The Potential of Folate-Positive Circulating Tumor Cells (FR+-CTC) as a Novel Diagnostic Biomarker for Non-Small Cell Lung Cancer: A Systematic Review of Clinical Trials

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Abstract:

Background: Non-small-cell lung cancer (NSCLC) is malignancy that remains the leading cause for cancer mortalities. Diagnosis is often made in advanced stages, hence, the unmet need for novel diagnostic methods. FR+-CTC is acknowledged as a potential diagnostic biomarker that detects NSCLC presence, distinguishing it from benign lung diseases and healthy individuals.

Purpose of Study: This study aims to investigate the potential of FR+-CTC to be utilized as an accurate, sensitive, and specific diagnostic biomarker for NSCLC.

Methods: This systematic review is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria. Studies were obtained from databases namely Wiley Online Library, MEDLINE, Science Direct, CENTRAL, and ProQuest. The outcome assessed includes summary receiver operating characteristics (sROC) evaluating diagnostic accuracy taking form of area under the curve (AUC) analysis. Risk of bias assessment is carried out using Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2).

Results: 5 studies confirm a higher amount of FR+-CTC in peripheral blood can be utilized as a diagnostic marker in NSCLC patients. Detection of FR+CTC in NSCLC diagnosis is superior to existing biomarkers with a sensitivity and specificity of 81.94% and 73.08%. FR+CTC presents the highest AUC (0.823; 95% CI, 0.773-0.874) compared to other biomarkers. FR +- CTC levels can differentiate the types of lung adenocarcinoma with acceptable sensitivity.

Conclusion: FR+-CTC detection is a reliable diagnostic method with the

highest degree of accuracy for diagnosing NSCLC compared to other biomarkers. FR+-CTC can also be utilized to predict possible malignancies, even in its early stages.

Keywords: Biomarkers, circulating tumor cells, diagnosis, folate receptor, lung cancer, NSCLC

Introduction:

Globally, lung malignancies remain a significant healthcare challenge within the field of respiratory medicine, as acknowledged that lung cancer remains the leading cause for cancer-related deaths.[1,2] Advancements in the understanding of non-small cell lung cancer (NSCLC) demonstrated that often, NSCLC is only diagnosed during the advanced stages, which results in a generally poor prognosis for patients.[3] Hence, there is an unmet need for novel diagnostic and screening methods. An accumulating body of evidence had displayed the potential of folate receptor-positive circulating tumour cells (FR^+-CTC) as an accurate diagnostic biomarker that detects the presence of NSCLC and is also able to accurately distinguish it from other benign lung diseases that similarly manifests in non-specific symptoms of NSCLC such as coughing, chest pain, hemoptysis hemoptysis, and dyspnea. It is detected in significantly greater amounts in NSCLC patients, which underpins its utilization as a diagnostic marker. In the long run, the ability to carry out an accurate, sensitive sensitive, and specific diagnostic test for NSCLC would concurrently contribute to the realization of SDG number 3, indicator 3.4.1, which aims to reduce mortality rate from cancer among other diseases. Hence, the focus of this review would be to systematically review the potential of FR^+-CTC as a diagnostic biomarker for NSCLC.

Methods

This section contains the data sources, search terms and strategies, selection criteria, number of studies found and included.

Search Strategy

For this review, literature search was conducted based off the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA). In obtaining the relevant studies, the following keywords was used: “folate receptor-positive" AND “non-small-cell lung cancer” OR “NSCLC” AND “Biomarker” altogether with appropriate Mesh terms and synonyms. The search strategy was carried out in PubMed/MEDLINE, Wiley Online Library, ProQuest, CENTRAL/Cochrane and Science Direct to disseminate articles that were published up until 6 April 2021.

Inclusion & Exclusion Criteria

Upon the creation of this review, the included studies possessed several inclusion criteria as follows: (1) Clinical trials (2) Studies that evaluate FR+-CTC altered expression to

distinguish between those who are, (3) either healthy, has a benign lung disease, or NSCLC. There is no limitation for age/gender/race. (4) Outcomes of: Area under the curve (AUC) value of ROC analysis; sensitivity & specificity; median FR^{+}-CTC value; as well as any defined threshold or FR^{+}-CTC cutoff levels. Meanwhile, the exclusion criteria applied includes: (1) literatures with irretrievable full text; (2) reviews, letters, commentaries & conference abstracts, (3) studies written in languages other than Bahasa Indonesia or English, (4) incomplete clinical trials.

