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Broodman, Ingrid; Lindemans, Jan; van Sten, Jenny; Bischoff, Rainer; Luider, Theo M

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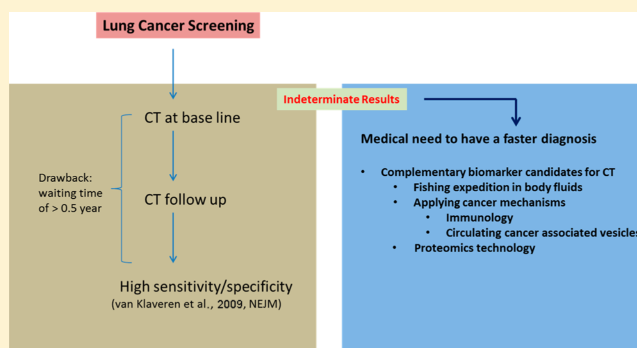
Serum Protein Markers for the Early Detection of Lung Cancer: A Focus on Autoantibodies

Ingrid Broodman,[†] Jan Lindemans,[†] Jenny van Sten,[‡] Rainer Bischoff,[*,§] and Theo Luider[‡]

[†]Department of Clinical Chemistry and [‡]Department of Neurology, Erasmus University Medical Center, Wytemaweg 80, 3015 CN Rotterdam, The Netherlands

[§]Analytical Biochemistry, Department of Pharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands

ABSTRACT: Lung cancer has the highest mortality rate among cancer patients in the world, in particular because most patients are only diagnosed at an advanced and noncurable stage. Computed tomography (CT) screening on high-risk individuals has shown that early detection could reduce the mortality rate. However, the still high false-positive rate of CT screening may harm healthy individuals because of unnecessary follow-up scans and invasive follow-up procedures. Alternatively, false-negative and indeterminate results may harm patients due to the delayed diagnosis and treatment of lung cancer. Noninvasive biomarkers, complementary to CT screening, could lower the false-positive and false-negative rate of CT screening at baseline and thereby reduce the number of patients that need follow-up and diagnose patients at an earlier stage of lung cancer. Lung cancer tissue generates lung cancer-associated proteins to which the immune system might produce high-affinity autoantibodies. This autoantibody response to tumor-associated antigens starts during early stage lung cancer and may endure over years. Identification of tumor-associated antigens or the corresponding autoantibodies in body fluids as potential noninvasive biomarkers could thus be an effective approach for early detection and monitoring of lung cancer. We provide an overview of differentially expressed protein, antigen, and autoantibody biomarkers that combined with CT imaging might be of clinical use for early detection of lung cancer.



KEYWORDS: *antibody, antigen, biomarker, computed tomography (CT) screening, early detection, lung cancer, next-generation sequencing (NGS), proteomics, tumor immunology*

LUNG CANCER INCIDENCE AND ETIOLOGY

Lung cancer is the most common cancer type. Worldwide, more than 1.8 million men and women were diagnosed with lung cancer in 2012.[1,2] In that year, an estimated 1.6 million died of lung cancer, accounting for one-fifth (19%) of all cancer deaths in the world.[1]

Cigarette smoking is the most important risk factor for lung cancer and accounts for about 80–90% of cases.[3,4]

Almost 70% of the people diagnosed with lung cancer are 65 or older. The median age at time of diagnosis is about 70 years.[5,6] Lung cancer is more common in men than in women. The male to female age-standardized incidence rates ratio is about 60% higher in men.[4] This high male to female ratio is mainly due to the higher prevalence of cigarette smoking in men than women.[7]

Survival rates of lung cancer patients vary depending on the stage of the cancer at diagnosis. The 5 year survival rate for lung cancer is about 15%.[4,8] However, the 5 year survival rate may increase up to 49% when lung cancer is diagnosed at an early stage.[5]

TYPES OF LUNG CANCER

Lung cancer, also known as carcinoma of the lung, is a malignant lung tumor formed by uncontrolled cell growth in the tissues of the lung, usually in the bronchi and the airways of the lungs. This growth may spread to a site distant from the lungs and produce metastatic tumors in brain, bone, liver, or adrenal glands.[4] Primary lung cancers are carcinomas that start in the lung and are derived from epithelial cells. The two main primary types of lung cancer are non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). About 85% of all lung cancers are NSCLC. The three main subtypes of NSCLC are adenocarcinoma (40%), squamous cell carcinoma (25–30%), and large cell carcinoma (10–15%).[5]

