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Review

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Review

Identification of biomarkers for the early detection of non-small cell lung cancer: a systematic review and meta-analysis

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Abstract

Lung cancer (LC) causes few symptoms in the earliest stages, leading to one of the highest mortality rates among cancers. Low-dose computerised tomography (LDCT) is used to screen high-risk individuals, reducing the mortality rate by 20%. However, LDCT results in a high number of false positives and is associated with unnecessary follow-up and cost. Biomarkers with high sensitivities and specificities could assist in the early detection of LC, especially in patients with high-risk features. Carcinoembryonic antigen (CEA), cytokeratin 19 fragments and cancer antigen 125 have been found to be highly expressed during the later stages of LC but have low sensitivity in the earliest stages. We determined the best biomarkers for the early diagnosis of LC, using a systematic review of eight databases. We identified 98 articles that focussed on the identification and assessment of diagnostic biomarkers and achieved a pooled area under curve of 0.85 (95% CI 0.82–0.088), indicating that the diagnostic performance of these biomarkers when combined was excellent. Of the studies, 30 focussed on single/antigen panels, 22 on autoantibodies, 31 on miRNA and RNA panels, and 15 suggested the use of circulating DNA combined with CEA or neuron-specific enolase (NSE) for early LC detection. Verification of blood biomarkers with high sensitivities (Ciz1, exoGCC2, ITGA2B), high specificities (CYFR21-1, antiHE4, OPNV) or both (HSP90α, CEA) along with miR-15b and miR-27b/miR-21 from sputum may improve early LC detection. Further assessment is needed using appropriate sample sizes, control groups that include patients with non-malignant conditions, and standardised cut-off levels for each biomarker.

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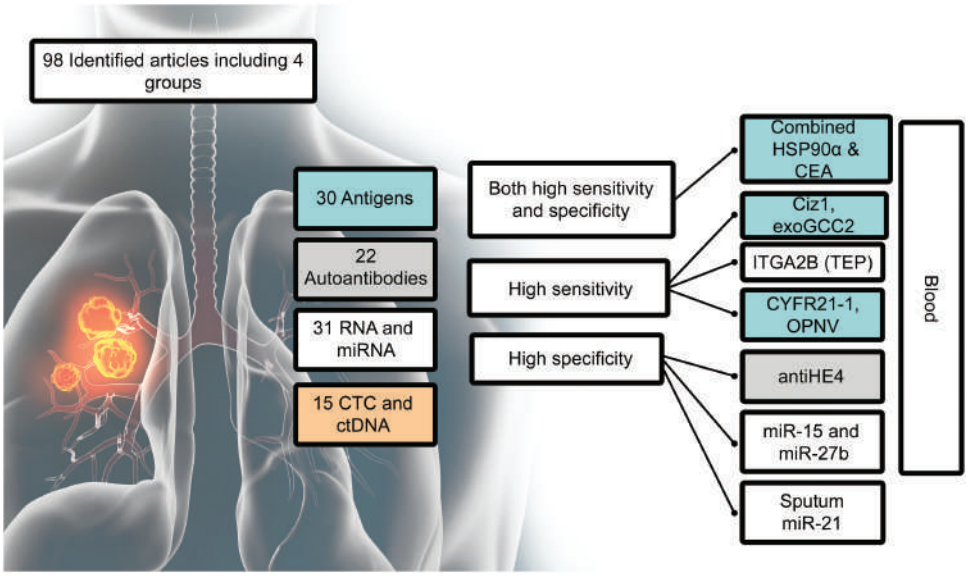
Graphical Abstract



Abbreviations: ADC, adenocarcinoma; AUC, area under curve; CEA, carcinoembryonic antigen; CI, confidence interval; CIZ1, CDKN1A Interacting Zinc Finger Protein 1; COPD, chronic obstructive pulmonary disease; CRP, c-reactive protein; CT, computer tomography; CTA, cancer testis antigen; CTC, circulating tumour cells; CYFRA21-1, cytokeratin 19 fragments; GCC2, GRIP And Coiled-Coil Domain Containing 2 protein; ITGA2B, integrin alpha 2b (tumour-educated platelets, TEP); LC, lung cancer; LDCT, low-dose computer tomography; MAGE, melanoma-associated antigen gene; NSCLC, non-small cell lung carcinoma; NSE, neuron-specific enolase; OPNV, osteopontin velocity; PET, positron emission tomography; PRISMA, preferred reporting items for systematic review; PROSPERO, prospective register of systematic review; QUADAS, quality assessment of diagnostic accuracy studies; ROB, risk of bias; SCC, squamous cell carcinoma; TA, tumour antigens; TAAb, tumour-associated antibodies.

Background

Lung cancer (LC) is one of leading causes of cancer-related mortality. It is the second most common cancer, accounting for 18.6% of all tumours (1,2) and affecting both genders with an annual incidence of 2 million worldwide. There are two main types of LC: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), the latter accounting for >80% of all LC cases. Two-thirds of patients are diagnosed at an advanced stage of disease, when surgical options are not recommended. Smoking is a major risk factor for LC and is associated with 80% of LC-associated mortality (3). Thus, late detection and poor prognosis in LC makes disease management challenging. The 5-year survival rate can be raised to 80% if diagnosed at an early stage, but it has remained stubbornly low at around 15% (1).

The common diagnostic methods of LC (4) include medical imaging computer tomography (CT), Positron Emission Tomography (PET) and fluorescence bronchoscopy as well as many biochemical and histological assays such as sputum and pleural cytology, and polymerase chain reaction (PCR). Although low-dose (LD) CT reduces LC mortality, it increases the number of nodules identified which are associated with a high frequency of false positives (up to 95%) due to the limited indicators of their propensity to become malignant. This time and cost implications including patients' psychological distress and exposure to radiation associated with imaging techniques.

A number of serological markers have been investigated for their ability to provide a LC diagnosis including cytokeratin 19 fragments (CYFRA21-1), carcinoembryonic antigen (CEA) and neuron-specific enolase (NSE) (5–7). CYFRA21-1 is the most sensitive biomarker for NSCLC, especially squamous cell tumours. However, CYFRA21-1 is also highly expressed in gastrointestinal, urological and gynaecological tumours, and in low amounts in some benign diseases (8,9), giving it a low specificity for NSCLC. CEA is also expressed in the foetal gastrointestinal epithelium, pancreas and liver in low concentrations (10). It is already used as a diagnostic tumour marker in colon cancer but is also highly expressed in adenocarcinomas such as gastric and pancreatic cancer (10,11). CEA levels are low in the early stages of SCLC but increase in 40–65% of NSCLC patients in the late stages with metastasis (12). NSE is a tumour marker of SCLC used in diagnosis, follow-up and prognosis, but it has low sensitivity and specificity (13). Some reports identified NSE expression in approximately 10–20% of NSCLC patients with an expression associated with tumour burden, number of metastatic sites and treatment response (14).

Tumour antigens (TAs) and tumour-associated antibodies (TAAbs) formed through immune responses against LC could be identifiable before the onset of symptoms. Thus, TAAbs may be valuable for the early diagnosis of LC as they are stable and persist in serum for a long time compared to TAs. The Early Cancer Detection Test (CDT)-Lung was developed by Oncimmune Inc and focusses on two TAAbs panels. A panel of six or seven AAbs were found to have sensitivities of 83% and 91% and specificities of 46% and 37%, respectively (15). Yang *et al.* (16) reviewed the use of autoantibodies as an early detection tool for LC diagnosis. Among those autoantibodies considered, the panel of p53, PGP9.5, SOX2, GAGE7, GBU4-5, CAGE and melanoma-associated antigen gene (MAGE)A1 had a sensitivity of 56.4% for the detection of early LC in 397 patients with lung nodules compared with 74 control individuals. This study recommended the panel could be combined with CT for early LC detection as it achieved high specificity of 95.80% (17).

This systematic review aims to report the diagnostic biomarkers being considered for use in the early detection

of NSCLC and includes the analysis of their sensitivities, specificities and area under curves (AUC) receiver operating characteristics (ROC) to help provide a current prioritisation list. This article considers four main groups of studies focussing on antigens, autoantibodies, miRNAs and circulating DNA in blood and sputum that may provide useful non-invasive biomarkers for the earlier detection of NSCLC.

Methods

This review was performed and reported according to the Preferred Reporting Items for Systematic Review (PRISMA) guidelines (18). The protocol (Supplementary Table 1) was registered on the international prospective register of systematic reviews (PROSPERO: CRD42022336488). Papers which did not acquire informed, written consent from all participants in their study were excluded.

Search strategy

CINAHL, MEDLINE, PubMed, Scopus, Web of Science, Cochrane library and Clinicaltrial.gov were searched from 1 January 1970 until 21st May 2023. Literature searches were performed using the following terms: (cancer* or tumour* or tumour* or neoplasm* or carcinoma* or malignancy*) AND (lung* or pulmonary) AND (antigen* OR protein* OR RNA* OR ctDNA* OR miRNA* OR cell surface marker* OR inflammatory cell*) AND (early detection OR early diagnosis OR early biomarker OR early marker). The initial search, removal of duplicates, title and abstract screening, and full-text reviews were performed by two authors independently.

Exclusion and inclusion criteria

Exclusion and inclusion criteria are detailed in the protocol (Supplementary Table 1). Briefly the inclusion criteria were primary research articles that had studied human adult LC in at least 10 patients. Both retrospective and prospective studies were eligible, including case–control and cohort studies. Biomarkers included single or biomarker panels found in blood, urine, sputum and pleural fluids for LC diagnosis. Cell line and animal studies, as well as prognostic and predictive biomarkers were excluded. Studies that did not report sensitivity and specificity and/or raw data were excluded as well.

Data extraction

Data extraction was performed by E.M. and D.G.M. using a pre-piloted data extraction form. Information extracted included author, year, country, population comparison groups, specimen type, name of biomarkers, technique used, sensitivity, specificity, AUC for stage I and II NSCLC.

Risk of bias

Risk of bias (ROB) was performed by two independent researchers using Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2). It was based on four domains: participant selection, index test, reference standard and timing and flow. Each prompt in the domain was assessed as 'yes' or 'no' or 'unclear'. The first three domains were also assessed for applicability. The study had low ROB/applicability concerns if all domains were rated low, unclear if there was at least one domain rated unclear, and had high ROB/applicability concerns if at least one domain was rated high (19). No study was excluded based on ROB. Figures were visualised using Robvis (20).

