New biochemical, immune and molecular markers in lung cancer: Diagnostic and prognostic opportunities

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Abstract

Lung cancer is one of the most common neoplasms and the leading cause of cancer-related deaths worldwide. Despite recent progress in understanding the pathomechanisms of lung cancer, it is frequently associated with late diagnosis, high incidence of metastases and poor response to treatment. Thus, there is extensive research in the field of biomarkers that aims to optimize management of lung cancer. The aim of this study was to review the current perspectives of a wide spectrum of circulating molecules that seem promising as new potential biomarkers of lung cancer. Among these, biochemical (active proteins), immunological (immunocompetent cells, cytokines, chemokines, and antibodies) and genetic (circulating tumor DNA, cell-free DNA and microRNA) markers are presented and discussed. The use of these markers would support the early detection of lung cancer and might be used for predicting disease progression, response of the disease to targeted therapies, monitoring the course of treatment, and developing individualized diagnostic and therapeutic strategies. Special attention was given to potential markers of nervous system involvement in the course of lung cancer, due to its prevalence and devastating impact. Limitations of the potential biomarkers are also outlined and future directions of investigations in this field highlighted, with the aim of improving the accuracy and practical utility of these biomarkers.

Key words: lung cancer, biomarkers, molecular, biochemical, immune

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Introduction

According to the GLOBOCAN estimates,[1] lung cancer was the 2nd most common type of cancer in terms of incidence in 2020, accounting for 11.4% of all newly diagnosed cancer cases. It was also by far the leading cause of death due to malignancies, accounting for 18% of all cancer mortality, which is almost double that of the 2nd most common cause, colorectal cancer. Moreover, it is estimated that lung cancer will remain at the top of both of these categories by 2040, with an expected growth of 58.8% in the number of cases and 63.8% in mortality.[2] From a histological point of view, lung cancer is typically divided into subtypes: small cell lung cancer (SCLC), adenocarcinoma, squamous cell carcinoma (SCC), and large cell carcinoma, the latter 3 usually being jointly referred to as non-small cell lung cancer (NSCLC).[3] The main symptoms of lung cancer, that can occur separately or in combination, are cough, dyspnea, pain, hemoptysis, aphonia or hoarseness, weight loss or asthenia, and superior vena cava syndrome, although an asymptomatic course at the time of diagnosis is not unusual.[4] Moreover, distant metastases are frequent, especially to the central nervous system. In the early stages of NSCLC, brain metastases are present in 0.6–3% of patients[5] and this increases up to 50% in the course of the disease.[6] In SCLC, brain metastases occur in about 10% of patients at the time of diagnosis and in additional 40–50% at later stages.[7]

Another type of nervous system involvement, resulting from immune-mediated responses to the presence of lung cancer antigens, are paraneoplastic neurological syndromes (PNS). Lung cancer, predominantly SCLC, is considered to be the most common malignancy associated with PNS.[8,9] From a clinical perspective, PNS can involve both the central and peripheral nervous systems, with the most commonly reported syndromes being peripheral neuropathy, followed by limbic encephalitis, subacute cerebellar degeneration, Lambert–Eaton syndrome, myopathy, encephalomyelitis,[8–10] and neuromyelitis optica.[11,12] Paraneoplastic neurological syndromes may develop a few years before the detection of cancer,[13,14] which highlights the potential of using such syndromes for early diagnosis.[15,16]

Treatment options for lung cancer can be applied alone or in combination and include surgery (for early stage disease), chemotherapy, radiotherapy, targeted therapy, and immunotherapy.[17–21] Despite recent advances in the diagnosis and treatment of lung cancer, the prognosis is still unfavorable. According to the tumor-node-metastasis (TNM)-based staging of lung cancer, the 5-year survival rate for NSCLC varies between 50% in clinical stage IA and 2% in clinical stage IV.[22] Small cell lung cancer is associated with even worse outcomes, such as a 5-year survival of 10% in the early stage of disease, with only 4.6% of patients diagnosed in the extensive stage surviving 2 years.[23] As a consequence of high mortality rates and frequency of metastases present at diagnosis, much of the recent research has focused on early diagnosis and identification of potential markers of disease progression, local infiltration and metastatic activity, as well as treatment response. The early diagnosis of lung cancer is based mainly on computed tomography imaging, confirmed using cytological and histopathological examination of specimens obtained during bronchoscopy or other invasive procedures.[24] However, the diagnostic process and prognosis may be complemented by additional biomarkers.

By definition, biomarkers are molecules or abnormal parameters that distinguish an individual with a particular disease from the studied population. Biomarkers can be detected in bodily fluids such as blood, serum, urine, sputum, pleural effusion, or cerebrospinal fluid.[25,26] Recently, biochemical, immune and molecular biomarkers have been recognized as the most promising and clinically relevant with regard to lung cancer, and they are being extensively investigated to evaluate their sensitivity and specificity.[27] An early detection of the dissemination of neoplastic processes and the establishment of risk factors for its occurrence are particularly important in terms of prognosis and therapeutic possibilities. Given the significant impact of nervous system involvement on disease burden, morbidity and mortality, the identification of its presence and selection of patients at increased risk of this complication are of great importance.

Objectives

The aim of this study was to review the current data on the role of new biochemical, immune and molecular markers in the diagnosis of lung cancer, and to evaluate its progression, with a focus on the involvement of nervous system in the course of disease. Ongoing research and its future directions in this field have been reviewed in view of potential implications for early detection of cancer, tailoring treatment plans based on prognosis, and monitoring the course of disease.

Materials and methods

A literature search was performed using the PubMed and Embase databases, covering the period from the beginning of 2010 until February 28, 2022, with a combination of the search terms: "lung cancer", "NSCLC", "SCLC", "biomarker", "biochemical", and "molecular". After excluding papers written in a language other than English, conference abstracts and duplicates from further screening, a total of 2745 original studies and review articles were retrieved. Full texts of eligible papers were analyzed for their relevance to the topic, as well as several further potentially relevant papers that were identified in reference lists from the texts. Initially, the literature search was conducted by the lead author, with the results reviewed

and verified by the other authors. This led to the identification and inclusion of 217 published studies that were considered the most relevant to the topic. The preparation of the study was conducted by following the Enhancing Transparency in Reporting the Synthesis of Qualitative Research (ENTREQ) checklist,[28] selected according to the Enhancing the QUAlity and Transparency of Health Research (EQUATOR) Network guidelines (https://www.equator-network.org).

Biochemical markers of lung cancer

Several biochemical biomarkers have already been implemented into lung cancer diagnostics and management, including carcinoembryonic antigen (CEA), cytokeratin 19 fragment marker (CYFRA 21-1), neuron-specific enolase (NSE), and cancer antigen 125 (CA-125).[29,30] However, the sensitivity and specificity of these markers are disputable, as their levels can be elevated in other diseases. As such, new candidate biomarkers have been proposed that are thought to have better accessibility and clinical utility, such as soluble intercellular cell adhesion molecule-1 (sICAM-1), which plays an important role in adhesion between host cells and cancer cells in the promotion of tumor growth. The overexpression of sICAM-1 was reported in lung cancer patients with lymph node and distant metastases, and was linked to shorter overall survival (OS) and progression-free survival (PFS).[31] Similarly, high levels of angiopoietin-2, an important factor involved in angiogenesis, were associated with lymph node metastases and a poorer prognosis.[32] Transforming growth factor beta (TGF-β),[33] glucose transporter 1 (GLUT1), which enhances the supply of glucose to tumor cells,[34] and urinary GM2 activator protein (GM2AP), a molecule involved in the induction of cancer invasion,[35] are other recently proposed predictors of poor outcome. Podoplanin, a potential inhibitor of tumor cell growth and self-renewal, was identified as a marker of lower malignancy in SCC and better prognosis in patients with this type of lung cancer.[36] In another meta-analysis, high serum levels of amyloid A, a protein correlated with an acute inflammatory response, were suggested as a discriminative marker, especially for the detection of SCC.[37] Among other potential biomarkers, tumor necrosis factor receptor-associated protein 1 (TRAP1) was overexpressed in patients with higher pathological TNM stage and lymph node metastases, and was correlated with a shorter disease-free survival.[38]

Immune markers of lung cancer

The development of lung cancer is associated with a changing profile of immune system activity, with a shift from type 1 T helper cell-derived signaling to type 2 T helper cell pathways. Furthermore, dendritic cell, natural killer (NK) cell and T helper cell activity has been shown to decrease, whilst regulatory T cell (Treg) activity has been seen to increase. Additionally, programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which are markers of checkpoint inhibition, have also been shown to increase. Meanwhile, tumor-specific antigens and tumor-associated antigens (TAAs) are expressed by neoplastic cells and evoke an immune response, such as the induction of antibody production. Therefore, these immune-mediated processes might be used as markers for detection and monitoring of lung cancer, or for predicting its activity.[39,40]

