Am J Cancer Res 2023;13(4):1594-1601
www.ajcr.us /ISSN:2156-6976/ajcr0148108

Brief Communication

The diagnostic value of circulating abnormal cells in early lung cancer

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Received November 29, 2022; Accepted April 6, 2023; Epub April 15, 2023; Published April 30, 2023

Abstract: Lung cancer is the leading cause of cancer-related deaths globally. Early detection of lung cancer can lead to more effective treatment and improved survival. Circulatory abnormal cells (CACs) with specific chromosomal variation may be used to diagnose lung cancer and to differentiate benign from malignant nodules. The value of CAC in precancer diagnosis, however, remains controversial. In this study, a systematic review and meta-analysis are conducted to clarify the diagnostic value of CAC in early-stage lung cancer. A systematic literature search was conducted using the following medical topic title terms and text-free words: "circulating genetically abnormal cells", "CACs", "liquid biopsy", "early lung cancer", "non-small cell lung cancer", "diagnostic accuracy", "sensitivity" and "specificity" in Science Direct, CNKI and Wanfang databases, respectively. Sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and area under the curve were analyzed by STATA15.0 (MP) software. Deek funnel plots were used to assess potential publication bias. Heterogeneity was tested using the I2 statistic and the Cochrane Q test. 7 major studies were included in this meta-analysis, and a total of 53728 participants were analyzed. In the diagnosis of early lung cancer, CAC had pooled sensitivity, specificity, and receiver operating characteristics of 0.80 (95% CI: 0.73-0.86), 0.85 (95% CI: 0.69-0.94), and 0.87 (95% CI: 0.84-0.90). The combined positive likelihood ratio, negative likelihood ratio, diagnostic odds ratio, and diagnostic score were 23.36 (95% CI: 7.33-74.46), 5.42 (95% CI: 2.37-12.43), 0.23 (95% CI: 0.16-0.35) and 3.15 (95% CI: 1.99-4.31) respectively. Publication bias was not detected. The CAC is effective at detecting lung cancer in its early stages.

Keywords: Pulmonary nodules, circulating genetically abnormal cells (CAC), early diagnosis of lung cancer

Introduction

As one of the most common malignant tumors in the world, lung cancer poses a serious threat to human life and health [1]. Most of the patients were diagnosed with advanced lung cancer. A timely diagnosis of lung cancer can have a significant impact on the survival and quality of life of patients [2, 3].

At present, the early diagnosis of lung cancer, however, requires a sensitive and specific non-invasive diagnostic method. A variety of clinical diagnosis methods are available for early lung cancer, including percutaneous biopsy, low-dose computed tomography (LDCT), liquid biopsy, and positron emission tomography (PET) [4-6].

At present, the most commonly used methods for evaluating pulmonary nodules are phlegm shedding cytology, chest CT, bronchoscopy, and pulmonary biopsy [4]. Each of these methods has its disadvantages or benefits. For example, commonly used chest CT scans have the advantage of being fast [5], however, for small, unclearly bounded nodules close to blood vessels, it is easy to miss the diagnosis [1]. Low-dose CT diagnosis of early lung cancer, however, has a high false positive rate [7], for pulmonary nodules smaller than 10 mm and benign pulmonary nodules, it often leads to a higher false positive rate in the diagnosis of pulmonary nodules, which will not only increase the economic burden of patients, but also cause unnecessary tension. Present invasive diagnos-

tic methods for early lung cancer have their own obvious shortcomings [8-10].

Due to their low sensitivity in early diagnosis, traditional non-invasive methods such as serum tumor biomarkers, circulating tumor cells (CTCs), and circulating nucleic acids cannot be used for large-scale screening [11-14]. Therefore, it is of great significance to develop a non-invasive detection method for early lung cancer with strong sensitivity and specificity.

Recently, CACs with specific chromosomal variants have been identified in lung cancer patients. Mutations in chromosome 3 (3p22.1, 3q29) and chromosome 10 (10q22.3, CEP10) lead to abnormal expression in peripheral blood mononuclear cells. We call these cells CACs. The CACs test overcomes the limitations of the CTC test because it does not rely on EpCAM [15]. Additionally, CAC is strongly associated with the early onset of lung cancer [16, 17].

We summarized the existing clinical data of CAC and found the following conclusions with clinical reference value. First, Mao-Song Ye et al. emphasized the diagnostic value of CAC in the diagnosis of pulmonary nodules smaller than 10 mm, and the diagnostic efficacy will be improved when CAC is combined with other risk factors [18, 22]. Xiao-Chang Qiu and Wei-Ran Liu found that CAC is more sensitive and specific than other single or combined tumor markers in diagnosing early-stage lung cancer [19, 21]. Ming-Xiang Feng et al. found that the type of nodules (pure ground glass, solid and mixed nodules), nodule size, single or multiple nodules had no effect on the diagnostic value of CAC [20]. Han Yang et al. found that the diagnostic efficacy of CAC combined with PANIDS was higher than that of CAC or PANIDS alone [23]. Ruth L. Katz et al. found that the number of CAC had no significant difference in the prognosis of lung cancer [24].

These studies thus suggest that CACs may be an efficient and specific biomarker for lung cancer diagnosis. A reliable auxiliary method for judging lung nodules is to detect CAC levels. Despite this, CACs testing is still in its infancy when it comes to clinical applications, especially when it comes to diagnosis. The sensitivity and specificity of CAC in early lung cancer diagnosis have varied considerably across studies. A meta-analysis of diagnostic accuracy might lead to more robust conclusions. Based on these studies, we performed a comprehensive analysis of CAC within the diagnostic range of lung cancer values.