Synthesis methods

Due to the wide range and combinations of biomarkers assessed and the limited information provided, a diagnostic test meta-analysis and the evaluation of the diagnostic performance of each biomarker was not possible. Instead, we conducted a random-effects meta-analysis by pooling the AUC and the corresponding 95% confidence intervals (CI) using Review Manager Version 5.4. A random-effects model was used as it accounted for between-study heterogeneity. Cochran's Q test and I^2 statistics were used to evaluate heterogeneity. Heterogeneity was considered unimportant when I^2 = 0–40%, moderate when I^2 = 30–60%, substantial when I^2 = 50–90% and considerable when I^2 = 75–100%. Heterogeneity was significant when $P < 0.10$ and I^2 value >50%. If there were more than 10 studies in the meta-analysis, heterogeneity was explored using sensitivity and subgroup analysis. Sensitivity analysis was done by excluding studies one by one. If the results remain consistent, they were robust. If results differed, they were treated with caution (21). Subgroup analysis was performed based on the type of biomarkers investigated [antigens, autoantibodies, miRNA and RNA, ctDNA and circulating tumour cells (CTC)] and the type of control. A statistically significant subgroup effect was defined as $P < 0.1$ (22). If there were more than 10 studies in a meta-analysis, publication bias was assessed by visual inspection of a funnel plot and conducting Egger's regression and Kendalls tau test (23,24).

If studies did not report AUC and 95% CI, a narrative synthesis of the diagnostic properties of the biomarkers were conducted, by categorising studies based on the type of biomarker assessed and the findings of each study were summarised (sensitivity, specificity and AUC if reported) (25).

Results

The database searches identified 7295 articles in total and 2474 duplicates were removed (Supplementary Table 2). 4636 articles were excluded based on title and abstract. After evaluating 185 full-texts, 98 articles were included in this systematic review (Figure 1, Table 1). The sample sizes varied across the included studies, ranging from 18 to 1479 LC cases. 30 studies investigated either single antigens or antigen panels and reported sensitivities ranging from 48 to 95% and most studies investigated blood biomarkers. 22 studies investigated autoantibodies, 31 studies focussed on miRNAs and RNA, and 15 studies explored CTCs and ctDNA in early-stage NSCLC. Six studies (Table 2) identified biomarkers that had a sensitivity and specificity of more than 90% but the average specificity and sensitivity of the biomarkers in each group were determined (Table 3). Antigens had the lowest values for both variables and the standard deviation (SD), and were not deemed to be the best option as biomarkers of NSCLC based on the literature examined. ctDNA and CTC had the highest values of sensitivity, and a high specificity, with the lowest SD of all of the groups suggesting these were the best options for the early (minimally-invasive) detection of NSCLC.

Risk of bias

86 studies had high ROB (Figure 2; Supplementary Table 3) most commonly due to the use of case–control study designs, causing the 'patient selection' domain to have high ROB. Three other domains also scored poorly, most notably 'flow

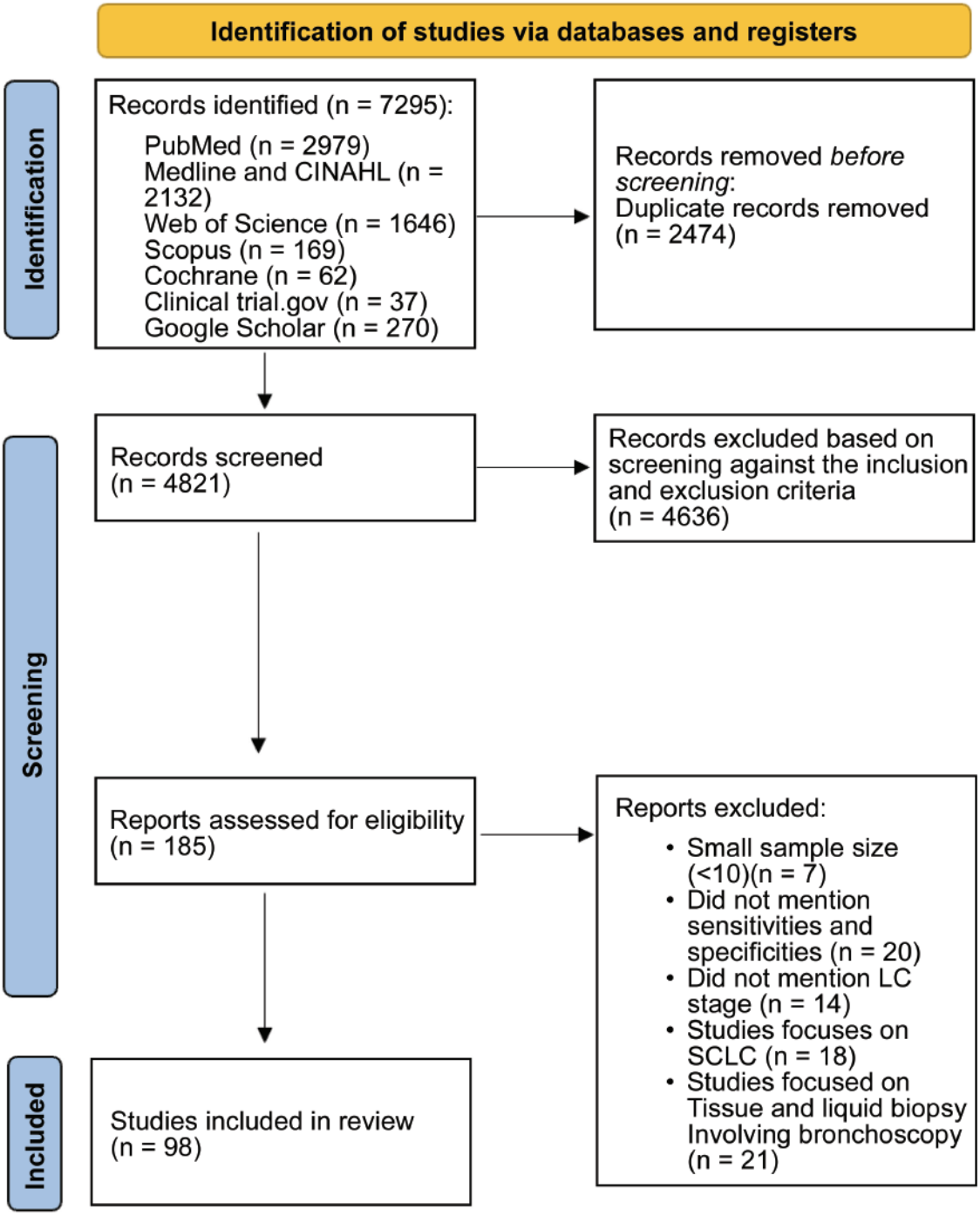


Figure 1. PRISMA-P flow diagram depicting the study selection process.

and timing'. Applicability concerns were low for all studies. Different methods of detection were used in each study and could have impacted the robustness of the results obtained. However, ELISA was the most common technique applied to analyses of these early LC biomarkers, mainly used for antibodies and antigens, while RT-PCR was used for miRNA and ctDNA detection.

Synthesis of the results—meta-analysis

Thirty-one studies reported adequate data to enable the pooling of AUC, and random-effects meta-analysis found that the pooled AUC was 0.85 (95% CI 0.82–0.088), indicating that the diagnostic performance of biomarkers for early NSCLC were excellent (26). However, the heterogeneity was also considerable (I^2 = 96%, P < 0.00001). Sensitivity analysis found that the pooled AUC remained consistent, indicating that the results were robust. Subgroup analysis found that there was no significant subgroup difference (I^2 = 51.8%, P = 0.10) based on the type of biomarker used (Figure 3A). Of the four types of biomarkers, pooled AUC for the autoantibodies subgroup was the lowest (pooled AUC = 0.80, 95% CI 0.72–0.88). Subgroup analysis based on the type of control showed that there was a significant subgroup difference in diagnostic performance (I^2 = 71.7%, P = 0.003), and the

Table 1. Characteristics of included studies

Author	Country	Sample size	Comparative group	Specimen	Name of protein(s) evaluated	Method of detection	Sensitivity %	Specificity %	AUC 95% CI
Antigens									
Ajona *et al.* (39)	Spain	78	NSCLC/indeterminate nodules	P	C4c, CYFRA 21-1, and CRP	ELISA	82	95	0.9
Bigbee *et al.* (88)	USA	56/30	NSCLC/indeterminate nodule	S	Prolactin, transthyretin, thrombospondin-1, E-selectin, C-C motif chemokine 5, macrophage migration inhibitory factor, plasminogen activator inhibitor, receptor tyrosine-protein kinase, erbb-2, cytokeratin fragment 21.1 and serum amyloid A	Luminex xMAP immunoassay	77.10	76.2	NG
Fahrmann *et al.* (89)	USA	1299	High risk	S	A four-marker protein panel (4MP) consisting of CA125, CEA, SPA, CYFRA21-1	Bead-based immunoassays	91.5	45.4	0.79
Farlow *et al.* (27)	USA	90/43	NSCLC/non-cancer	S	TNF-α, CYFRA 21-1, IL-1ra, MMP-2, MCP-1 and sE-selectin	Luminex xMAP immunoassay	99	95	0.979
Gasparri *et al.* (30)	Italy	46/41	NSCLC/high-risk	S	ARSA, PRKCA, ACTR3B, and CD59	MS	94.83	93.56	0.98
Goebel *et al.* (87)	Multiple—UK, Russia, Ukraine	1479	NSCLC/HC	P	CA-125, CEA, CYFRA21-1, EGFR/HER1/ErBB1, Gro-Pan, HGF, IL-10, IL-12p70, IL-16, IL-2,IL-4, IL-5, IL-7, IL-8, IL-9, Leptin, LIF, MCP-1, MIF, MIG, MMP7,MP9, MPO, NSE, PDGF-BB, Rantes, Resistin, sFasL, SAA,sCD40-ligand, sICAM-1, TNFRI and sTNFRII	Multiplex immunoassay platform	80	95	0.963
Higgins *et al.* (33)	UK and USA	35/170/160	LC/inflammatory diseases	T/P	Ciz1	SDS-PAGE	95	74	0.958
Jeong *et al.* (31)	Korea	70/16	NSCLC/HC	P	Exosomal GCC2	ELISA	90	75	0.844
Joseph *et al.* (34)	USA	1182	NSCLC/nodules	P	OPNV	ELISA	80	88	0.88
Jung *et al.* (43)	Korea	200/150	LC/control group	S	EGFR1, MMP7, CA6, KIT, CRP, C9 and SERPINA3	Proteomic	75	91.70	0.82/0.77
Kupert *et al.* (90)	USA	145	NSCLC/BN/HC	P	Secretory phospholipase A2-IIa	ELISA	48–67	86	0.68–0.86
Lai *et al.* (91)	China	201/112/94	NSCLC/HC/Nodules	S	CEA, Cyfra21-1, CST1	ELISA	88.4	89.1	0.92
Li *et al.* (92)	China	37/11	NSCLC/HC	P	MDK, WFDC2, and CXCL14	Luminex technology	NG	NG	0.96
Li *et al.* (93)	China	98/100	NSCLC/BLD	S	CA153 + CA125 + CEA + TNF – alpha + hs – CRP	Immunoluminescence analyser	66.82	93.51	NG

