Original Article

Detection of occult tumor cells in regional lymph nodes is associated with poor survival in pN_0 non-small cell lung cancer: a meta-analysis

Zhicheng He[1], Yang Xia[1], Shaowen Tang[2], Yijiang Chen[1], Liang Chen[1]

[1]Department of Thoracic Surgery, Jiangsu Province Hospital, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China; [2]Department of Epidemiology and Biostatistics, School of Public Health, Nanjing Medical University, Nanjing 210029, China

Contributions: (I) Conception and design: Z He, L Chen; (II) Administrative support: Y Chen, L Chen; (III) Provision of study materials or patients: Z He, Y Xia, L Chen; (IV) Collection and assembly of data: Z He, Y Xia, S Tang; (V) Data analysis and interpretation: Z He, S Tang, L Chen; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Liang Chen, MD. Department of Thoracic Surgery, Jiangsu Province Hospital, the First Affiliated Hospital of Nanjing Medical University, No. 300 Guangzhou Road, Nanjing 210029, China. Email: clbright0909@njmu.edu.cn.

Background: patients of pN_0 non-small cell lung cancer (NSCLC) with occult tumor cells (OTCs) in regional lymph nodes (LNs) are reported to have controversial prognostic outcomes.

Method: We pooled pN_0 NSCLC patients with OTCs in LNs and compared with those without OTCs. Patient characteristics, hazard ratios (HRs) and 95% confidence intervals (CIs) for overall survival (OS) and/or disease-free survival (DFS) were analyzed. HR greater than 1 conferred an increased hazard for patients with OTCs.

Results: Eighteen articles were finally enrolled in the meta-analysis and 15 studies provided sufficient data for extracting HRs for OS, resulting to 5 articles available for DFS analysis. The combined HRs of OS was 2.22 (95% CI, 1.87 to 2.64) and 2.4 (95% CI, 1.71 to 3.36) for analysis of DFS. The similar trend was obtained in the subgroup analyses regarding detection methods and study type. Interestingly, even in the analysis of mean numbers of LNs dissection (MLND) intraoperatively, both subgroups (LNs/Pts. <12 and ≥12) illustrated significant HRs of OS (HR: 3.13, 95% CI, 2.17 to 4.52 in LNs/Pts. <12 subgroup and HR: 2.09, 95% CI, 1.63 to 2.68 in LNs/Pts. ≥12). The combined HR of OS in this section was 2.37 (95% CI, 1.63 to 2.68). No publication bias was detected in all the meta-analysis sections. The prognosis of patients with OTCs is inferior to those without OTCs in the terms of OS and DFS regardless of detection methods, study types and MLND.

Conclusions: The prognosis of patients with OTCs is inferior to those without OTCs in the terms of OS and DFS regardless of detection methods, study types and MLND.

Keywords: Non-small cell lung cancer (NSCLC); early stage; occult tumor cells (OTCs); hazard ratio (HR); meta-analysis

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Introduction

Lung cancer remains the leading cause of cancer deaths in the United States, accounting for about 27% of all estimated cancer death in 2014 (1). The current staging of lung cancer plays a critical role in the prognosis of this disease. Lymph node (LN) metastasis is the vital prognostic factor in regional lung cancer. However, the accurate identification of the status of LNs remains an elusive goal (2). Data are available indicating the modest 5-year survival (73%) reported in the earliest stage of non-

small cell lung cancer (NSCLC) (denoted as stage I_A by the International Association for the Study of Lung Cancer) (3). These documents suggest that operable patients might have occult tumor cells (OTCs) by the time of operation and a proportion of these patients are understaged by the routine hematoxylin-eosin staining staging procedure (4). At any rate, OTCs in regional LNs are identified more frequently than expected in those patients diagnosed as N_0 by conventional methods.

At present, immunohistochemistry staining (IHC) and reverse transcription-polymerase chain reaction (RT-PCR) analysis for epithelial markers can be used to identify OTCs in NSCLC (2), serving as morphological and non-morphological methods respectively. Variable terminology and definitions have used to define such small metastatic foci, including micrometastasis, isolated tumor cell, disseminated tumor cell, minimal residual disease and OTC. We chose the term of "OTC" because it globally depicts metastases that are not diagnosed by standard hematoxylin-eosin pathologic methods. Hermanek and his colleagues (5) proposed that, by using IHC, micrometastases are clusters of tumor cells with greatest diameter between 0.2 and 2 mm, while isolated tumor cells are defined as single tumor cells or small clusters of cells measuring small than 0.2 mm in greatest diameter. In addition, RT-PCR is also utilized for the detection of OTCs from solid tumors and to quantify the expression of tumor markers and genes associated with tumors (6).