Methods

Retrieval strategy

For this study, the results retrieved from the databases up to November 2022 were considered. The following medical subject headings and text terms included “circulating genetically abnormal cells”, “CAC”, “CACs”, “liquid biopsy”, “early lung cancer”, “non-small cell lung cancer”, “diagnostic accuracy”, “sensitivity and specificity” to conduct a systematic literature search in the Science Direct, CNKI and Wan fang databases, respectively. Even though all included papers were in English, there were no language restrictions for a more comprehensive analysis.

Inclusion and exclusion criteria

The following criteria are required for further meta-analysis: (1) Peer-reviewed articles. (2) Patients with early-stage lung cancer (excluding those based on in vitro or animal models). As negative controls, we used patients with non-malignant pulmonary nodules and healthy volunteers.

We incorporated the following exclusion criteria: (1) One study had a sample size of less than 20. (2) The data in this study were too small to calculate sensitivity and specificity. The following studies were excluded: conference abstracts, case reports, qualitative findings reports, systematic reviews, and letters to the editor.

Data extraction

Two investigators independently extracted the data from all eligible studies: author, publication time, cut-off, sample size, TP, FP, FN, TN, Specificity, and sensitivity. In case of disagreement, seek the opinion of a third examiner and resolve the disagreement by consensus or discussion. We independently assessed the methodological quality and risk of bias of the included studies using the Diagnostic Accuracy Study Quality Assessment 2 (QUADAS-2) tool.

Statistical methods

All analyses were performed with Stata/MP software (version 15). The diagnostic accuracy

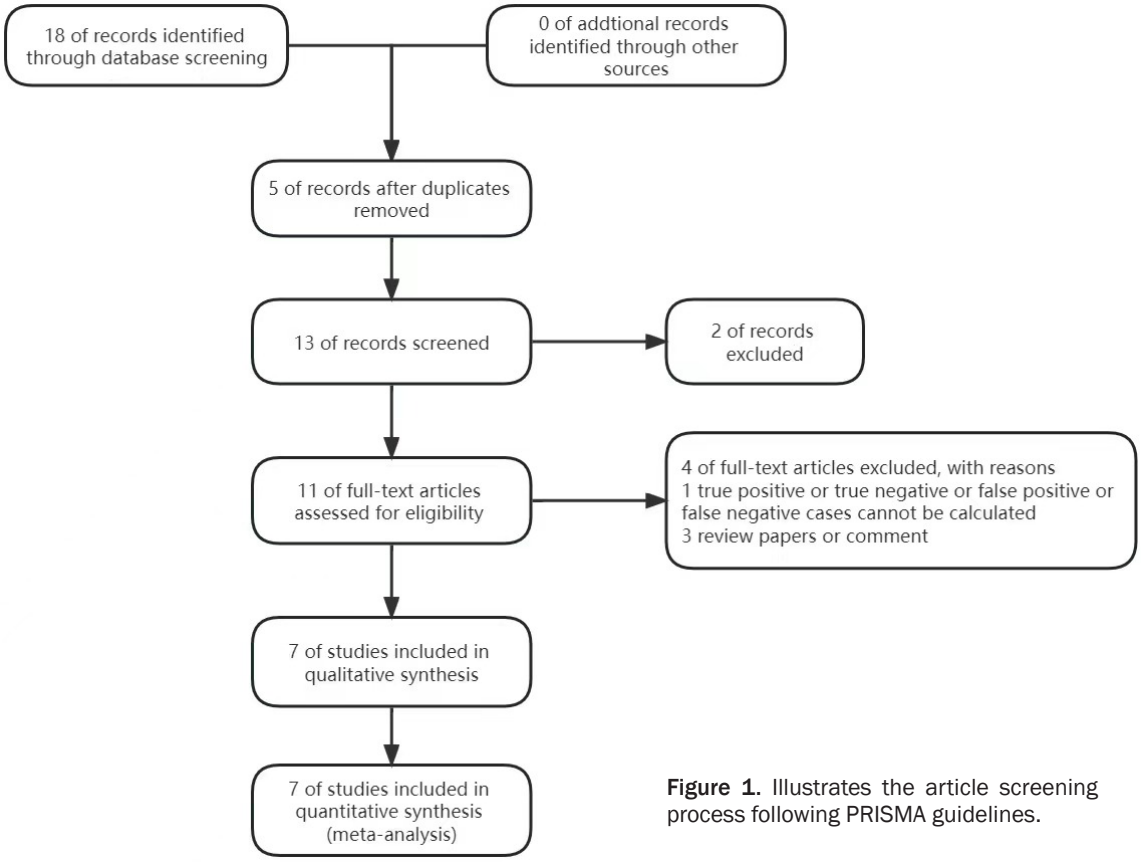

Figure 1. Illustrates the article screening process following PRISMA guidelines.

indicators included sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio (DOR). Using the summarized receiver operating characteristic (sROC) curve, the area under the curve (AUC) was calculated to visualize the diagnostic accuracy. To assess inter-study heterogeneity (other than threshold effects) and inter-study inconsistency, we calculated the Cochran Q statistic and the inconsistency index (I^2), and set the significance level of the corresponding *P* value to P = 0.05. Due to the expected between-study heterogeneity, the DerSimonian and Laird methods were applied for random-effects analysis model (REM), as it provides a more conservative estimate of pooled data. To identify publication bias, we assessed it by visual inspection of the funnel plots of Deek. All hypothesis tests were considered statistically significant if the *P*-value was < 0.05.

Results

Results of search and inclusion studies

In total, 18 related papers were found through a systematic search. A total of 7 eligible articles on CAC for the diagnosis of early lung cancer were included in this meta-analysis [18-24]. A description of the screening process for the studies included in this review can be found in **Figure 1**.

Features and quality assessment of the included literature

The meta-analysis included seven related studies, and **Table 1** shows the true positive, false positive, true negative, and false negative results of each study. Supplementary Table 1 shows the specimen type, sex, nodule location and CAC quantitative information. Two of the clinical articles about CAC are related to pathological indexes (Supplementary Table 2).