Table 1. Continued

Author	Country	Sample size	Comparative group	Specimen	Name of protein(s) evaluated	Method of detection	Sensitivity %	Specificity %	AUC 95% CI
Ma *et al.* (26)	China	318/239 769/493	NSCLC stage I/ HC	Urine	MDH2	ELISA	70.13 68.92	66.11 58.22	0.768 0.723
Meng *et al.* (94)	China	60/15	NSCLC/HC	S	EpCAM and CEA	Ratiometric biosensor for exosome	93.3	86.7	0.916
Nolen *et al.* (95)	USA	172	LC/high risk	S	MIF, TTR, THSP, sVCAM-1 and tPAI-1	Multiplexed bead-based immunoassays	70/74	90/93	0.85/0.894
Pakvisal *et al.* (96)	Thailand	76/12/53	NSCLC/BLD/ HC	S	C5AR1, CLEC4A and NLRP3 specific to CD3	Flow cytometry	71.5	70	NG
Pio *et al.* (97)	Spain	56/22	NSCLC/BLD	Sputum/ BAL	Complement factor H	ELISA	80-sputum 82-BAL	88-sputum 77-BAL	NG
Sun *et al.* (98)	China	1223	NSCLC/BPC/OC/ HC	S	IDH1	ELISA	63.3/55	86.8/86.3	0.907/0.788
Sun *et al.* (65)	China	71/62	NSCLC/BLD	Sputum	A proliferation-inducing ligand (APRIL)	Immunocytochemistry	82	97	NG
Song *et al.* (99)	Korea	30/15	NSCLC/HC	P	p53-anti-p53-autoantibody complex	Labelled immunoassay	81.6	93.3	NG
Wang *et al.* (36)	China	350/411	NSCLC/control (BLD, HC)	S	MIC-1, Cyfra21-1, CA125 and CEA	Immunoassay/ELISA	84.40	90	0.957
Wang *et al.* (38)	China	132/48/92	LC/BLD/HC	S	NSE + CEA + CYFRA21-1	Electrochemical luminescence	75.76	88.57	0.63
Wieskopf *et al.* (100)	France	161/97	LC/BD	S	CYFRA 21-1	Immunoradiometric assay	59	94	0.85
Wu *et al.* (101)	Taiwan	102/84	ADC/HC	P	Beta-1,4-galactosyltransferase 1, CD44 antigen, eukaryotic initiation factor 4A-I, galectin-1, mucin-16, protein disulfide-isomerase A3, and vimentin	LC-MRM-MS assay	97.2	61	0.76
Yang *et al.* (28)	China	370/110	NSCLC stage I/ BLD	S	Ferritin, CA125, CEA, NSE and CYFRA21-1	Electrochemiluminescence	92.97	90	0.95
Yu *et al.* (102)	China	513	Nodules	S	ACSL4	ELISA	65.1	90.2	0.762
Yuan *et al.* (29)	China	175/160	LC/HC	P	HSP90α, CEA	ELISA	95.63	99.97	0.996
Zhang *et al.* (103)	China	78/44	NSCLC/BLD	S	CEA, Cyfra21-1, miR3149 and miR-4769.3p	Flow fluorescence immunoanalyser, qPCR	88.46	81.82	0.898
Autoantibodies									
Chen *et al.* (45)	China	458	NSCLC/ non-malignant nodules/HC	S	MAGEA1, PGP9.5, SOX2, and TP53	ELISA	71.8	89	0.89
Doseeva *et al.* (52)	USA	230/150	NSCLC/BLD	S	One autoantibody marker (NY-ESO-1) and three Ags (CEA, CA-125, and CYFRA 21–1)	Luminex xMAP technology	74/77	80/80	0.81/0.85

Table 1. Continued

Author	Country	Sample size	Comparative group	Specimen	Name of protein(s) evaluated	Method of detection	Sensitivity %	Specificity %	AUC 95% CI
Du *et al.* (17)	China	397	LC/nodules	S	Seven TAAs (p53, PGP9.5, SOX2, GAGE7, GBU4-5, CAGE and MAGEA1)	ELISA	56.53	91.60	NG
Ezzatifar *et al.* (104)	Iran	190/30	NSCLC/HC	S	Nucleolin	ELISA	85	96.67	0.948
Farlow *et al.* (40)	USA	16/196	NSCLC/COPD/ non-malignant nodules/NC	S	IMPDH, phosphoglycerate mutase, ubiquillin, Annexin I, Annexin II, and HSP70-9B	Proteomic/Luminex-based 'direct-capture' immunobead assays	94.8	91.1	0.964
Hua *et al.* (105)	China	83/26	NSCLC/BLD	S	7-TAAbs (P53, PGP9.5, SOX2, GAGE7, GBU4–5, MAGEA1 and CAGE)	ELISA	55.44	87.5	0.65
Huo *et al.* (106)	China	121/34/100	NSCLC/HC/ nodules	S	7AAb (GAGE7, CAGE, MAGEA1, SOX2, GBU4-5, PGP9.5, and p53)	ELISA	45.5	85.3	0.66
Lastwika *et al.* (107)	USA	20/10/250	LC/nodules	T/P	IgG: EPB41L3, ANKRD36B, FGCR2A, and LINGO1; IgM: S100A7L2	ELISA	50	70	0.74/0.78
Li *et al.* (64)	USA	30/30	NSCLC/control	Sputum	DDX6, ENO1, and 14–3-3 ζ (protein zeta)	Array/ELISA	81	83	0.87
Liu *et al.* (108)	China	211/200	NSCLC/HC	P	CD25-MUC1-VEGFR1	ELISA	49.6	95	0.883
Lowe *et al.* (109)	USA	600	AAH and SCD	S	AAH: LTBP1*, BMI1*, GAGE7*, AGBL5 HES1*	SEREX	86	78	0.81/0.88
Jiang *et al.* (41)	China	150 744	LC/HC/BLD	S	7AAb (TP53, NPM1, FGFR2, PIK3CA, GNA11, HIST1H3B, and TSC1)	Protein array/ELISA	94.4 89.4	84.9 78.2	0.897 0.838
Mu *et al.* (110)	China	633/147	NSCLC/BLD	S	7-TAAbs + SCCA + CYFRA21-1	Chemiluminescence immunoassay	37.76	81.84	0.648
Ouyang *et al.* (111)	China	443 569	NSCLC/HC/BLD	S	7 AAB, CEA, CYFRA 21-1	ELISA	52.26 44.02	77.46 83	0.686 0.668
Pan *et al.* (42)	China	69/30/25 88/36/18	NSCLC stage I/ HC/BLD	S	IgA autoantigens (i.e. BCL7A, and TRIM33 and MTERF4) and three IgG autoantigens (i.e. CTAG1A, DDX4 and MAGEC2)	ELISA	73.5 68.2	>85 87	0.503 0.673
Ren *et al.* (112)	China	2008	LC/patients (GGNs) and/or solid nodules	S	p53, GAGE7, PGP9.5, CAGE, MAGEA1, SOX2 and GBU4-5	ELISA	59/62	90	0.781

Table 1. Continued

Author	Country	Sample size	Comparative group	Specimen	Name of protein(s) evaluated	Method of detection	Sensitivity %	Specificity %	AUC 95% CI
Song *et al.* (49)	Korea	170	NSCLC/HC	P	CYFRA 21-1–anti-CYFRA 21-1 autoantibody immune complex (CIC) and free CYFRA 21-1	Labelled immunoassay	76	87.5	NG
Yang *et al.* (13)	China	(42)61/24/29	LC/BLD/HC	S	HE4	ELISA	54.76	96.23	0.848
Zang *et al.* (51)	China	176/140	LC/HC	S	CEA, CA125, Annexin A1-Ab, and Alpha enolase-Ab	Multiplexed serum immunoassays	86.5	82.3	0.897
Zhang *et al.* (113)	China	68/68	ADC/HC	S	CEA, 5 IgM AAB (TSHR, ERBB2, survivin, PIK3CA, and JAK2)	ELISA	56.63	93.98	0.744
Zhong *et al.* (35)	USA	46 102	Stage I NSCLC and risk-matched control	P	PXN, SEC15L2, BAC clone RP11-499F19, XRCC5, and MALAT1	Phage library/Affymetric array	100 91.3	95.7 91.3	0.99
miRNAs and RNA									
Cazzoli *et al.* (114)	USA	30/105	LC/BD/HC	P	miR-151a-5p, miR-30a-3p, miR-200b-5p, miR-629, miR-100, and miR-154-3p	RT-PCR	97.5/96	72/60	0.76
D'Ambrosi *et al.* (115)	Netherlands	30/27/3	NSCLC/HC/ Nodules	B	2 circRNAs (circSLC8A1 and circCHD9) and 3 mRNAs (PSMB9, RUNX1, and LILRB1)	RNAseq	85	86	0.96
Dong *et al.* (116)	China	300	NSCLC/HC	P	CEA, miR-1247-5p, miR-301b-3p, and miR-105-5p	RT-PCR	88.4	64.7	0.815
Dong *et al.* (117)	China	290/105	NSCLC/HC	P	CEA, TEP SNORD55	RT-PCR	66.3	90	0.828
Dou *et al.* (118)	USA	50/35/29 44/32/51	ADC I,II/BLD/ HC	P	hsa-miR-101-3p/hsa-miR-126-5p	Sequencing/PCR	81.1/70.4	78.1/72.7	0.82/0.742
Duan *et al.* (119)	China	12/120	NSCLC/HC	S	miR-492, miR-590-3p, and miR-631	RT-PCR	86.7	71.7	0.828
Fan *et al.* (120)	China	128/193	NSCLC/BPD	S	Five miRNA ratios-(miR-15b-5p/miR-146b-3p, miR-20a-5p/miR-146b-3p, miR-19a-3p/miR-146b-3p, miR-92a-3p/miR-146b-3p, and miR-16-5p/miR-146b-3p)	RT-PCR	70	90	0.79
Gupta *et al.* (121)	USA	67/65 59/60	NSCLC/HC	Sputum	Three lncRNAs (SNHG1, H19, and HOTAIR)	qRT-PCR	82.09 81.36	89.23 88.33	0.80
Hennessey *et al.* (54)	USA	50/130	NSCLC/HC	S	miR-15b and miR-27b	RT-PCR	100	84	0.98
Jiang *et al.* (122)	China	35/15	NSCLC/HC	P	miR-152-3p and miR-1277-5p	qRT-PCR	73.3	86.7	0.79