About 10–15% of lung cancers are SCLC. SCLC often starts in the larger airways, the primary (main) and secondary (lobar) bronchi. It is the most aggressive type of lung cancer, grows more quickly than NSCLC, and often metastasizes to other parts of the body early in the development of the disease.[5] Most of the SCLC patients have widespread metastasis at the time of diagnosis. SCLC is often associated with paraneoplastic

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syndromes (PNS).[5] The 5 year survival for SCLC (6%) is lower than that for NSCLC (21%).[5,6] Nearly all cases of SCLC are due to cigarette smoking.[4,5,9]

DIAGNOSIS AND STAGING

Lung cancer tumors usually grow for many years without causing any signs or symptoms during the early stages of the disease. About 5−10% of the lung cancer patients are diagnosed with lung cancer at an early stage when still asymptomatic during a physical examination for an unrelated medical problem or after a routine chest X-ray. Unfortunately, most symptomatic lung cancer patients are diagnosed with lung cancer at an advanced stage. Chest X-ray only produces a flat, 2D image of the lungs and usually detects large tumors but may miss smaller or hidden tumors and does not exclude lung cancer.[10,11] Computed tomography (CT) uses a combination of many X-ray images taken in a circle around the chest and computer technology to produce highly detailed 3D images of the internals of the lungs. CT scans are able to detect smaller tumors than routine chest X-ray analyses and also determine the size, shape, and location of the tumor because of the 3D measurement. The final determination of whether a tumor is malignant and definitive diagnosis of the type of lung cancer can only be made by examining of a tissue sample by a pathologist. This tissue sample can be obtained by bronchoscopy, sputum cytology, or fine needle aspiration biopsy.[4,5]

Lung cancer staging is based on a system that describes the growth and extent of spread of the cancer to other parts of the body. The TNM system classifies patients in five stages: 0 (in situ), I, II, III, or IV. Patients with a higher stage number have a poorer prognosis and lower survival rate.[12]

LUNG CANCER SCREENING

The purpose of lung cancer screening is to detect lung cancer at an early and still curable stage to improve the survival rate of lung cancer patients. Survival rate improves significantly with early detection of the disease, with a respective 5 year survival rate increasing from 2 to 7, 19, 25, 36, and 43% for stages IV, IIIB, IIIA, IIB, IIA, and IB and to 50% for stage IA.[12] Surgery offers the best chance to cure early-stage NSCLC patients. Because these patients are usually asymptomatic, only 15% of all diagnoses of lung cancer are from stage I.[4] In contrast, CT screening detected 48−85% of lung cancers at stage I.[11,13] Therefore, CT screening is performed on apparently healthy, asymptomatic people at high risk of lung cancer such as current smokers and former smokers.

RANDOMIZED SCREENING STUDIES

Randomized screening studies for early detection of lung cancer in high-risk individuals are ongoing. An overview of the main large-scale lung cancer screening studies is presented in Table 1. These studies compare CT screening with chest X-ray or usual care. The aim of these lung cancer screening studies is to reduce mortality by 20−25% by detection at an early and still curable stage. Three trials in Europe, the DANTE (Detection and Screening of Early Lung Cancer by Novel Imaging Technology and Molecular Essays), DLCST (Danish Lung Cancer Screening Trial), and MILD (Multicentric Italian Lung Detection) trials, reported no significant reduction in lung cancer mortality.[14−18]

The largest study, the NLST (U.S. National Lung Screening Trial) study, reported a significant lung cancer mortality reduction of 20.3% in high-risk individuals who were screened annually with CT compared with those who were screened annually by chest X-ray.[19,20] At present, the NELSON, ITALUNG, LUSI, and the UKLS screening studies (Table 1) are still ongoing. When data of all randomized screening studies become available, a definitive conclusion of the effectiveness of CT screening can be drawn.