A range of immunocompetent cells have been identified within lung tumors, with their type and distribution in the nest or stroma of the tumor found to have prognostic significance. The prevalence of Tregs, M2 macrophages and immature dendritic cells was associated with poor survival, while the presence of $CD8^+$ T cells, $CD4^+$ T cells, M1 macrophages, and NK cells was linked to better outcomes.[41] Some of these findings were specific for particular types of lung cancer, with high intratumoral neutrophil density being correlated with poor prognosis in patients with adenocarcinoma, contrary to the patients with SCC.[42] A similar analysis of bronchoalveolar lavage fluid demonstrated material obtained from the affected lung that contained an increased number of neutrophils and a predominance of $CD8^+$ T cells, Tregs and M2 macrophages.[43]

Measures of the systemic inflammatory response from peripheral blood, including monocyte count and neutrophil-to-lymphocyte ratio (NLR), might also serve as predictors of tumor development, especially its propensity to metastasize.[44] Indeed, a meta-analysis of 14 studies revealed that a high NLR was associated with shorter OS in NSCLC and SCLC patients.[45] Additionally, flow cytometry studies have demonstrated that $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ ratio, and NK cells were all decreased, and inversely correlated with the progression of clinical stage in NSCLC, while Tregs increased parallel to cancer progression.[46]

Emphasis has been put on measuring serum cytokine and chemokine levels, which reflect the inflammatory processes related to the development of the cancer, in both NSCLC[47] and SCLC.[48] Higher serum levels of interleukin (IL)-6 and IL-8 predicted a risk of lung cancer up to several years before the diagnosis,[49] whilst the expressions of IL-8 with IL-6 and IL-6 with IL-17 were shown to be negative prognostic factors for early-stage lung cancer.[50] In addition, elevated levels of IL-17 in the serum of SCLC patients correlated with a propensity to metastasize and a shorter OS.[51] Angiogenesis inhibitors IL-20, and IL-22, which promotes tumor growth, were also found to be prognostic factors of lung cancer outcomes. High serum levels of IL-20 adversely correlated with time to cancer progression, and lower levels of IL-22 in the bronchoalveolar lavage of NSCLC patients were associated with worse rates of survival.[52] Studies on the prognostic value

of chemokines have demonstrated that high levels of C-C motif chemokine ligand 2 (CCL2), CCL19 and C-X-C motif chemokine ligand 16 (CXCL16), and low levels of CCL5 were linked with better survival. In contrast, high levels of CXCL8 and C-X-C motif chemokine receptor 4 (CXCR4) were associated with worse survival rates.[53]

Autoantibodies to TAAs may be detectable in the asymptomatic stage of cancer and persist in high levels in serum, which indicates their potential use as biomarkers for early detection of lung cancer.[54] The Early CDT®-Lung is a panel test for the presence of 7 autoantibodies against TAAs (p53, NY-ESO-1, CAGE, GBU4–5, SOX2, HuD, and MAGE A4) that is currently used in patients with a high risk of lung cancer. The test was validated in large cohorts of NSCLC and SCLC patients and demonstrated high overall specificity, but a rather low sensitivity in SCLC, which was even lower in NSCLC.[40] The presence of relevant autoantibodies may also have a prognostic value in lung cancer, with 1 study reporting on patients with NSCLC who were positive for antineural and antinuclear antibodies, and showed better rates of survival.[55] Another study reported a panel of 4 antibodies (MAGEA1, PGP9.5, SOX2, and TP53) that were overexpressed in NSCLC and correlated negatively with OS.[56] In another report, levels of an antibody against human DNA-topoisomerase I were significantly higher in the NSCLC group than in the controls, though the prognosis was worse in the antibody-negative group.57

Studies on the tumor-related immune environment have identified antibodies against PD-1 and its ligand (PD-L1) as a potential therapeutic option in lung cancer. Thus, the value of PD-L1 was investigated as a predictive biomarker for the efectiveness of therapy with anti-PD1/PD-L1 agents. Higher levels of PD-L1 expression were shown to be associated with a more effective treatment and longer survival, although results have not been consistent across studies.[58] At the same time, a high expression of CTLA-4 predicted worse survival in NSCLC but was not validated as a predictive marker of the response to the anti-CTLA-4 treatment.[41] Other markers have been sought, with Zhou et al. constructing a panel of 5 tumor-associated autoantibodies (p53, BRCA2, HuD, TRIM21, and NY-ESO-1) designed to predict the response to immune checkpoint inhibitors.[59] Panel positivity was found to be indicative of a better response and longer PFS. In the previous work, the same association was established for NY-ESO-1 and XAGE1 serum antibodies,[60] anti-nuclear antigens, extractable nuclear antigens, and anti-smooth cell antigens.[61] In another study, SIX2 autoantibodies were consistently upregulated in the non-responder group.[62] A panel comprising 13 antibodies showed high accuracy in predicting poor outcome in pre-operative samples of NSCLC patients (stage I–IIIa).[63] Concordant prognostic utility was confirmed for an antibody against cyclin Y.[64] Moreover, autoantibody status was suggested to be helpful not only in predicting clinical outcome, but also in assessing the risk of immune-related adverse events during treatment.[65]

Different types of biomarkers can be combined to further improve their diagnostic and predictive value, with 1 report establishing a panel of markers that could identify patients at risk of lymph node metastases.[66] The panel included tumor necrosis factor alpha (TNF-α), tumor necrosis factor-receptor I (TNFR1) and macrophage inflammatory protein-1α (MIP-1α), along with 3 autoantibodies that target ubiquilin-1, hydroxysteroid-(17-β)-dehydrogenase and triosephosphate isomerase. Validation of the panel using a classification algorithm revealed a sensitivity of 94% and specificity of 97%. A meta-analysis on advanced lung cancer inflammation index, which is a prognostic score that considers body mass index (BMI), serum albumin and NLR, revealed a significant correlation between the score and OS and PFS.[67] The prediction of survival in SCC patients was proposed on the basis of 4 immunological markers, including monocyte ratio, NLR, PD-L1 immunostaining score, and PD-1-positive stained tumor-infiltrating lymphocyte counts.[44] A large study was performed comprising patients with NSCLC, treated with PD-1/PD-L1 inhibitors, in order to establish the potential efficacy of 2 combined biomarkers, defined as Lung Immune Prognostic Index (LIPI). This index measured derived neutrophil/(leukocyte minus neutrophil) ratio and lactate dehydrogenase levels in order to predict the resistance to immune checkpoint inhibitors.[68] Both of these factors were independently associated with worse OS and PFS in patients treated with immune checkpoint inhibitors, while no such correlation was observed in a group treated with chemotherapy only. Neutrophil-to-lymphocyte ratio was shown to correlate not only with shorter OS but also with the presence of Kirsten rat sarcoma viral oncogene homologue (*KRAS*) and epidermal growth factor receptor (*EGFR*) mutations.[69] Lung Immune Prognostic Index[70] and NLR[71] have also been investigated for their prognostic value in patients with SCLC. The aforementioned potential biochemical and immune biomarkers of lung cancer are summarized in Table 1.