Table 1. Continued

Author	Country	Sample size	Comparative group	Specimen	Name of protein(s) evaluated	Method of detection	Sensitivity %	Specificity %	AUC 95% CI
Kim *et al.* (123)	Canada	21/10	NSCLC/HC	BAL/ sputum	5 miRNAs (miR-21, miR-143, miR-155, miR-210, and miR-372)	qRT-PCR	85.7 BAL 67.8 sputum	100-BAL 90-sputum	NG
Li *et al.* (57)	China	64/40	NSCLC/HC	S	CEA + Exo-GAS5	RT-PCR	89.06	90.00	0.919
Li *et al.* (124)	USA	35/40	NSCLC stage I/ HC	Sputum	miR-31 and miR-210	Digital PCR	80.6	91.7	0.89
Lin *et al.* (125)	USA	135 126	Indeterminate nodules	P	11 (miR-21–5p miR-103a-3p miR-126–3p miR-135a-5p miR-145–5p miR-141–3p miR-193b-3p miR-200b-3p miR-205–5p)	Microarray and droplet digital PCR (ddPCR)	89.90 73.5	90.90 75.5	0.91
Ma *et al.* (126)	USA	1272 111	Indeterminate nodules	B	miRs-19b-3p and -29b-3p	qRT-PCR	80.30 72.6	89.40 81.9	0.91
Razzak *et al.* (127)	Canada	21/10	NSCLC/HC	Sputum	miR-21, miR-210, and miR-372	qRT-PCR	67	90	NG
Reis *et al.* (55)	Canada	54/40	Early NSCLC/HC	P	miR-16-5p, miR-92a-3p, miR-451a	RT-PCR	84	100	0.87
Roa *et al.* (128)	Canada	24/6	NSCLC/HC	Sputum	miR-21, miR-155, miR-210, miR-143, miR-372	qRT-PCR	83.3	100	NG
Su *et al.* (69)	China	117/174	NSCLC stage I/ control (PN)	Sputum	2 miRNAs (miR-31 and miR-210) and methylation of 2 genes (RASSF1A and 3OST2)	qRT-PCR	87.3	90.3	0.93
Su *et al.* (68)	USA	117/103	NSCLC stage I/ control (PN)	Sputum	miRs-21, 31, and 210 + small nucleolar RNA (snoRDs-66 and 78)	qRT-PCR	89	89	0.94
Tulinský *et al.* (129)	Czech	60/60	NSCLC/HC	P	miR-126, miR-143, miR-145, let-7a and let7g	qRT-PCR	75–85	75–85	0.90–0.93
Wang *et al.* (130)	China	165/118	NSCLC/HC	P	SNORD42B and SNORD111	qRT-PCR	61.8	77.1	0.719
Wang *et al.* (131)	China	82	pulmonary nodules both benign and malignant	P	miRNA-17, miRNA-146a, miRNA-200b, miRNA-182, miRNA-155, miRNA-221, miRNA-205, miRNA-126, miRNA-7, miRNA-21, miRNA-145, and miRNA-210	RT-PCR	50	92.9	0.896
Wu *et al.* (132)	China	100/100	NSCLC/HC	P	miR-340 and miR-450b-5p	qRT-PCR	78.33	77.5	0.862
Wu *et al.* (133)	China	48/48/32	NSCLC I/II/HC/ LBL	S	Four serum miRNAs including miR-21-5p, miR-141-3p, miR222-3p, and miR-486-5p, and 2 serum exosomal miR-146a-5p and miR-486-5p	qRT-PCR	85.42	92.50	0.96

Table 1. Continued

Author	Country	Sample size	Comparative group	Specimen	Name of protein(s) evaluated	Method of detection	Sensitivity %	Specificity %	AUC 95% CI
Xie *et al.* (66)	USA	23/17	NSCLC/cancer free	Sputum	mir-21	qRT-PCR	47.82	100	NG
Xing *et al.* (67)	USA	67/55	SCC/HC	Sputum	miR-205, miR-210 and miR-708	Microarray/qRT-PCR	73	96	0.87
Xing *et al.* (134)	USA	122/136/155	Indeterminate solid nodules	Sputum	miR205/miR708/miR375/ miR200b/miR182/miR155/ miR372 miR143 (miRs21, 31, and 210)	RT-PCR	82.93/82.09/80.52	87.84/88.41/86.08	0.919
Xing *et al.* (56)	China	17/534	NSCLC/control (BN/HC)	S	ITGA2B belongs TEP	RNA-seq/ q-PCR and ddPCR	92.8/91.2	78.6/56	0.892
Yu *et al.* (135)	USA	64/58	NSCLC/HC	Sputum	miR-21, miR-486, miR-375 and miR-200b	qRT-PCR	69.22	81.7	0.83
Zhou *et al.* (136)	China	15	ADC	P	SNORD60	qRT-PCR	74.2	75.3	0.828
ctDNA and CTC									
Abou-Zeid *et al.* (137)	Egypt	25/25	NSCLC/HC	P	HOXA9, SOX2, HV2	qRT-PCR	88	100	0.958
Carozzi *et al.* (59)	Italy	1356	LC/smokers/ ex-smokers	P/sputum	MSI/LOHs loci, with the loci 1 to 5 (3p14.2, 3p21-p23, 3p26.1, 3p13, 5q15) and 7 to 9 (9p22-p23, 9p21, 13q12.3)	PCR	90	71	NG
Chen *et al.* (138)	China	161	Nodules	P	CDO1, SOX17 and HOXA7	QMSP	90	71	NG
Chen *et al.* (139)	China	41/10	NSCLC/HC	B	EpCAM and Folate receptor alpha (FRα)	Immunomagnetic separation method	75.61	90	NG
Gao *et al.* (140)	China	89	Nodules	S/P/T	APC, RASSF1A	QMSP	56.9	90.3	0.81
Leung *et al.* (141)	UK	211	NSCLC/HR	P/T	ctDNA (EGFR, KRAS, and TP53 mutation)	RT-PCR	75	89	NG
Hulbert *et al.* (142)	Netherlands	150/60	NSCLC stage I IIA/BLD	P/sputum	P-CDO1, TAC1, and SOX17 Sputum-(TAC1, HOXA17, and SOX17)	SMART-MSP	93-P 98-sputum	62-P 71-sputum	0.77-P 0.89
Hubers *et al.* (143)	Netherlands	159/154	NSCLC stage I IIA/BLD	Sputum	TAC1, HOXA7, SOX17	QMSP	67.1 42.5	89.5 96.5	0.69
Paci *et al.* (144)	Italy	151/79	NSCLC/HC	B	Amplification of hTERT	qRT-PCR	85.8	46.8	0.79
Su *et al.* (69)	China	117/174	NSCLC stage I/ control (PN)	Sputum	RASSF1A, 3OST2 and PRDM14	QMSP	82.9 45.3	76.4 86.2	0.79 0.68
Wan *et al.* (145)	China	48	NSCLC	B	NOTCH1, IGF2, EGFR, and PTCH1	CellCollector® in vivo CTC capture	65.85	62.5	NG
Xue *et al.* (146)	USA	(31)72/26	NSCLC/control	P	FR + CTC	immunomagnetic leukocyte depletion/PCR	74.19	73.08	0.8221

Table 1. Continued

Author	Country	Sample size	Comparative group	Specimen	Name of protein(s) evaluated	Method of detection	Sensitivity %	Specificity %	AUC 95% CI
Yang *et al.* (60)	China	50	Nodules	P	Methylation of 8 genes (CDH13, WT1, CDKN2A, HOXA9, PITX2, CALCA, RASSF1A, and DLEC1)	QMSP	72	91	NG
Zhong *et al.* (63)	China	18	Solid nodules	S	(CEP8) CTC, CA125 or NSE	Electrochemiluminescence/ FISH	83	100/83 with NSE	NG

The number between () represents number of NSCLC patients with stages I and II.
AAH, Atypical adenomatous hyperplasia; ARSA, Arysulfatase A; AUC, area under curve; B, blood; BAL, bronchioalveolar lavage; BN, benign nodules; BPC, benign pulmonary condition; BLD, benign lung diseases; Bmi-1, B-lymphoma Moloney murine leukaemia virus insertion region-1; C4c, complement-derived fragment; CI, confidence interval; CRP, C-reactive protein; CTC, circulating tumour cells; Ciz1, nuclear matrix-associated DNA replication factor; CXCL14, C-X-C motif chemokine ligand 14; ddPCR, droplet digital PCR; FR, folate receptor; FOXL2, fork-head box L2 gene; HC, healthy control; HE4, Human epididymis secretory protein 4; HES1, mammalian hairy and Enhancer-of-split homologues 1; IDH1, isocitrate dehydrogenase 1; ILra, interleukin-1ra; LTBP1, Latent Growth Factor Beta Binding Protein; MCP1, monocyte chemotactic protein-1; MDH2, malate dehydrogenase 2; MDK, Midkine; MIC-1, Macrophage inhibitory cytokine-1; MIF, macrophage migration inhibitory factor; MMP2, matrix metalloproteinase-2; MSI/LOH, genomic instability loss of heterozygosity/microsatellite instability; ncRNA, non-coding RNA; NG, not given; NSCLC, non-small cell lung cancer; OC, other cancers; OPV, OPN velocity; P, plasma; PTGER4, prostaglandin E receptor 4 gene; q-PCR, quantitative real-time PCR; QMSP, real-time quantitative methylation-specific polymerase chain reaction; SCD, squamous cell dysplasia; S, serum; SHOX2, methylation of short stature homeobox 2 gene; sVCAM-1, soluble vascular cell adhesion molecule; T, tissue; TC, training cohort; TB, tuberculosis; TEP, tumour-educated platelets; THSP, thrombospondin; TNF-α, tumour necrosis factor α; tPAI-1, tissue plasminogen activator inhibitor 1; TTR, transthyretin; VC, validation cohort; WFDC2, WAP four-disulphide core domain 2.

biomarkers performed the least accurately in differentiating early NSCLC from benign lung diseases (pooled AUC = 0.74, 95% CI 0.67–0.81) (Figure 3B). There was also no significant subgroup difference based on the source of biomarker (I^2 = 0%, P = 0.95), suggesting that the diagnostic performance of biomarkers was similar regardless of the source of biomarker (Figure 3C). The funnel plot appeared asymmetric. Kendalls tau (P = 0.009) and Egger's test (P = 0.003) were significant, indicating that publication bias may be present (Figure 3D). The findings of this meta-analysis should be interpreted with caution as each study investigated different individual biomarkers. There were limited studies with data suitable for meta-analysis, hence this meta-analysis was not representative of all studies encompassed by the systematic review. However, we provide preliminary evidence that current NSCLC biomarkers can generally be expected to perform well diagnostically.

Taking all data into consideration, the subgroup of miRNA and RNA biomarkers showed the highest specificity (0.91) (Table 3), followed by antigens (0.86), DNA and CTC (0.84), and finally, autoantibodies (0.77), although, results of DNA and CTC showed more statistical robustness as their I^2 value was lower.

Antigens as biomarkers for NSCLC

The most sensitive and specific antigens biomarkers, with values over 90% (Table 2), were discovered by Farlow *et al.* (27), when they analysed different combinations of biomarkers based on TNF-α, CYFRA 21, interleukin-1ra, MMP-2, monocyte chemotactic protein-1 and sE-selectin achieving 99% sensitivity and 95% specificity. Yang *et al.* (28), Yuan *et al.* (29) and Gasparri *et al.* (30) used different antigen combinations; but did not achieve the same sensitivity and specificity as Farlow *et al.* (27), except for the specificity, 99%, in the combination of HSP90α and CEA described by Yuan *et al.* (29). Ma *et al.* (26) showed that malate dehydrogenase 2 (MDH2) was detected in urine with sensitivities of 70.13%, 68.92% and specificities of 66.11%, 58.22% in training and validation cohorts, respectively (26).