Data Extraction & Study Outcomes

3 independent reviewers extracted data, with any discrepancies adjudicated through consensus. The details extracted from the reviewed studies includes (1) authors and year of publication; (2) disease characteristic of the population; (3) study characteristics: location, designdesign, and sample size; (4) details regarding sampling method and method to analyze FR^{+}-CTC levels. The main outcomes assessed is a summary of the receiver operating characteristics (ROC) curve that demonstrates the diagnostic accuracy of a diagnostic procedure, defined from the area under the curve (AUC). whereas an AUC <0.5 = not useful, 0.5-0.6 = bad, 0.6-0.7 = sufficient, 0.7-0.8 = good, 0.8-0.9 = very good, and 0.9-1.0 = excellent.

Risk of Bias Assessment

Risk of bias assessment was conducted using QUADAS-2. Among all the reviewed studies, 2 showed unclear risk of bias for patient selection, 2 showed unclear risk of bias for index test, and two studies showed unclear risk of bias for reference standard. These are due to the lack of clear explanation regarding certain parts of the methodology of the study. Regarding applicability concerns, all studies showed to have low risks. The full methodological quality assessment is displayed in **Table 1.**

Table 1. Summary of Quality Assessment of Included Studies using QUADAS-2

Study	RISK OF BIAS				APPLICABILITY CONCERNS		
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Yu ; 2013	☺	☺	?	☺	☺	☺	☺
Ding; 2018	?	☺	☺	☺	☺	?	☺
Chen; 2015	☺	?	☺	☺	☺	☺	☺
Xue; 2018	?	☺	?	☺	☺	☺	☺
Zhou; 2019	☺	?	☺	☹	☺	☺	☺

☺ Low Risk ☹ High Risk ? Unclear Risk

Results:

In this section, the authors should include data found from sources and organized systematically i.ei.e., chronologically, thematically, methodologically, etc. Moreover, they should also include an analysis of primary study results based on current medical principles. Include

summary of results from included studies as well as your own analysis and evaluation of the articles. Avoid using personal opinions; be objective in the analysis.

Search Results

Search results from the 5 international databases yielded 172 studies. These were then initially screened through their title & abstract relevancy, and study type relevancy, which resulted in 11 studies and 8 studies after exclusion of duplicates. Full-text screening was then conducted resulting in further exclusion of 3 studies due to their incompatible study design. Complete visualization of the comprehensive selection process is attached as **Figure 1.**