Circulating tumor DNA and circulating tumor cells as lung cancer markers

Circulating tumor DNA

Circulating tumor DNA (ctDNA) enters the bloodstream predominantly as a result of necrosis and apoptosis of tumor cells, although there is also evidence that it can be actively released by viable cells and several other processes.[72] It usually constitutes a small fraction (0.1–1%) of cell-free DNA (cfDNA) in plasma[73]; however, its level reflects tumor activity and expansion and can be much higher in patients with a more advanced disease.[74] There is increasing interest in using ctDNA in the diagnosis of various types

Table 1. Biochemical and immune markers of lung cancer

Biomarker	Study	Type of study	Study group	Outcomes
sICAM-1 (serum)	Wu et al.[31]	meta-analysis	23 studies – out of them, 7 investigated prognostic value (915 LC patients)	LC detection, more advanced stage, lymph node metastases, distant metastases, shorter PFS and OS
Angiopoietin-2 (serum)	Xu et al.[32]	meta-analysis	20 studies – out of them, 7 investigated prognostic value (575 LC patients)	more advanced stage, lymph node metastases, shorter OS
Transforming growth factor beta (tissue and plasma)	Li et al.[33]	meta-analysis	8 studies – 579 LC patients	poor prognosis
Glucose transporter 1 (tissue)	Zhang et al.[34]	meta-analysis	26 studies – out of them, 10 investigated prognostic value (1731 LC patients)	differential diagnosis of SCC, more advanced stage, lymph node metastases, shorter OS, disease-specific and DFS
GM2 activator protein (serum, urine and lung tissue)	Potprommanee et al.[35]	observational	Serum and urine from 133 LC patients and 143 NSCLC lung tissue samples	LC detection, shorter OS and DFS
Podoplanin (tissue)	Hu et al.[36]	meta-analysis	8 studies – 725 SCC patients	better differentiation of SCC, longer OS and PFS
SAA (serum)	Biaoxue et al.[37]	meta-analysis	9 studies – 1392, including 960 LC patients	LC detection, especially SCC
TRAP1 (tissue)	Kuchitsu et al.[38]	observational	64 adenocarcinoma patients	more advanced stage, lymph node metastases, shorter DFS, worse response to platinum-based chemotherapy
Intratumoral $CD66b^+$ TANs density	Rakaee et al.[42]	observational	536 NSCLC patients (289 SCC, 201 adenocarcinoma, 46 large cell carninoma)	longer disease-specific survival in SCC, shorter disease-specific survival in adenocarcinoma
NLR, monocyte ratio, PD-L1 immunostaining score and, PD-1-positive stained tumor-infiltrating lymphocyte counts	Jiang et al.[44]	observational	156 SCC patients (104 in training, and 52 in validation group)	shorter OS
NLR	Yin et al.[45]	meta-analysis	2734 LC patients (2433 NSCLC, 301 SCLC)	shorter OS
$CD3^+$, $CD4^+$, $CD4^+/CD8^+$, and NK cells – downregulation Treg – upregulation (serum)	Wang et al.[46]	observational	153 NSCLC patients	NSCLC detection
IL-2, IL-4, IL-6, IL-8, TNF-α, MIP-1α (serum)	Hardy-Werbin et al.[48]	observational	84 SCLC patients	low IL-4, MIP-1α – more advanced stage IL-8 – shorter OS IL-2 – sensitivity to ipilimumab IL-6, TNF-α – resistance to ipilimumab
IL-6, IL-8 (serum)	Pine et al.[49]	observational	270 LC patients in the study group and 532 in the validation group	increased risk of LC development
IL-6, IL-8 and combined IL-6/IL-8 classifier (serum)	Ryan et al.[50]	observational	548 LC patients	shorter OS
IL-17, VEGF (serum)	Lin et al.[51]	observational	76 SCLC patients	both – LC detection, number of metastases IL-17 – more advanced stage, shorter OS
HGF, IL-20, IL-22 (serum and BALF)	Naumnik et al.[52]	observational	46 NSCLC patients (10 adenocarcinoma, 25 SCC, 11 large cell carcinoma)	HGF, IL-22 – NSCLC detection serum HGF, BALF IL-22 – shorter OS serum IL-20 – shorter PFS
Antineural and antinuclear antibodies (serum)	Blaes et al.[55]	observational	61 NSCLC patients (29 adenocarcinoma, 32 SCC)	longer OS
Panel of 4 antibodies: MAGEA1, PGP9.5, SOX2, and TP53 (serum)	Chen et al.[56]	observational	401 participants in training set, including 177 NSCLC patients and a validation set of 57 NSCLC patients	NSCLC detection, shorter OS
Human DNA-topoisomerase I antibody (serum)	Wu et al.[57]	observational	127 NSCLC patients (70 adenocarcinoma, 57 SCC)	NSCLC detection, longer OS
Panel of 5 autoantibodies: p53, BRCA2, HUD, TRIM21, and NY-ESO-1 (plasma)	Zhou et al.[59]	observational	166 NSCLC patients (37 in discovery cohort and 129 in validation cohort)	better response to immune checkpoint inhibitors: higher ORR, longer PFS

Table 1. Biochemical and immune markers of lung cancer – cont.

Biomarker	Study	Type of study	Study group	Outcomes
NY-ESO-1 and XAGE1 antibodies (serum)	Ohue et al.[60]	observational	88 NSCLC patients (13 in discovery and 75 in validation cohort)	good response to anti-PD-1 therapy: higher ORR, longer OS and PFS
ANA, ENA and ASMA antibodies	Giannicola et al.[61]	observational	92 NSCLC patients (55 adenocarcinoma, 31 SCC, 6 undefined)	good response to anti-PD-1 therapy: longer OS and PFS
SIX2 autoantibody (plasma)	Tan et al.[62]	observational	50 NSCLC patients (17 in discovery cohorts 1 and 2, 16 in verification and 17 in validation cohort)	worse response to anti-PD-1 therapy: higher plasma level in non-responders
Panel of 13 antibodies (serum)	Patel et al.[63]	observational	157 NSCLC patients (83 adenocarcinoma, 74 SCC; 111 in training and 46 in validation cohort)	shorter OS
Anti-CCNY antibody (serum)	Ma et al.[64]	observational	264 NSCLC patients (134 adenocarcinoma, 130 SCC)	shorter OS in postoperative patients
Rheumatoid factor, antinuclear, antithyroglobulin and antithyroid peroxidase antibodies (serum)	Toi et al.[65]	observational	137 NSCLC patients (86 non-squamous NSCLC, 51 SCC)	higher rate of immune-related adverse events, higher ORR, longer PFS
Biomarker panel: TNF-α, TNF-RI, MIP-1α, and autoantibodies against Ubiquilin-1, hydroxysteroid-(17-β)-dehydrogenase and triosephosphate isomerase (serum)	Patel et al.[66]	observational	127 NSCLC patients (81 adenocarcinoma, 32 SCC, 14 undefined; 20 in training and 107 in validation cohort)	lymph node metastases
Low ALI (serum)	Zhang and Chen[67]	meta-analysis	8 studies – 1587 LC patients	shorter OS and PFS
LIPI	Mezquita et al.[68]	observational	466 NSCLC patients treated with ICIs (270 adenocarcinoma, 159 SCC (remaining 37 patients were classified as 'NSCLC- other' in the original study); 161 in test and 305 in validation cohort)	worse response to immune checkpoint inhibitors: shorter OS and PFS
NLR	Seitlinger et al.[69]	observational	2027 NSCLC patients	shorter OS, detection of EGFR/*KRAS* mutations
LIPI	Sonehara et al.[70]	observational	171 SCLC patients	shorter OS and PFS
NLR	Lu et al.[71]	meta-analysis	20 studies – 5141 SCLC patients	more advanced stage, shorter OS and PFS
S100B and S100B autoantibody (serum)	Choi et al.[194]	observational	128 LC patients (61 adenocarcinoma, 40 SCC, 13 SCLC and 14 other), 150 NSCLC patients	detection of brain metastases
	Chen et al.[195]	observational	150 NSCLC patients	detection of brain metastases, shorter OS and PFS
ProApolipoprotein A1 (serum)	Marchi et al.[196]	observational	103 LC patients	detection of brain metastases
high NLR, platelet-to-lymphocyte radio and C-reactive protein	Sert et al.[197]	observational	208 NSCLC patients (41 adenocarcinoma, 124 SCC, 43 undefined)	development of brain metastases
NLR	Koh et al.[198]	observational	260 NSCLC patients (194 adenocarcinoma, 66 other)	detection and development of brain metastases
Lower mean platelet volume	Li et al.[199]	observational	476 NSCLC patients (113 adenocarcinoma, 119 other)	detection of brain metastases
Fibrinogen, platelet count	Zhu et al.[200]	observational	275 NSCLC patients	shorter OS and poor prognosis in patients with brain metastases
Purkinje cell cytoplasmic antibody type 2 (serum)	Gadoth et al.[212]	observational	96 patients (including lung cancer)	PNS detection
Anti-Hu (serum)	Graus et al.[213]	observational	196 SCLC patients	higher response rate, longer OS
	Gozzard et al.[214]	observational	238 SCLC patients	longer OS
	Monstad et al.[215]	observational	200 SCLC patients	not associated with survival

SAA – serum amyloid A; TRAP1 – tumor necrosis factor receptor-associated protein 1; NLR – neutrophil-to-lymphocyte ratio; PD – programmed cell death protein; NK – natural killer; TNF-α – tumor necrosis factor alpha; IL – interleukin; VEGF – vascular endothelial growth factor; BALF – bronchoalveolar lavage fluid; TNF-R1 – tumor necrosis factor receptor 1; MIP-1α – macrophage inflammatory protein-1α; LIPI – Lung Immune Prognostic Index; LC – lung cancer; NSCLC – non-small cell lung cancer; SCC – squamous cell carcinoma; ICIs – immune checkpoint inhibitors; PFS – progression-free survival; OS – overall survival; ORR – objective response rate; EGFR – epidermal growth factor receptor; KRAS – Kirsten rat sarcoma viral oncogene; DFS – disease-free survival; TAN – tumor-associated neutrophil; HGF – hepatocyte growth factor; ANA – anti-nuclear antigen, ENA – extractable nuclear antygen; ASMA – anti-smooth cell antygen; ALI – advanced lung cancer inflammation index.