Meta-analysis of benign and malignant pulmonary nodules diagnosed by CAC

Heterogeneity analysis: Using the random effect model, the effect sizes were combined, and the results showed increased heterogeneity (Q = 58.92, I^2 = 89.8%) (**Figure 2A**). All diagnostic odds ratios, sensitivity, specificity, positive like-

Table 1. Analyze the characteristics of the file

Author	Year	n	Cut off	TP	FP	TN	FN	Sensitivity, %	Specificity, %
Ruth L. Katz	2020	207	≥ 3	95	0	100	12	88.8	100
Han Yang	2022	53	≥ 3	45	12	22	14	76.3	64.7
Wei-Ran Liu	2020	310	> 1	156	15	63	76	67.2	80.8
Ming-Xiang Feng	2021	205	≥ 3	145	8	29	23	86.3	78.4
Xiao-Chang Qiu	2021	63	≥ 3	45	3	10	5	90	76.9
Mao-Song Ye	2022	728	≥ 3	414	53	145	116	78.11	73.23
Mao-Song Ye	2021	125	> 2	57	6	38	24	70.4	86.4

TP, true positive; FP, false positive; TN, true negative; FN, false negative.

lihood ratios, and negative likelihood ratios I2 were above 50%.

Subgroup analysis: Our results suggest significant heterogeneity of non-threshold effects in these seven studies. We used subgroup analyses to try to identify sources of heterogeneity. The subgroup included "year of publication" and "country", and the results of the subgroup analysis showed that the intra-group heterogeneity of the United States and China was not significant in the subgroup analysis by country group, and the heterogeneity was significant after combining, indicating that countries may be a potential source of heterogeneity (**Figure 2B** and **2C**).

Combination effect analysis: The combined sensitivity, specificity and AUC were 0.80 (95% CI: 0.73-0.86), 0.85 (95% CI: 0.69-0.94) and 0.87 (95% CI: 0.84-0.90), respectively (**Figure 3** and Supplementary Figure 1). According to Supplementary Figures 2, 3, the pooled positive likelihood ratio, negative likelihood ratio, diagnostic odds ratio, and diagnostic score for CAC were 5.42 (95% CI: 2.37-12.43), 0.23 (95% CI: 0.16-0.35), 23.36 (95% CI: 7.33-74.46), and 3.15 (95% CI: 1.99-4.31). According to Supplementary Figure 4, the 95% confidence set is located in the lower left quadrant, suggesting circulating abnormal cells are more accurate in diagnosing lung nodules.

Fagan nomogram analysis: Simulating the clinical situation was predicted at 50%. Modeling the clinical situation used a 50% prediction probability. There was an 84% posterior probability of a positive test result, while the negative likelihood ratio was 0.23 and the negative posterior probability was 19 (**Figure 4**).

Publication bias

The Deek funnel plot (Supplementary Figure 5) shows that the slope of the coefficient is 0.76, indicating that there is no publication bias in the included studies.

Discussion

As a noninvasive biomarker for disease monitoring, CAC has gained increasing importance as a means of diagnosis. Due to the large differences between studies, the clinical significance of CAC in patients with early lung cancer remains controversial. A meta-analysis of published studies evaluated the clinical utility of CAC. CACs are highly sensitive and accurate in detecting lung lesions in the early stages of lung cancer. It has been shown that aggressive tumors have fewer cohesive cells, resulting in more CAC and possible spread and metastasis [25]. Considering the early onset of abnormal tumors [26], CACs can be seen in both precancerous and malignant lesions, highlighting their value in lung cancer early detection.

Wei-Ran Liu et al. found that early NSCLC patients had a higher number of CACs than healthy people and considered CAC testing to be clinically meaningful for the therapeutic efficacy of surgery in NSCLC patients [21]. Han Yang et al. have shown that CAC has a better diagnostic effect in combination with PANIDS [23]. Mao-Song Ye et al. proposed that CAC has a high diagnostic value in the diagnosis of pulmonary nodules smaller than 10 mm, and when combined with other clinical risk factors, the predictive ability of CAC will be improved [22]. Ming-Xiang Feng et al. found that the number of CAC may increase with the development of the disease [20]. However, there are differences among studies on whether the diagnosis of pulmonary nodules by CAC is affected by nodule type and age, which may be related to the classification criteria and statistical methods of nodule types. The results of Han Yang et al. showed that CAC had a weak correlation with