Jeong *et al.* (31) demonstrated that the exosomal GRIP And Coiled-Coil Domain Containing 2 (GCC2) could be a biomarker for early NSCLC. When derived from NSCLC exosomes, GCC2 was upregulated, but it has also been found to be increased in other cancers such as those affecting the liver, which are also associated with poor prognosis (32). However, the isolation of exosomes from the blood is a time-consuming process and requires high quality controls to ensure the purity of these molecules is of the standard required for further analyses (31). Higgins *et al.* (33) focussed on the CDKN1A Interacting Zinc Finger Protein 1 (Ciz1), which is a nuclear matrix protein. Its expression was restricted to tumours and not found in normal tissues with a specificity of 71% for early NSCLC and a false positive rate of 50%. This suggested that Ciz1 had a limited capacity to differentiate NSCLC stage among high-risk sub-groups.

Osteopontin (OPN), a secreted phosphoprotein, was increased in NSCLC, however, it could not differentiate benign lung diseases from pulmonary carcinoma probably due to its roles in wound healing and tissue remodelling. Joseph *et al.* (34) found that OPN concentrations in plasma changed as a function of time (OPN velocity; OPNV) and acted as a biomarker for early NSCLC with 80% sensitivity and 88% specificity. Suggesting that OPNV velocity could be useful for the

Table 2. Studies with sensitivity and specificity >90%

Group	Study	Sensitivity TC/VC	Specificity TC/VC	AUC	Biomarker panel
Antigens	Farlow *et al.* (27)	99	95	0.979	TNF-α, CYFRA 21-1, interleukin-1ra, MMP-2, monocyte chemotactic protein-1 and sE-selectin
	Yang *et al.* (28)	92.97	90	0.95	Ferritin, CA125, CEA, NSE and CYFRA21-1
	Yuan *et al.* (29)	95.63	99.97	0.996	HSP90α and CEA
	Gasparri *et al.* (30)	94.83	93.56	0.98	ARSA, PRKCA, ACTR3B and CD59
Autoantibodies	Zhong *et al.* (35)	100/91.3	95.7/91.3	0.99	Paxillin, SEC15L2, BAC clone RP11-499F19, XRCC5 and MALAT1
	Farlow *et al.* (40)	94.8	91.1	0.964	IMPDH, phosphoglycerate mutase, ubiquillin, Annexin I, Annexin II and HSP70-9B

TC, training cohort; VC, validation cohort.

Table 3. Averages of the sensitivity and specificity of all possible lung-cancer biomarkers

	Sensitivity % ± SD	Specificity % ± SD
Antigens	77.2 ± 10.1	86.08 ± 17.5
Antibodies	79.4 ± 15.2	77.33 ± 5.8
miRNA	79.83 ± 8.9	90.33 ± 12.5
ctDNA and CTC	81.43 ± 5.7	84.15 ± 4.8

detection of early NSCLC especially in the context of indeterminate nodules. Zhong *et al.* (35) examined patients with stage I NSCLC versus those patients in high-risk groups for expression of a biomarker panel that included paxillin (PXN), SEC15L2, BAC clone RP11-499F19, XRCC5 and MALAT1. They demonstrated a sensitivity of 100% and specificity of 95.7% in the training cohort, and a sensitivity and specificity of 91.5% in the validation group (35). This panel represents a promising approach to complement a CT scan for early NSCLC diagnosis based on a predictive accuracy of 91%. The validation cohort contained 102 patients including 40 patients with indeterminate nodules, 56 patients with autoimmune diseases and six patients with prevalence cancers. Rigorous validation was required including sample analysis during tumour transformation as eight occult cancers were misclassified as normal whilst using this panel. The protein panel and its association with NSCLC requires further investigation using different types of cancers and controls as the proteins did not show significant homology to the complete sequence and small differences in amino acids may indicate they belong to different parent proteins than they were assigned to in the GenBank database.

Farlow *et al.* (27) identified six markers: tumour necrosis factor α (TNF-α), CYFRA 21-1, interleukin-1ra (IL-1ra), matrix met alloproteinase-2 (MMP-2), monocyte chemotactic protein-1 (MCP-1) and sE-selectin. Only CYFRA 21-1 was a well-characterised biomarker for NSCLC diagnosis whilst TNF-α and IL-1ra were known inflammatory mediators that were non-specific to cancer. The sensitivity and specificity of this panel was high for early LC detection, at 99% and 95%, respectively, with 66/74 patients being correctly identified stage I and II LC when compared to 43 non-malignant lung conditions that were used as controls. However, the panel had a 47% false positive rate and failed to differentiate between NSCLC and inflammatory diseases such as chronic obstructive pulmonary disorder and pneumonia which share similar inflammatory mediators. To improve the specificity, tumour-associated autoantibodies could also be used (27). Wang *et al.* (36) used a panel of four-markers including MIC-1, CYFRA21-1, CA125 and CEA and showed high sensitivity and specificity for LC diagnosis. There was a higher sensitivity of 90.4% for adenocarcinomas and 92.1% for squamous cell carcinoma (in NSCLC), compared with 83.9% for SCLC. This panel also had a lower sensitivity for early stages (84.4%) compared to the sensitivity (89.5%) for disease diagnosis at all stages. The panel was tested using an independent group of patients, demonstrating a sensitivity and specificity of 88.4% and 93.1%, respectively. However, there was a false positive rate of 56.4% in the blinded samples from patients with benign tumours and tuberculosis (36).

Combining serum ferritin, shown to play a prognostic role in advanced hepatic cancer (37), with NSCLC markers (CA125, CEA, NSE and CYFRA21-1) improved the diagnostic performance of the panel for early NSCLC diagnosis to a sensitivity of 92.97% and specificity of 90% in elderly patients (28). This panel requires verification in a larger cohort and in younger members of the patient population. Wang *et al.* (38) found that the addition of haptoglobin to the clinical LC biomarkers CEA, NSE and CYFRA21-1 improved their diagnostic performance, especially for CYFRA21-1 and Hp for squamous LC. Ajona *et al.* (39) showed that C4c, CYFRA 21-1 and C-reactive protein (CRP) are potential biomarkers of early NSCLC. However, this panel was derived from a limited selection of circulating proteins using retrospective samples. The standardisation and calibration of assays, especially for C4c, and larger prospective studies with different control groups are essential to test diagnostic accuracy.

Autoantibodies as biomarkers for NSCLC

Autoantibodies biomarkers with the highest sensitivities and specificities were paxillin combined with SEC15L2, BAC clone RP11-499F19, XRCC5 and MALAT1 (35) while Farlow *et al.*, found that a six-autoantibody panel (IMPDH, phosphoglycerate mutase, ubiquillin, Annexin I, Annexin II, and HSP70-9B) achieved an AUC of 0.964, a sensitivity of 94.8%, and a specificity of 91.1%. The overall misclassification rate was 7% within the patient population analysed (n = 196). Further validation of this finding will require the use of an asymptomatic cohort containing a suitable control such as smokers, as well as healthy and cancer patients (40). Inflammatory conditions such as COPD induce specific

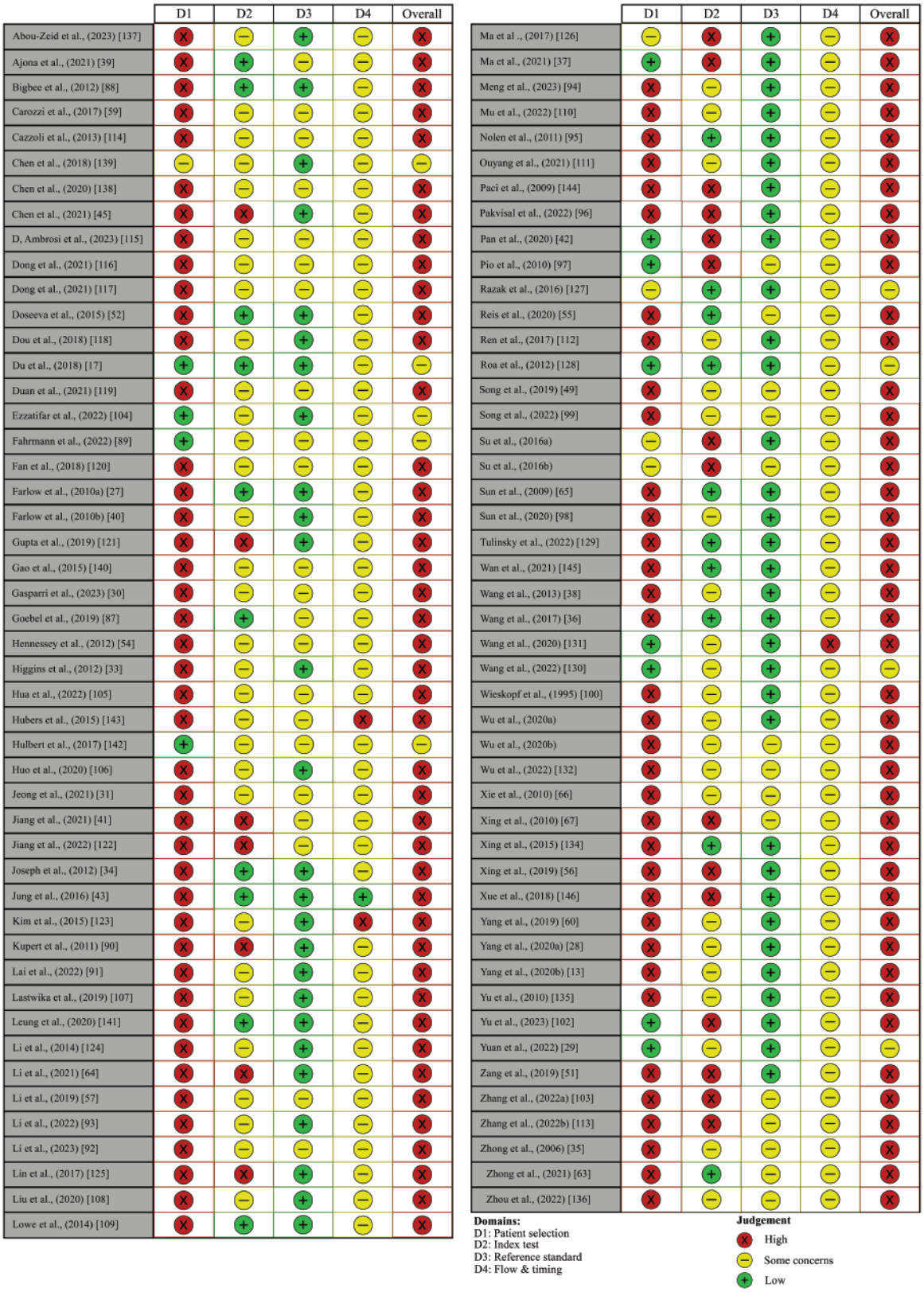


Figure 2. Risk of bias assessment of the selected articles. (**A**) Risk of bias rating for each study and (**B**) for each domain across all studies.

autoantibody production that may lead to the misclassification of patients having NSCLC. Whether these autoantibodies are produced during or before carcinogenesis still needs to be explored (40). Jiang *et al.* (41) stated their seven-TAAb panel showed the highest sensitivity and specificity for early NSCLC, at 94.4% and 82.7%, respectively. A limitation of this study included the small sample number of patients with early NSCLCs (72 patients with stage I and II) and