of neoplasms, including lung cancer, and for monitoring the course of disease.[75] The method of obtaining ctDNA from plasma, known as liquid biopsy,[76] is considered a promising alternative to standard tissue biopsy. This noninvasive and safe technique may be easily implemented in all patients, even those for whom a traditional biopsy is not possible, and it enables avoiding complications such as pneumothorax, hemorrhage and air leaks.[77,78]

Rapid advances in molecular techniques that used to detect cancer-specific mutations in cfDNA, such as polymerase chain reaction (PCR) or next-generation sequencing, have offered new perspectives of on implementing liquid biopsies into clinical practice.[79] In a large analysis of data from over 8000 lung cancer patients, ctDNA profiling revealed somatic alterations in 86%, and identified driver oncogene mutations in 48.4% of them.[80] Furthermore, ctDNA profiling has been used to distinguish between benign and malignant lung tumors, and to detect lung cancer at an early stage. Indeed, the assay based on deep sequencing detected 63% of stage I and 83% of stage II lung cancers, respectively.[81]

In a study by Liang et al., the analysis of DNA methylation patterns was performed using tissue samples from patients with lung nodules in order to distinguish between malignant and benign tumors.[82] A predictive model based on 9 methylation markers for ctDNA was then applied to plasma samples, with a sensitivity of 79.5% and specificity of 85.2% for detecting lung cancer. Regarding its subtypes, the sensitivity was 73.9% for adenocarcinoma and 100% for SCC. This difference may be explained by higher intensity of necrotic processes observed in SCC tissue, which results in a greater release of ctDNA into the bloodstream, therefore being eligible for analysis. Existing data support the utility of ctDNA methylation analysis in detecting early-stage lung cancer,[83] and a subsequent study on a large group of lung cancer patients is being conducted to develop a ctDNA methylation classifier for incidental lung nodules.[84] Longitudinal methylation profiling along with somatic mutation analysis in patients with NSCLC have also shown prognostic potential in assessing the risk of recurrence.[85]

With regard to its prognostic value, the level of ctDNA was found indicative of lymph node involvement in resectable NSCLC.[86] Other investigators collected tissue and plasma samples from NSCLC patients before and after surgery in order to identify driver mutations in genes, including *EGFR*, *KRAS*, *TP53*, *BRAF*, *PIK3CA*, and *ERBB2*.[87] Out of 46.3% of plasma samples which were positive for ctDNA before tumor resection, a significant decrease in mutation frequency was noticed, from 8.88% before surgery to 0.28% after the procedure. Furthermore, ctDNA was more prominent in stage Ia and Ib cancers than in more advanced stages. In a follow-up study of surgically treated lung cancer patients, targeted mutations were present in 93% of patients before surgery and in 54% at some point after surgical resection. Interestingly, all of the patients with ctDNA still detectable after surgery experienced progression of the disease, while those without ctDNA remained disease-free.[88]

Use of ctDNA in detecting minimal residual disease was demonstrated in a study where multiplex-PCR assay panels were used to screen for ctDNA in plasma samples of early-NSCLC patients, pre- and postoperatively.[89] A sample was considered ctDNA-positive if at least 2 pre-established single nucleotide variants were detected. Circulating tumor DNA was found in 48% of pre-operative samples, and the detection rate was substantially higher for SCC (97%) than for adenocarcinoma (19%). Again, this discrepancy may be due to less extensive necrotic processes in the latter. Moreover, significant correlations were observed between the results of postoperative ctDNA profiling and the occurrence of clinical relapse or resistance to chemotherapy.[90–94] The use of ctDNA profiling has also been researched in SCLC, although to a lesser extent. In a Chinese study, SCLC patients with higher ctDNA levels had significantly shorter PFS and OS.[95] This relationship between ctDNA detection and poor prognosis has been also been observed in other research.[96] The potential role of ctDNA in SCLC detection and progression monitoring was further strengthened by a large ctDNA analysis in over 10,000 cancer patients. In this group, the highest detectability of ctDNA in all cancer types was in SCLC, reaching 91.1%.[97]

The role of ctDNA profiling is also gaining attention in tailoring and monitoring of lung cancer treatment, and several liquid biopsy tests have been developed for this purpose.[98–100] This method can be used before applying adjuvant chemotherapy, which is considered an option in NSCLC, to identify eligible patients.[101] Based on recent understanding of the mechanisms of resistance to tyrosine kinase inhibitors (TKIs),[102] ctDNA analysis may be a promising tool in this area. Circulating tumor DNA analysis has also been used to investigate resistance mechanisms in patients with NSCLC treated with rociletinib, a 3rd generation EGFR inhibitor.[103] Multiple resistance mechanisms to the drug were present in 46% of patients, while at least 1 such mechanism was found in 65% of them, with *MET* copy number gain being the most common, as it was found in 26% of the patients. In another experiment, researchers were able to identify driver and resistance mutations through next generation sequencing of ctDNA, even when tissue sequencing was not successful.[104] Furthermore, there is also some evidence for the detection of *T790M* mutation in ctDNA profiling in patients with *T970M*-negative tissue.[105] These observations support the potential of ctDNA not only as a supplementary method, but also as an independent screening tool that could be applied in the planning of individualized treatment strategies.

Detectable *EGFR* mutations in cfDNA were associated with a longer PFS in response to treatment with erlotinib, a TKI, while its persistence in a follow-up plasma analysis resulted in shorter PFS and OS.[106] In another study comprising patients treated with erlotinib, *EGFR T790M* mutations linked to TKI treatment resistance were detectable

in cfDNA even before disease progression.[107] Several other studies have also underlined the potential role of ctDNA profiling in the detection of resistance mutations as a part of disease monitoring.[108–113] Changes in ctDNA profile demonstrated a good predictive value in a study by Nabet et al., where plasma samples were analyzed in patients with advanced lung cancer treated with immune checkpoint inhibitors.[114] A significant (at least 50%) drop in detectable ctDNA levels at 4 weeks after the initial treatment was considered a molecular response and helped identify patients with durable clinical benefit, defined as PFS of at least 6 months. Similar results were found by other authors, underlining the association between ctDNA decrease and better PFS and OS.[115–118] Accordingly, baseline and post-treatment ctDNA indicated worse clinical outcomes.[119–122] Circulating tumor DNA has also been investigated in the evaluation of tumor mutation burden (TMB), a novel predictive marker reflecting the total number of existing mutations, which is thought to be predictive of the response to PD-1 and PD-L1 inhibitors. It was hypothesized that patients with a higher burden of somatic mutations would benefit from immune checkpoint inhibitors due to a better recognition of neoantigens. This beneficial effect was confirmed with tumor tissue analyses of NSCLC patients treated with pembrolizumab,[123] nivolumab or ipilimumab.[124] The evaluation of blood-based TMB, assessed with ctDNA genetic profiling, revealed complementary findings. Non-small cell lung cancer patients with high blood-based TMB treated with atezolizumab showed a better response to the therapy in 1 study,[125] while in another report,[126] higher TMB was found to correlate with shorter PFS and OS in NSCLC. These diverse results point to potential limitations of ctDNA analysis, such as a small possible range of mutations that can be detected using liquid biopsy. Future improvements to the method should include establishing validated sequencing panels and cut points.[127]

As a potential marker of lung cancer diagnosis and progression, ctDNA was also compared to previously known biomarkers and showed a higher detection rate and positive predictive value than CYFRA21-1, CEA, NSE, SCC, CA-125, and CA19-9.[87] Concordant results were obtained in a similar study, where plasma samples were taken before, during and after surgery.[128] In this study, the sensitivity of ctDNA detection was higher than for protein tumor markers (63.2% compared to 49.3%), and a significant drop in the average ctDNA mutation frequency after surgery was also reported.