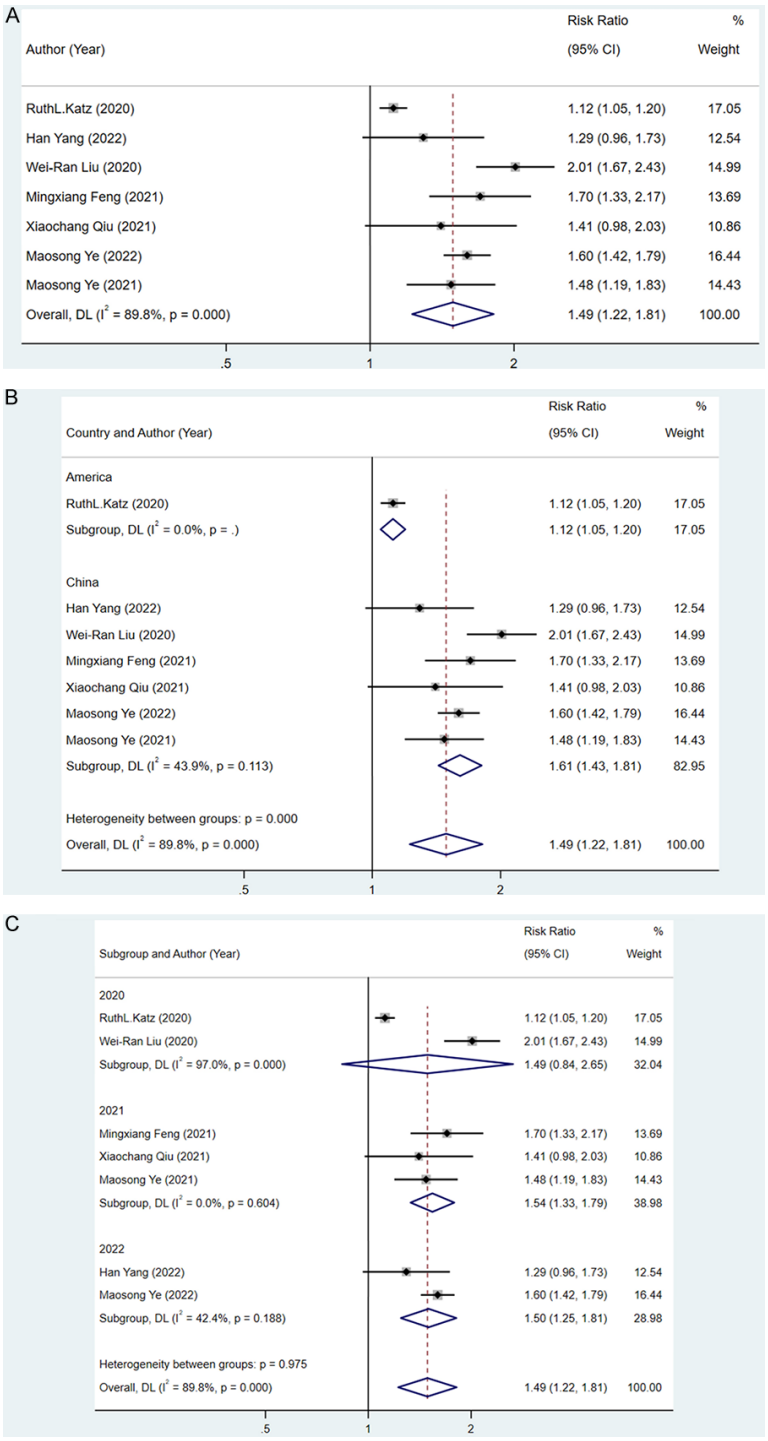

Figure 2. Forest plot of Heterogeneity analysis and subgroup analysis. A. Forest plot of Heterogeneity analysis CI = confidence interval, Point estimates are shown as brown squares and 95% CIs are shown as error bars. B. Forest plot of subgroup analysis (country) CI = confidence interval, Point estimates are shown as brown squares and 95% CIs are shown as error bars. C. Forest plot of subgroup analysis (year) CI = confidence interval, Point estimates are shown as brown squares and 95% CIs are shown as error bars.

age, and in Wei-Ran Liu's study, the positive result of CAC was not correlated with age, which may be due to Wei-Ran Liu et al.'s use of age as a dichotomous variable, resulting in reduced detection efficiency [21, 23]. Therefore, whether CAC count is related to age and nodule type still needs further study.

In addition, each study has its limitations, such as small sample size and adenocarcinoma bias in most studies. Therefore, whether CAC count is related to the pathological type, treatment factors and effect of its combined diagnosis model of lung nodules need to be further explored and supported by sufficient clinical sample size.

For pulmonary nodules smaller than 10 mm, the diagnosis is more difficult. Mao-Song Ye mentioned that compared with traditional lung cancer markers, CACs has better diagnostic value in the diagnosis of pulmonary nodules smaller than 10 mm. When the cut off value was > 2, the sensitivity and specificity of CAC were 70.5% and 86.4%, respectively. Diagnostic efficacy of CAC (AUC = 0.824, 95% CI = 0.746-0.886) significantly higher than CEA (AUC = 0.520, 95% CI = 0.429-0.610), SCC (AUC = 0.537, 95% CI = 0.446-0.627), NSE (AUC = 0.519, 95% CI = 0.428-0.609), Pro-GRP (AUC = 0.516, 95% CI = 0.425-0.606), CYFRA21-1 (AUC = 0.511, 95% CI = 0.420-0.602) and any other biomarkers (AUC = 0.512, 95% CI = 0.421-0.602) [22].

So far, lung cancer testing in patients with lung cancer Pulmonary nodules ≤ 10 mm are still a diagnostic challenge. When lung nodules are smaller than 10 mm in size, clinicians face the dilemma of whether to perform additional imaging or

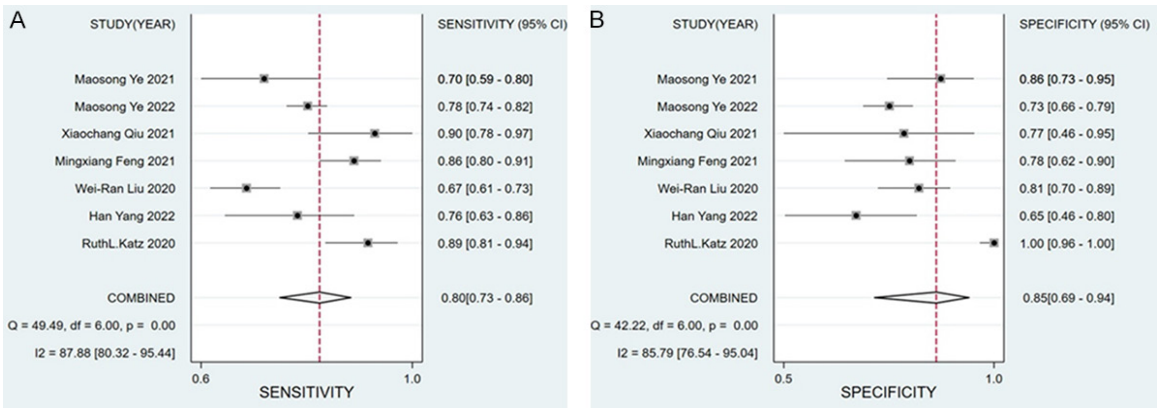

Figure 3. Combined sensitivity and specifity forest plot. Point estimates are shown as brown squares and 95% CIs are shown as error bars. A. Comprehensive sensitivity forest map; B. Compound specific forest map; CI = confidence interval.