Up to now, studies specifically focusing on the prognostic importance of OTCs in pN_0 lung cancer are limited. Some previous investigations have stated that the presence of OTCs in LNs are clinically relevant in NSCLC and confer worse prognosis (7-9). However, others documented that OTC has no negative impact on pN_0 NSCLC patients with regards to overall survival (OS) or disease-free survival (DFS) (10). Hence, whether, or to what extent, the hypothesis of the relatively poor prognosis in pN_0 NSCLC patients correlating with the existence of OTCs in regional LNs requires to be verified. This study aims to provide meta-estimates comparing pN_0 NSCLC patients with OTCs to those without OTCs in regional LNs in terms of OS and DFS.

Materials and methods

Search strategy and selection criteria

We systematically searched PubMed and Chinese Biological Medicine databases up to Dec. 12th 2014 using the following key words: "micrometastasis" or "occult tumor cell" or "skip metastasis" or "microdissemination" or "isolate tumor cell" or "small tumor deposit", "lung cancer" "NSCLC" and "lymph node". We also searched the Cochrane library (http://www.cochrane.org) with the same terms within the same period. Furthermore, we reviewed all reference lists of relevant articles and review articles for additional studies that met our inclusion criteria. No language restrictions and time limits were applied to the initial search.

Two reviewers (Z. He and Y. Xia) independently screened all the titles and abstracts for eligibility and retrieved full articles for further assessments. We defined eligible studies as patients with complete clinical records, data on follow-up and pathologically conformed and completely removed primary pN_0 NSCLC. We also required documented validation of molecular detection of OTC using any form of IHC or RT-PCR. Adequate documents of the compared groups (OTC group, representing patients of pN_0 with OTC and control group, representing those without OTC) were also required to extract hazard ratios (HRs) and 95% confidence intervals (CIs). We excluded studies that totally enrolled less than 20 patients or pooled less than 10 in the OTC group. Furthermore, we also excluded studies that focused on small-cell lung cancer (SCLC) or mixed with SCLC. Additionally, studies that mainly documented OTC in distant sites of body like blood circulation or bone marrow were not eligible for this analysis. In case of multiple publications from the same author or institution with identical or overlapping study population recruitment more than 30%, the most informative and recent study was included. The details of selection flow were summarized in *Figure 1*.

In the assessment of risk of bias, the Newcastle-Ottawa Scale was utilized to evaluate all the cohort studies. This scale uses a maximum of nine stars to assess a study in three domains (section of participants, comparability of both groups, and the ascertainment of outcomes).

Data extraction

Two authors (Z. He and Y. Xia) independently extracted the following data from the pooled articles. Discrepancies were resolved by discussion with two senior authors (Y. Chen and L. Chen). A standardized form was developed to extract data from each enrolled studies, including author, publication year, study year, study type (prospective or