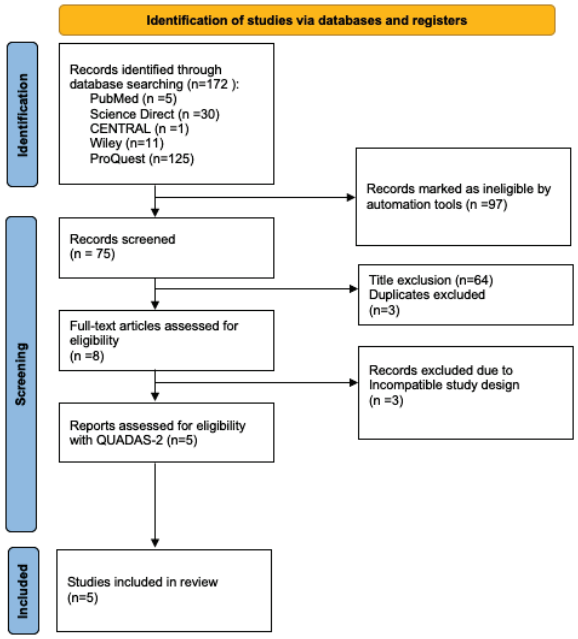

Figure 1. PRISMA Flow Chart of Search Strategies

Characteristics of Included Studies

5 included studies assessed the diagnostic accuracy of FR^{+}-CTC as a diagnostic biomarker for patients with NSCLC. All ofAll the studies were conducted in various regions of China.[4-8] Of the 5 studies, 3 were single-blinded, prospective trial,[5,6,7] 1 was a double-blinded, prospective, single center trial,[4] and 1 study was a single-blinded, prospective, multi-centered trial.[8] All 5 studies defined "malignant" lesions as patients with NSCLC subtypes such as adenocarcinoma or squamous cell carcinoma. The results were compared to a control group consisting of a mixture of healthy patients and patients with benign lung diseases. All 5 studies used 3 mL of peripheral blood for samples and are FR^{+}-CTC profiling was conducted using Ligand-targeted polymerase chain reaction (LT-PCR). Data was gathered from 1702 volunteers, (1158 were NSCLC patients, 437 were benign lung disease patients, 107 were healthy patients). A comprehensive summary of characteristics of each study are presented in **Table 2.**

Discussion:

Current State of Lung Cancer Diagnostics

The clinical diagnosis and management of NSCLC is currently largely based pathological findings and clinical symptoms, which would then further categorize NSCLC into different clinical stages, from stages.[9,10] Due to a rapidly growing body of evidence, guidelines regarding lung cancer was were established, and the most recent being the 8th American Joint Committee on Cancer stage classification for lung cancer. This guideline utilized the TNM criteria to assess the clinical stages of lung cancer, in which the T criteria assessed the size of the primary tumor, N assesses invasion to neighboring lymph node, and M assesses the presence of metastasis. The subsequent treatment plan and survival is then based largely on this clinical staging.[11] Interestingly, how pathological, clinical, and sometimes radiological findings are staged into different groups still varies from one established guideline to another, and it is acknowledged, that the accuracy of these clinical staging, are generally low (50-60%).[9]

The Diagnostic Properties of FR+-CTC

FR^+-CTC is a diagnostic marker where it assesses whether folate receptors (FR) are present in circulating tumour cells (CTC) cancer patients.[12] CTCs are tumour cells that shed off from primary tumours and into the vasculature, indicating an intermediate stage tumour. The ultimate goal of CTCs is to eventually assert dominance over healthy cells within the body through metastasis, and metastasis and inflict extensive cell deaths and mutations.[13] FR are membrane glycoproteins usually found on the CTC surface, hence making them a possible biomarker for the presence of CTCs in the circulation.[12,14] Minimally invasive, liquid biopsies are usually taken to assess the quantification of FR^+-CTCs in patients with suspected cancer, usually through qPCR methodologies. With little amounts of cells producing FRs on their surface, FR^+-CTC detection is substantially more reliable for diagnosis of cancer.[12]

The Potential of FR+-CTC as a Biomarker to Diagnose Malignant Lung Cancer, and as a Screening Indicator

CTCs have the considerable potential to be acknowledged as a standard screening test and be used for molecular characterization of a tumor. Results of studies have confirmed that a higher amount of

FR$^+$-CTC in peripheral blood is associated with adverse prognosis in NSCLC patients.[15] Detection of CTCs in NSCLC has been challenging due to the rarity in circulation; hence, it is therefore critical that sensitive and specific CTC detection methods are generated to be used as a potential molecular marker not only for early detection of NSCLCs but also for assessing aspects of prognosis such as the possibility for metastasis.[16] Currently, fields focusing on medical technology have been showing their ability to monitor CTC in patients with advanced lung cancer. For example, a study by Krebs et al. Discovered that CTCs that were acknowledged by CellSearch System can perform as a novel prognostic factor in patients with NSCLC.[4] The previous statement can is supported by Chen et al. which stated that a doubled number of patients with NSCLC presented with high CTC levels compared to patients with benign lung disease and healthy donors. Furthermore, FR expression was upregulated in about 75.7% of patients with NSCLC, indicating that FR may be a precise potential target for detecting CTCs in lung cancer patients.[4] Xue et al., found that the performance of FR$^+$CTC in the diagnosis of lung cancer is proven to have a sensitivity and specificity of 81.94% and 73.08% for the entire study cohort.[6] Further analysis of FR-positive CTC detection in patients with distinct pathological varieties of lung adenocarcinoma shows a sensitivity of 60%, 73.2%, 73.9%, and 75% in patients with adenocarcinoma in situ (AIS), minimally invasive adenocarcinoma (MIA), invasive glands, and IA variants, respectively. Conjointly, its efficacy for detection also has a satisfactory diagnostic validity of $P < 0.05$.[5,8]

