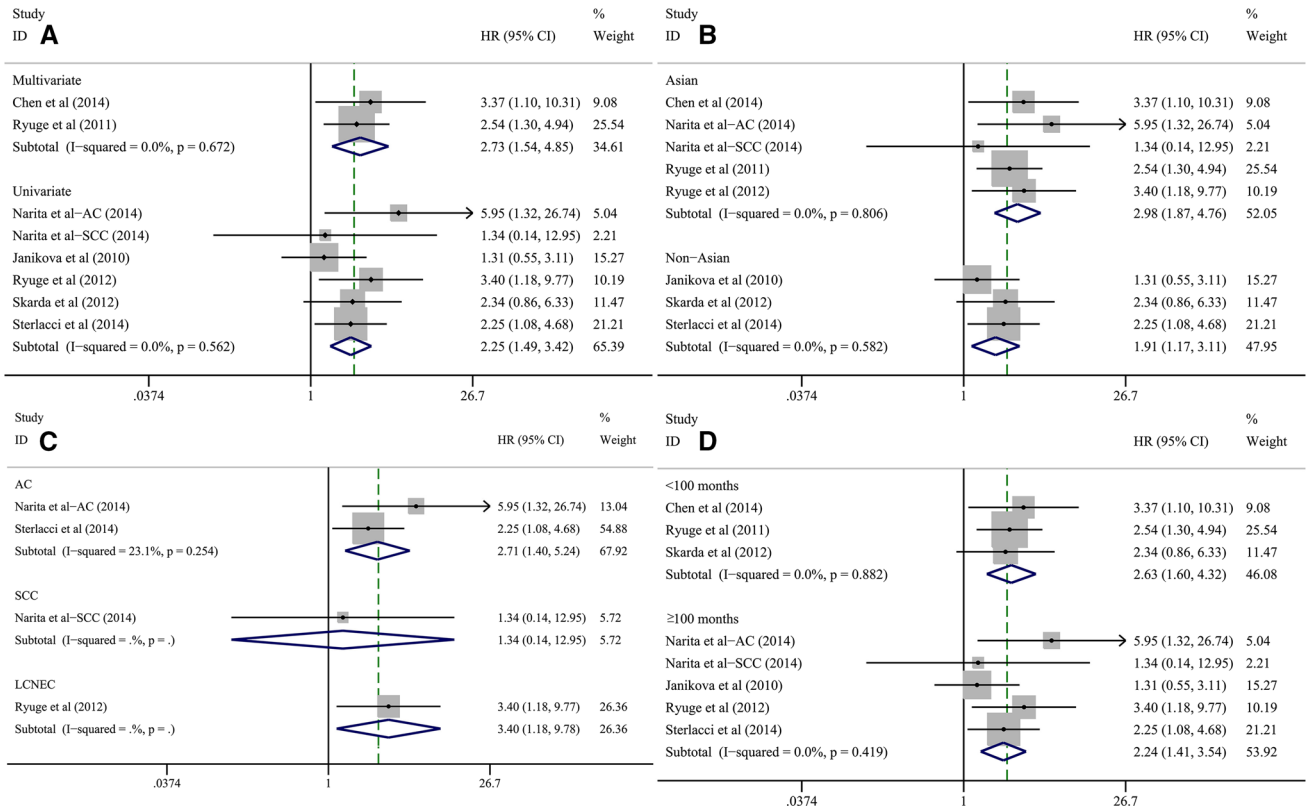

Fig. 5 Subgroup analysis for the prognostic value of Nestin expression in patients with NSCLC stratified by **a** statistical analysis, **b** the origins of patients, **c** histological subtypes and **d** follow-up periods. *AC* adenocarcinoma, *CI* confidence interval, *HR* hazard ratio, *LCNEC* large cell neuroendocrine carcinoma, *NSCLC* non-small cell lung cancer, *SCC* squamous cell carcinoma

Sensitivity analysis

Sensitivity analysis was conducted to verify the stability of the pooled HR. As shown in the forest plot derived from sensitivity analysis, none of the individual HRs was out of the estimated ranges (Fig. 6). That means no substantial variation would be revealed between the adjusted outcomes and primary outcomes if excluding each study sequentially. Thus, the strong robustness of our meta-analysis was confirmed.

Publication bias

No evidence for significant publication bias was detected by either Egger's test or Begg's test in the assessments for the association between Nestin expression and clinicopathological characteristics of NSCLC. The derived outcomes are listed in Table 2 (plots not shown). Meanwhile, both Egger's test and Begg's test indicated that there was no significant publication bias within the relationship between Nestin expression and poor OS of NSCLC (Egger's test: $P = 0.61$; Begg's test: $P = 0.54$; Fig. 7).