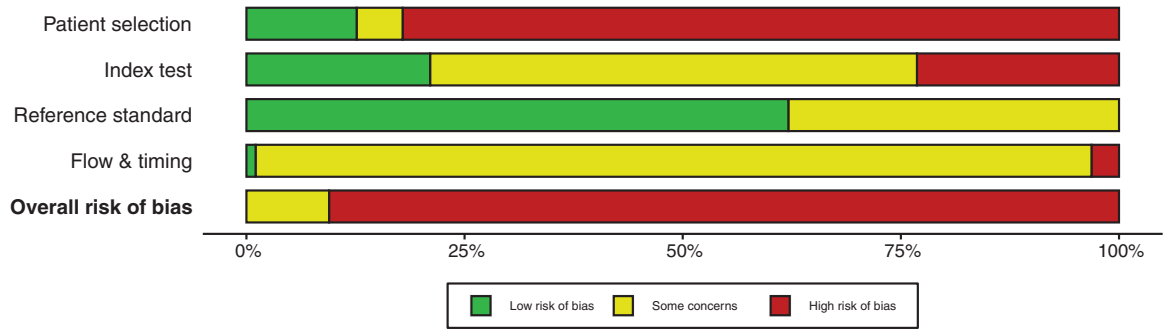


Figure 2. Continued

A

Study or Subgroup	AUC	SE	Weight	AUC IV, Random, 95% CI
1.1.1 Antigens				
Ajona et al., 2021 [39]	0.905	0.0332	3.0%	0.91 [0.84, 0.97]
Fahrmann et al., 2022 [89]	0.795	0.0128	3.4%	0.80 [0.77, 0.82]
Gasparri et al., 2023 [30]	0.9864	0.0069	3.4%	0.99 [0.97, 1.00]
Goebel et al., 2019 [87]	0.893	0.0214	3.2%	0.89 [0.85, 0.93]
Joseph et al., 2012 [34]	0.882	0.0791	1.9%	0.88 [0.73, 1.04]
Kupert et al., 2011 (BPN) [90]	0.685	0.0536	2.5%	0.69 [0.58, 0.79]
Lai et al., 2022 [91]	0.9135	0.0222	3.2%	0.91 [0.87, 0.96]
Meng et al., 2023 [94]	0.916	0.0602	2.4%	0.92 [0.80, 1.03]
Ouyang et al., 2021 (BLD) [111]	0.656	0.0265	3.1%	0.66 [0.60, 0.71]
Pio et al., 2010 [97] BAL	0.855	0.0536	2.5%	0.85 [0.75, 0.96]
Pio et al., 2010 [97] Sputum	0.925	0.0179	3.3%	0.93 [0.89, 0.96]
Sun et al., 2020 (validation) [98]	0.788	0.0393	2.9%	0.79 [0.71, 0.87]
Yuan et al., 2022 [29]	0.996	0.002	3.4%	1.00 [0.99, 1.00]
Subtotal (95% CI)			**38.3%**	**0.87 [0.82, 0.92]**
Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 515.82$, df = 12 ($P < 0.00001$); $I^2 = 98\%$				
Test for overall effect: $Z = 33.92$ ($P < 0.00001$)				
1.1.2 Autoantibodies				
Doseeva et al., 2015 [52]	0.805	0.0281	3.1%	0.81 [0.75, 0.86]
Hua et al., 2022 [105]	0.6539	0.0488	2.6%	0.65 [0.56, 0.75]
Li et al., 2021 [64]	0.8667	0.0399	2.9%	0.87 [0.79, 0.94]
Yang et al., 2020a [13]	0.848	0.0367	2.9%	0.85 [0.78, 0.92]
Subtotal (95% CI)			**11.6%**	**0.80 [0.72, 0.88]**
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 13.33$, df = 3 ($P = 0.004$); $I^2 = 78\%$				
Test for overall effect: $Z = 20.19$ ($P < 0.00001$)				
1.1.3 miRNA and RNA				
D'Ambrosi et al., 2023 [115]	0.96	0.03	3.1%	0.96 [0.90, 1.02]
Duan et al., 2021 [119]	0.8275	0.0395	2.9%	0.83 [0.75, 0.90]
Gupta et al., 2019 [121]	0.8	0.0357	3.0%	0.80 [0.73, 0.87]
Li et al., 2019 [57]	0.9195	0.0355	3.0%	0.92 [0.85, 0.99]
Su et al., 2016a [141] miRNA	0.89	0.0281	3.1%	0.89 [0.83, 0.95]
Tulinsky et al., 2022 (miR-126) [129]	0.913	0.0316	3.0%	0.91 [0.85, 0.97]
Wu et al., 2020b [133]	0.96	0.0152	3.3%	0.96 [0.93, 0.99]
Wu et al., 2022 [132]	0.862	0.023	3.2%	0.86 [0.82, 0.91]
Xing et al., 2010 [67]	0.789	0.039	2.9%	0.79 [0.71, 0.87]
Yu et al., 2010 [135]	0.834	0.034	3.0%	0.83 [0.77, 0.90]
Subtotal (95% CI)			**30.4%**	**0.88 [0.84, 0.92]**
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 45.98$, df = 9 ($P < 0.00001$); $I^2 = 80\%$				
Test for overall effect: $Z = 42.43$ ($P < 0.00001$)				
1.1.4 DNA and CTC				
Chen et al., 2020 [45]	0.885	0.023	3.2%	0.89 [0.84, 0.93]
Hubers et al., 2015 [143]	0.685	0.0434	2.8%	0.69 [0.60, 0.77]
Hulbert et al., 2017 [142] plasma	0.77	0.0459	2.7%	0.77 [0.68, 0.86]
Hulbert et al., 2017 [142] sputum	0.89	0.0459	2.7%	0.89 [0.80, 0.98]
Paci et al., 2009 [144]	0.79	0.0408	2.8%	0.79 [0.71, 0.87]
Su et al., 2016a [69] DNA methylation	0.795	0.0383	2.9%	0.80 [0.72, 0.87]
Xue et al., 2018 [146]	0.8222	0.0517	2.6%	0.82 [0.72, 0.92]
Subtotal (95% CI)			**19.7%**	**0.81 [0.75, 0.86]**
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 22.00$, df = 6 ($P = 0.001$); $I^2 = 73\%$				
Test for overall effect: $Z = 28.25$ ($P < 0.00001$)				
Total (95% CI)			**100.0%**	**0.85 [0.82, 0.88]**
Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 899.77$, df = 33 ($P < 0.00001$); $I^2 = 96\%$				
Test for overall effect: $Z = 50.93$ ($P < 0.00001$)				
Test for subgroup differences: $Chi^2 = 6.23$, df = 3 ($P = 0.10$), $I^2 = 51.8\%$				

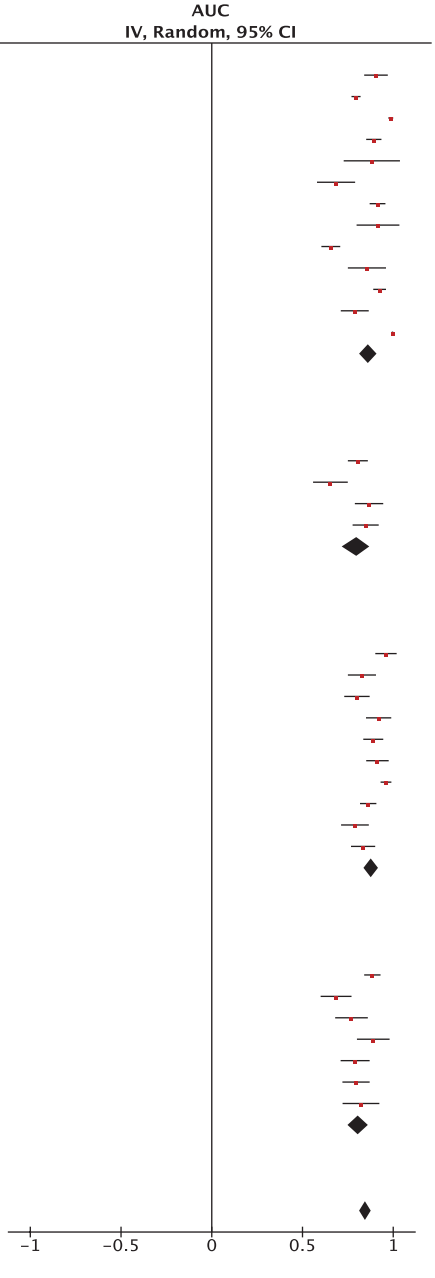


Figure 3. Meta-analysis of selected studies. (**A**) Forest plot for meta-analysis of AUC and 95% CI, and subgroup analysis based on type of biomarker. (**B**) Forest plot for subgroup analysis based on type of control. (**C**) Forest plot for subgroup analysis based on source of biomarker. (**D**) Funnel plot for publication bias assessment.