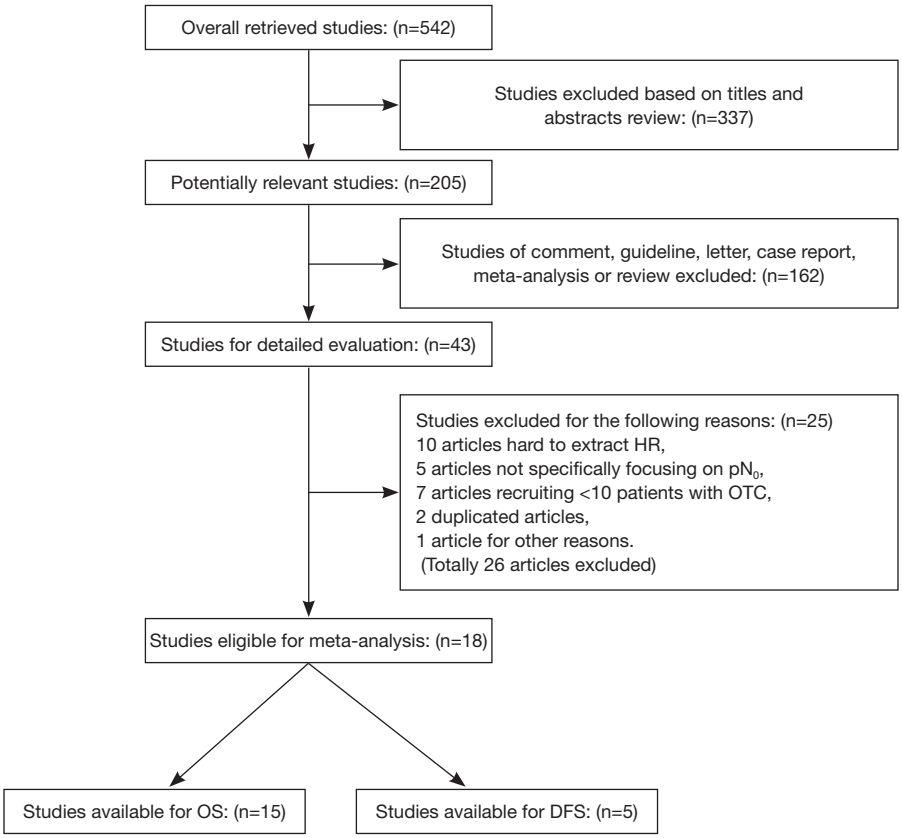

Figure 1 Flow chart of article selection. OTC, occult tumor cell; OS, overall survival; DFS, disease-free survival.

retrospective), numbers of patients participating in each study, population ethnicity, numbers of patients with OTC, mean numbers of LNs dissected (MLND), incidence of positive LNs in all harvested ones, detection method (IHC or RT-PCT), definition of OTC using a certain assay and prognostic outcome (OS and/or DFS).

Statistical methods

Time to event data were synthesized using HRs and 95% CIs. In all HR calculations, the group without OTC was regarded as the reference. Primary outcomes included OS and/or DFS, which were used to analyze the prognosis of patients with OTC compared to those without OTC. As summary statistics were not directly given in any of the articles to allow direct calculation of the HRs and CIs, we extracted statistics from Kaplan-Meier curves and estimated HRs and 95% CIs according to the algorithm proposed by Tierney (11). When the P value was available, the HR and variance were estimated using P value (Mantel Hansel, log rank, or Cox regression) and events in each arm observed-expected.

When the P value was absent, the HR was calculated using survival curve, and the curve was divided into time periods as suggested by Parmar *et al.* (12). Less than 20% of total events were included in each time period, and time periods were different between each study depending on event rates. From the curve data, variance, observed minus expected (O-E) value, and HR were calculated. Minimum and maximum follow-up data, which were either identified directly from data reported or estimated from the survival curve using methods suggested by Tierney *et al.* (11), were collected to estimated HRs accurately.

For each meta-analysis result, I-squared statistic was performed to assess the heterogeneity among the included trials. When the heterogeneity was statistically significant (I-squared >50%), a random effect model was applied for analysis. Otherwise, a fixed effect model was used. In the subgroup analysis, heterogeneity between groups was absent when P value was more than 0.05.

Both the Begg's and Egger's bias indicators were used to test the effect of publication bias. A two-tailed P value less than 0.05 was considered statistically significant. All the

statistical tests used in our meta-analysis were performed using STATA version 11.5 (Stata Corporation, College Station, TX, USA).

This meta-analysis was in consistence with the PRISMA statement for reporting systematic reviews and meta-analysis of studies that evaluated healthcare interventions (13).

Results

We pooled 542 potentially relevant articles from our research of the published literatures. Totally, 499 articles were excluded, resulting in 43 for detailed evaluations (*Figure 1*). Of the 43 articles, 10 presented insufficient information for extracting HRs, 5 not exclusively recruiting pN_0 patients (14-18). Moreover, 7 studies enrolled less than 10 cases in the OTC group (19-25). Two duplicated articles (26,27) with the same authors were less informative than 2 enrolled articles (7,28). Additionally, 1 article presenting an obscure definition in the OTC identification was excluded (29), of which, microarray analysis software was used to identify a variety of gene patterns that specifically correlated with LNs metastases and eventually the top 50 most significant genes were used to distinguish the OTC-positive and OTC-negative in LNs. Finally, 18 articles were eligible for meta-analysis and only 2 studies (7,8) documented sufficient data for extracting HRs for both OS and DFS, 13 and 3 studies merely for OS and DFS analysis, respectively.

The 18 enrolled articles used as data sources for the present meta-analysis included 1,951 patients: 481 in the OTC group (24.65%). Study size ranged from 31 to 580 patients. The incidence of OTC in the LNs that were negative by routine histopathology reached as high as 29.1% (9). However, another study presented the lowest incidence of OTC of 1.7% from the same population ethnicity (Japanese) (30). Fourteen pooled studies were based on the retrospective data except for 4 prospective ones. While IHC method was used in 14 studies with RT-PCT technique in 4 studies. In addition, 13 studies provided sufficient data for analyzing HRs of OS in the subgroup analyses (LNs/Pts. <12 and ≥12). The detailed characteristics of the included clinical trials were summarized in the *Table 1*.