THE NELSON STUDY

The NELSON study (Nederlands-Leuvens Longkanker Screeningsonderzoek), a Dutch-Belgian Lung Cancer Screening trial, is the world's second largest randomized lung cancer computer tomography screening trial and differs from the NLST study by

Table 1. Main Large-Scale Randomized Controlled Lung Cancer Screening Trials[a]

trial	initiation/completion	*N*	design	screens	♂ (%)	age (y)	pack (y)	quit (y)[b]
DANTE[15,16,21]	2001	2472	CT vs none	5	100	60−74	≥20	<10
Italy	2009							
NLST[19,20]	2002	53 454	CT vs CXR	3	59	55−74	≥30	<15
USA	2011							
ITALUNG[22]	2004	3206	CT vs none	4	65	55−69	≥20	<10
Italy	ongoing							
NELSON[23−25]	2004	15 822	CT vs none	5	84	50−75	≥15[c]	≤10
Netherlands/Belgium	ongoing							
DLCST[17,26]	2004	4104	CT vs none	5	55	50−70	≥20	<10
Denmark	2011							
MILD[18]	2005	4099	CT vs none	5	66	≥49	≥20	<10
Italy	2012							
LUSI[27,28]	2007	4052	CT vs none	5	65	50−69	≥15[c]	≤10
Germany	ongoing							
UKLS[29,30]	2011	4055	CT vs none	pilot study	75	50−75	NA[d]	NA
United Kingdom	ongoing							

[a]CXR, chest X-ray; DANTE, Detection and Screening of Early Lung Cancer by Novel Imaging Technology and Molecular Essays; DLCST, Danish Lung Cancer Screening Trial; ITALUNG, Italian lung study; CT, computed tomography; MILD, Multicentric Italian Lung Detection trial; *N*, patient number; NA, not applicable; NELSON, Dutch-Belgian Lung Cancer Screening Trial (Dutch acronym); NLST, National Lung Screening Trial; LUSI, German Lung Screening and Intervention trial; UKLS, U.K. Lung Screening trial; y, years; ♂, male. [b]Quit smoking. [c]Inclusion criteria ⩾15 cigarettes per day for 25 y or ⩾10 cigarettes per day for 30 years. [d]Inclusion criteria ≥5% risk of lung cancer in 5 y.

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screening interval, referral policy, and a control arm wherein individuals receive no screening (usual care).[25,31] The NELSON trial started in 2003. The main purpose of the trial was to investigate whether CT screening leads to a reduction of lung cancer mortality of at least 25% at 10 years of follow-up in a high-risk population. Current or former smokers between 50 and 75 years of age and with a smoking history of at least 15 cigarettes per day for at least 25 years or at least 10 cigarettes per day for at least 30 years were included in the trial. A total of 15 822 participants were randomized (1:1) to a screen or a control arm. The screen arm received CT screening at baseline (first screening round), 1 year later (second screening round), 3 years later (third screening round), and 5.5 years later (fourth screening round), whereas the control arm received no CT screening.[23] Screening results were considered positive for (part) solid lung nodules with a volume of >500 mm^3 (>9.8 mm in diameter) and indeterminate for (partly) solid lung nodules with a volume of 50 to 500 mm^3 (4.6 to 9.8 mm in diameter). Participants with an initial indeterminate screening result received a follow-up CT scan 3 months later to classify their final screening test result as negative or positive based on nodule volume doubling time (VDT).[24,32] If the nodule had a VDT of <400 days, the final screening result was considered positive. Participants with a positive screening result were referred to a pulmonologist for a diagnostic follow-up according to the national guideline.[33] If lung cancer was diagnosed, the participant was offered treatment and went off screening. Participants with a negative screening result re-entered the trial and underwent a second-round CT scan 12 months later.

The first three screening rounds resulted in 493 positive results, and 200 (40.6%) participants were diagnosed with lung cancer.[31] Lung cancers in the NELSON trial were more frequently detected at an early stage (70.8% stage I) than at an advanced stage (8.1% stage IIB-IV) compared with other screening trials.[24,25,31] While the NLST defined any solid nodule with a diameter ≥4 mm as a positive screening result,[19,20] the NELSON trial considered only solid lung nodules with a volume >500 mm^3 (>9.8 mm in diameter) or VDT < 400 days as a positive screening result. By using these more stringent criteria, the positive predictive value was higher in the NELSON trial (40.6%) than in the NLST trial (3.6%). Consequently, the percentage of false-positive results was substantially lower in the NELSON trial (59.4%) than in the NLST (96.4%) trial.[19,25,31] Lung cancer mortality results of the NELSON trial are upcoming. The first results on mortality reduction after 10 year follow-up are expected in 2016.

