REVIEW

Editorial Process: Submission:02/01/2024 Acceptance:06/14/2024

Tumor Educated Platelets as a Biomarker for Diagnosis of Lung cancer: A Systematic Review

Vaishali Walke[1]*, Saikat Das[2], Amol Mittal[3], Amit Agrawal[3]

Abstract

Introduction: Lung cancer is one of the commonest cause of cancer associated mortality worldwide. Platelets have emerged as key players in cancer development and progression by supporting tumor growth, and dissemination. In the present systematic review, we analyzed RNA transfer between cancer cells and platelets and explored potential role of different platelet RNA profiles as onco-signature in diagnosis, subtyping, disease progression and treatment monitoring in carcinoma lung carcinoma. **Materials and Methods:** The study followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines and Cochrane Manual of Systematic Reviews and Meta-analysis that included seven studies on patients with lung cancer, with data on tumor-educated platelets, and control group. The outcome measured was based on sensitivity, specificity, and ROC. PUBMED, SCOPUS, Central Cochrane Registry of Controlled Trials and Science Direct databases were searched using specific search terms until October 2023. QUADAS - 2 tool was used to assess quality, risk of bias and applicability concerns. **Results:** The analysis revealed AUC > 70% for different platelet mRNAs, with sensitivity and specificity of more than 60 %. AUC and sensitivity were highest for ITGA2B (AUC 0.922; sensitivity 92.8%). lncRNA GTF2H2-1 was the most specific platelet RNA. On QUADAS-2 tool, 3/7 articles were unclear in reference standards, patient flow timing, and 1/7 had high bias in both aspects. For applicability, 1/7 studies were unclear in reference standards, and 2/7 in index tests. **Conclusion:** TEP RNA can aid in early diagnosis of lung cancer and of proven utility in its early-stage detection. TEP RNA can also monitor disease progression and treatment response.

Keywords: Tumor-educated platelets- biomarkers- lung cancer

Asian Pac J Cancer Prev, **25 (6)**, 1911-1920

Introduction

Non-small cell lung cancer (adenocarcinoma and squamous cell carcinoma) accounts for the majority of lung cancer. It is one of the most common malignant tumors worldwide and has shown a significantly rising trend in terms of morbidity and mortality in recent years [1]. Most of these patients present in advanced stage due to delayed diagnosis. Conventionally, lung cancer is detected through various radiological investigations including chest X-rays, sputum cytology, positron-emission tomography (PET), low-dose computed tomography (CT), and magnetic resonance imaging. Despite continuous improvement in therapeutic strategies, the 5-year survival rate for lung cancer and NSCLC is still only 5–20% [2], since approximately two-thirds of lung cancer patients either present with locally advanced disease or may have distant metastasis at the time of diagnosis [3]. Hence, the concept of liquid biopsy is being readily explored as an alternative to tissue-based diagnosis being non-invasive and provide quick results. Platelets are the second most abundant cell type in peripheral blood with 7-10 days' lifespan. Despite the small size and lack of nuclei, platelets contain an ample repertoire of biomolecules, including functional ribosomes, signaling proteins, and different types of RNA, such as messenger RNA (mRNA), micro-RNA (miRNA), long non-coding RNA and circular RNA (circ RNA) [4]. Interestingly, platelet transcriptome does not completely reflect its parental megakaryocyte profile and they actively respond to local and systemic pathological conditions such as cancer by altering their transcriptome. The dynamicity of the membrane makes platelets sequester RNA or modify their nucleic acid content in response to external stimuli through both direct (through the membrane system) and indirect (through vesicles) mechanisms leading to alterations of the platelet transcriptome. Tumor cells thus may exploit these mechanisms to educate platelets and potentially leave a signature that may help in diagnosis. In the process of interacting and responding with cancer cell signals, platelets alter their RNA profile through three primary avenues: sequestration of tumor-specific biomolecules,

*[1]Pathology and Lab Medicine, All India Institute of Medical Sciences, Bhopal, India. [2]Radiation Oncology, All India Institute of Medical Sciences, Bhopal, India. [3]Neurosurgery, All India Institute of Medical Sciences, Bhopal, India. *For Correspondence: vaishali.patho@aiimsbhopal.edu.in*

tumor-specific splice events, and megakaryocyte alteration [4]. Thus tumor-educated platelets (TEPs) are functional cells in blood circulation with a distinct microenvironment associated with tumor-driven phenotype [3]. In addition to differentially splicing mRNA, noncoding RNAs can also vary in TEPs. Lnc RNAs are a group of RNA molecules of size of approximately 200 nucleotides that do not encode protein, but instead regulate gene expression through epigenetic modifications, intracellular transport, RNA splicing, and transcriptional regulation [4,5]. The present systematic review was carried out to examine the role of TEP in the early diagnosis of primary lung cancer and its subtypes, to analyse the various forms of RNA profile from TEP playing a role in the diagnosis of lung carcinoma and to study the role of TEP in tumor progression and monitoring. This will be helpful in further exploration of role of this biomarker in lung cancer diagnostics.

Materials and Methods

The protocol is registered with PROSPERO 2023 CRD42023408063 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42023408063. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [6] and the Cochrane Manual of Systematic Reviews and Meta-analysis was used for the study [7]. The study participants were patients diagnosed with primary lung carcinoma (with or without metastatic disease) on tissue (histopathology). These studies reported data regarding tumor educated platelets in blood samples. The study consisted of control group where subjects without any clinical or radiological evidence of malignancy were included and the outcome was measured as sensitivity, PPV and ROC. The authors searched PUBMED, SCOPUS, Central Cochrane Registry of Controlled Trials (The Cochrane Library) and Science Direct databases with search terms till Oct 2023. (Table 1) and in addition, the reference list of included studies was evaluated for potentially eligible studies. All studies were screened by two reviewers independently to determine if they satisfied the criteria; a third one was involved when the disagreement occurred. The inclusion criteria were decided before the literature search was performed. Studies included if published in English language peer-reviewed literature. The type of study included was observational (case-control). Case series, case reports, letters, editorials, comments, animal studies and studies from non-English literature were excluded. The final set of included articles was assessed by one reviewer (VW) who extracted data from all the studies, using a piloted data extraction form. A second reviewer (AA) independently extracted data from a subset of the included studies to evaluate the reproducibility of data extraction. The details included were study authors, year, country, inclusion criteria, exclusion criteria, sample size for each group, age, gender, and reported outcomes. For missing data authors were contacted and disagreements were resolved with consensus. The other data retrieved from the reports included methodological quality, participant characteristics, laboratory methods and outcome data such as sensitivity & specificity. Study design (cross-sectional / case-control, blinding (single / double-blind vs unblinded reference standard results) potential for verification bias, (complete vs partial / differential verification of index test results by reference standard. For missing data authors were contacted and disagreements were resolved with consensus. QUADAS -2 tool was used to assess the quality, risk of bias and applicability concerns [8,9].