B

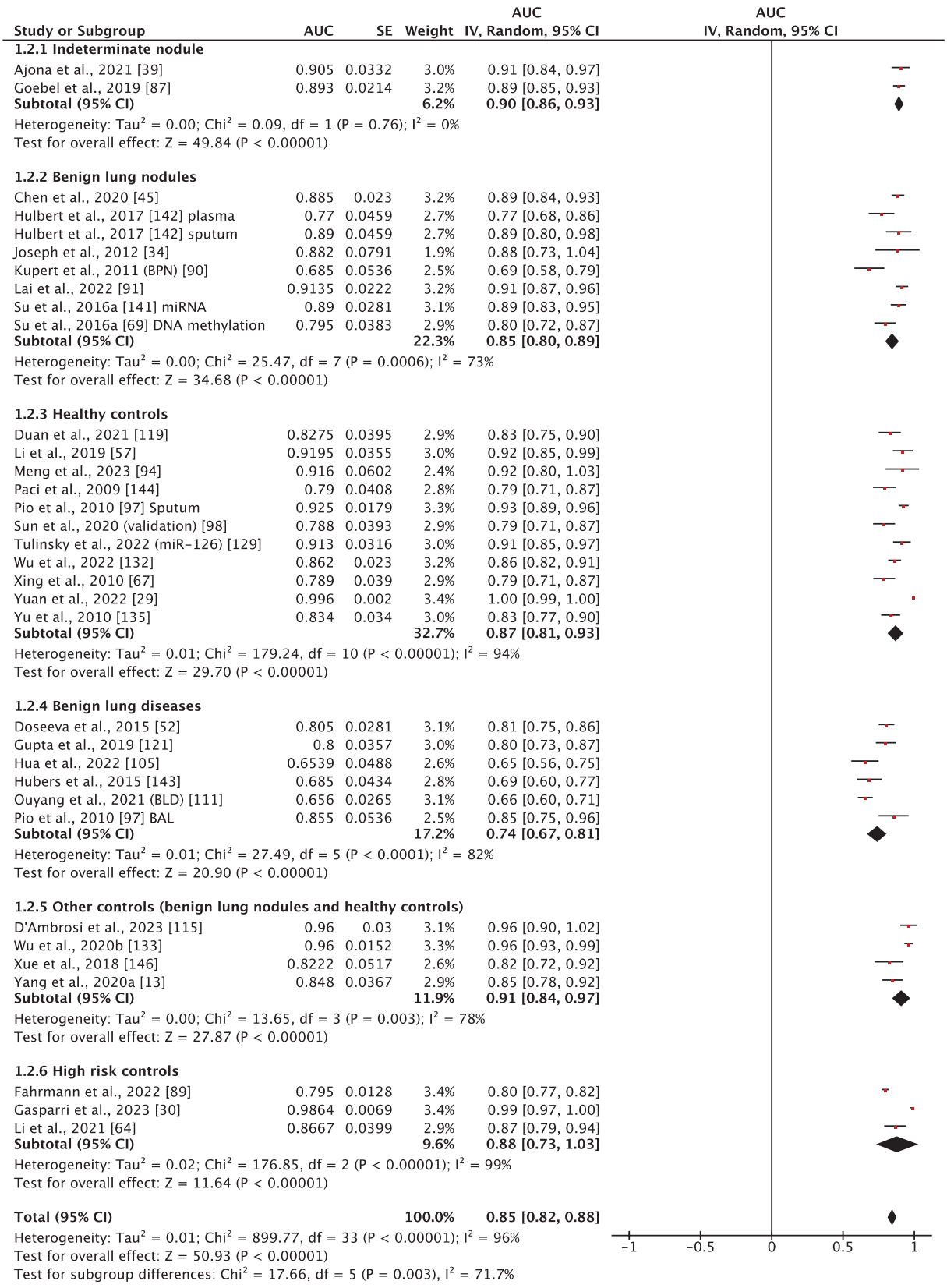


Figure 3. Continued

the diagnostic performance of the panel for malignant rather than benign lung nodules will require further assessment.

Pan *et al.* (42) suggested a panel of six autoantibodies could enable the early detection of NSCLC with sensitivities and specificities of 73.5%/68.2% and >85%/87 for the training and validation cohorts, respectively. The six autoantigens were shown to be highly expressed in NSCLC by immunohistochemistry with positive scores of 66.7%, 61.6%,

C

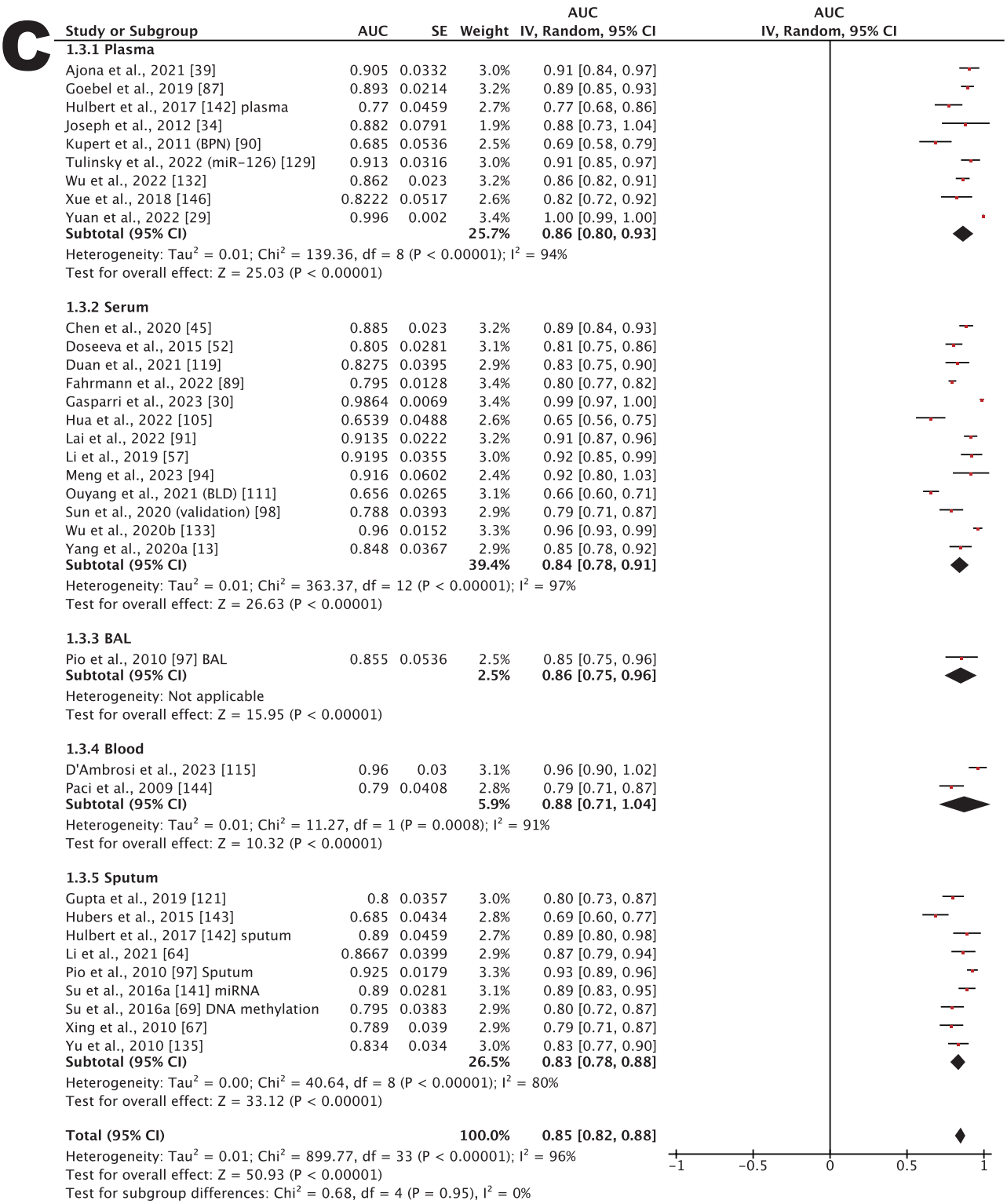


Figure 3. Continued

58.3%, 58.3%, 26.6% and 36.7% for BCL7A, TRIM33, MTERF4, CTAG1A, DDX4 and MAGEC2, respectively (43). Some of these genes are known to be mutated in NSCLC especially the cancer testis antigens (CTAs) CTAG1A, DDX4 and MAGEC2. Inclusion of CTAs can increase both the specificity and sensitivity of a biomarker panel by virtue of their elevated levels in disease and restricted expression in healthy tissues.

One drawback of this study, like most published biomarker studies, is that it is a single-centre study, that would benefit from the utilisation of a larger cohort size. Considering the genetic variation between ethnicities, and the impact of the environment on LC development, we note that in this study, economic progress and air pollution may have impacted the biomarkers that are relevant to LC in East Asia (44).

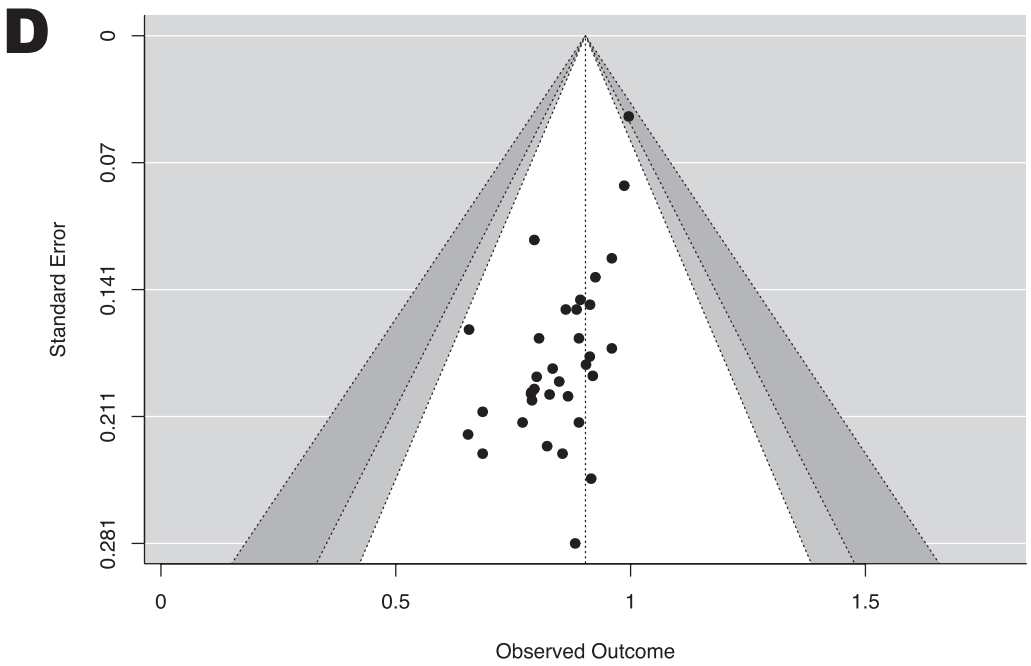


Figure 3. Continued

Chen *et al.* (45) focussed on the autoantibodies that are associated with cancer-stem cell-like (stem) signatures. Only SOX2 expression was associated with tumour stage. MAGEA1 is a CTA that is overexpressed in NSCLC and is associated with necrosis. 70–85% of NSCLC patients have upregulated MAGEA1, A3 and B2 due to global promoter hypomethylation (46). MAGEA1 and MAGEA3/4 have been found to be expressed in 17% and 44% of NSCLC samples, respectively (47). MAGEA3/4 was found more often in squamous cell carcinoma $P < 0.001$ while MAGEA1 was found more frequently in adenocarcinoma. Determining MAGE transcript levels in urine and sputum may be useful for biomarker discovery in LC (48). However, MAGEA1 levels were also increased in other cancers such as breast and gastric tumours (47). Thus, this panel is not recommended to identify the type of cancer but could aid in early diagnosis of NSCLC when combined with a CT scan. The presence of autoantibodies against these CTAs may predict poorer survival but further studies are required to validate the utility of this panel for early LC diagnosis with a larger number of participants (45).

Song *et al.* (49) found that the ratio of anti-CYFRA 21-1 autoantibody immune complex (CIC) and free CYFRA 21-1 had a sensitivity of 76.0%, 80.0%, 76.9% and 50.0% for the detection of stage I, II, III and IV LCs, respectively. Therefore, this could be applied to the identification of asymptomatic patients in a seemingly healthy population. A similar finding was made in colon cancer as CYFRA21-1 is a fragment of cytokine 19 that is overexpressed in epithelial cancers (50).