Impact of OTC on OS/DFS of enrolled pN_0 NSCLC patients

There were totally 15 studies involved in the analysis of the OS with 5 studies available in DFS. The combined HR of OS was 2.22 (95% CI, 1.87 to 2.64), while the combined HR of DFS was 2.4 (95% CI, 1.71 to 3.36) (*Figure 2*). These data indicated that the prognosis of patients with OTC was inferior to those without OTC with regard to OS and DFS.

The statistics for heterogeneity showed results of I-squared were 38.1% and 27.4% in OS and DFS analysis accordingly (*Figure 2*). Thus the fixed-effects model was use for HR of OS and DFS.

There was no publication bias detected using both the Begg's and Egger's bias indicators at both sections of analyses (P=0.100 for OS including 15 articles, and P=0.199 for DFS including 5 articles, respectively, seeing *Figure 3*).

Impact of OTC on OS/DFS of pN_0 NSCLC patients using IHC or RT-PCR technique

With the intention of reducing the heterogeneity among studies, we initially divided them into two groups according to the different methods in the detection of OTC: IHC and RT-PCR analysis. In 11 studies for OS analysis using IHC method, the combined HR was 2.13 (95% CI, 1.76 to 2.58) and these combined studies were homogeneous (I-squared =34.9%). While in 4 studies for OS using RT-PCT technique, the combined HR for OS was 2.71 (95% CI, 1.79 to 4.12) with moderate heterogeneity (I-squared =51.5%). Notably, Between-group heterogeneity was lower (P=0.304) (*Figure 2A*). Likewise, of 5 studies available for DFS analysis, the same trend was obtained in both IHC and RT-PCR subgroups, but presenting significant heterogeneity between subgroups (P=0.048) (*Figure 2B*).

Impact of OTC on OS/DFS of pN_0 NSCLC patients based on different study type

Of all the 15 studies available for OS analysis, 12 retrospective studies presenting the HR of 2.39 (95% CI, 1.94 to 2.93) agreed with 3 prospective ones with HR of 1.87 (95% CI, 1.36 to 2.57). Both of these combined studies were of homogeneity (I-squared =40.5% and 20.6%, respectively) (*Figure 2C*). Five studies provided valuable data for DFS analysis illustrating the same trend in 3 retrospective and 2 prospective studies. Detailed information was available in *Figure 2D*.

Impact of OTC on OS of pN_0 NSCLC patients undergoing different MLND (LNs/Pts. <12 or ≥12)

Totally, 13 studies documented sufficient information for

Table 1 General characteristics of enrolled studies

Author	Publication year	Study year	Study type	Patient (No.)	Ethnicity	OTC (No.)	LNs/ Pts.**	Positive LN (%)	Detection method	Definition of OTC	Endpoint
Dai	2013	2005.3–2007.5	Retro.	49	Chinese	16	3.6	22.70	RT-PCR	*FHIT*/*CDKN2A* mRNA deletion	OS&DFS
Rusch	2011	NA	Pros.	580	Worldwide*	130	NA	20	IHC	CK positive	OS&DFS
Yamashita	2010	1993–2000.3	Retro.	117	Japanese	34	12.3	29.10	IHC	CK positive	OS
Ouyang	2008	1996.1–2003.12	Retro.	78	Chinese	21	NA	NA	IHC	CK positive	OS
Li	2008	2000.1–2002.1	Retro.	89	Chinese	21	4.5	9	RT-PCR	MUC1 mRNA expression	OS
Rena	2007	1998.1–2005.12	Retro.	87	Italian	14	8.0	2.70	IHC	CK positive	DFS
Roh	2004	1994–2002	Retro.	35	Korea	14	12.5	5.50	IHC	CK positive	OS
Wang	2003	1998.1–1998.12	Retro.	58	Chinese	16	4.2	9.50	RT-PCR	MUC1 mRNA expression	OS
Harden	2003	1995–1999	Pros.	73	American	13	3.5	NA	RT-PCR	DAPK/APC methylation positive	OS
Osaki	2002	1993.9–2000.4	Retro.	115	Japanese	32	21.1	1.70	IHC	CK positive	OS
Kawano	2002	1986–1998	Retro.	49	Japanese	13	37.1	NA	IHC	CK positive	OS
Gu	2002	1987.1–1990.12	Retro.	49	Japanese	22	9.7	7.46	IHC	CK/p53 positive	OS
Wu	2001	1981.1–1996.12	Retro.	103	Japanese	21	14.0	3.40	IHC	CK positive	OS
Osaki	2001	1991.4–1993.7	Retro.	67	Japanese	19	NA	NA	IHC	CK positive	DFS
Ohta	2001	1986–1996	Retro.	181	Japanese	44	17.0	NA	IHC	CK positive	OS
Kubuchok	1999	1989.10–1991.12	Pros.	65	German	10	5.5	6.20	IHC	Ber-EP4 positive	OS
Dobashi	1997	1987–1990	Retro.	31	Japanese	14	10.2	8.30	IHC	p53 positive	OS
Passlick	1996	1989.10–1991.12	Pros.	65	German	10	5.5	6.20	IHC	Ber-EP4 positive	DFS