Results

Search Results

The search strategy as described in methods yielded 28 Trials in the Cochrane database, 60 articles in PubMed NLM database, 87 articles in Scopus database, and 12 entries in Science Direct database. A total of 187 records were identified, 10 were duplicate entries in multiple databases and hence were removed from screening. Out of the 177 remaining articles, 160 records were excluded after reading out the relevant abstract on the basis of exclusion criteria such as language, type of studies, animal studies, TEP etc. and these were also not addressing the research question. Seventeen articles were finally selected for a full-text review. After a detailed analysis of the full text of these articles, ten articles were excluded with reasons (Supple Table A). A systematic review was then conducted on the remaining seven articles that fulfilled the inclusion criteria. The above results are shown as a PRISMA flow diagram (Figure 1) [6]. The description of included studies are outlined in Tables 2 & 3 [3, 10-15]. In a proof-of-concept study of D Ambrosi et al., symptomatic patients with NSCLC showed significant downregulation of tumor-educated platelet circ RNA, particularly nuclear-receptor-interacting protein 1 (NRIP1) gene which is located on chromosome 21. The study found 411 circ RNA out of 4732 RNAs differentially expressed in patients with NSCLC. The results were statistically significant. Out of 411 differentially expressed circRNAs, ~80% displayed downregulation while the remaining were upregulated. The source of TEP was a peripheral blood sample drawn from patients with NSCLC (one day before surgery) and control (asymptomatic) group. The control group and patient were matched in terms of confounding factors viz. age and gender. The results obtained from RNA sequencing were validated using quantitative RT-PCR. In addition, the statistically significant downregulation of NRIP1 gene correlates with metastasized and late stage of NSCLC. The authors concluded that circRNA transcriptome modifications could serve as a potential biomarker for early diagnosis, detection, and progression of non-squamous cell lung cancer. Several other studies have established the role of NRIP1 gene in gastric, cervical, and ovarian cancers [16-18]. Their limitations included were small sample size, and loss of follow-up of control group due to data anonymity [10].

In the study by Dong et al. [11] authors investigated the role of tumour-educated platelets small nuclear RNA (snRNA) in patients with lung cancer and normal healthy volunteers were selected for the study. The TEP snRNA was harvested from the subject's peripheral blood using RT-PCR. The TEP snRNA U1, U2, and U5

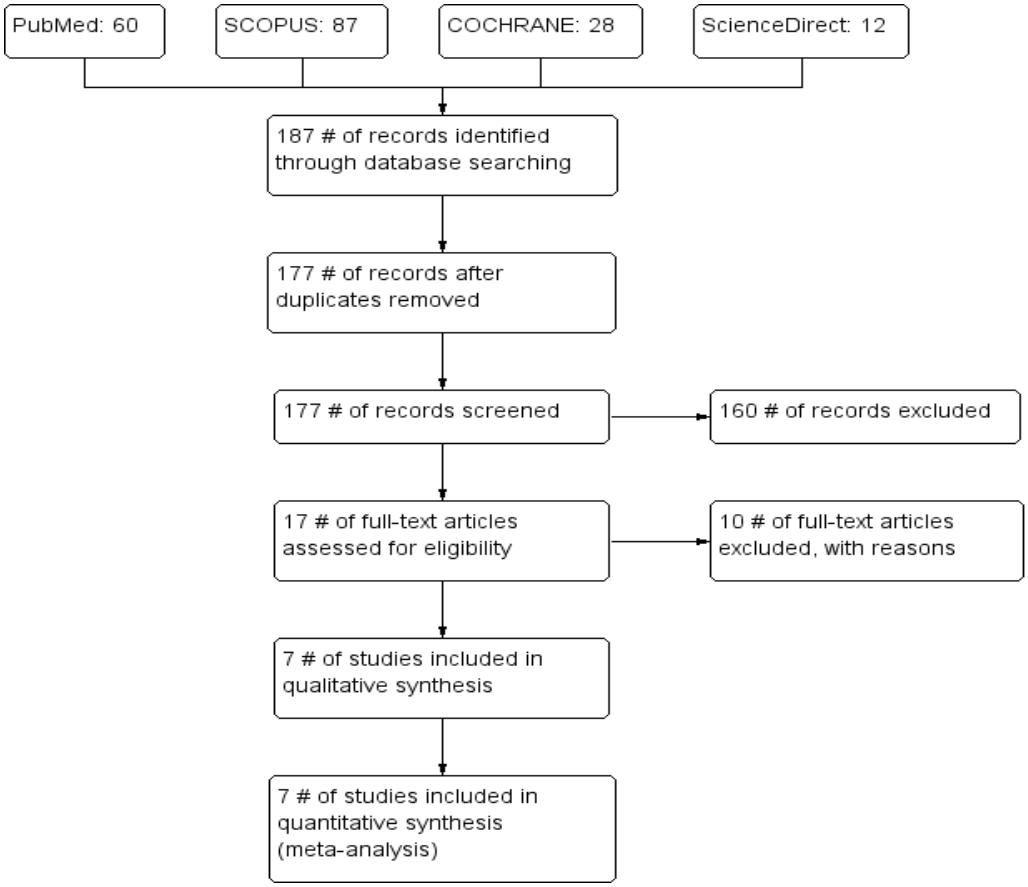

Figure 1. The PRISMA Flow Diagram Depicts the Distribution of Studies with Reference to Inclusion & Exclusion Criteria

were downregulated in patients with lung cancer. In addition, the clinicopathologic features correlated with the snRNA. The TNM staging and metastasis were linked with the spliceosome complex. Moreover, these snRNAs were associated with factors such as gender, smoking, histology type, and tumour size demonstrating their role in tumorigenesis and metastasis. In patients with early-stage cancer, all three snRNA were significantly decreased highlighting their role in early diagnosis. The authors evaluated the diagnostic efficacy of these biomarkers using the ROC curve which revealed an AUC of 0.840, sensitivity of 85.9%, and specificity of 70.15 respectively. The combination of U1, U2, and U5 with traditional biomarkers such as carcinoembryonic antigen (CEA) improved the diagnostic efficiency for cancer progression. The TEP snRNA was altered after receiving chemotherapy