Circulating tumor cells

Apart from ctDNA, the so-called "liquid biopsy" techniques may also reveal circulating tumor cells (CTCs) that originate from primary or metastatic tumors.[129] As CTC numbers in plasma are very low, they may be detected by means of various methods, including immunomagnetic separation with EpCAM- or CD45-based assays, PCR or telomerase-based assays, as well as cellular isolation with size-dependent filters.[130] A recently published meta-analysis, including 21 studies with almost 4000 participants, demonstrated high pooled sensitivity and specificity of CTCs in lung cancer detection.[131] There is also some evidence of CTC role as a potential marker of lung cancer progression and dissemination, as a higher abundance of detectable CTCs before the commencement of treatment resulted in shorter OS and PFS in NSCLC patients.[132] In another meta-analysis,[133] the presence of CTCs was shown to be associated with response to chemotherapy and prognosis. Patients who were CTC-positive at baseline or who converted to CTC-positive during treatment, presented with lower rates of disease control, as well as worse OS and PFS. Irrespective of their correlation with survival rates, CTCs were also associated with lymph node metastasis.[134]

The analysis of CTC number at baseline and at different time points in the course of SCLC was referred to for the prediction and monitoring of the response to chemotherapy.[135] Circulating tumor cells obtained from plasma samples may be also used for the detection of specific mutations related to lung cancer, such as EGFR[136] and KRAS,[137] with a higher sensitivity than ctDNA. Moreover, specific gene rearrangements can be detected in CTCs with promising acurracy. In patients with lung adenocarcinoma, anaplastic large-cell lymphoma (*ALK*) gene rearrangement and ALK protein expression in CTCs were concordant with findings from tumor tissue,[138] which has been confirmed by other researchers.[139,140] A rearrangement of repressor of silencing 1 (*ROS1*) is another example of chromosomal aberrations detectable in CTCs, with biopsy-confirmed gene fusion in NSCLC patients.[141] Dynamic changes in the number of CTCs with aberrant ALK-fluorescence in situ hybridization patterns, such as *ALK* copy number gain, might serve as predictive markers of the response to treatment, as these aberrations are considered to be one of the mechanisms underlying acquired resistance to crizotinib (an ALK and ROS1 inhibitor). A decrease in CTCs with *ALK* copy number gain during treatment with crizotinib was linked to a longer PFS.[142]

Apart from the lack of standardized methods of analysis, CTCs appear to have other limitations similar to ctDNA evaluation. These include low detection rate, especially in patients with an early stage of the disease, and an unclear influence of tumor heterogeneity and its localization on liquid biopsy findings. Table 2 and Table 3 summarize the results of studies concerning ctDNA and CTCs in lung cancer, respectively.

MicroRNA as a lung cancer marker

MicroRNAs (miRNAs) are noncoding small molecules, comprising approx. 21 nucleotides. They are considered post-transcriptional regulators of gene expression. They

achieve this by binding to the 3′-UTR of target messenger RNA, which results in repressing translation or promoting messenger RNA deadenylation and degradation.[143] Due to their biological role, miRNAs are thought to be important in cancer initiation and progression, as they can influence both oncogenes and tumor suppressor genes.[144] Furthermore, a potential role of miRNA in the diagnostics and treatment of lung cancer has been recently highlighted.[145] Considering the availability of miRNA expression in cancer tissue and bodily fluids, especially in serum, it can be easily measured using liquid biopsy.[145] A growing popularity of miRNA research has led to the development of diagnostic panels which may be used complementarily in the early detection of malignant lung lesions and are constantly being improved.[146,147]

A signature panel of 15 miRNAs was able to differentiate between patients with lung cancer and those with non-tumor lung disease, other systemic diseases, and healthy controls, with a sensitivity of 82.8% and a specificity of 93.5%.[148] Other authors used a panel of 2 miRNAs (miRs-31-5p and 210-3p) detected in sputum and 1 miRNA (miR-21-5p) from plasma, that reached sensitivity and specificity in the detection of lung cancer of 85.5% and 91.7%, respectively.[149] Even greater sensitivity and specificity (99% for both) was achieved by a combination of miR-1268b and miR-6075, and was validated in a group of over 3000 participants and maintained its performance regardless of TNM stage or histological type of tumor.[150] A number of specific miRNAs have also proven to be valuable in the early diagnosis of NSCLC,[151] distinguishing NSCLC from SCLC[152] and specific types of NSCLC.[153]

Numerous miRNAs are efficient in the prognosis of disease progression and resistance to treatment. The analysis of miRNA expression in advanced NSCLC cases revealed 17 miRNAs significantly associated with 2-year survival rate.[154] At the same time, the downregulation of miR-590-5p was linked to lower median survival rates in a cohort of NSCLC patients,[155] while the upregulation of miR-25 was higher in NSCLC patients compared to the control group, but also correlated negatively with OS and relapse-free survival.[156] In a separate analysis, patients with adenocarcinoma and SCC with high expression of miR-25-3p had shorter OS, regardless of tumor histology.[157] A meta-analysis on the prognostic value of the downregulation of miR-126 highlighted its relationship to unfavorable outcomes of NSCLC.[158] Others reported an association between miR-153,[159] miR-494,[160] miR-519d[161] and more advanced clinical stage, presence of lymph node metastases, and worse OS in NSCLC patients. Similar results regarding a poor prognosis in NSCLC patients were reported for the downregulation of miR-184,[162] miR-185,[163] miR-770,[164] and miR-30a-5p,[165], and the upregulation of miR-23b-3p, miR-10b-5p and miR-21-5p,[166] miR-31,[167] miR-378,[168] miR-942, and miR-601.[169] On the contrary, a high expression of miR-3195 resulted in longer OS,[170] while miR-21 and miR-4257 were established as predictors of NSCLC recurrence.[171] In patients with SCLC, the upregulation of miR-92b and miR-375 was related to chemotherapy resistance and shorter PFS.[172] At the same time, miR-422a and miR-135a showed a strong association with metastases to lymph nodes in lung cancer patients,[173,174] lower expression of miR-139-5p was found in NSCLC patients with bone metastases,[175] and miR-375-3p was also proposed to be a possible biomarker of SCLC metastatic activity.[176]

Expression profiles of miRNA may also serve as markers for treatment response,[170] with higher expression of miR-1249-3p observed in individuals who responded well to chemotherapy. Changes in serum levels of various miRNA panels have been used to predict worse sensitivity to chemotherapy.[177,178] Additionally, in a cohort of early-stage NSCLC patients, the expression of miR-216b was significantly increased after a successful tumor resection.[179] Profiling of miRNA may also be indicative of the response to radiotherapy[180,181] or immunotherapy, with patients who significantly overexpressed miR-320b-d before the treatment with PD-1/PD-L1 inhibitors not responding well to the therapy. In the same group, a decrease in miR-125b-5p was observed in those who presented with only a partial response.[182] In NSCLC patients, miR-504 expression differed significantly depending on *EGFR* mutation status.[183] An experimental miRNA panel was also tested for discrimination between *ALK*-positive and *ALK*-negative lung cancers,[184] which is relevant to immunotherapy treatment options. The miRNAs were also proposed as markers of resistance to EGFR-TKI therapy,[185] as shorter OS was reported in patients with high serum levels of miR-30b and miR-30c treated with erlotinib.[186] Furthermore, miR-30c expression patterns showed utility in predicting cardiotoxicity in patients treated with bevacizumab,[187] which indicates the potential of this method for stratifying the risk of adverse events for particular therapies. Further attempts to improve diagnostic and prognostic accuracy of miRNA in lung cancer patients include combining this method with other commonly used biomarkers, such as CEA and CYFRA21-1.[188,189]

Although miRNA profiling has gained much interest in recent years, its application in clinical practice still has some limitations. Methodological discrepancies within study design and technological details of tools applied can be seen throughout the studies on miRNA in lung cancer, which prevents consistent conclusions.[190] Another issue to be addressed is a lack of specificity of candidate miRNAs, as there is a large number of these being examined in various types of cancer, and these miRNAs are involved in the regulation of multiple biological pathways. Furthermore, miRNA expression can be affected by disease stage and the treatment used,[191,192] which has to be considered in the clinical interpretation of research findings. Therefore, there is a need for further studies on representative groups of patients with the use of consistent methodology,