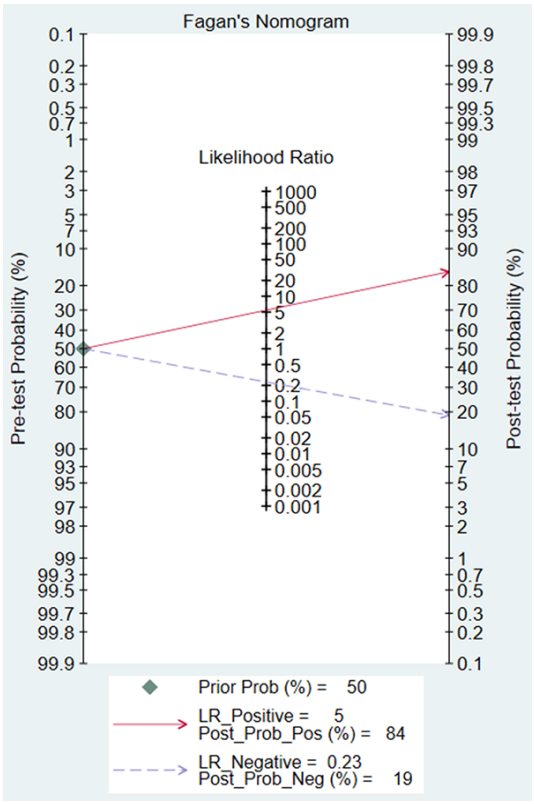

Figure 4. Accuracy of Fagan nomogram CAC in the diagnosis of early cancer. Fagan drew an axis with the prior log ratio on the left, the middle axis represents the log-likelihood ratio, and the right axis represents the posterior log ratio.

even invasive surgery due to the low specificity of CT [27]. In the early stages of lung cancer, cells with genetic abnormalities in the blood stream and sputum can be detected. The abnormal cells detected show chromosomal and genetic abnormalities in the tumor suppressor genes and proto-oncogenes common in lung cancer patients. In early lung cancer screening, specific detection of CACs may have higher auxiliary diagnostic value than circulating tumor cells (CTC) [28]. Because CTC is characterized by surface markers, if the markers of CTC cannot be detected, the sensitivity will be greatly reduced.

There are several limitations to our meta-analysis. Several studies have revealed marked heterogeneity in the collected data. There is potential for CAC to be used as a new biomarker for early detection, but many criteria have not yet been defined. Firstly, it is impossible to identify all the sources of heterogeneity. Secondly, this analysis may have been biased by including English-only studies. Since no publication bias was observed in this study, we believe that this bias should be relatively small. As a third factor, different cut-off values will likely result in heterogeneity between the studies. Further research is needed to assess the predictive value of CAC for some particular genetic variants or non-epithelial lung cancers. CAC could be used as a diagnostic biomarker for lung cancer in its early stages. This diagnostic tool can improve patient outcomes and quality of life by providing timely access to treatment.

Conclusion

Conclusion: CACs are more valuable than traditional tumor markers in the detection of early lung cancer, as well as in the detection of small nodular carcinomas on CT.

Acknowledgements

This research was funded by Project of Henan Science and Technology 2018 (pro-ject#2018-020006) to HM.

Disclosure of conflict of interest

None.

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References

[1] Xie S, Wu Z, Qi Y, Wu B and Zhu X. The metastasizing mechanisms of lung cancer: recent advances and therapeutic challenges. Biomed Pharmacother 2021; 138: 111450.

[2] Duma N, Santana-Davila R and Molina JR. Non-small cell lung cancer: epidemiology, screening, diagnosis, and treatment. Mayo Clin Proc 2019; 94: 1623-1640.

[3] Hirsch FR, Scagliotti GV, Mulshine JL, Kwon R, Curran WJ Jr, Wu YL and Paz-Ares L. Lung cancer: current therapies and new targeted treatments. Lancet 2017; 389: 299-311.

[4] Lee C, Guichet PL and Abtin F. Percutaneous lung biopsy in the molecular profiling era: a survey of current practices. J Thorac Imaging 2017; 32: 63-67.

[5] Wille MM, Dirksen A, Ashraf H, Saghir Z, Bach KS, Brodersen J, Clementsen PF, Hansen H, Larsen KR, Mortensen J, Rasmussen JF, Seersholm N, Skov BG, Thomsen LH, Tønnesen P and Pedersen JH. Results of the randomized Danish lung cancer screening trial with focus on high-risk profiling. Am J Respir Crit Care Med 2016; 193: 542-551.

[6] Humphrey LL, Deffebach M, Pappas M, Baumann C, Artis K, Mitchell JP, Zakher B, Fu R and Slatore CG. Screening for lung cancer with low-dose computed tomography: a systematic review to update the US preventive services task force recommendation. Ann Intern Med 2013; 159: 411-420.

[7] Aberle DR, Adams AM, Berg CD, Black WC, Clapp JD, Fagerstrom RM, Gareen IF, Gatsonis C, Marcus PM and Sicks JD. Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 2011; 365: 395-409.

[8] Silva CL, Perestrelo R, Silva P, Tomás H and Câmara JS. Implementing a central composite design for the optimization of solid phase microextraction to establish the urinary volatomic expression: a first approach for breast cancer. Metabolomics 2019; 15: 64.