Table 1. The Summary of the Database Search Strategy for the Review Process

Database	Search terms
PubMed	(("cysts"[MeSH Terms] OR "cysts"[All Fields] OR "cyst"[All Fields] OR "neurofibroma"[MeSH Terms] OR "neurofibroma"[All Fields] OR "neurofibromas"[All Fields] OR "tumor s"[All Fields] OR "tumoral"[All Fields] OR "tumorous"[All Fields] OR "tumour"[All Fields] OR "neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "tumor"[All Fields] OR "tumour s"[All Fields] OR "tumoural"[All Fields] OR "tumourous"[All Fields] OR "tumours"[All Fields] OR "tumors"[All Fields]) AND ("educability"[All Fields] OR "educable"[All Fields] OR "educates"[All Fields] OR "education"[MeSH Subheading] OR "education"[All Fields] OR "educational status"[MeSH Terms] OR ("educational"[All Fields] AND "status"[All Fields]) OR "educational status"[All Fields] OR "education"[MeSH Terms] OR "education s"[All Fields] OR "educational"[All Fields] OR "educative"[All Fields] OR "educator"[All Fields] OR "educator s"[All Fields] OR "educators"[All Fields] OR "teaching"[MeSH Terms] OR "teaching"[All Fields] OR "educate"[All Fields] OR "educated"[All Fields] OR "educating"[All Fields] OR "educations"[All Fields]) AND ("blood platelets"[MeSH Terms] OR ("blood"[All Fields] AND "platelets"[All Fields]) OR "blood platelets"[All Fields] OR "platelet"[All Fields] OR "platelets"[All Fields] OR "platelet s"[All Fields] OR "plateletes"[All Fields])) AND (clinicaltrial[Filter] OR randomized controlled trial[Filter]) Filters: from 1000/1/1 - 2023/10/7
SCOPUS	TITLE-ABS-KEY (tumor AND educated AND platelets) AND (LIMIT-TO (DOCTYPE,"ar"))
COCHRAE	27 Trials matching tumor-educated platelets in Title Abstract Keyword
Science Direct	Title, abstract, keywords: tumor educated platelets (only research articles were included)

Table 2. Characteristics of Individual Studies, Emphasizing Their Methodologies

Study author (year)	Country	Stages of Lung Cancer	Additional Marker	Specimen	Method / Threshold	Type of Study	Sample Size
D'Ambrosi, 2021 [10]	Amsterdam, Netherlands	Early &Late-stage Lung Cancer	-	Blood platelets	RNA sequencing CircRNA diagnosis using accurate circRNA finder (ACFI) suite Differential transcript analysis - thromboSeq software Validation of circRNA using quantitative RT-PCR	Proof of Concept Study	N= 12. NSCLC = 6, Control = 6 Total circRNA = 411 Upregulation 84 Downregulation 327
Dong, 2020 [11]	China	Early &Advanced stage disease (Metastasis)	CEA	Blood platelets	Low-speed centrifugation, RNA isolation SnRNA U1, U2, U5 levels detection quantitative real-time polymerase chain reaction (qRT-PCR) Exosomes isolation ultracentrifugation & identification by qNano. qRT PCR performed using Ultra SYBR mixture and light cycler 480q PCR system	Prospective case-control study	N = 770. Control = 361 Lung cancer = 382
Dong, 2021 [12]	China	Early & Advanced stage disease (Metastasis)	CEA	Blood platelets	SNORic datasets to see the expression of snoRNAs between NSCLC and normal tissues.	Retrospective observational study	NSCLC = 290
Li, 2021 [3]	China	Early & Advanced stage disease	CEA Cyfra 21-1 NSE	Blood Platelets	Platelet extraction- low-speed centrifugation Platelet mRNAselection – microarray validation- qPCR	Retrospective case-control study	Lung Cancer 329 Control 300
Liu, 2019 [13]	China	Early stage NSCLC	-	Blood Platelets	Platelet extraction- low-speed centrifugation Total RNA extraction - Trizol Platelet mRNAselection – microarray validation- qPCR	Prospective Cohort study	NSCLC 225 Control 185
Luo, Chang 2018 [14]	China	Early & Advanced stage disease (Metastasis)	EGFR	Plasma & platelet	Platelet isolation from GEO datasets, RNA isolation & cDNA formation, RT PCR for lncRNA expression detection, RTPCR for EGFRvIII detection.	NA ? Prospective	Three GEO datasets to screen five lncRNA
Xing, 2019 [15]	China	Stage 1 NSCLC	CEA	Blood Platelets	Stages: Candidates selection, testing, validation, and prognostic analysis. Initial screening - RNA-seq,Validation - q-PCR. Measurement of platelet mRNA. Diagnostic panel verified with absolute quantification methodology: ddPCR and RPL32. Creationof a nomogram – prediction of the OS rate of NSCLC.	Diagnostic & prognostic Study? Prospective design	Screening phase: NSCLC 9 & C-8 Preliminary screening NSCLC 22 Benign Pulmonary nodule 10 Control 15. Test cohort NSCLC 152. BPN 109 ;Control 97 Independent validation cohort NSCLC 91; BPN 32, Control 53

suggesting a potential impact of the treatment on their expression. They concluded that the TEP snRNA is a potential biomarker for lung cancer. It also serves as a guide for monitoring lung cancer progression. The limitations included a small sample size [11].