Table 2. Circulating tumor DNA (ctDNA) in lung cancer

Biomarker	Study	Type of study	Study group	Outcomes
ctDNA profiling	Mack et al.[80]	observational	8388 NSCLC patients (4142 adenocarcinoma, 4246 not specified)	detection of driver and resistance mutations
ctDNA profiling	Peng et al.[81]	observational (clinical trial No. NCT03081741)	136 LC patients (100 adenocarcinoma, 28 SCC, 1 SCLC, 7 other)	LC detection
ctDNA methylation patterns	Liang et al.[82]	observational	132 LC patients in validation cohort	LC detection
ctDNA methylation profiling	Yang et al.[83]	observational	39 LC patients	LC detection
ctDNA methylation profiling	Li et al.[85]	obervational	65 NSCLC patients (49 adenocarcinoma, 11 SCC, 5 other)	higher risk of relapse
VAF level of ctDNA	Zhang et al.[86]	observational (cohort from TRACERx clinical trial No. NCT01888601)	95 NSCLC patients (55 adenocarcinoma, 32 SCC, 8 other; 58 in training, and 37 in validation cohort)	lymph node metastases
ctDNA profiling before and after surgery	Guo et al.[87]	observational	41 NSCLC patients (33 adenocarcinoma, 6 SCC, 1 neuroendocrine tumor and 1 large cell carcinoma)	response to treatment
ctDNA profiling	Chaudhuri et al.[88]	observational	40 LC patients (37 NSCLC, 3 SCLC)	detection of minimal residual disease, shorter OS and PFS
ctDNA profiling	Abbosh et al.[89]	observational (cohort from TRACERx clinical trial No. NCT01888601)	100 LC patients	higher risk of relapse
ctDNA profiling	Waldeck et al.[90]	observational	21 NSCLC patients	higher risk of relapse
ctDNA profiling	Kuang et al.[91]	observational (cohort from GASTO 1035 clinical trial No. NCT03465241)	38 NSCLC patients (23 adenocarcinoma, 6 SCC, 9 other)	shorter RFS, chemotherapy resistance
ctDNA profiling	Xia et al.[92]	observational	330 NSCLC patients	shorter RFS, increased RFS in patients who recieved adjuvant therapies
ctDNA profiling	Qiu et al.[93]	observational	103 NSCLC patients (60 adenocarcinoma, 38 SCC, 1 adenosquamous carcinoma, 1 atypical carcinoid, 3 large cell neuroendocrine carcinoma)	shorter RFS, increased RFS in patients who recieved adjuvant chemotherapy
ctDNA profiling	Zhang et al.[94]	observational	14 LC patients (7 adenocarcinoma, 2 SCC, 5 SCLC)	higher risk of relapse, chemotherapy resistance
ctDNA profiling	Nong et al.[95]	observational	22 SCLC patients	shorter OS and PFS
ctDNA profiling	Herbreteau et al.[96]	observational (cohort from IFCT-1603 clinical trial No. NCT03059667)	68 SCLC patients	shorter OS and PFS, longer OS in patients with low ctDNA abundance treated with atezolizumab
ctDNA profiling	Chabon et al.[103]	observational (cohort from clinical trials No. NCT01526928 and No. NCT02147990)	43 NSCLC patients	detection of resistance mutations to rociletinib (EGFR inhibitor)
ctDNA profiling	Thompson et al.[104]	observational	102 NSCLC patients	detection of driver and resistance mutations
ctDNA profiling	O'Kane et al.[105]	observational	72 NSCLC patients	detection of resistance mutations, shorter PFS
ctDNA profiling	Mok et al.[106]	observational (cohort from FASTACT-2 clinical trial No. NCT00883779)	305 NSCLC patients	*EGFR* mutations detection, longer PFS in EGFR-positive patients treated with erlotinib
ctDNA profiling	Oxnard et al.[107]	observational	13 NSCLC patients	resistance mutations detection, treatment response to erlotinib
ctDNA profiling	Kim et al.[108]	observational	81 adenocarcinoma patients	detection of *EGFR* mutations, higher number of metastases, shorter PFS, shorter duration of disease control by EGFR-TKIs

Table 2. Circulating tumor DNA (ctDNA) in lung cancer – cont.

Biomarker	Study	Type of study	Study group	Outcomes
ctDNA profiling	Dono et al.[109]	observational	42 adenocarcinoma patients	detection of *T790M* mutation
ctDNA profiling	Beagan et al.[110]	observational	20 adenocarcinoma patients with *EGFR T790M* mutation	response to osimertinib (TKI) – higher ctDNA level in nonresponders
ctDNA profiling	Boysen Fynboe Ebert et al.[111]	observational (clinical trial No. NCT02284633)	225 NSCLC patients with *EGFR* mutations: 82 treated with osimertinib (80 adenocarcinoma, 2 SCC)	response to osimertinib (TKI) – longer PFS, higher ORR and disease control rates in patients with clearing of ctDNA after treatment initiation
ctDNA profiling	Lei et al.[112]	observational	98 NSCLC patients with *EGFR* uncommon mutation	detection of resistance mutations to icotinib (TKI)
ctDNA profiling	Provencio et al.[113]	observational	228 NSCLC patients with *EGFR* mutation (210 adenocarcinoma, 18 other)	response to TKI – longer OS and PFS in patients with clearing of ctDNA after treatment initiation
ctDNA profiling	Nabet et al.[114]	observational	99 NSCLC patients (85 non-squamous LC, 14 SCC)	response to ICI – longer PFS in patients with significant drop of ctDNA after single ICI cycle
ctDNA profiling	Goldberg et al.[115]	observational	28 NSCLC patients (27 non-squamous LC, 1 SCC)	response to ICI – longer OS and PFS in patients with a significant drop of ctDNA after treatment
ctDNA profiling	Ricciuti et al.[116]	observational	62 NSCLC patients (56 non-squamous LC, 6 SCC)	response to pembrolizumab – higher ORR, longer OS and PFS in patients with significant drop of ctDNA after treatment
ctDNA profiling	Hellmann et al.[117]	observational	31 NSCLC patients (28 non-squamous, 3 SCC)	response to ICI – longer PFS in patients with undetectable ctDNA after treatment
ctDNA profiling	Guo et al.[118]	observational	64 NSCLC patients (28 non-squamous LC, 36 SCC)	response to treatment (surgery+chemotherapy) – longer OS in patients with decreasing ctDNA and methylated DNA
ctDNA profiling before and after surgery	Peng et al.[119]	observational	77 NSCLC patients (40 adenocarcinoma, 30 SCC, 7 other)	response to treatment – shorter OS and RFS in ctDNA-positive pre- and postoperative patients
ctDNA profiling	Giroux Leprieur et al.[120]	observational	23 NSCLC patients, 15 included in analysis (10 non-squamous LC, 5 SCC)	response to nivolumab – longer PFS in patients with significant drop of ctDNA after treatment
ctDNA profiling	Lee et al.[121]	observational	57 adenocarcinoma patients	bone metastases detection, response to TKI – shorter PFS
ctDNA profiling	Roosan et al.[122]	observational	370 NSCLC patients (345 adenocarcinoma, 13 SCC, 12 other)	detection of somatic mutations, longer PFS in patients with low ctDNA level
ctDNA tumor mutational burden	Gandara et al.[125]	observational (samples from POPLAR trial No. NCT01903993 and OAK trial No. NCT02008227)	211 samples from NSCLC patients (POPLAR study) and 583 samples from NSCLC patients (OAK study)	response to atezolizumab – longer OS and PFS
ctDNA tumor mutational burden	Chae et al.[126]	observational	136 NSCLC patients (99 adenocarcinoma, 27 SCC, 10 other)	response to ICIs – shorter OS and PFS
ctDNA profiling	Chen et al.[128]	observational	76 NSCLC patients (59 adenocarcinoma, 17 SCC)	detection of cancer-specific mutations
ctDNA profiling	Ma et al.[205]	observational	21 NSCLC patients	detection of cancer-specific mutations in CSF
ctDNA profiling	Huang et al.[206]	observational	35 adenocarcinoma patients	detection of *EGFR* mutations in patients with CNS metastases
ctDNA profiling	Belloum et al.[207]	observational	56 NSCLC patients (49 adenocarcinoma, 5 SCC, 2 other)	detection of cancer-specific mutations in patients with metastases
ctDNA profiling	Aldea et al.[208]	observational (clinical trial No. NCT02666612)	247 LC patients (230 adenocarcinoma, 5 SCC, 12 other)	detection of cancer-specific mutations in patients with metastases

VAF – variant allele frequency; RFS – relapse-free survival; LC – lung cancer; NSCLC – non-small cell lung cancer; SCC – squamous cell carcinoma; SCLC – small cell lung cancer; EGFR – epidermal growth factor receptor; OS – overall survival; PFS – progression-free survival; TKI – tyrosine kinase inhibitor; ORR – objective response rate; ICIs – immune checkpoint inhibitors; CSF – cerebrospinal fluid.