Consistent with previously stated, CTC levels distinguishes lung cancer from nonmalignant lung disease with a consistently high AUC of 0.813 in the validation set, which was higher than the plasma tumor markers. To extensively investigate how FAR$^+$CTC detection compares with currently used tumor biomarkers in patients with NSCLC, Yu et al. compared its diagnostic efficiency with the current clinical biomarkers, including NSE, CEA, CA125, cyfra 21-1, and SCC Ag. Providentially, FR$^+$CTC detection method displays superior AUC (0.823; 95% CI, 0.773-0.874) compared with the other biomarkers. Moreover, a larger study by Chen et al. also confirmed a supporting result of the comparison, where FR$^+$CTC displayed the highest AUC (0.815) and significant sensitivity (75%) and specificity (85%). Accordingly, these results indicate that CTCs could satisfactorily identify NSCLC patients

with a greater degree of accuracy compared to current biomarkers, even in its early stages.[4]

The potential of FR$^+$-CTCs to assess tumor metastasis is also an issue of notable importance which makes up for the deficiencies of other detection methods. The use of CTCs as a liquid biopsy is favorable for serial assessment of metastasis during the disease in a real-time manner via an uncomplicated form of blood draw. CTCs levels in patients with stage III-IV lung adenocarcinoma turned out to be higher than in stage I–II patients.[3] Concerning previous reports, the FR$^+$-CTC count in lung cancer patients with a >3 cm nodule size was notably higher than those with a ≤3 cm nodule size.[7] Hence, these results proved the clinical significance of FR$^+$-CTC as a sensitive and reliable diagnostic assay for lung cancer metastasis screening that could drive further work-up decisions.

Strength and Limitations

Strengths of this systematic review includes the implementation of blinding, with 4 studies from Xue et al, Ding et al, Yu et al, and Zhou et al using the single-blinded method, while 1 study, by Chen et al uses double-blinded method.[4-8] Another strength also included in this review is the uniform use of LT-PCR for FR$^+$-CTC quantification across all studies.[4-8] Study results also investigates the diagnostic accuracy of FR$^+$-CTC to distinguish NSCLC patients from healthy and benign lung disease populations, in addition to comparing AUCs with several established reference standard. Finally, all the reviewed studies show generally low risk of bias in terms of flow and timing and applicability concerns. To our knowledge, this is the first systematic review that investigates the diagnostic accuracy of FR$^+$-CTC to distinguish NSCLC patients from a healthy/benign lung disease population.

The current study has certain limitations. Generalizability of the results is low as all studies are conducted in China. A larger one might be needed for the results to be globally representative. Furthermore, there is a relatively small unclear risk of bias in terms of patient selection, index test & reference standard, as the said studies did not specifically address the full extent of research methodology.

Conclusion:

Advancements in a wide variety of biomarkers have been investigated to predict diagnosis and prognosis; unfortunately, NSCLC, one of the causes of cancer-related death worldwide, is only often discovered at

an advanced stage when treatments have only narrowed efficacy.

In the five gathered studies we reviewed, we identified the potential of folate receptor-positive circulating tumor cells (FR+-CTC) as an absolute diagnostic biomarker with high sensitivity, specificity, and AUC for diagnosing NSCLC and is also able to differentiate it from other benign lung diseases in a precise manner. The folate receptor (FR), a cell-surface receptor glycoprotein, although also exhibited in multiple cancers-- no cells expressing FR have been recognised in the circulatory system except for CTCs and activated monocytes. Hence, as FR expression was found to be upregulated in roughly 75.7% of patients with NSCLC, FR may be a specific potential target for detecting CTCs in a patient with NSCLC. Moreover, CTC detection in NSCLC also revealed significant correspondence between disease stages and CTC numbers, as CTC levels in patients with stage IV lung cancer were significantly higher than those with earlier stages. Taking everything into account, it is apparent that our results claim FR+-CTC as a reliable biomarker that would be clinically valuable for early diagnosis of NSCLC and treatment response assessment. Further investigation with a more extensive sample size is obligated to evaluate the diagnostic effectiveness of FR+CTC in subjects with large nodule sizes.