Dong et al. [12] in their study included 209 patients of NSCLC and 105 healthy controls. It evaluated the role of TEP SNORD55 as a marker for the diagnosis of lung cancer. The study concluded that the TEP SNORD55 was significantly downregulated in patients with lung cancer. In addition, TEP SNORD55 helps in detecting early-stage lung cancer NSCLC. The lung cancer patients with thrombocytosis showed more downregulation of SNORD55 as compared with patients with low platelets. The ROC curve for SNORD55 as a potential biomarker showed good sensitivity and specificity (79.3 & 68.3%). The authors reinforced the combined diagnostic efficacy of SNORD55 and CEA for diagnosing lung cancer. The ROC curve showed an AUC of 0.8 and specificity of 90 % which is greater than SNORD55 alone. In addition, SNORD55 helps in the early diagnosis of lung squamous and adenocarcinoma. The limitation included small sample size, single-centre study, lack of external validation of SNORD55 as a biomarker, absence of longitudinal data, retrospective nature of the study, and cost-effectiveness of SNORD55. In addition, the study did not compare the platelets and tissues from the same donor because they were not matched, so the differences between them could not be directly observed [12]. The study by Liu et al. [13] evaluated the role of TEP mRNA in the diagnosis and early detection of lung cancer in 410 subjects (225 cancer patients, 185 controls). The authors included three platelet mRNA viz. MAX, MTURN, and HLA-B. The MTURN mRNA was significantly upregulated in female

Table 3. Characteristics of Individual Studies Emphasizing Diagnostic Accuracy of TEP in Diagnosis of Lung Cancer

Study	Lung Cancer - Histology	RNA	TEP	AUC	Sensitivity	Specificity	Cut-Off	p-value	Expression
D'Ambrosi,2021[10]	NSCLC	circ RNA	NRIP1	NA	NA	NA	FDR 0.05	0.0302	Downregulation
Dong, 2020 [11]	NSCLC, Adenocarcinoma, SCC, Small cell lung cancer	snRNA	U1	0.769	74.6	66.5	NA	0.001	Downregulation
			U2	0.84	81.4	74.2	NA	0.001	Downregulation
			U5	0.809	90.1	63.7	NA	0.001	Downregulation
			U1, U2, U5	0.84	85.9	70.1	NA	NA	Downregulation
			snRNA + CEA	0.809	64.9	87.7	NA	NA	Downregulation
Dong, 2021 [12]	NSCLC, Adenocarcinoma, Squamous Cell carcinoma	snoR-NA	SNORD55	0.803	79.3	68.3		p < 0.0001	Downregulation
Li, 2021 [3]	Adenocarcinoma, Squamous cell carcinoma, small cell	lncRNA	GTF2H2-1	0.807	68.3	81.7	5.8875	0.0001	Downregulation
			RP3-466P17.2	0.788	77.8	67.8	15.82	0.0001	Downregulation
			lnc-ST8SIA4-12	0.725	73.7	69.8	10.8025	0.0001	Upregulation
			linc-GTF2H2-1, RP3-466P17.2, and lnc-ST8SIA4-12	0.921	82.6	87.1	NA	0.0001	NA
			GTF2H2-1 + NSE + CEA + Cyfra21-1	0.899	76.6	85	NA	0.0001	NA
Liu, 2019 [13]	Adenocarcinoma, Squamous cell carcinoma, small cell	mRNA	MAX	0.734	60.6	81.7	NA	0.0001	Upregulation
			MTURN					0.0001	Upregulation
			HLA B					0.0016	Upregulation
Luo, 2018 [14]	NSCLC, Adenocarcinoma, Squamous Cell carcinoma	lncRNA	MAGI2-AS3					0.0001	Downregulation
			Adenocarcinoma	0.853	-	-	-	0.0001	Downregulation
			SCC	0.892	-	-	-	0.0001	NA
			ZFAS1					0.0001	
			Adenocarcinoma	0.78	-	-	-	0.0001	
			SCC	0.744	-	-	-	0.0001	
			MAGI2-AS3 + ZFAS1						
			Adenocarcinoma	0.908	-	-	-	0.0001	
			SCC	0.919	-	-	-	0.0001	
Xing, 2019 [15]	NSCLC	mRNA	ITGA2B	0.922	92.8	78.6	0.001759	0.001	Upregulation
			SELP	0.799	75.7	77.2	0.003346	0.001	Upregulation
			ITGA2B + CEA	0.957	90.1	86.9	0.29188	NA	

Table 4. Characteristics of Individual Studies Emphasizing Diagnostic Accuracy of TEP in Early Diagnosis of Lung Cancer

Study	RNA	TEP	AUC	Sensitivity	Specificity	Cut-Off	p-value
D'Ambrosi, 2021 10	circ RNA	NRIP1*					0.098
Dong, 2020 11	snRNA	U1	0.669	90	38.5	-	0.0001
		U2	0.805	78.8	74.2	-	0.0001
		U5	0.752	86.3	63.7	-	0.0001
		U1, U2, U5	0.826	93.8	60.7	-	-
		snRNA + CEA	0.809	64.9	87.7	-	-
Dong, 2021 12	snoRNA	SNORD55	0.784	91.2	49.7	-	0.0001
		Early Adenocarcinoma	0.759	89.7	49.7	-	0.0001
		Early SCC	0.854	68.4	93.1	-	0.0001
		SNORD55 + CEA	-	-	-	-	-
Li, 2021 3	lncRNA	GTF2H2-1	0.761	59.6	81.7	5.8875	0.0001
		RP3-466P17.2	0.771	87.2	55.9	15.36	0.0001
		lnc-ST8SIA4-12	0.768	83	68.3	10.8625	0.0001
		linc-GTF2H2-1, RP3-466P17.2 & lnc-ST8SIA4-12	0.895	93.6	69.8	-	0.0001
		GTF2H2-1 + NSE + CEA + Cyfra21-1	-	-	-	-	-
Liu, 2019 13	mRNA	MAX	0.787	72.7	85.4	-	0.0115
		MTURN					0.0001
		HLA B					0.007
Luo, 2018 14	lncRNA**	MAGI2-AS3					
		Adenocarcinoma					
		SCC					
		ZFAS1					
		Adenocarcinoma					
		SCC					
		MAGI2-AS3 + ZFAS1					
		Adenocarcinoma					
		SCC					
Xing, 2019 15	mRNA	ITGA2B	0.94	96.4	78.6	-	0.001
		SELP	0.846	83.9	77.2	-	0.001
		ITGA2B + CEA	0.955	91.1	86.9	-	0.001