Table 3. Circulating tumor cells (CTCs) in lung cancer

Biomarker	Study	Type of study	Study group	Outcomes
CTCs	Zhao et al.[131]	meta-analysis	21 studies – 3997 participants, including 2714 LC patients	LC detection
CTCs	Jiang et al.[132]	meta-analysis	10 studies – 1002 NSCLC patients	high abundance of CTCs – shorter OS and PFS
CTCs	Wu et al.[133]	meta-analysis	8 studies – 453 LC patients	lower disease control rate, shorter OS and PFS in CTC-positive patients at baseline and during chemotherapy
CTCs	Wang et al.[134]	meta-analysis	20 studies – 1576 NSCLC patients	lymph node metastases, more advanced stage, shorter OS and PFS
CTCs	Jiang et al.[135]	meta-analysis	16 studies – 1103 SCLC patients	shorter OS and PFS in patients with high pre-treatment CTC level, shorter OS in patients with high CTCs level after treatment
CTCs	Liu et al.[136]	meta-analysis	8 studies – 170 NSCLC patients	*EGFR* mutations detection
CTCs	Shen et al.[137]	meta-analysis	12 studies – 1131 LC patients	*KRAS* mutation detection
CTCs	Ilie et al.[138]	observational	87 adenocarcinoma patients	*ALK* gene rearrangement detection
CTCs	Pailler et al.[139]	observational	32 NSCLC patients	*ALK* gene rearrangement detection
CTCs	Tan et al.[140]	observational	26 NSCLC patients	*ALK* gene rearrangement detection
CTCs	Pailler et al.[141]	observational	8 NSCLC patients	*ROS1* gene rearrangement detection
CTCs	Pailler et al.[142]	observational	39 NSCLC patients	response to crizotinib – longer PFS in patients with decrease in CTC number with *ALK*-CNG

LC – lung cancer; NSCLC – non-small cell lung cancer; PFS – progression-free survival; OS – overall survival; ORR – objective response rate; EGFR – epidermal growth factor receptor; KRAS – Kirsten rat sarcoma viral oncogene; SCLC – small cell lung cancer; ALK – anaplastic lymphoma kinase; ROS1 – repressor of silencing 1; CNG – copy number gain.

in order to ensure reproducibility and generalizability of results. Studies investigating miRNA in lung cancer are presented in Table 4.

Markers of nervous system involvement in the course of lung cancer

Markers of brain metastases

Calcium binding protein B (S100B), synthesized in astrocytic terminal processes,[193] is an established marker of blood–brain barrier disruption. The detection of S100B protein along with anti-S100B autoantibody allows to distinguish between lung cancer patients with or without brain metastases, with a sensitivity of 89% and a specificity of 58%.[194] Furthermore, the evidence of an association between serum S100B level and brain metastases with subsequently worse prognosis has been shown by other researchers.[195] However, low specificity for S100B is a main barrier to its implementation, as its abnormal expression has also been reported in patients with cerebrovascular disease. Nonetheless, pro-apolipoprotein A-1 levels, measured using proteomic techniques, appear to be a more specific marker as it was increased in lung cancer patients with brain metastases, regardless of cerebrovascular disease.[196]

Among other potential markers, high NLR, platelet-to-lymphocyte ratio and C-reactive protein (CRP) levels were suggested to indicate the development of brain metastases in lung cancer patients after definitive radiotherapy or radiotherapy combined with chemotherapy.[197] High NLR ($\geq$4.95) and lower mean platelet volume were associated with an increased risk of brain metastases in patients with NSCLC.[198,199] At the same time, high plasma fibrinogen concentration and platelet count correlated with shorter OS in NSCLC patients already diagnosed with brain metastases.[200]

The miRNA has also emerged as a relevant marker of brain metastases in lung cancer, with the overexpression of miR-330-3p noted in the serum of NSCLC patients with brain metastases, when compared to those without dissemination to the central nervous system.[201] Significantly lower expression of miR-330 was found in lung cancer patients who had undergone whole-brain radiation therapy, and these patients proved to be radiation-sensitive.[202] Furthermore, serum levels of miR-21 before and after radiotherapy in lung cancer patients with brain metastases were significantly correlated with OS.[203] The expression of miR-483-5p and miR-342-5p in serum and cerebrospinal fluid differed between patients with leptomeningeal and brain parenchymal metastases.[204]

The detection of ctDNA in the cerebrospinal fluid of patients with lung cancer brain metastases displayed higher mutation detection rates than peripheral blood samples.[205] Sensitivity of detecting *EGFR* mutations in ctDNA from plasma or cerebrospinal fluid was comparable, while *T790M* mutations were more prevalent in plasma samples.[206] With regard to the location of metastases, *EGFR*,

Table 4. MicroRNAs (miRNAs) in lung cancer

Biomarker	Study	Type of study	Study group	Outcomes
the miR-Test (13 miRNAs; serum)	Montani et al.[146]	observational (cohort from COSMOS clinical trial No. NCT01248806)	calibration set: 24 patients (12 with LC); validation set: 1008 patients (36 with LC); clinical set: 74 LC patients	LC detection
miRNA panel (24 miRNAs; plasma)	Sozzi et al.[147]	observational (cohort from MILD clinical trial No. NCT02837809)	939 participants, including 69 LC patients	LC detection
miRNA panel (15 miRNAs; whole blood)	Fehlmann et al.[148]	observational	3102 participants, including 606 LC patients	LC detection
miRNA panel (miR-31-5p and miR-210-3p in sputum and miR-21-5p in plasma)	Liao et al.[149]	observational	132 NSCLC patients (74 adenocarcinoma, 58 SCC; 76 in training and 56 in testing cohort)	NSCLC detection
miR-1268b and miR-6075 (serum)	Asakura et al.[150]	observational	1566 LC patients (1217 adenocarcinoma, 221 SCC, 23 SCLC, 105 other; 208 in discovery cohort and 1358 in validation cohort)	LC detection
miR-145 (serum and plasma)	Tao et al.[151]	meta-analysis	9 studies – 1394 NSCLC patients	NSCLC detection
serum miRNA panel (miR-9-5p, miR-21-5p, miR-223-3p, CEA, CYFRA21-1, and SCC)	Yang et al.[189]	observational	104 NSCLC patients (59 adenocarcinoma, 40 SCC, 5 other)	NSCLC detection
panel of 3 miRNAs: miR-17, miR-190b and miR-375 (plasma)	Lu et al.[152]	observational	1132 participants (456 high-risk individuals, 315 adenocarcinoma, 224 SCC, 137 SCLC; 106 in discovery cohort, 565 in training cohort and 461 in validation cohort)	discrimination between SCLC and NSCLC
6 miRNAs: miR-211-3p, miR-3679-3p, miR-4787-5p, miR-3613-3p, miR-3675-3p, and miR-5571-5p (tissue and plasma)	Pu et al.[153]	observational	40 NSCLC patients (27 adenocarcinoma, 13 SCC)	discrimination between NSCLC subtypes
panel of 17 miRNAs (serum)	Wang et al.[154]	observational	391 NSCLC patients (214 adenocarcinoma, 87 SCC, 90 other; 8 in screening, 192 in training and 191 in testing set)	higher risk of death, shorter median survival time
miR-590-5p (plasma; downregulation)	Khandelwal et al.[155]	observational	80 NSCLC patients (18 adenocarcinoma, 41 SCC, 21 mixed)	NSCLC detection, shorter OS
miR-25 (serum)	Li et al.[156]	observational	128 NSCLC patients (102 adenocarcinoma, 26 SCC)	NSCLC detection, more advanced stage, lymph node metastases, shorter OS and RFS
miR-126 (tissue and plasma; downregulation)	Sun et al.[158]	meta-analysis	8 studies investigated prognostic value (1102 NSCLC patients)	shorter OS
miR-494 (tissue and serum)	Zhang et al.[160]	observational	90 NSCLC patients (55 adenocarcinoma, 35 SCC)	more advanced stage, lymph node metastases, shorter OS and disease-free survival
miR-519d (tissue and serum; downregulation)	Wang et al.[161]	observational	130 NSCLC patients (40 adenocarcinoma, 90 SCC)	NSCLC detection, more advanced stage, lymph node and distant metastases, shorter OS
miR-184 (downregulation), miR-191 (both in serum)	Ding et al.[162]	observational	100 NSCLC patients (82 adenocarcinoma, 13 SCC, 5 large cell carcinoma)	NSCLC detection, shorter OS
miR-185 (serum; downregulation)	Liu et al.[163]	observational	146 NSCLC patients (88 adenocarcinoma, 58 SCC)	LC detection, more advanced stage, lymph node metastases, shorter OS

Table 4. MicroRNAs (miRNAs) in lung cancer – cont.