Worldwide*, including White, Hispanic/Latino, Black/African American, Asian and American Indian/Alaska Native; LNs/Pts.**, the mean numbers of LNs dissection in each patient intraoperatively. *FHIT* and *CDKN2A*, two tumor suppressor genes; CK, cytokeratin; MUC1, a cell surface glycoprotein, a specific marker for epithelial tissues; OS, overall survival; DFS, disease-free survival; Retro, retrospective study; Pros, prospective study; Pts., patients; LN, lymph node; RT-PCT, reverse transcription-polymerase chain reaction; IHC, immunohistochemistry staining.

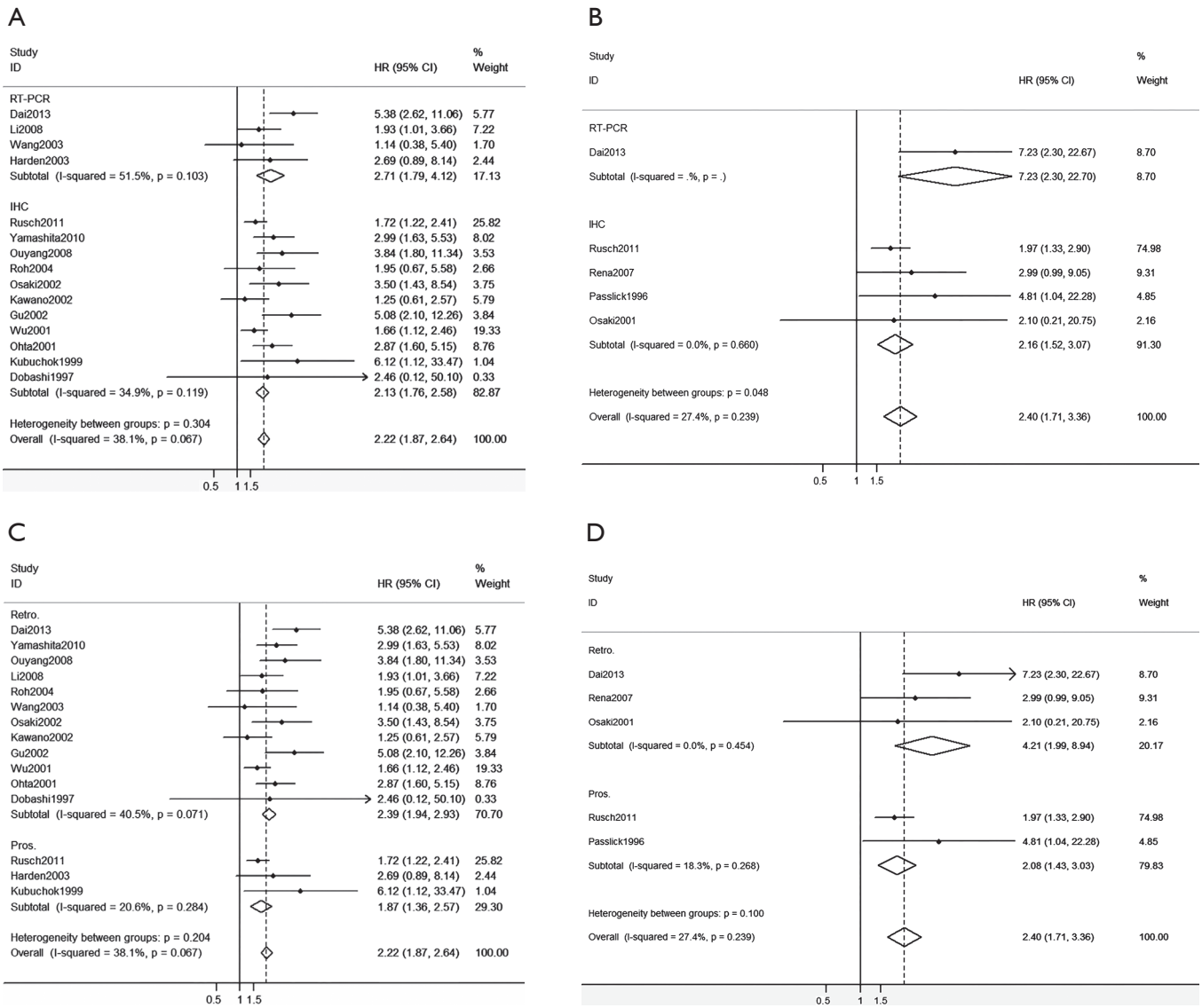

Figure 2 HR analysis of OS (A) and DFS (B) for patients with OTC *vs.* without OTC and sub-analysis according to different detection methods (RT-PCT and IHC), and HR analysis of OS (C) and DFS (D) for patients with OTC *vs.* without OTC and sub-analysis according to different study types (retrospectively and prospectively). OTC, occult tumor cells; OS, overall survival; DFS, disease-free survival.

HR of OS analysis regarding MLND (*Figure 4*). Patients with OTC were more than three times likely to have the probability to death comparing with those without OTC in LNs/Pts. <12 subgroup (HR: 3.13, 95% CI, 2.17 to 4.52). Interestingly, the general trend for lower HR favored LNs/Pts. ≥12 subgroup (HR: 2.09, 95% CI, 1.63 to 2.68), but this was not significantly different between subgroups (P=0.072). Neither of the subgroups illustrated heterogeneity in each pooled estimates (I-squared: 28.7% and 28.8%, respectively).

In this section involving 13 studies, significant difference was absent in the publication bias analysis (P=0.330) (*Figure 5*).

Discussion

We review 18 cohort studies, most of which showed the evidence that pN_0 patients with OTCs in regional LNs, even receiving complete tumor resection, had an associated higher risk of recurrence or death compared with those