Studies also combined autoantibodies with antigens as biomarkers for NSCLC. Zang *et al.* (51) found that combining autoantibodies, with LC antigens, improved the diagnostic performance of this biomarker panel, with a sensitivity of 86.5% and specificity of 82.3%. Both alpha enolase and annexin A1 (autoantibodies investigated in this study) are upregulated in LC and were suggested to be biomarkers for NSCLC staging. This study did not include non-malignant lung diseases and was not externally validated. Doseeva *et al.* (52) found that a panel of one autoantibody marker and the detection of three Ags had a sensitivity and specificity of 74% and 80% in the training cohort, and a higher sensitivity of 77% but the same specificity (80%) in the validation cohort. Five out of eight cases of false positives were COPD patients but COPD is known as independent risk factor of LC (53). However, this study also lacked samples from patients with benign nodules (52).

miRNAs and mRNAs as biomarkers for NSCLC

miRNA are short sequences of non-coding RNA of 19–22 nucleotides that are involved in the control of gene transcription. miRNAs have also been described as possible biomarkers for LC with high sensitivity. The highest was miR-15b and miR-27b with a 100% sensitivity and 84% specificity (54) while miR-16-5p, miR-92a-3p and miR-451a can facilitate an early LC diagnosis (55). Further studies should include large numbers of patients and controls from ethnic groups to which these panels are needed. The specificities of each miRNA were also high, ranging from 72 to 92.5% for all 46 miRNAs examined in 15 selected articles.

Xing *et al.* (56) identified mRNA from tumour-educated platelets (ITGA2B) with a sensitivity of 92.8% and specificity of 78.6% in the training cohort. In the validation cohort, the specificity decreased significantly to 56%. Li *et al.* (57) found that CEA and exo-GAS5 also showed a high diagnostic performance for stage I NSCLC with a sensitivity of 84.21% and a specificity of 90%. Growth arrest-specific transcript 5 (GAS5) (58) has been shown to be decreased in both tissue and plasma from NSCLC patients and its expression was associated with NSCLC tumour size (58). lncRNA GAS5 expression is downregulated in NSCLCs while GAS5 expression in secreted exosomes was upregulated in NSCLCs. The diagnostic significance of exosomic GAS5 was higher in tumour tissue than in circulating GAS5 serum levels but also more difficult and time-consuming to determine.

DNA and CTC as biomarkers for NSCLC

Five different loci from the microsatellite instability (MSI)/loss of heterozygosity (LOH) loci family had a sensitivity of 90% and a specificity of 71% for early NSCLC (59). In contrast, Yang *et al.* (60) showed the highest specificity (91%) for eight methylated genes, with a 72% of sensitivity. Chromosome enumeration probe 8 (CEP8) is one of the CTCs produced by NSCLC tissue (61) and it's expression was associated with diagnosis and prognosis of NSCLC with high sensitivity and specificity at 83.3% and 98.6%, respectively (62). CEP8 combined with CA125 increased the detection of NSCLC from 83 to 100% for sensitivity. Combining CEP8 and NSE achieved a sensitivity and specificity of 83% each. The limitation of this study included the small sample size of 18 solid nodules, the absence a control group and a lack of result validation. CEP8 performance and reference values also need to be established for LC (63).

Sputum biomarkers in lung cancer

Sputum is produced directly by the upper and lower respiratory tract and can serve as a surrogate sample for the diagnosis of LC. LC tissue can affect the biological components of sputum and detection of overexpressed genes in sputum can help diagnose LC (64,65). Single antigen, A proliferation-inducing ligand (APRIL), has high specificity of 97% and sensitivity of 82% following detection by immunocytochemistry (65). Three sputum TAAbs were developed as a biomarker panel for the diagnosis of LC, regardless of stage, site, and histologic type, with 81% sensitivity and 83% specificity (64). miR-21 has low sensitivity of 48% but an absolute 100% specificity in 23 NSCLC samples compared to 17 controls (66). Panels of miR-205, miR-210 and miR-708 had a diagnostic sensitivity of 73% and specificity of 96% of distinguishing patients with squamous cell carcinoma from healthy controls (67). Su *et al.* (68) found that three miRNAs combined with two small nucleolar RNA had an AUC-ROC of 0.94 when distinguishing NSCLC patients from cancer-free subjects. Subsequently Su *et al.* (69) found that a combination of two miRs and the methylation of two genes had sensitivity of 87% and specificity of 90% when identifying stage I NSCLC compared with controls.

Discussion

Our review found that well-performing single biomarkers for early NSCLC diagnosis included Ciz1 (sensitivity: 95%) and exosomal GCC2 (sensitivity: 90%) with a slightly lower specificity of 71% for CIZ1 and 75% for exosomal GCC2, respectively. Tumour-educated blood platelets (ITGA2B) also had high sensitivities in both the training (92.8%) and validation cohorts (91.2%) but low specificity. In contrast, CYFRA 21-1 and anti-HE4 had high specificity for LC (95% each). OPNV had a sensitivity of 80% and a specificity of 88% as a biomarker for early LC. Biomarker panels (Table 1) had high sensitivity and specificity (greater than 90%). Combining biomarkers was more likely to facilitate the early detection of NSCLC, especially, when antigens or autoantibodies were combined with miRNAs. Early detection is the holy grail of NSCLC diagnoses as it offers the opportunity to significantly increase survival rates, aid in the management of the disease, and reduce overall healthcare costs. CYFRA21-1 is a prognostic biomarker for advanced NSCLC, predominately found in lung tissues that correlates with tumour size, lymph node involvement and the stage of the disease. Lower baseline levels of CYFRA21-1 were associated with both longer overall survival and failure free survival ($P < 0.0001$ and $P = 0.0003$) (70). Wang *et al.* (71) found that serological levels CYFRA21-1 combined with other markers delivered different sensitivities and specificities, depending on the sample size. A commonly used combination of CYFRA21-1, CEA and NSE for NSCLC detection revealed a very low sensitivity of 31% in contrast to its very high specificity of 96%. These assays have the drawback of low sensitivity especially in the early stages of NSCLC, whilst the same panel of biomarkers have shown high sensitivity at advanced stages. Therefore, this combination could not be recommended for use in early detection in clinical practice. However, the high concentration of these biomarkers in body fluids/levels in tissues are poor prognostic indicators. Thus, these three biomarkers could be used to predict relapse before the onset of clinical symptoms as their concentration can be used to monitor therapy response/resistance.

In the early stages of NSCLC, patients are primarily asymptomatic but a low tumour presence can elicit TAs/TAAbs that are detectable at higher levels in liquid biopsies, compared to samples from healthy individuals (72). Thus, changes in the immune response can be detected in blood before clinical symptoms appear. Different molecules such as proteins and miRNA have been shown to be very sensitive biomarkers, which are cost effective and safe compared to imaging techniques such as CT scans that are associated with an increased risk of cancer due to radiation, require specialist training and are expensive compared to blood tests. miRNAs in blood have a higher sensitivity for LC diagnosis compared to miRNAs in sputum as the oral cavity contains many enzymes such as those that degrade these small molecules (73). Although sputum represents a source for LC biomarkers and is considered a non-invasive technique (64), it requires patient co-operation and capacity to deliver spontaneous samples. Sputum collection and analysis can be highly variable, dependent on factors such as the quality of the collected sputum and the techniques used in the laboratory which can impact the reliability and reproducibility. miRNAs lack the specificity needed for early LC diagnosis as they are expressed in many cancers and healthy tissues. Due to this and their reduced stability, miRNAs are not suggested for clinical use (73). In contrast, antigens are frequently used as markers for disease diagnosis with the aid of imaging techniques (11,74).

Biomarkers can be diagnostic, predictive or indicative (75,76). Biomarker discovery is largely dependent on an analytic validation for measuring biomarkers in body fluids. Blood is mostly used to detect molecular changes associated with LC after depleting the abundant proteins; leaving the biomarkers of interest that are usually present in very low concentrations. The stability of biomarkers is a crucial factor as it affects reproducibility and analytic validation procedures (77,78). In addition to study design and population selection should also be considered. Sample size should be statistically valid as a low number of participants exaggerates the diagnostic performance of biomarkers. The required sample sizes should be calculated to achieve 95% confidence levels and 80% power for purpose of testing the validity of the biomarker (79). Moreover, age- and gender-matched controls

should be considered. Ideally, biomarkers must be highly sensitive and specific for cancer diagnosis. However, there is no marker in clinical practice that possesses both 100% sensitivity and specificity. The use of biomarkers have been proposed in addition to imaging techniques, which would have a greater benefit-to-risk ratio compared to markers or imaging alone (80).

Biomarker research should be optimised by developing a common workflow. Identifying the optimal cut-off point of biomarkers is required for their application in the clinical setting. Most biomarkers in this review utilised retrospective designs and samples from tissue banks. Ideal biomarker studies should have a prospective design such as randomised controlled trials, with a large sample size ensuring that the study is able to achieve adequate precision following the Standards for Reporting of Diagnostic Accuracy Studies (STARD) guidelines and examine populations with disease and compare them to age- and sex-matched controls (81). This would reduce false positives associated with CT results and thus overtreatment and side effects from unnecessary interventions (82). Although a change in biomarker expression may not reflect true clinical benefit, it has been associated with pathway modulation (83). Biomarker translation into clinical practice is a challenging mission and even with approved markers such as CEA for colon cancer diagnosis, it's sensitivity is not ideal as it is expressed in other cancers and non-malignant conditions (84).

Sensitivities and specificities are dependent on the biomarker selected and the LC types studied. Biomarker assays require both robustness and reproducibility to be applied for clinical use (85). Studies with validation cohorts are more robust than studies with only a testing group (86). For example, Xing *et al.* (56) showed that the variations in results were due to a difference in the number of participants and controls with a range of non-malignant conditions being used to determine the specificity of the biomarkers. Goebel *et al.* (87) examined 21 candidate biomarkers including antigens and cytokines using a multiplex immunoassay but many were excluded even with >80% sensitivity and >95% specificity as the assay lacked reproducibility and was difficult to perform using such a large number of biomarkers. Developing an optimal multiplex test is required to validate the findings of this study and to examine its functionality and clinical use (87).

This systematic review has several limitations. We only included articles in English and some quantitative studies could not be included as they did not adequately report the diagnostic performance of the biomarkers investigated. There was also considerable variability across studies in terms of timing, participants and control groups, sampling, and biomarker detection methods. Included studies assessed a combination of biomarkers, which commonly were not validated in multicentre studies hence we were unable to make firm conclusions on their diagnostic accuracy, nor conduct a meta-analysis for each biomarker. Future studies should report their findings adequately, following the STARD guidelines for the construction of 2-by-2 tables for diagnostic meta-analysis, and minimally also including the 95% CI of diagnostic effect measures (81). Future research of NSCLC biomarker diagnosis should emphasise the validation of biomarkers so that they can be translated into clinical use and impact patient treatment and care.

Highlights

- Identification of 98 articles that found biomarkers for early lung cancer.
- Pooled area under curve of 0.86 indicated an excellent diagnostic performance.
- Four types of biomarkers were identified—antigens, autoantibodies, RNAs and circulating DNA.
- Biomarkers with high sensitivities/specificities can improve early detection.

Supplementary material

Supplementary data are available at *Carcinogenesis* online.

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Author contributions

E.M., S.H. and B.G. designed the study. E.M., D.M. and S.Q.Y. performed the Systematic Literature Review, analysed the data and made the figures. E.M., S.Q.Y. and B.G. wrote the paper. S.Q.Y. and M.H. assessed the risk of bias. All authors read and approved the final manuscript.

Availability of data and materials

All data generated or analysed during this study are included in this published article. Any additional information/data is available on reasonable request to the corresponding author.

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