*Significant down-regulation in advanced stages (p-value 0.03). The difference between early stage & late stage was not significant (p -value 0.098); **Role of TEP has been described in an early diagnosis of lung adenocarcinoma and squamous cell carcinoma

patients with lung cancer. The three sets of mRNAs were upregulated in cases with lung cancer and patients with early lung cancer. The combined AUC for these three mRNA was 0.734 serving as a promising novel biomarker for diagnosis of lung cancer. In addition, the three-mRNA showed reduced expression in lung cancer patients post chemotherapy suggesting a role of these biomarkers in the assessment of lung cancer progression and responsiveness to the chemotherapeutic regimen. However, the results need to be analysed considering the limitations in mind. The sample size is small, and the authors did not analyse the effect of MTURN expression in non-smoker lung cancer patients [13]. A study by Liu et al, [3] examined the role of TEP long non-coding RNA (lncRNA) in early diagnosis and as a potential biomarker for lung cancer. The study enrolled 329 lung cancer patients & 300 healthy controls. The platelet qualitative analysis and validation were confirmed using microarray and PCR. More than 1500 lncRNA displayed upregulation and downregulation as compared to healthy controls. However, GTF2H2-1, RP3-466P17.2, and lnc-ST8SIA4-12, were selected for further validation. Out of three, lnc-ST8SIA4-12 was upregulated and linc-GTF2H2-1 and RP3-466P17.2 showed significant downregulation in patients with lung cancer. The ROC curve was plotted for individual lncRNA. The RP3-466P17.2 was most sensitive whereas GTF2H2-1 was more specific. The combined diagnostic efficacy of all the three lncRNA was higher when compared with individual TEP RNA (AUC 0.921, 82.6% sensitivity, and 87.1% specificity). For diagnosis of early lung cancer, the sensitivity and specificity of lnc-ST8SIA4-12 were highest (83%, and 68.3% respectively). In addition, the diagnostic efficacy was improved when these lncRNA were combined with CEA. Moreover, the lncGTF2H2-1 was linked with cancer progression and had a negative correlation with tumor size and TNM staging. The author

concluded that lncRNA GTF2H2-1, RP3-466P17.2, and lnc-ST8SIA4-12 possess reasonable diagnostic efficacy for the prediction of lung cancer. The tests employed were non-invasive and lncRNA was found in abundance. However, the study has several limitations such as a small sample size, and failure to account for differences in the lncRNA expression to platelets ageing [3].

The study Luo CL et al. [14] assessed the role of long non-coding RNA as a diagnostic biomarker for patients with lung cancer. These lncRNAs exhibited significantly different expression in patients with NSCLC as compared with healthy controls. The authors selected five lncRNA from the GEO dataset. The study revealed a significant downregulation of MAGI2-AS3 and ZFAS1 in patients with NSCLC. In addition, lncRNA MAGI2-AS3 directly correlated with the TNM staging, lymph node, and distant metastasis. Moreover, the combined diagnostic efficacy of MAGI2-AS3 and ZFAS1 was higher (AUC 0.919) as compared to the single lncRNA. The authors concluded that TEP RNA is an important diagnostic biomarker for the diagnosis of NSCLC [14].

The study of Xing S et al, [15] examined the role of TEP RNA ITGA2B in the diagnosis of NSCLC. The study enrolled 17 patients – nine patients with NSCLC and eight as healthy controls. The TEP RNA served as a robust diagnostic marker in the prediction of patients with NSCLC (AUC 0.922). The efficacy was higher as compared with CEA (AUC 0.922, CEA AUC 0.785). The rate of ITGA2B positivity was higher in patients CEA negative NSCLC. Moreover, the combined efficacy of CEA and ITGA2B was higher when compared with a single TEP RNA biomarker. In addition, the ITGA2B helped in differential diagnosis of NSCLC concerning benign nodules, and healthy controls as compared to SELP and CEA. The patients with higher platelet IGTA2B mRNA expression had poorer OS as compared with patients with low expression. The author also constructed a nomogram model using CEA and TEP mRNA ITGA2B which showed reliable accuracy in predicting the OS in patients with NSCLC. The study concluded that TEP RNA ITGA2B is a potent biomarker for diagnosing NSCLC in CEA-negative patients and with stage 1 NSCLC [15].

Risk of Bias Assessment

The QUADAS-2 tool was used to assess the risk of bias in the included studies. All these studies did not report any bias in terms of patient selection. However, in terms of applicability, two out of seven studies were unclear in terms of index tests. For assessment of the risk of bias, three studies were unclear in terms of reference standards and patient flow and timing. One study reported high bias both in reference standards and flow and timings. In terms of applicability, only one study out of seven was unclear regarding reference standards. (Table 4) and Suppl data (Figure A and B)

Discussion

The five-year survival rate is less than 20% for NSCLC and most of the patients are diagnosed at a late stage of the disease. The TEP RNA helps a clinician in an early diagnosis of lung cancer and thereby an early management of lung cancer patients. Over the past few years, genomic explorations have led to the discovery of various blood-based non-invasive methods of cancer diagnostics and monitoring called Liquid biopsy [16]. Liquid biopsy overcomes the problem of accessibility to the tumor tissue and is much less invasive, which allows for more frequent and real-time monitoring of tumor dynamics. It potentially allows for a more comprehensive molecular profile as it is more reflective of clonal heterogeneity. The era of precision cancer medicine has heightened the need for high-quality diagnostic material [3]. Hence, liquid biopsy represents a potential complement to surgical diagnosis in the current landscape and may eventually become an alternative in the era of personalized medicine. In recent years, the sequencing technologies developed have found that tumor tissue can release small numbers of tumor cells, ct DNA, ct RNA, exosomes, and tumor-educated platelets (TEPs) [18]. Tumor-educated platelets have emerged as rich bio-sources of cancer-related RNA profiles in liquid biopsies and are applicable for early cancer detection. Interaction between blood platelets and cancer cells plays an important role in various steps of cancer development & progression. This crosstalk led to changes in the platelet RNA content and education of tumor platelets. These biomarkers can be used for diagnosis, screening and disease prognosis, treatment selection & disease progression. It was also shown in recent studies that platelets have higher circRNAs and are differentially expressed in different types of cancer, and they play a role in several steps of tumor initiation proliferation, progression and chemo resistance. Platelet-derived RNA has recently been promoted as a putative diagnostic tool for various pathologies, including cancer in general and NSCLC in particular [17]. Since then, rapid advances in the sensitivity and depth of RNA sequencing have taken this concept to the forefront of translational medicine [4,17]. Interestingly, normalization of the platelet proteome following tumor resection highlights the potential of this approach not only as a diagnostic tool but also in monitoring therapy and disease recurrence [18]. In addition to providing an initial diagnosis, platelet RNA has the potential to coordinate and predict outcomes from therapeutic regimens. Although platelet levels of tumor-derived mutant RNA often lie beneath the detection limit of conventional reverse transcription polymerase chain reaction (RT-PCR) and RNA-sequencing approaches. Newer technologies such as droplet-digital PCR can analyse tumor RNAs within plasma with improved accuracy and sensitivity. Such advances are likely to improve the detection of mutant RNAs within TEPs [19]. Many studies have evaluated the promising role of TEP RNA as a potential blood-based biomarker for the diagnosis of lung carcinoma. Based on these considerations the present systematic review is carried out to understand the potential role of different TEP RNA signatures as a blood-based biomarker in the diagnosis of primary lung cancer, and its subtypes of all the available literature to obtain updated evidence on the role of TEP RNA in early detection, typing and progression of lung cancer. Although circulating RNA is found to be