More than 6600 serum samples of the NELSON trial were collected at baseline. Because the NELSON trial can provide prospectively collected samples at baseline of patients with early stage lung cancer, matched with healthy smoker controls, as well with subjects with benign pulmonary nodules, the well-controlled population of the NELSON trial constitutes an ideal set for the design of a case-control study on early lung cancer biomarker discovery.

■ BIOMARKERS

There is a medical need for additional biomarkers for early detection of lung cancer, as CT screening leaves 15−52% of cases undetected at baseline.[11,13] Furthermore, CT screening has a high rate of false-positive rate due to the high prevalence of benign pulmonary nodules in the population. This results in unnecessary follow-up CT scans, additional tests, biopsies, or even surgery. In the NLST, 24% of subjects with benign nodules underwent an unnecessary surgical procedure (thoracotomy, thoracoscopy, or mediastinoscopy).[19] These invasive follow-up procedures are costly and may harm patients.[34,35] Alternatively, false-negative and indeterminate results may harm patients due to the delayed diagnosis and treatment of lung cancer.

Biomarkers in blood could be a noninvasive, cost-effective tool to stratify individuals at high risk of lung cancer who would benefit from CT screening and guide subsequent therapy. These biomarkers may be used for early diagnosis, optimal treatment selection, and prognosis. They may not only reduce the number of unnecessary invasive procedures but also lead to the earlier resection of malignant nodules, which will substantially improve the prognosis of the patient. Unfortunately, there is still no clinically relevant blood biomarker available for lung cancer, although various groups have proposed proteins, mostly in panels of antigens or autoantibodies. In searching for a clinically relevant biomarker, it is vital to understand the biological processes of lung cancer. Lung cancer cells have defects in their regulatory processes that maintain normal cell proliferation and homeostasis. Critical changes in their cell physiology lead to cancer growth. Lung cancer cells are insensitive to growth-inhibitory signals and show escape from apoptosis, unlimited replication, sustained angiogenesis, tissue invasion, and metastasis.[36] Transformation from a normal to a malignant lung epithelial cell arises after a series of genetic and epigenetic changes, eventually leading to invasive lung cancer by clonal expansion.[37] The molecular composition of lung cancer is complex and heterogeneous, which leads to variable biological, histological and clinical presentations. Various oncogenes, tumor suppressor genes, signaling pathway components, and other cellular processes are involved in the molecular pathogenesis of lung cancer.[4,38] These molecular processes can lead to the release of various lung cancer associated components, which are not necessarily related to each other, such as tumor DNA, tumor cell fragments, and various mutated or overexpressed proteins into body fluids. Therefore, identification of these lung-cancer-associated components in body fluids as potential biomarkers is a way to detect lung cancer at an early stage and allow more appropriate treatment at that early stage, resulting in a better prognosis. Serum or plasma are considered the most suitable body fluid derivatives for biomarker discovery and diagnosis because they form a minimally invasive and easily accessible source.

■ LUNG-CANCER-ASSOCIATED PROTEINS

Lung cancer is often associated with the differential expression of several proteins, which may be potential biomarkers for lung cancer. Table 2 represents a selection of lung cancer-associated proteins as potential blood-based biomarkers for lung cancer that have been described in literature.

Well-known and clinically used lung cancer protein biomarkers in serum are carcinoembryonic antigen (CEA), CYFRA 21-1 (cytokeratine 19 fragment), neuron-specific enolase (NSE), progastrin-releasing peptide (ProGRP), and squamous cell carcinoma antigen (SCCA). Although these proteins are elevated in serum of a fraction of lung cancer patients, they are not sensitive or specific enough to detect lung cancer in a clinically relevant way or to have enough value as biomarker for the diagnosis of asymptomatic patients with lung cancer.[49,50]

Lung tumor cells may secrete or release small amounts of tumor-associated proteins at an early stage of lung cancer. Detection of these lung cancer-associated proteins in biological