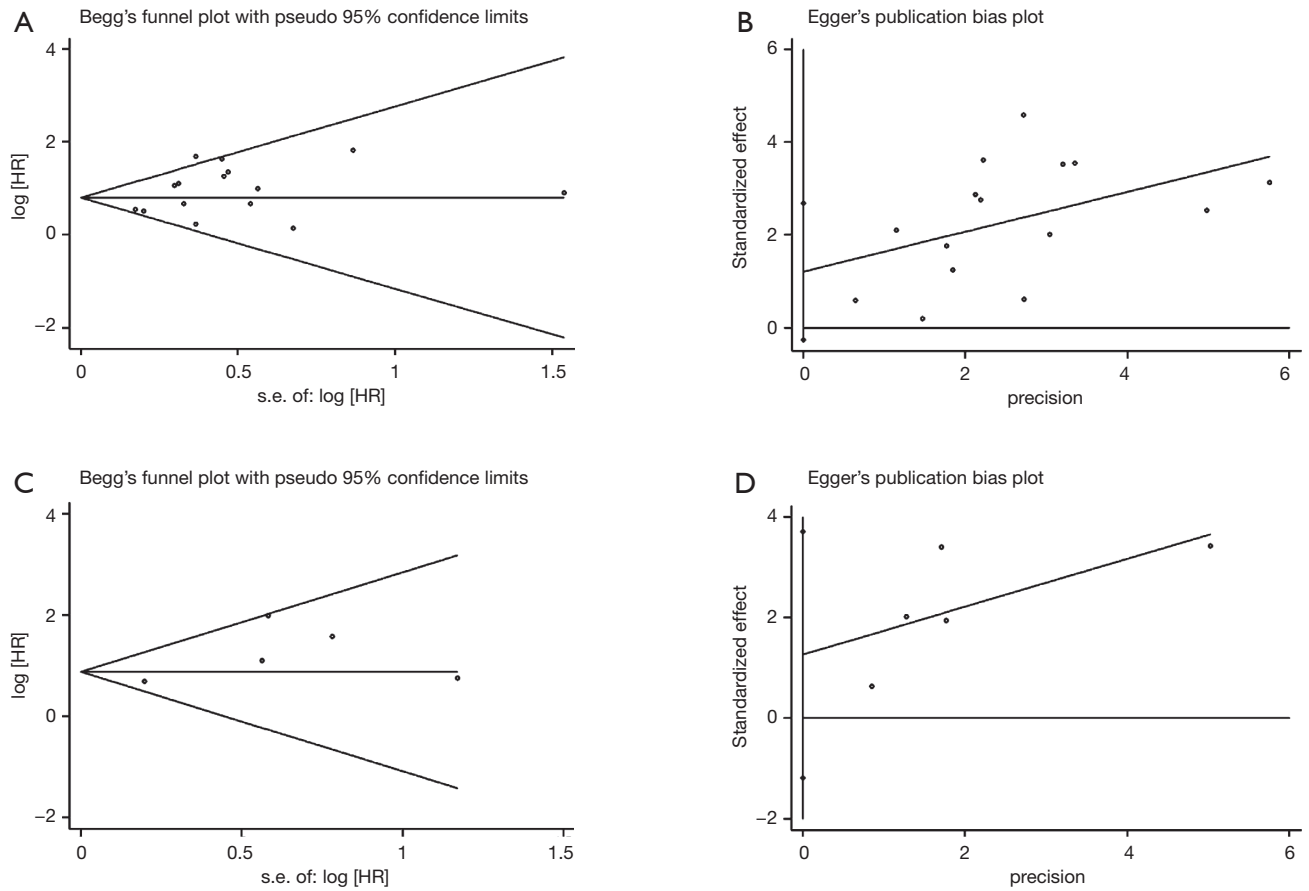

Figure 3 Publication bias detection using both Begg's (A) and Egger's (B) bias indicators in the analysis involving 15 articles available for OS meta-analysis (P=0.100), and publication bias detection using both Begg's (C) and Egger's (D) bias indicators in the analysis involving 5 articles available for DFS meta-analysis (P=0.199). OS, overall survival; DFS, disease-free survival.

without OTCs (7-9,28,30-40). This seemingly undisputed conclusion was challenged by several documents which indicated that the presence of OTCs had no significant effect on the OS or DFS (10,41,42). Hence, we could not arbitrarily reach the extent of the impact of OTCs in regional LNs on survival of pN_0 NSCLC prior to the meta-analysis.

In the meta-analysis, the prognosis of patients with OTCs was inferior to those without OTCs in the terms of OS and DFS regardless of detection methods, study types and MLND per patients intraoperatively. This emphasizes the importance of the status of regional LNs, which has a primary impact on the management of NSCLC patients (43). The detection of LN occult micrometastatic tumors cells provides a precise assessment of tumor staging and has powerful clinical implications for completely resected pN_0 NSCLC patients. Currently, postoperative adjuvant chemotherapy is a not accepted as a routine standard therapy for those patients because of its uncertain results for the improvement of patients' prognosis. Additionally, nodal micrometastasis does not only reflect lymphogenous spread but also it may