[9] Wisnivesky JP, Henschke CI and Yankelevitz DF. Diagnostic percutaneous transthoracic needle biopsy does not affect survival in stage I lung cancer. Am J Respir Crit Care Med 2006; 174: 684-688.

[10] Yamagami T, Kato T, Iida S, Hirota T and Nishimura T. Percutaneous needle biopsy for small lung nodules beneath the rib under CT scan fluoroscopic guidance with gantry tilt. Chest 2004; 126: 744-747.

[11] Chen M and Zhao H. Next-generation sequencing in liquid biopsy: cancer screening and early detection. Hum Genomics 2019; 13: 34.

[12] Paoletti C and Hayes DF. Circulating tumor cells. Adv Exp Med Biol 2016; 882: 235-258.

[13] Sorber L, Zwaenepoel K, Deschoolmeester V, Van Schil PE, Van Meerbeeck J, Lardon F, Rolfo C and Pauwels P. Circulating cell-free nucleic acids and platelets as a liquid biopsy in the provision of personalized therapy for lung cancer patients. Lung Cancer 2017; 107: 100-107.

[14] O'Flaherty L, Wikman H and Pantel K. Biology and clinical significance of circulating tumor cell subpopulations in lung cancer. Transl Lung Cancer Res 2017; 6: 431-443.

[15] Jiang F, Yin Z, Caraway NP, Li R and Katz RL. Genomic profiles in stage I primary non small cell lung cancer using comparative genomic hybridization analysis of cDNA microarrays. Neoplasia 2004; 6: 623-635.

[16] Katz RL, He W, Khanna A, Fernandez RL, Zaidi TM, Krebs M, Caraway NP, Zhang HZ, Jiang F, Spitz MR, Blowers DP, Jimenez CA, Mehran RJ, Swisher SG, Roth JA, Morris JS, Etzel CJ and El-Zein R. Genetically abnormal circulating cells in lung cancer patients: an antigen-independent fluorescence in situ hybridization-based case-control study. Clin Cancer Res 2010; 16: 3976-3987.

[17] Feng M, Lin M, Zhou H, Zhu Y, Chen B, Ye X, Huang C, Zhang J and Bai C. Clinical utility of circulating genetically abnormal cells within low-dose computed tomography lung cancer screening: a correlative MCPND trial study. J Clin Oncol 2020; 38: e15536.

[18] Ye M, Tong L, Zheng X, Wang H, Zhou H, Zhu X, Zhou C, Zhao P, Wang Y, Wang Q, Bai L, Cai Z, Kong FS, Wang Y, Li Y, Feng M, Ye X, Yang D, Liu Z, Zhang Q, Wang Z, Han S, Sun L, Zhao N, Yu Z, Zhang J, Zhang X, Katz RL, Sun J and Bai C. A classifier for improving early lung cancer diagnosis incorporating artificial intelligence and liquid biopsy. Front Oncol 2022; 12: 853801.

[19] Qiu X, Zhang H, Zhao Y, Zhao J, Wan Y, Li D, Yao Z and Lin D. Application of circulating genetically abnormal cells in the diagnosis of early-

stage lung cancer. J Cancer Res Clin Oncol 2022; 148: 685-695.
[20] Feng M, Ye X, Chen B, Zhang J, Lin M, Zhou H, Huang M, Chen Y, Zhu Y, Xiao B, Huang C, Katz RL and Bai C. Detection of circulating genetically abnormal cells using 4-color fluorescence in situ hybridization for the early detection of lung cancer. J Cancer Res Clin Oncol 2021; 147: 2397-2405.
[21] Liu WR, Zhang B, Chen C, Li Y, Ye X, Tang DJ, Zhang JC, Ma J, Zhou YL, Fan XJ, Yue DS, Li CG, Zhang H, Ma YC, Huo YS, Zhang ZF, He SY and Wang CL. Detection of circulating genetically abnormal cells in peripheral blood for early diagnosis of non-small cell lung cancer. Thorac Cancer 2020; 11: 3234-3242.
[22] Ye M, Zheng X, Ye X, Zhang J, Huang C, Liu Z, Huang M, Fan X, Chen Y, Xiao B, Sun J and Bai C. Circulating genetically abnormal cells add non-invasive diagnosis value to discriminate lung cancer in patients with pulmonary nodules ≤ 10 mm. Front Oncol 2021; 11: 638223.
[23] Yang H, Chen H, Zhang G, Li H, Ni R, Yu Y, Zhang Y, Wu Y and Liu H. Diagnostic value of circulating genetically abnormal cells to support computed tomography for benign and malignant pulmonary nodules. BMC Cancer 2022; 22: 382.
[24] Katz RL, Zaidi TM, Pujara D, Shanbhag ND, Truong D, Patil S, Mehran RJ, El-Zein RA, Shete SS and Kuban JD. Identification of circulating tumor cells using 4-color fluorescence in situ hybridization: validation of a noninvasive aid for ruling out lung cancer in patients with low-dose computed tomography-detected lung nodules. Cancer Cytopathol 2020; 128: 553-562.
[25] Yendamuri S, Vaporciyan AA, Zaidi T, Feng L, Fernandez R, Bekele NB, Hofstetter WL, Jiang F, Mehran RJ, Rice DC, Spitz MR, Swisher SG, Walsh GL, Roth JA and Katz RL. 3p22.1 and 10q22.3 deletions detected by fluorescence in situ hybridization (FISH): a potential new tool for early detection of non-small cell lung cancer (NSCLC). J Thorac Oncol 2008; 3: 979-984.
[26] Zojer N, Dekan G, Ackermann J, Fiegl M, Kaufmann H, Drach J and Huber H. Aneuploidy of chromosome 7 can be detected in invasive lung cancer and associated premalignant lesions of the lung by fluorescence in situ hybridisation. Lung Cancer 2000; 28: 225-235.
[27] Sadate A, Occean BV, Beregi JP, Hamard A, Addala T, de Forges H, Fabbro-Peray P and Frandon J. Systematic review and meta-analysis on the impact of lung cancer screening by low-dose computed tomography. Eur J Cancer 2020; 134: 107-114.
[28] Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L, Smith MR, Kwak EL, Digumarthy S, Muzikansky A, Ryan P, Balis UJ, Tompkins RG, Haber DA and Toner M. Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature 2007; 450: 1235-1239.