Biomarker	Study	Type of study	Study group	Outcomes
miR-770 (serum; downregulation)	Sun et al.[164]	observational	196 NSCLC patients	more advanced stage, lymph node metastases, shorter OS
miR-23b-3p, miR-10b-5p, miR-21-5p (plasma exosomal)	Liu et al.[166]	observational	10 adenocarcinoma patients in discovery cohort and 196 NSCLC patients in validation cohort (115 adenocarcinoma, 73 SCC, 8 other)	shorter OS
miR-378 (serum exosomal)	Zhang and Xu[168]	observational	103 NSCLC patients (53 adenocarcinoma, 46 SCC, 4 other)	more advanced stage, lymph node metastases, shorter OS, higher rate of radiotherapeutic response
miR-942 and miR-601 (serum)	Zhou et al.[169]	observational	125 NSCLC patients in validation cohort	NSCLC detection, more advanced stage, lymph node metastases, shorter OS and RFS
miR-3195, miR-1249-3p (both in serum)	Kumar et al.[170]	observational	75 NSCLC patients (42 adenocarcinoma, 33 SCC)	miR-1249-3p – adenocarcinoma detection, higher rate of complete and partial response to chemotherapy miR-3195 – longer OS
miR-21 and miR-4257 (plasma exosomal)	Dejima et al.[171]	observational	201 NSCLC patients (138 adenocarcinoma, 55 SCC, 8 other; 6 in discovery and 195 in validation cohort)	shorter RFS in surgically treated patients
miR-92b and miR-375 (plasma)	Li et al.[172]	observational	63 SCLC patients (including discovery and validation cohorts)	shorter PFS, a significant decrease in patients who responded to chemotherapy
miR-422a (lymph node tissue, plasma)	Wu et al.[173]	observational	77 LC patients (26 in training and 51 in validation cohort; 35 adenocarcinoma, 36 SCC, 6 SCLC)	lymph node metastases
miR-135a (serum)	Zou et al.[174]	observational	117 NSCLC patients (59 adenocarcinoma, 58 SCC)	lymph node metastases
miR-375-3p (plasma exosomal)	Mao et al.[176]	observational	126 SCLC patients (2 validation cohorts of 57 and 69 patients, respectively)	lymph node and distant metastases
miR-216b (serum exosomal; downregulation)	Liu et al.[179]	observational	105 NSCLC patients (45 adenocarcinoma, 60 SCC)	LC detection, more advanced stage, lymph node metastases, shorter OS and DFS
miR-96 (plasma exosomal)	Zheng et al.[180]	observational	52 NSCLC patients	shorter OS, higher levels in patients with radiotherapy-resistant NSCLC
panel of 11 miRNAs (serum)	Sun et al.[181]	observational (cohort from 4 clinical trials)	80 NSCLC patients	radiotherapy resistance – shorter OS
miR-320b-d, miR-125b-5p (plasma exosomal)	Peng et al.[182]	observational	30 NSCLC patients	worse response to PD-1/PD-L1 inhibitors – lower rate of complete and partial response
miR-504 (plasma)	Szpechcinski et al.[183]	observational	66 NSCLC patients (56 adenocarcinoma, 10 other)	detection of *EGFR* mutations
miR-28-5p, miR-362-5p, miR-660-5p (plasma; downregulation)	Li et al.[184]	observational	6 NSCLC patients in screening cohort and 73 NSCLC patients in validation cohort	detection of *ALK* mutations miR-660-5p – increased level after crizotinib treatment in responders miR-362-5p – shorter PFS
miR-21 (plasma)	Li et al.[185]	observational	25 non-squamous NSCLC patients	resistance to immunotherapy – miR-21 level higher at the time of acquired resistance to TKI than at baseline
miR-30b and miR-30c (plasma)	Hojbjerg et al.[186]	observational	29 adenocarcinoma patients with *EGFR* mutations	shorter PFS and OS in patients treated with erlotinib
miR-30c (serum)	Zhou et al.[187]	observational	80 NSCLC patients (47 adenocarcinoma, 23 SCC, 10 adenosquamous NSCLC)	cardiotoxicity during bevacizumab therapy
miR-762 (serum)	Chen et al.[188]	observational	148 NSCLC patients (84 adenocarcinoma, 64 SCC)	more advanced stage, lymph node metastases, shorter OS and RFS in patients treated with gefitinib

Table 4. MicroRNAs (miRNAs) in lung cancer – cont.

Biomarker	Study	Type of study	Study group	Outcomes
miR-330-3p (serum)	Wei et al.[201]	observational	122 NSCLC patients (95 adenocarcinoma, 18 SCC, 9 other)	detection of brain metastases
	Jiang et al.[202]	observational	258 LC patients with brain metastases (149 adenocarcinoma, 61 SCC, 48 other)	radiation sensitivity – lower survival rate and median survival time
miR-21 (serum)	Zhu et al.[203]	observational	200 LC patients with brain metastases (97 adenocarcinoma, 55 SCC, 48 large cell carcinoma)	radiation sensitivity – shorter OS
miR-483-5p and miR-342-5p (serum exosomal and CSF exosomal)	Xu et al.[204]	observational	38 LC patients (25 adenocarcinoma, 13 other)	detection of leptomeningeal metastases

CEA – carcinoembryonic antigen; CSF – cerebrospinal fluid; LC – lung cancer; NSCLC – non-small cell lung cancer; SCC – squamous cell carcinoma; SCLC – small cell lung cancer; EGFR – epidermal growth factor receptor; OS – overall survival; PFS – progression-free survival; RFS – relapse-free survival; DFS – disease-free survival; PD – programmed cell death protein; ALK – anaplastic lymphoma kinase; TKI – tyrosine kinase inhibitors.

KRAS, *BRAF*, or *ERBB2* mutations in plasma ctDNA could be detected in half of the patients with isolated brain metastases,[207,208] and ctDNA positivity was associated with a higher risk of extra central nervous system dissemination.

Markers of paraneoplastic neurological syndromes

Specific autoantibodies related to the development of PNS can be regarded as markers of the involvement of nervous system in the course of lung cancer.[14] Onconeural antibodies, directed against intracellular antigens, are already well recognized and commonly used in practice. Most prevalent among these antibodies are anti-Hu, anti-Yo, anti-Ri, anti-CV2, anti-Tr, anti-amphiphysin, and anti-Ma/Ta,[8] with anti-Hu, anti-Ri, anti-CV2, and anti-amphiphysin being closely linked to lung cancer.[209] Recent years have also seen advances in establishing the role of antibodies against cell-surface or synaptic antigens, such as antibodies against N-methyl-D-aspartate receptors, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, the γ-aminobutyric acid receptor-B, leucine-rich glioma-inactivated protein 1, and contactin-associated protein-like 2.[210]

Several other autoreactive antibodies and related antigens were investigated for their use in the detection of PNS in lung cancer patients, including phosphodiesterase 10A and Purkinje cell cytoplasmic antibody type 2 antibodies.[211,212] Apart from early detection of lung cancer, some of these antibodies were investigated in terms of their prognostic value. Indeed, some reports have indicated that the presence of anti-Hu antibodies in patients with SCLC was associated with limited stage of the disease and satisfactory response to therapy[213] or longer median survival,[214] although this correlation was not clear.[215] Despite their clinical utility, onconeural and cell-surface antibodies have limited sensitivity and specificity, they are not detectable in every patient with PNS,[216] and may be associated with different kinds of neoplasms.[217] Thus, further exploration in this field seems warranted.

Conclusions

In this review, we outlined the recent development in the research on of potential biochemical, immunological and molecular markers of lung cancer that have shown promising sensitivity and/or specificity. Molecular markers are associated with improved understanding of complex tumor genetics, while immunological markers have provided a more thorough insight into the tumor-related immune environment, thus opening new perspectives in diagnosis and effective management of lung cancer. Diagnostic markers that enable an early detection of the disease may be valuable in supporting existing screening methods, while markers with predictive potential could contribute to the identification of patients at high risk of recurrence or with the propensity for metastases, with scope for the development of individualized monitoring and treatment strategies. With the advent of new treatment options such as immune checkpoint and kinase inhibitors, the recognition of mechanisms of resistance to targeted therapy and emerging decisions about personalized treatment strategies appear as key elements of lung cancer management, with the use of relevant biomarkers being indispensable. Further investigation should be aimed at improving the accuracy and specificity of these markers, perhaps by combining them into panels.

Circulating biomarkers seem particularly promising for practical use as their detection is non-invasive, safe and easily repeatable. However, the majority of molecules

investigated still need thorough validation, with standardization of techniques and assays used, and established cut-off values. Furthermore, a systematic comparative analysis of efficacy should be performed for findings from liquid biopsy and from tumor tissue studies. Finally, the implementation of markers into clinical practice needs more supportive evidence, preferably from clinical trials.

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