be a signal of early phase of hematogenous systemic tumor cells dissemination (7). Finally, the incidence OTC as high as 29.1% in LNs highlights that many of these cancers could not be curable by operation alone. This identification of subgroups of patients with different outcomes could help physicians tailor accurate postoperative management strategies and stratify patients who would probably benefit most from aggressive chemotherapy (44).

One previously published systematic review has estimated the relationship between the presence of OTCs and the prognosis of the early stage lung cancer based on eight included studies but mixed with IHC and RT-PCT detection methods (45). Concerns have been addressed that

RT-PCT may be overly sensitive and may include false-positive cases and consequently magnify the role of OTCs in the prognosis of NSCLC (2). Thus we imperatively conducted the meta-analysis to stratify the potentially distinctive roles of RT-PCR and IHC in OTCs detection. In the subgroup analysis, HR in the RT-PCR subgroup was comparable to that in IHC in term of OS. However, the statistically significant difference of HRs for DFS was obtained in both groups. These probably reflected diversity in case selection, heterogeneity in histopathology, or the fact that studies have been small and retrospective.

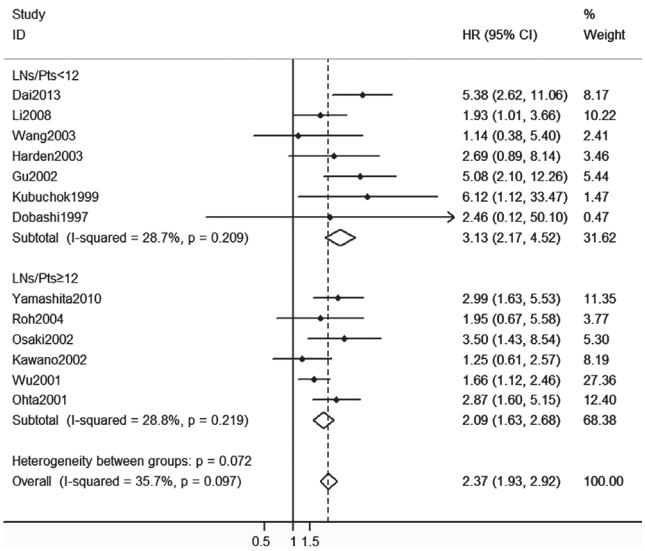

Figure 4 HR analysis of OS for patients with OTC *vs.* without OTC and sub-analysis according to LNs/Pts. (<12 and ≥12). OTC, occult tumor cells; OS, overall survival; LN, lymph node; Pts., patients.