highly abundant in human platelets, there are few studies which mention the examination of circ RNA content of platelets derived from patients diagnosed with NSCLC, there is a wide range and types of mRNA being evaluated for the clinical management of patient of NSCLC. Also, the pooled effect of studies available in the literature regarding its applicability as a biomarker needs to be assessed concerning sensitivity, specificity and accuracy.

In a study conducted by D'Ambrosi et al, [10] the mRNA NRIP1 was examined for its role as a coregulator that activates or represses different receptor transcription factors. The study found that there is a downregulation of circ NRIP1 in NSCLC and the later stages (metastasis), making it a potential candidate for lung cancer diagnosis and progression. Although the study evaluated the associations of NRIP1 with other factors such as smoking and histological subtypes of NSCLC, they were found to be statistically insignificant [10].

Dong X et al, [11] conducted a study on patients with lung cancer to examine the levels of TEP SnRNA U1, U2, and U5. Their findings indicated that the levels of SnRNA were reduced in patients with lung cancer and that this downregulation was associated with disease progression. Additionally, this downregulation was observed in patients with advanced stages of the disease and those who had metastasized [11].

A study carried out by Dong et al. [12] examined the use of TEP SNORD55 as a biomarker for diagnosing non-small cell lung cancer (NSCLC). The study also looked at the expression of SNORD55 in patients with lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). The results showed that the expression of SNORD55 was significantly increased in both LUAD and LUSC tissue samples, even in early-stage disease. This suggests that SNORD55 plays a role in the development of cancer. As a diagnostic marker, TEP SNORD55 was found to be significantly decreased in NSCLC compared to healthy controls. The accuracy of TEP SNORD55 to distinguish early-stage NSCLC from healthy controls was assessed using the area under the curve (AUC), sensitivity, and specificity. The AUC was 0.784, sensitivity was 91.2%, and specificity was 49.7%. However, combining snoRNA with CEA significantly improves the diagnostic efficacy of cancer progression, as well as early diagnosis of both adenocarcinoma and squamous cell carcinoma. The AUC for the combined markers was 0.828, sensitivity was 66.3%, and specificity was 90.0%. The study has found that SNORD55 RNA is effective in diagnosing Lung Adenocarcinoma and SCC, with an AUC of 0.791 and 0.826, respectively. The study also highlights the importance of mRNA in accurately identifying the origin of pan-cancer and providing real-time information on the status of tumor cell gene variation. The authors observed that mRNA is upregulated in tumor tissue and downregulated in TEP, which can be explained by the fact that TEP RNA formation is affected by megakaryocytes rather than directly from the tumor. However, the study notes that there were no paired samples of tissue and blood for evaluating mRNA SNORD55 [12].

Li et al. [3] studied the differentially expression of long non-coding RNAs (lncRNAs) in lung cancer patients as compared to the healthy controls. They screened 1502 upregulated and 1859 downregulated lncRNAs using Microarray and validated their findings using qPCR technique. The study selected 20 upregulated and 10 downregulated mRNAs for further validation. After an analysis of the data, three TEP lncRNAs (lncRNA GTF2H2-1, RP3-466P17.2, and lncST6SIA4-12) were found to be differentially expressed in lung cancer patients as compared to healthy controls. They confirmed their findings by Sanger sequencing, using a Ct value of > 35 as the critical level. The study found that TEP lncRNAs GTF2H2-1 and RP3-466P17.2 were downregulated with an AUC of 0.781 and 0.788, respectively, while lncST6SIA4-12 was upregulated in lung cancer patients with an AUC of 0.725. This upregulation was observed in early-stage lung cancer patients as well with an AUC of 0.704, 0.771, and 0.768. The integration of these lncRNAs with CEA level, Cyfra 21-1, and NSE had an AUC of 0.899, which could be used to distinguish advanced-stage from early-stage lung cancer. The study also found that the expression of TEP lincRNA GTF2H2-1 was related to smoking history, tumor length, Tumor stage (T stage), Lymph node stage (LN stage), and TNM stage. The expression of TEP RP3-466P17.2 was related to smoking history and pathological type, while the expression of TEP lnc-ST8SIA4-12 was not related to any of the clinical parameters. There was no significant difference in the TEP between healthy controls and benign diseases. The TEP expression of linc-GTF2H2 and RP3-466P17.2 decreased, while the expression of lnc-ST8SIA4-12 increased in early lung cancer as compared to healthy controls. The study observed that a combination of four biomarkers has a high diagnostic capacity for lung cancer, with an AUC of 0.899, a sensitivity of 76.6%, and a specificity of 85.0%. They inferred that the TEP linc GTF2H2-1 biomarker was significantly different between early and late-stage lung cancer and could be used to predict the lung cancer progression. This biomarker was inversely related to T-stage progression and LN metastasis. The study also found that this biomarker could be used as an independent risk factor for advanced T stage and TNM stage, but not for LN or distant metastasis [3].