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Table 2. Characteristics and Performance of Blood-Based Proteins as Potential Biomarkers for Lung Cancer[a,b]

				stage (%)								
reference	proteins	N	remarks subjects	I	II	III	IV	Tx	sensitivity (%)	specificity (%)	AUC	method
Li et al.[39]	13 peptide panel	143	D, IPNs[c]	-	-	-	-	100	93	45	0.82	MRM-MS
		104	V, IPNs[c]	-	-	-	-	100	90	27	0.60	
Patz et al.[40]	CEA, AAT, SCCA[d]	509	D, PNs	41	13	26	16	4	82	84	NA	LCBA
		399	V, PNs	46	18	21	13	2	80	89	NA	
Pecot et al.[41]	clinical data + CT data + MALDI-MS signature[e]	100	IPNs[f]	-	-	-	-	100	NA	NA	0.57	
									NA	NA	0.67	
									NA	NA	0.72	MALDI-MS
Bigbee et al.[42]	10 protein panel	60	NSCLC, SC	-	-	-	-	100	73	93	NA	Luminex
Diamandis et al.[43]	Penatraxin-3	383	LC, SC	14	2	4	2	78	37	90	0.60	ELISA
Takano et al.[44]	Nectin-4	295	NSCLC, HC	27 (I-IIIA)		73 (III-B-IV)		-	54	98	NA	ELISA
Ostroff et al.[45]	12 protein panel	985	D, NSCLC, SC	47	15	38	0	-	91	84	0.91	Aptamers
		341	V, NSCLC, SC	49	14	35	2	-	89	83	0.90	
Patz et al.[46]	CEA, RBP4, AAT, SCCA	100	D, LC, HC	40	4	30	26	-	89	85	NA	ELISA
		97	V, LC, HC	33	6	39	22	-	78	75	NA	
Yildiz et al.[47]	MALDI-MS signature[e]	182	D, NSCLC, SC	39 (ES)		61 (LS)		-	67	89	0.82	MALDI-MS
		106	V, NSCLC, SC	40 (ES)		60 (LS)		-	58	86	0.82	
Gao et al.[48]	CRP, SAA, MUC1	80	LC, SC	-	-	-	-	100	71	93	NA	protein microarray

[a]Note: Data are listed by most recent publication first (2013−2005). [b]I, stage I; II, stage II; III, stage III; IV, stage IV; AUC, area under the curve; D, discovery set; ELISA, enzyme-linked immunosorbent assay; ES, early stage: NSCLC I, II and limited SCLC; HC, healthy controls; IPNs, indeterminate pulmonary nodules; LC, lung cancer (NSCLC and SCLC); LCBA, lung cancer biomarker (Immuno)assay; LS, late stage: NSCLC III, IV and extensive SCLC; MRM-MS, multiple-reaction-monitoring mass spectrometry; MS, mass spectrometry; N, patient number; NA, not applicable; PNs, benign and malignant nodules; SC, smoking controls; Tx, tumor stage unknown (or not described), V, validation set. [c]Nodule size 10−20 mm. [d]Logistic regression model based on LBCA data and nodule size. [e]Signature of seven features. [f]5−20 mm.

samples has been proposed to support early diagnosis, prognosis, and optimal treatment of lung cancer. However, the large dynamic range of other proteins in blood-derived samples, which extends over 10 orders of magnitude, and the high abundance of albumin (55%) in serum or plasma is a major problem to detect these low-abundant proteins by liquid chromatography−mass spectrometry.[51] Depletion of high-abundant proteins or targeted enrichment of lung-cancer-associated proteins are the main strategies to overcome this problem and to enhance the detection of these low-abundant proteins. New DNA-based aptamers have been developed that contain chemically modified nucleotides that bind to different low-abundant proteins with high affinity. Ostroff et al. used an aptamer-based proteomic assay in a multicenter case-control study of 291 NSCLC cases and 1035 smoking controls.[45] They developed a panel of 12 proteins (cadherin-1, CD30 ligand, endostatin, HSP90α, LRIG3, MIP-4, pleiotrophin, PRKCI, RGM-C, SCF-sR, sL-selectin, and YES) that was able to distinguish 213 NSCLC cases (62% stage I−II) from 772 controls with 91% sensitivity and 84% specificity. This panel was tested on a validation set consisting of 78 NSCLC cases (63% stage I−II) and 263 controls, including patients with COPD and benign nodules reaching a sensitivity of 89% at a similar specificity of 83% and an AUC of 0.90. The reason that this panel is not clinically implemented might be because sensitivity and specificity are still too low.