Discussion

To the best of our knowledge, this is the first systematic meta-analysis to demonstrate the clinicopathological and prognostic significance of Nestin expression in patients with NSCLC. In this meta-analysis, we concluded that Nestin expression was significantly associated with the unfavorable conditions of differentiation degree, lymphatic metastasis, TNM stage and tumor size of NSCLC. On the contrary, no significant relationship was observed between Nestin expression and histological subtypes, gender, age or smoking status in patients with NSCLC. Remarkably, positive Nestin expression was significantly associated with the lower OS, indicating that Nestin could be an effective biomarker for predicting the poor prognosis of NSCLC. Further analysis indicated that such correlation was still prominent in the subgroups stratified by statistical analysis, the origins of patients and follow-up periods, and in AC cases and LCNEC cases. However, Nestin expression showed no significant predictive value for poor prognosis in SCC cases.

Nestin was firstly discovered as a neuronal stem cell biomarker in 1985 [34]. In 1990, Lendahl et al. [7] re-

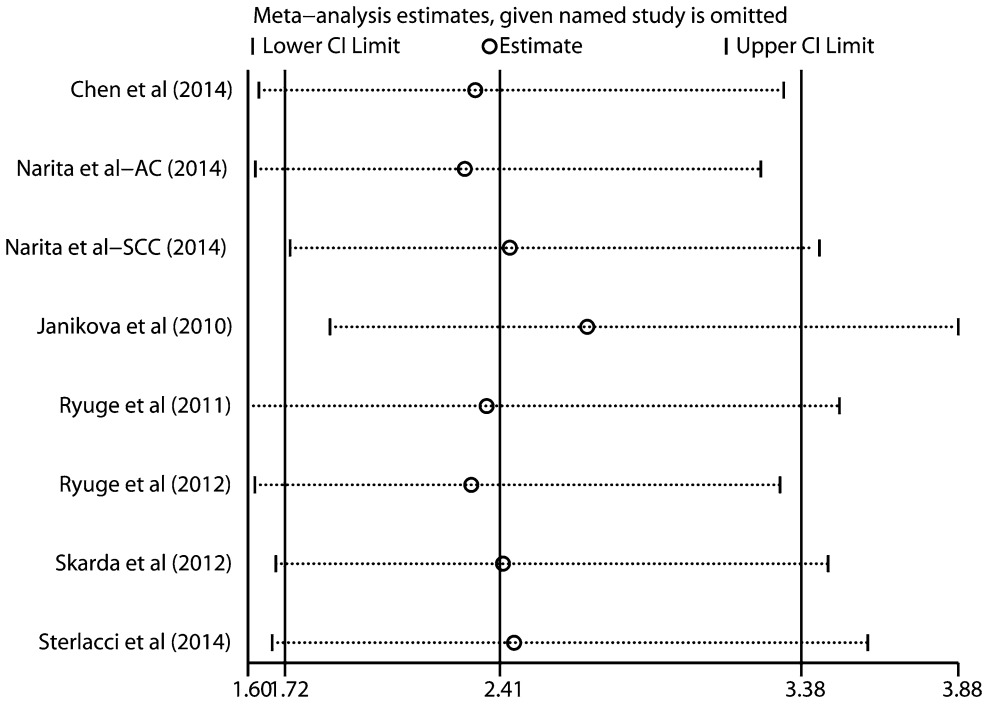

Fig. 6 Sensitivity analysis of the association between Nestin expression and the prognosis of NSCLC. *AC* adenocarcinoma, *CI* confidence interval, *NSCLC* non-small cell lung cancer, *SCC* squamous cell carcinoma