The TEP mRNA expression in lung cancer was examined by Liu et al [13] in which authors used microarray to screen TEP mRNA and qPCR for validation in a larger cohort. Three platelet mRNA sets were selected (MAX, MTURN, and HLA-B) and evaluated for their diagnostic efficacy and chemotherapy response prediction. The study found that all three-platelet mRNA were significantly higher in lung cancer patients compared to healthy controls. In early-stage lung cancer patients, these platelet mRNA values were even higher than in healthy donors. Furthermore, the MAX and MTURN mRNA values were significantly decreased post-surgery, which indicates a close correlation with tumor burden. The study concluded that MTURN mRNA could be a useful parameter for early detection of lung cancer, especially in females. The AUC for TEP MTURN was 0.825 with a sensitivity of 84.7% and specificity of 72.5%. Low mRNA levels were also associated with a favorable

first chemotherapy response, making it a promising noninvasive biomarker. The authors noted that the study had limitations, including a small sample size (225 patients, with only about 40 having lung cancer) and a lack of detailed information on chemotherapy. Additionally, the correlation with non-smokers could not be assessed in the study [13].

The study of Luo et al. [14] aimed to explore the correlation between lncRNA and EGFRvIII in patients with lung NSCLC. The researchers first screened five lncRNA with potential diagnostic significance from three gene databases based on the expression of tumor tissue from patients with squamous cell carcinoma and adenocarcinoma. Later, they examined the expression pattern and diagnostic value of these five lncRNA in platelets and plasma derived from patients with NSCLC to explore the role of TEP in the diagnosis of NSCLC and its subtypes. The authors noted a moderate correlation between plasma and platelet mRNA. These mRNAs have a better ability to distinguish between NSCLC and control groups. The study also found a negative correlation between the expression level of ZFASI and TNM stage, while MAGI2-A S3 expression was significantly correlated with TNM Stage, lymph node and distant metastasis. The early diagnosis of lung cancer, especially NSCLC, can supplement clinical-oncologic decision-making. Another important observation of the study was that platelets and plasma revealed the same kind of EGFRvIII mutation, a tumor-specific target with no difference [14].

Xing et al. [15] conducted a study on patients with lung cancer and found that two markers, TEP mRNA ITGA2B and SELP, were higher in NSCLC patients compared to controls. The measurement of ITGA2B mRNA was found to have better diagnostic value than CEA in diagnosing NSCLC, particularly in stage I cases. In contrast, SELP mRNA had an inferior diagnostic value compared to ITGA2B. The results suggest that ITGA2B plays a crucial role in distinguishing malignant versus benign disease in CEA-positive cases. Additionally, high TEP ITGA2B was identified as an independent risk factor for poorer prognosis in overall survival among NSCLC patients. The integration of ITGA2B, CEA, and clinical stage was successfully used to predict OS in NSCLC patients and showed improved prognostic accuracy. The study demonstrates the diagnostic and prognostic relevance of TEP ITGA2B as a platelet RNA marker for NSCLC in test and independent validation cohorts. However, there are some limitations to the study, including NSCLC bias, control and selection bias [15].

The present review was carried out on seven studies which aim to identify the role of tumor-educated platelets RNA in early diagnosis of patients with lung cancer. All the included studies employed different platelet RNA and hence conclusive evidence regarding the diagnostic efficacy of TEP RNA for lung cancer remains low. The analysis of these studies revealed an AUC of 70% and more for different types of platelet RNA used as a biomarker for diagnosing lung cancer. In addition, the sensitivity and specificity of different platelet RNA was more than 60 per cent in all the included studies. The AUC and sensitivity were highest for the ITGA2B for diagnosing lung cancer (AUC 0.922; sensitivity 92.8%). The lncRNA GTF2H2-1 was most specific platelet RNA for diagnosing lung cancer. All the studies recruited patients with non-small cell lung cancer including adenocarcinoma and squamous cell carcinoma. Different authors have utilised different sets of platelet RNA including but not limited to lncRNA, snRNA, mRNA, snoRNA, and circRNA. Different platelet RNA was expressed differently. The majority of TEP RNA were downregulated whereas few showed upregulation. In addition, the TEP RNA helped in diagnosing early-stage lung cancer as was evident from the study analysis. The AUC of all studies for different TEP RNA was above 65 per cent. The least sensitive TEP RNA was lncRNA GTF2H2-1 with a sensitivity of 59.6% and the maximum sensitivity was ITGA2B (96.4%) for early lung cancer diagnosis. U1 snRNA was the least specific with a specificity of 38.5% and SNORD55 snoRNA was most specific for the diagnosis of early squamous cell carcinoma (SCC) with a specificity of 93.1%. The analysis also pointed towards the combined usage of TEP RNA and CEA antigen for an early diagnosis of lung cancer with improved sensitivity and specificity.

In conclusion, TEP RNA can either be up or downregulated. They help in the early diagnosis of lung cancer (NSCLC, adenocarcinoma, squamous cell carcinoma). TEP RNA has been proven to be useful in early-stage diagnosis of lung cancer. It is a non-invasive technique for diagnosing lung cancer. TEP RNA can monitor the progression of the disease as well as the response to the chemotherapy regimen. It acts as a valuable guide for clinical-oncological decision-making. The limitations of this review include low level of evidence, heterogeneity of the included studies, and utilization of different platelet RNA across the included studies which results in an indefinite conclusion on the role of specific platelet RNA in the diagnosis of lung cancer. Although the individual studies have demonstrated statistically significant results for each different platelet RNA, the pooled analysis could not be conducted given the different types of RNA included across different studies. However, the future of liquid biopsy looks promising. Further clinical trials are necessary to establish the role of this platelet RNA in the diagnosis of lung cancer. Limitations: However, a definitive type of TEP RNA could not be found due to low level of evidence, heterogeneity of included studies, and utilization of different platelet RNA. Moreover, pooled analysis could not be conducted given the different types of RNA included across different studies. The future of liquid biopsy looks promising and further clinical trials are necessary to establish the role of platelet RNA in diagnosis of lung cancer.

Author Contribution Statement

All authors contribution as per CRediT, author statement are as follows: Vaishali Walke (VW): Conceptualization, methodology, writing and reviewing manuscript. Saikat Das (SD): Validation, review manuscript, curating of data. Amol Mittal (AM): Draft manuscript preparation, curating of data. Amit Agawal (AA): Methodology, review manuscript, supervision.