Li et al. used immunoaffinity columns for the depletion of high-abundant proteins. They developed and validated a 13-protein blood-based classifier using multiple-reaction-monitoring mass spectrometry (MRM-MS) in a retrospective study consisting of 52 NSCLC and 52 benign controls. Their classifier distinguished benign from early-stage (IA) NSCLC nodules with a sensitivity of 90% but quite low specificity of 27%.

Table 2 gives an overview of different discovery and validation studies by various research groups. Unfortunately, none of these studies reaches overall sensitivities and specificities to reliably discriminate lung cancer patients from controls, notably for early-stage lung cancer. In addition, most of the proposed lung cancer proteins were not validated between lung cancer cases and controls that were matched for smoking habit, which is the most relevant group for screening purposes. None of the proteins in Table 2 is currently in use or developed as a biomarker for the early detection of lung cancer.

■ IMMUNOLOGICAL BIOMARKERS

The presence of tumor cells can activate the immune system to respond to tumor-specific antigens or to tumor-associated antigens.[52,53] Tumor-specific antigens (TSAs) are only expressed in tumor cells, whereas tumor-associated antigens (TAAs) are expressed differently by tumor cells and normal cells. The immune system not only protects the host against the development of

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primary tumors but may also, strangely enough, promote development of primary tumors. This process, also known as cancer immunoediting, consists of three phases: elimination, equilibration, and escape. Immunosurveillance occurs during the elimination phase, during which the immune system recognizes tumor cells as foreign cells and tries to eliminate them. Tumor cells that survive this phase enter into the equilibrium phase. In the equilibrium phase, tumor cells are mutated to tumor cell variants with increased resistance to immune attack. The equilibrium phase is assumed to be the longest of the three phases and may continue for several years. Tumor cell variants start to grow in an uncontrolled manner and eventually will be detected in the escape phase.[54,55] These tumor cells express TAAs that distinguish them from normal cells. Most of the TAAs are overexpressed, mutated, misfolded, or aberrantly processed proteins that initiate an autoreactive immune response.[52,56,57] Post-translational modifications (PTMs) of TAAs, such as acetylation, glycosylation, oxidation, phosphorylation, and proteolytic cleavage, may contribute to the immune response by creating a neo-epitope or by improving self-epitope presentation and affinity to the major histocompatibility complex (MHC) or the T-cell receptor.[52,56,58] Identification of tumor-associated antigens and autoantibodies to these antigens may provide an opportunity for early detection of lung cancer.[59]

■ ANTIBODIES AS BIOMARKERS

Autoantibodies to TAAs may be potential biomarkers for early detection of lung cancer. First, autoantibodies may be detectable in the asymptomatic stage of lung cancer, up to 5 years prior to detection by CT screening.[60,61] Second, in contrast with antigens, autoantibodies are stable and persist in serum for a relatively long period of time at rather high levels,[52] while tumor-associated antigens may only occur transiently at very low levels due to temporary changes in only a few (pre)neoplastic cells. However, the immune system is very sensitive in detecting these very low levels of TAAs and may respond by producing high-affinity T cells and autoantibodies.[62] Such an autoantibody response to a tumor-associated antigen may endure over years. Thus autoantibodies may be more easily detectable at an earlier stage than their corresponding TAAs.

Human IgGs are large molecules (~150 kDa) and composed of four polypeptide chains, two identical heavy chains (50 kDa) and two identical light chains (25 kDa). Each light chain has a variable (V_L) and constant (C_L) region. The heavy chains have three different constant regions (C_H1, C_H2, and C_H3) and one variable region (V_H). The first constant region and variable region of the heavy chain, together with the constant and variable part of the light chain, form the antigen binding fragment (Fab). The other two constant regions (C_H2 and C_H3) of the heavy chain form the Fc fragment. Three hypervariable complementarity-determining regions (CDR1, CDR2, and CDR3) in the variable regions of the heavy and light chains form the antigen-binding site. All CDRs in combination determine the specificity of the immunoglobulin to the antigen. During B-cell development and maturation, V, D, and J germline genes are rearranged to form a specific VDJ germline combination. These rearranged genes further diversify by somatic hypermutations to increase antibody affinity.[63−67] In both light and heavy chains, the diversity of CDR3 is even further enhanced by insertions and deletions of nucleotides. The high diversity of CDR3 makes it the key part of antigen recognition. It is the region that most directly interacts with the antigen.[68] The estimated potential diversity in immunoglobulins ranges from 10^{13} to more than 10^{50}.[66,69] Despite this large range, there is evidence of a repertoire bias, which means that specific germline genes are preferred in the repertoire of immunoglobulins during the immune response to a particular antigen.[70,71] Antigen-specific immunoglobulin sequences may thus be shared among different lung cancer patients and could serve as biomarkers for lung cancer.