Supplementary Table 1. Specimen type, sex, nodule location, CAC quantitative information

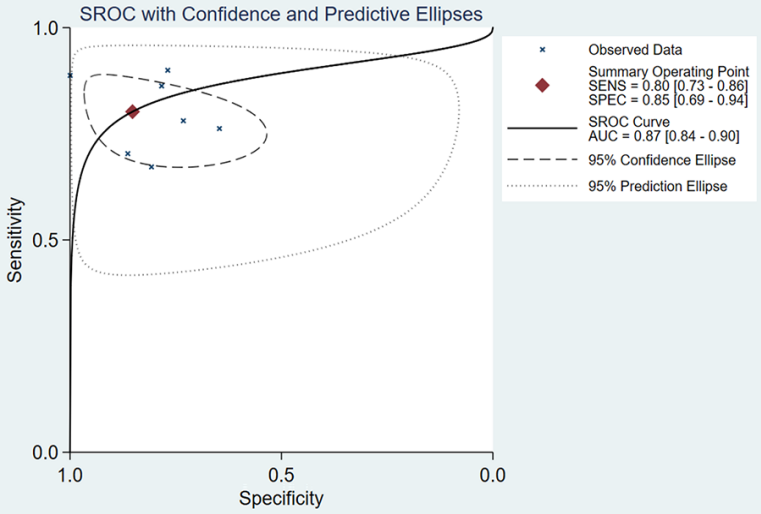
Type of the liquid (specimen)	Characteristic of CAC		Diagnostic efficacy of CAC in benign and malignant pulmonary nodules			REF
	Gender (male/female)	Location of the nodules (Upper lobe/Non-Upper lobe)	AUC	95% CI	*P*	
Peripheral venous blood	39/54 (P = 0.003)	59/34 (P = 0.848)	0.779	(0.587-0.806)	< 0.001	[23]
Peripheral venous blood	133/177 (P = 0.969)	/	0.769	(0.716-0.822)	< 0.001	[21]
Peripheral venous blood	33/30 (P = 0.612)	/	0.837	(0.810-0.864)	< 0.001	[19]
Peripheral venous blood	363/365 (/)	404/324 /	0.765	(0.727-0.803)	< 0.001	[22]
Peripheral venous blood	47/78 (/)	69/56 /	0.824	(0.746-0.886)	/	[18]
Peripheral venous blood	97/108 (/)	/	0.823	(0.741-0.906)	< 0.001	[20]
Peripheral venous blood	94/113 (/)	/	0.986	/	/	[24]

AUC, area under the curve; CI, confidence interval.

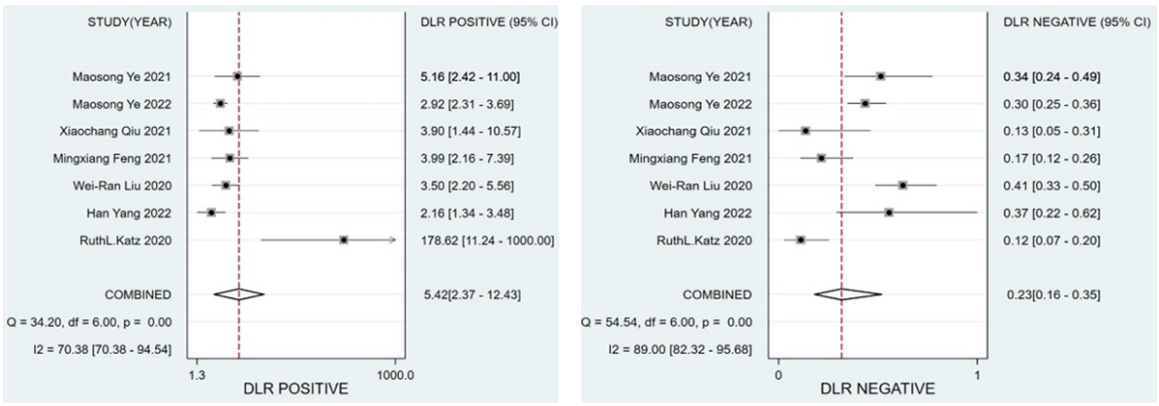
Supplementary Table 2. Pathological types of the files

Author	Pathological types	CACs positive	CACs negative	*P*-value	REF
Wei-Ran Liu	AIS and MIA	52 (71.2%)	21 (28.8%)	0.592	[21]
	IAC	98 (65.8%)	51 (34.2%)		
	Nonadenocarcinoma	6 (60.0%)	4 (40.0%)		
Xiao-Chang Qiu	Adenocarcinoma[a]	41 (89.1%)	5 (10.9%)	1.0	[19]
	Squamous cell carcinoma	2 (100%)	0 (0%)		
	SCLC	2 (100%)	0 (0%)		