Acknowledgements

The authors declare that: the article Systematic Review.

Approval

No human or animal subjects involved in the study, no IHEC approval required.

PRISMA guidelines followed and the protocol is registered with Cochrane database. The study protocol was registered with PROSPERO 2023 CRD42023408063 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42023408063. The study is not a part of any students thesis.

Conflict of interest

The authors have no conflict of interest.

Abbreviations

AUC- Area under curve
CEA: Carcino embryonic antigen
EGFR: Epidermal growth factor receptor
LUSC: Lung Squamous Cell Carcinoma
LUAC: Lung Adenocarcinoma
NSCLC: Non Small Cell Carcinoma
PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses
TEP: Tumor educated platelets
RNA: Ribonucleic Acid
mRNA: Messenger RNA
miRNA: Micro RNA
snRNA: Small nucleolar RNA
lncRNA: long noncoding RNA
TNM stage: Tumor, lymph node Metastasis stage
LN: LymphNode
PPV: Positive Predictive value
OS: Overall survival

References

1. Adjei AA. Lung cancer worldwide. J Thorac Oncol. 2019;14(6):956. https://doi.org/10.1016/j.jtho.2019.04.001.
2. Jones PA, Baylin SB. The epigenomics of cancer. Cell. 2007;128(4):683-92. https://doi.org/10.1016/j.cell.2007.01.029.
3. Li X, Liu L, Song X, Wang K, Niu L, Xie L, et al. Tep linc-gtf2h2-1, rp3-466p17.2, and lnc-st8sia4-12 as novel biomarkers for lung cancer diagnosis and progression prediction. J Cancer Res Clin Oncol. 2021;147(6):1609-22. https://doi.org/10.1007/s00432-020-03502-5.
4. Roweth HG, Battinelli EM. Lessons to learn from tumor-educated platelets. Blood. 2021;137(23):3174-80. https://doi.org/10.1182/blood.2019003976.
5. Spizzo R, Almeida MI, Colombatti A, Calin GA. Long non-coding rnas and cancer: A new frontier of translational research? Oncogene. 2012;31(43):4577-87. https://doi.org/10.1038/onc.2011.621.
6. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The prisma 2020 statement: An updated guideline for reporting systematic reviews. BMJ. 2021;372:n71. https://doi.org/10.1136/bmj.n71.
7. Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA. Cochrane Handbook for Systematic Reviews of Interventions. 2 nd Edition. Chichester (UK):John Wiley & amp; Sons; 2019.
8. Whiting P, Rutjes AWS, Reitsma JB, Bossuyt PMM, Kleijnen J. The development of quadas: A tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC Med Res Methodol 2003;3(1):25. 2003;3(1):25. https://doi.org/10.1186/1471-2288-3-25.
9. Whiting PF, Weswood ME, Rutjes AW, Reitsma JB, Bossuyt PN, Kleijnen J. Evaluation of quadas, a tool for the quality assessment of diagnostic accuracy studies. BMC Med Res Methodol. 2006;6:9. https://doi.org/10.1186/1471-2288-6-9.
10. D'Ambrosi S, Visser A, Antunes-Ferreira M, Poutsma A, Giannoukakos S, Sol N, et al. The analysis of platelet-derived circrna repertoire as potential diagnostic biomarker for non-small cell lung cancer. Cancers (Basel). 2021;13(18). https://doi.org/10.3390/cancers13184644.
11. Dong X, Ding S, Yu M, Niu L, Xue L, Zhao Y, et al. Small nuclear rnas (u1, u2, u5) in tumor-educated platelets are downregulated and act as promising biomarkers in lung cancer. Front Oncol. 2020;10:1627. https://doi.org/10.3389/fonc.2020.01627.
12. Dong X, Song X, Ding S, Yu M, Shang X, Wang K, et al. Tumor-educated platelet snord55 as a potential biomarker for the early diagnosis of non-small cell lung cancer. Thorac Cancer. 2021;12(5):659-66. https://doi.org/10.1111/1759-7714.13823.
13. Liu L, Song X, Li X, Xue L, Ding S, Niu L, et al. A three-platelet mrna set: Max, mturn and hla-b as biomarker for lung cancer. J Cancer Res Clin Oncol. 2019;145(11):2713-23. https://doi.org/10.1007/s00432-019-03032-9.
14. Luo CL, Xu ZG, Chen H, Ji J, Wang YH, Hu W, et al. Lncrnas and egfrviii sequestered in teps enable blood-based nsclc diagnosis. Cancer Manag Res. 2018;10:1449-59. https://doi.org/10.2147/CMAR.S164227.
15. Xing S, Zeng T, Xue N, He Y, Lai YZ, Li HL, et al. Development and validation of tumor-educated blood platelets integrin alpha 2b (itga2b) rna for diagnosis and prognosis of non-small-cell lung cancer through rna-seq. Int J Biol Sci. 2019;15(9):1977-92. https://doi.org/10.7150/ijbs.36284.
16. Sheng M, Dong Z, Xie Y. Identification of tumor-educated platelet biomarkers of non-small-cell lung cancer. Onco Targets Ther. 2018;11:8143-51. https://doi.org/10.2147/ott.S177384.
17. Calverley DC, Phang TL, Choudhury QG, Gao B, Oton AB, Weyant MJ, et al. Significant downregulation of platelet gene expression in metastatic lung cancer. Clin Transl Sci. 2010;3(5):227-32. https://doi.org/10.1111/j.1752-8062.2010.00226.x.
18. Sabrkhany S, Kuijpers MJE, Knol JC, Olde Damink SWM, Dingemans AC, Verheul HM, et al. Exploration of the platelet proteome in patients with early-stage cancer. J Proteomics. 2018;177:65-74. https://doi.org/10.1016/j.jprot.2018.02.011.
19. Best MG, Sol N, In 't Veld S, Vancura A, Muller M, Niemeijer AN, et al. Swarm intelligence-enhanced detection of non-small-cell lung cancer using tumor-educated platelets. Cancer Cell. 2017;32(2):238-52.e9. https://doi.org/10.1016/j.ccell.2017.07.004.