■ LUNG-CANCER-ASSOCIATED AUTOANTIBODIES

During tumor development, lung cancer patients produce specific autoantibodies to TAAs that are potential biomarkers for lung cancer. Table 3 represents a list of autoantibodies to TAAs as potential blood-based biomarkers for lung cancer that have been described in literature.

Although autoantibodies are an active area of research, this work has not yet led to clinically relevant molecular biomarkers. While all of these studies reported autoantibodies to TAAs, none of the proposed autoantibodies found application as biomarker in the clinic. These autoantibodies studies have limitations. First, most of the studies described in Table 3 lack adequate clinical validation. Second, most proposed markers are not specific for lung cancer. For instance, Annexin, CAGE, CEA, HER2, MUC1, NYESO-1, and p53 also arise in other cancers and autoimmune diseases. Furthermore, because of the heterogeneity of lung cancer, it is not likely that an autoantibody to any single tumor-associated antigen will detect all types of lung cancer. Because different target antigens are involved in the immune response to the different tumors, it is more likely that autoantibodies to an antigen panel will detect the different types of lung cancer. The *Early*CDT-Lung is currently used as an aid to risk assessment and the early detection of lung cancer in high-risk patients. This blood test measures autoantibodies to a panel of seven TAAs (p53, NY-ESO-1, CAGE, GBU4−5, SOX2, HuD, and MAGE A4) and was validated in large cohorts including early- and late-stage NSCLC and SCLC. This autoantibody panel showed an overall specificity of 91% but a rather low sensitivity of 37% in NSCLC and 55% in SCLC (Table 3), respectively.[61,74,80−82,88] Another limitation of most methods in Table 3 is that the antigen or antigen panel must be known at the start of the study. Therefore, the development of a sensitive and specific autoantibody detection method that does not require prior knowledge of the antigens offers opportunities to explore the complete inventory of autoantibodies and possibly the corresponding tumor-associated antigens.

To reach the highest sensitivity and specificity and to cover the histological heterogeneity of lung cancer, we propose that a panel of peptide sequences derived from the antigen-binding site of autoantibodies has potential as a screening test for early-stage lung cancer. While antibody diversity is huge, selection pressure during B-cell development may restrict the antibody diversity. Antibodies are subjected to selection pressure after rearrangement and affinity maturation.[70,71] Different studies have demonstrated that it is possible to identify similar or identical autoantibody sequences among different individuals.[93−97] Specific sequences of high-affinity antibodies can be expressed in response to low levels of tumor-associated antigens in early-stage lung cancer and may serve as biomarkers for the early detection of lung cancer.

In initial studies, we were able to detect early-stage lung cancer with an antibody-derived peptide panel with sensitivities

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Table 3. Characteristics and Performance of Blood-Based Autoantibodies to TAAs as Potential Biomarkers for Lung Cancer[a,b]