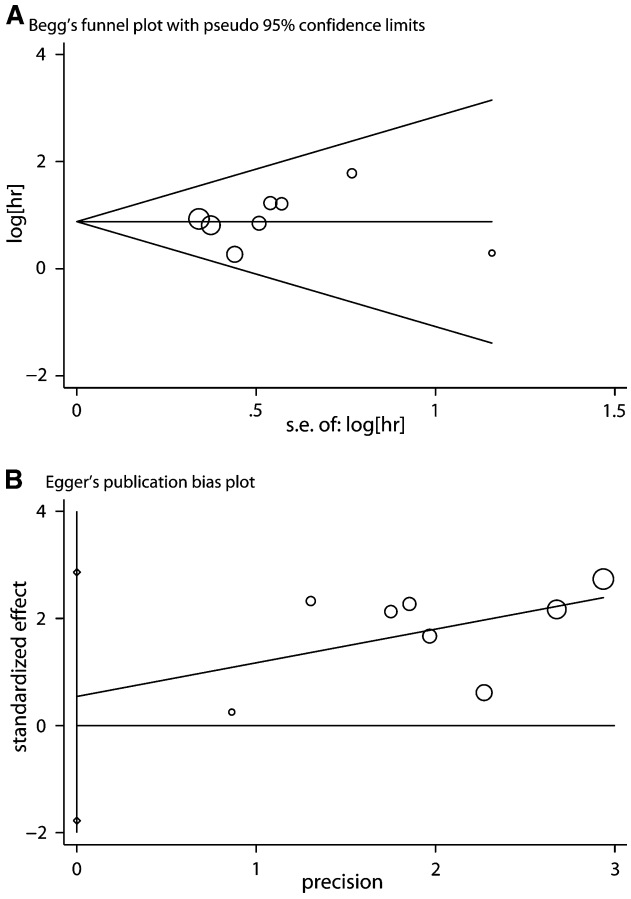

Fig. 7 Publication bias of the association between Nestin expression and the prognosis of NSCLC detected by **a** Begg's test and **b** Egger's test. *HR* hazard ratio, *NSCLC* non-small cell lung cancer

classified Nestin into class VI IFP. As an IFP, Nestin consists of a central α-helical rod domain, a short N-terminus and a special long C-terminus. It contains more than 1600 amino acids with the molecular weight of 177.4 kDa [35]. Nestin has been commonly detected in various embryonic cells including those in skeletal muscle, cardiac muscle and central nervous system [34]. A Number of experimental evidences have shown that the abundant expression of Nestin in the endothelium of embryonic capillaries can significantly contribute to angiogenesis [36, 37]. The underlying mechanisms may involve the participation of Nestin in cytoskeletal formation of immature precursors [38]. A large decrease in Nestin expression is detected in mature adult tissues, but Nestin can be re-expressed in adult tissues after some pathological changes, such as oncogenesis [15, 37]. Remarkably, Nestin is rarely expressed in the mature vascular tissues of cancers, indicating that Nestin can be an angiogenesis-specific marker for malignances [20, 21].

One major problem in oncological treatments is drug resistance. CSCs have the abilities of tumourigenicity, self-renewal and plasticity, which can affect the efficacy of chemotherapy and radiation on cancer treatments [39]. Nestin is regarded as a putative biomarker for identifying CSCs from both mesenchymal tumors and epithelial tumors. Many studies have shown that Nestin is generally co-expressed with some other CSC markers in the epithelial cells of tumor tissues [8]. Janikova et al. [14] analyzed the co-expression of Nestin and CD-133 in the archived paraffin sections from 121 NSCLC cases using double

immunofluorescence staining. Finally, the co-expression of Nestin and CD-133 was detected in 17 % of the cases but only positive in <1 % of the cells. It indicated that Nestin/CD-133-positive cells could be a novel marker for lung CSCs. Besides, Narita et al. [13] discovered that Nestin up-regulation could increase cell proliferation, migration and sphere formation in AC cell lines. Thus, Nestin may also potentially serve as a novel therapeutic target for treating NSCLC.

Previous laboratorial studies have provided the evidence suggesting the clinicopathological and prognostic roles of Nestin expression in some most common tumors, including NSCLC [11–18], breast cancer [19, 40–42], gastric cancer [21, 43], esophageal cancer [44] and prostate cancer [45]. A significant relationship between Nestin expression and unfavorable survival outcomes was commonly identified in various cancers. As a specific filament marker of neural stem cells, a latest systematic review performed by Lv et al. [46] has clarified that Nestin may be an important predictor for poor clinicopathological and prognostic features of glioma. Similarly, our meta-analysis quantitatively integrated all of the current evidences to systematically demonstrate the prognostic significance of Nestin expression and its relationship to the clinicopathological characteristics of NSCLC. The pooled outcomes showed that Nestin up-regulation was significantly associated with the unfavorable conditions for some pathological characteristics of NSCLC including differentiation degree, lymphatic metastasis, TNM stage and tumor size. These results were supported by the majority of included studies and verified the contribution of Nestin to tumor invasion and proliferation to some extent. However, we discovered a huge controversy among the correlation between Nestin expression and histological subtypes of NSCLC, resulting in a high heterogeneity across the included studies ($I^2 = 91.1$ %, $P < 0.001$).

The earliest study describing the significantly higher Nestin expression in ACs ($P < 0.001$) was reported by Chen et al. [12]. It was supported by their subsequent report enrolling a larger number of samples ($P = 0.001$) [11]. Skarda et al. [17] analyzed the clinical data of 114 NSCLC cases and discovered that Nestin was expressed more frequently in ACs than in SCCs, but without the statistical significance ($P = 0.25$). On the contrary, another study reported by Ryuge et al. [15] showed the significantly higher expression of Nestin in SCCs compared to ACs ($P = 0.001$). As Skarda et al. [17] described, such discordances might be caused by the difference in antibodies, which led to the divergences in epithelial cell staining. Our meta-analysis could not resolve this issue because the currently available studies were not enough for a detailed subgroup analysis. Thus, the accurate examination of Nestin expression in NSCLC subtypes is very necessary in future studies.