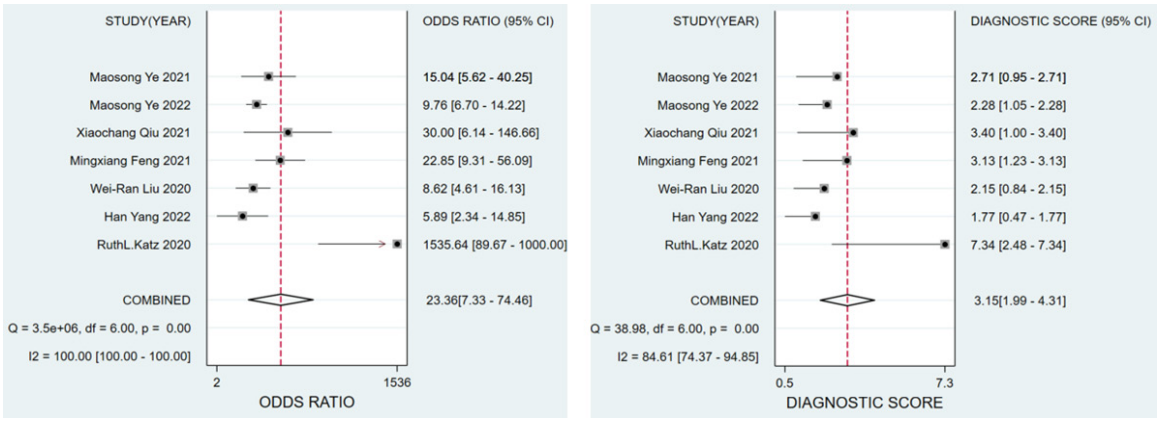
[a]Pathological types included 5 cases of adenocarcinoma in situ, 8 cases of minimally invasive adenocarcinoma, and 33 cases of invasive adenocarcinoma. AIS, adenocarcinoma in situ; IAC, invasive adenocarcinoma; MIA, minimally invasive adenocarcinoma.



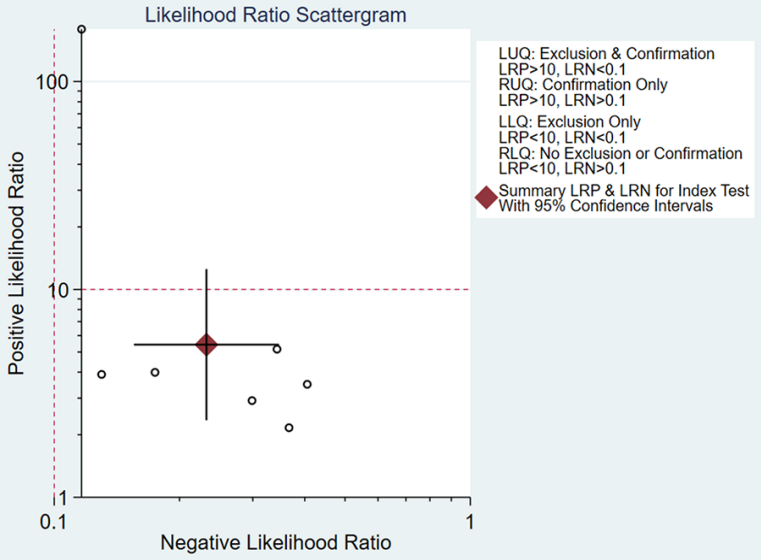
Supplementary Figure 1. Receiver operating characteristics and combined area under curve are summarized. The confidence ellipse indicates that the mean values of sensitivity and specificity are more likely to be in this region. The prediction ellipse indicates that individual values of sensitivity and specificity are more likely to be in that region. AUC = Area under the curve.



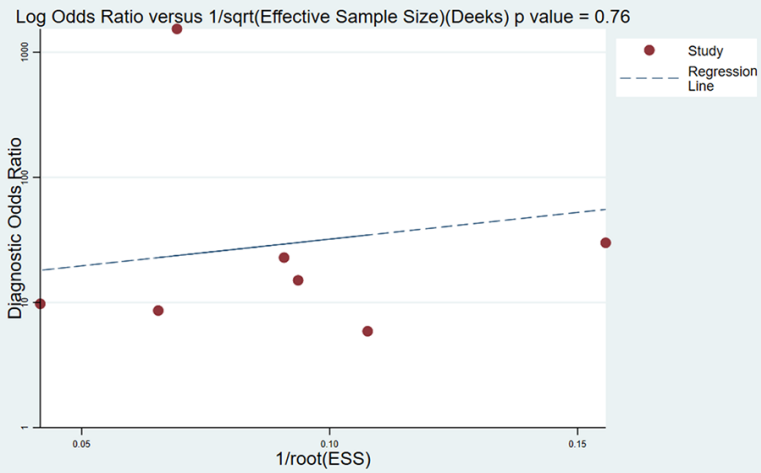
Supplementary Figure 2. Combined likelihood ratio forest graph (LR+, LR-). Point estimates are shown in brown boxes and 95% CIs are shown in error bars. CI = confidence interval. LR+ = positive likelihood ratio, LR- = negative likelihood ratio, positive likelihood ratio is the ratio of true positive rate to false positive rate of screening results. Negative likelihood ratio is the ratio of false negative rate to true negative rate of screening results.



Supplementary Figure 3. Forest plot of combined diagnostic score and diagnostic odds ratio. Point estimates are shown as brown squares and 95% CIs are shown as error bars. CI = confidence interval. The diagnostic ratio was the ratio of PLR to NLR-, that is, the ratio of positive likelihood to negative likelihood.



Supplementary Figure 4. Scatter plot of the distribution of likelihood ratios (LR+/LR-) and combined estimates for each study. LUQ = upper left quadrant, likelihood ratio positive > 10, likelihood ratio negative < 0.1: exclusion and confirmation; RUQ = upper right quadrant, likelihood ratio positive > 10, likelihood ratio negative > 0.1: confirmed only; LLQ = lower left quadrant, likelihood ratio positive < 10, likelihood ratio negative < 0.1: excluded.



Supplementary Figure 5. Deep funnel plots show publication bias. Each solid circle represents one study included in the meta-analysis. The middle line represents the pooled diagnostic odds ratio (DOR).