In view of surgical procedure for these patients potentially bearing OTCs in regional LNs, the ACOSOG Z0030 study group recommends that the mean numbers of LNs resected during lobectomy be ≥12 (46). Likewise, removal of 11 to 15 LNs confers better prognostic outcomes in the early stage NSCLC patients undergoing lobectomy (47). In the current meta-analysis, MLND varied from 3.5 to 37.1 based on different cohorts of patients. To clarify the potential heterogeneity in LNs dissection in the meta-analysis, we conducted a subgroup analysis to assess HRs of OS according to MLND (LNs/Pts. <12, or ≥12). The trend of worse prognostic outcome was presented in the LNs/Pts. <12 subgroup compared to that in the LNs/Pts. ≥12, despite of lacking statistically significant difference. Furthermore, both of the subgroup analyses showed a robust evidence for the prognostic value of OTCs in the early stage operable NSCLC.

It is universally accepted that retrospective studies are prone to bias as they particularly rely on data collected for another purpose (48), while the prospective cohort studies are relatively reliable and robust. However, in this study, patients with OTCs in LNs deemed higher risk of death or recurrence than those without OTCs regardless of the study design.

Despite of the conclusion we have obtained above, our research also had several limitations. Firstly, the selection of the controls varied between studies. It excluded the studies that totally enrolled less than 20 patients or less than 10 in the OTC group and those lacking sufficient survival

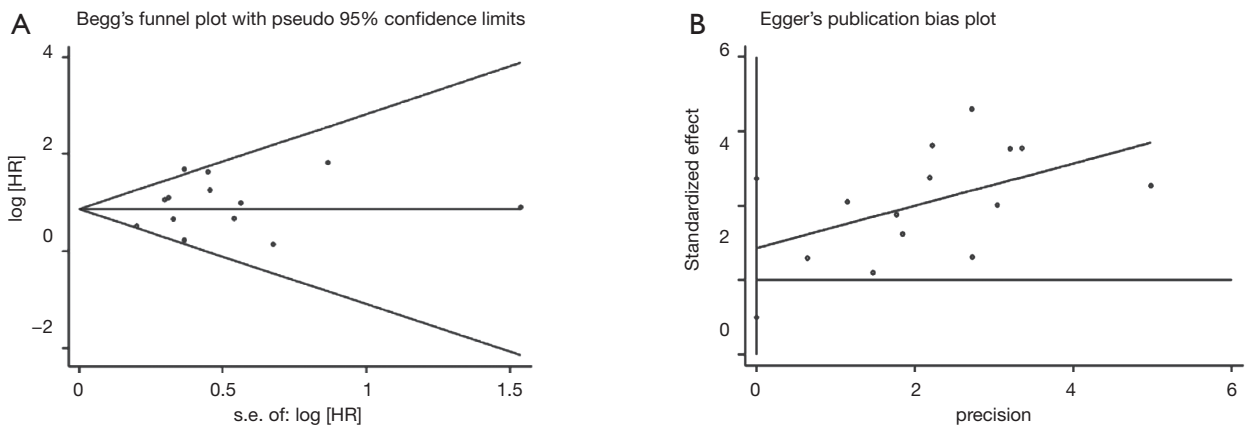

Figure 5 Publication bias detection using both Begg's (A) and Egger's (B) bias indicators in the analysis for OS involving 13 articles with sufficient data available for LNs/Pts. subgroup meta-analysis (P=0.330). OS, overall survival; LN, lymph node; Pts., patients.

data (e.g., HR, CI or survival curve). Secondly, there was statistical heterogeneity, probably originating from the differences in the characteristics of patients, normalization controls, technical platforms, the cut-off values or any other technical issues. Thirdly, the present meta-analysis was limited to the articles published up to December 2014, indicating the possibility that some relevant unpublished studies, which may have met the inclusion criteria, were missed. Finally, the available data included in this meta-analysis were primarily originated from certain ethnicities like Japanese, Chinese and German. Thus, a study of large cohort, multicenter and long follow-up may be required to clarify the prognostic value of OTCs in LNs in resected pN_0 NSCLC patients.

In conclusion, the correlation of OTCS in LNs and survival of pN_0 NSCLC has direct clinical implications. The controversy of the extent of LNs dissection in the procedures of pN_0 stage NSCLC has be clearer. Furthermore, improved detection techniques could be expected and finally a subgroup of patients who will potentially most benefit from adjuvant therapy might be identified.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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