The overall pooled HR outcomes indicated that positive Nestin expression could significantly predict the low OS in patients with NSCLC. However, we discovered that Nestin expression was significantly correlated with the poor prognosis in AC cases and LCNEC cases but not in SCC cases. We speculated that the limited evidence might cause huge negative effects on deriving the statistically significant results because only 47 SCC cases from one study were available for subgroup analysis [13]. The majority of our included studies just performed an overall survival analysis on all of the enrolled samples rather than a separated analysis for each subtype of NSCLC [12, 14, 15, 17]. Therefore, we recommend that performing a detailed survival analysis in a large number of SCC cases and AC cases, respectively, can help to further verify and modify our findings from subgroup analysis in the future.

We have tried our best to perform a comprehensive and detailed meta-analysis on the available investigations by now. However, we realize that the data incorporated into this meta-analysis are mainly derived from univariate analysis instead of multivariate analysis. On the one hand, none of the eight eligible studies [11–18] reported any statistic from multivariate analysis to assess the relationship between Nestin expression and clinicopathological characteristics of NSCLC. On the other hand, only two included studies [11, 15] reported the multivariate HR statistics to evaluate the prognostic value of Nestin expression in NSCLC. Multivariate analysis using logistic regression or Cox proportional hazards model is generally applied to eliminate the bias risks from other confounding factors in observational studies. Therefore, the validity of our summarized outcomes might be attenuated by the insufficient elimination of major confounders in most included studies, such as TNM stage, lymphatic metastasis and differentiation degree. Considering this inherent limitation of observational studies, our findings appearing the prognostic significance of Nestin expression in patients with NSCLC should better be further confirmed and modified by new studies without the bias risks from other confounding factors.

In addition, a huge heterogeneity existed within the cutoff definitions for positive staining of Nestin from different included studies was another one issue worthy of our attentions. The cutoff values for positive Nestin expression varied largely from 5 % staining to 50 % staining of cancer cells across the included studies, which might bring some unavoidable biases to the assessments for the prognosis of NSCLC [11–18]. According to an international consensus on the methodology and criteria of evaluation, a semi-quantitative "histoscore" considering both the percentage of positive cancer cells and staining intensity was generally used in half of the included studies [11, 12, 14, 17]. However, Sterlacci et al. [18] chose the median percentage

of positive cells as their cutoff value because the determination of cutoff definitions by receiver operating characteristic analysis was less relevant in a setting. Two studies reported by Ryuge et al. [15, 16] determined a >5 % positive staining of cancer cells was considered as significant because >5 % positive cancer cells were usually recognized in most Nestin-positive cases. Given above reviews, a "histoscore" semiquantitatively combined with the percentage of positive cancer cells and Nestin intensity seemed to be more recommended by the consensual methodology [47]. However, unified criteria based on the "histoscore" for positive staining of Nestin still remained a debate according to the current evidence. Therefore, a universally accepted cutoff definition for positive expression of Nestin will be urgently required to further clarify its prognostic roles in the subsequent investigations. More high-quality studies with consistent evaluation criteria will help to improve the validity of integrated outcomes in the updated meta-analysis in the future.

Limitations

Finally, several limitations in this meta-analysis should be acknowledged. First, most of the included studies in our meta-analysis just provided the available data based on univariate analysis instead of comprehensive multivariate analysis. The validity and accuracy of our summarized outcomes might be more or less decreased by the bias risks from some insufficiently eliminated confounding factors. Second, the cutoff values for positive Nestin expression varied across different studies, possibly causing some biases in pooled analysis. Third, far fewer than 20 studies were included into our meta-analysis, which might reduce the efficacy of both Begg's test and Egger's test and thus cause deviations from the actual publication bias. Four, we did not further evaluate the prognostic roles of the combinations of Nestin and other CSC markers in patients with NSCLC. Lastly, although no language restriction was imposed in the present meta-analysis, more additional papers may be identified by searching through some other non-English databases.

Conclusions

In conclusion, our meta-analysis demonstrates that Nestin may be an independent predictive biomarker for poor prognosis and clinicopathological characteristics in patients with NSCLC. Some limitations still exist in this meta-analysis. More studies with adequate eliminations of the bias risks from other confounding factors are necessary for further verifying and modifying our discoveries in the future.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required.

Human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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