Declarations

Ethics approval and consent to participate

Not applicable.

Availability of data and material

Not applicable.

Conflict of interests

The authors report no relationships that could be construed as a conflict of interest.

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Authors' contributions

Garry Soloan (Conceptualization, Methodology, Writing - Original Draft, Visualization, Project administration), Kieran Pasha Ivan Sini (Writing-Original Draft, Investigation, Visualization) and Raisa Zalfa Meutia Abubakar (Writing - Original Draft, Investigation, Resources)

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Appendix

Table 2. Summary of Study Characteristics

Author; Year	Study Location	Study Design	Study Population					Sample source	Platform	Study Outcomes							
			Sample Size	Age	Characteristics of study population		Characteristics of Control Group			Median FR+-CTC (Units/3 mL)	*p*	AUC (95% CI)	Sensitivity	Specificity	FR+-CTC Cutoff Levels	Sensitivity	Specificity
Chen, 2015	Shanghai	Prospective, double-blinded, single center clinical trial	756	N/A	Training Set	Healthy (n= 28)	Patients who are healthy or with benign lung disease	3 mL of peripheral blood	LT-PCR	5,72	N/A	0.815 (0.772-0.853)	72,46%	88,65%	8,93	74,40%	86,60%
						Benign lung disease (n=113)				6,6	In comparison to healthy controls : 0.314						
						NSCLC (n=236)				11,64	In comparison to healthy controls & benign lung disease: <0.001						
					Validation Set	Healthy (n =28)				5,95	N/A	0.813 (0.770 - 0.851)	76,37%	82,39%			

						Benign lung disease (n=114)				6,95	In comparison to healthy controls : 0.335						
						NSCLC (n=237)				12,41	In comparison to healthy controls & benign lung disease : <0.001						
Ding; 2018	Suzhou	Single-blinded Prospective clinical trial	200	N/A	Malignant SPN (n=50)		Patients with Benign SPN	3 mL antecubital venous blood	LT-PCR	9,79	In comparison to benign SPN as control: <0.001	0.836 (0.770-0.902)	N/A	N/A	8,35	70,20%	79,30%
					Benign SPN (n=30)					6,66	N/A						
					Lung Cancer (n=120)					10,65	In comparison to benign SPN as control: <0.001						

Xue; 2018	Sichuan	Single-blinded Prospective clinical trial	98	61	NSCLC (n=72)	Twenty-four patients with benign lung diseases and two healthy volunteers	Three milliliters of peripheral blood sample	LT-PCR	10,71	In comparison with control: <0.001	0.8221 (0.7208–0.9235)	74,19%	73,08%	8,7	81,94%	73,08%
				52	Control group (n=26)											
Yu; 2013	Beijing	Blinded Prospective Clinical Trial	266	N/A	Healthy (n=49)	Patients who are healthy or with benign lung disease	3 mL blood samples	LT-PCR	5,71	N/A	0.823 (0.773 - 0.874)	73,20%	84,10%	8,64	73,20%	84,10%
					Benign lung disease (n= 64)				6,74	In comparison to healthy controls : 0.105						
					NSCLC (n = 153)				10,82	in comparison to benign lung disease & healthy control : <0.001						
Zhou; 2019	Wuhan, Shanghai	Blinded Prospective multi-center trial	382	60	Training Set	NSCLC (n=181)	Patients with benign lung disease	3 mL peripheral blood samples	LT-PCR	9,9	Comparison of malignant vs benign lung diseases : <0.001	0.781 (0.698–0.864)	78,60%	78,40%	8,3	78,60%	78,40%

						Other types of malignant tumour(n=16)											
				57		Benign (n=63)				6,8							
				57	Validation Set	NSCLC (n=88)				9,9		0.792 (0.668–0.917)	82,70%	68,80%		82,70%	68,80%
						Other types of malignant tumour(n=5)											
				54		Benign (n=29)				6,8							

Notes: Legend: Non-small-cell lung cancer (NSCLC), Area under the curve (AUC), Ligand-targeted polymerase chain reaction (LT-PCR), Solitary pulmonary nodules (SPN)