reference	antibodies to TAAs	N	remarks subjects	stage (%) I	II	III	IV	Tx	sensitivity (%)	specificity (%)	AUC	method
Wang et al.[72]	5 AABs panel[c]	264	LC, SC	-	-	-	-	100	30	89	NA	ELISA
	5 AABs panel[d]	307	LC, BNs	-	-	-	-	100	30	88	NA	
Doseeva et al.[73]	NY-SO-1 (and CEA, CA125, CYFRA 21-1)[e]	230	D, NSCLC, SC	77	20	3	0	-	74	80	0.81	Luminex
		150	V, NSCLC, SC	32	35	20	13	-	77	80	0.85	
Jett et al.[74]	6 AABs panel[f]	752	LC, SC	39	11	21	15	13	46	83	NA	ELISA
	7 AABs panel[g]	847	LC, SC	39	11	21	15	13	37	91	NA	
Jia et al.[75]	p53, NY-ESO-1, Livin, Ubiquilin, BIRC, p62, PRDX	98	LC, SC	-	-	-	-	100	80	60	0.82	Luminex
Wang et al.[76]	ANXA1IgG	499	NSCLC, SC	45	19	32	4	-	24	90	0.64	ELISA
	DDX53IgG	499	NSCLC, SC	45	19	32	4	-	14	90	0.52	
Lowe et al.[77]	9 marker panel	200	D, AAH, SC	-	-	-	-	100[h]	92	90	0.87	phage-display + protein microarray
		300	V, AAH, SC	-	-	-	-	100[h]	82	70	0.81	
	13 marker panel	200	D, SCD, SC	-	-	-	-	100[h]	98	96	0.96	
		300	V, SCD, SC	-	-	-	-	100[h]	86	78	0.88	
Zhang et al.[78]	Anti-p16 IgA	497	NSCLC, SC	44	19	33	4	-	10	90	0.46	ELISA
	Anti-p16 IgG	446	NSCLC, SC	47	17	32	4	-	20	90	0.57	
Pedchenko et al.[79]	6 scFv panel IgM	43	NSCLC, SC	86	14	0	0	-	80	87	0.88	FMAT
Ye et al.	Anti-CD25	486	NSCLC, SC	45	18	33	4	-	35	90	0.70	ELISA
Chapman et al.[80]	6 AABs panel[f]	776	LC, SC	-	-	-	-	100	40	82	NA	LLC-LIMS
	7 AABs panel[g]	836	LC, SC	-	-	-	-	100	47	90	NA	
Lam et al.[81]	p53, NY-ESO1, CAGE, GBU4−5, Annexin I, SOX2[f]	1,254	LC, SC	60 (ES)		26 (LS)		14	34	87	NA	ELISA
Boyle et al.[82]	p53, NY-ESO-1, CAGE, GBU4−5, Annexin I, SOX2[f]	481	D, LC, SC	12 (ES)		70 (LS)		18	39	89	0.63	ELISA
		538	V, LC, SC	63 (ES)		10 (LS)		27	37	90	0.64	
Rom et al.[83]	c-myc, Cyclin A, Cyclin B1, Cyclin D1, CDK2, survivin	194	LC, SC	-	-	-	-	100	81	97	0.91	ELISA

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Table 3. continued

reference	antibodies to TAAs	N	remarks subjects	stage (%) I	II	III	IV	Tx	sensitivity (%)	specificity (%)	AUC	method
Farlow et al.[84]	IMPDH, Ubiquillin, phosphoglycerate mutase, Annexin I, Annexin II, HSP70–9B	196	NSCLC, SC	66	13	18	3	-	95	91	0.97	Luminex Immunoassay
Yao et al.[85]	DKK1	180	NSCLC, HC	-	-	-	-	100	62	84	NA	ELISA
Wu et al.[86]	OLFM1	180	NSCLC, HC	-	-	-	-	100	92	92	0.96	phage display + ELISA
Leidinger et al.[87]	1827 peptide clones	127	LC, HC	47	4	38	4	7	98	97	0.81	phage-display
Murray et al.[88]	p53, NY-ESO-1, CAGE, GBU4–5, Annexin I, SOX2[f]	481	LC, SC	12 (ES)	70 (-L-S)	18	34	91	NA	ELISA		
Qiu et al.[89]	Annexin I, LAMR1, 14–3–3 theta	170	NSCLC, SC	-	-	-	-	100[i]	51	82	0.73	protein micro array
Leidinger et al.[90]	20 peptide clones	79	NSCLC, HC	46	31	15	3	5	93	93	0.98	phage-display
Chapman et al.[61]	p53, NY-ESO-1, CAGE, GBU4–5, c-myc, HER2, MUC1	154	LC, HC	5	4	15	37	39	76	92	NA	ELISA
Pereire-Faca et al.[91]	14–3–3 theta, Annexin I, PGP 9.5	37	LC, SC	-	-	-	-	100[i]	55	95	0.84	Western blot + protein microarray
Zhong et al.[60]	Paxillin, SEC15L2, BAC cloneRP-11-499F19, XRCC5, MALATI1	46	D, NSCLC, SC	100	0	0	0	-	91	91	0.99	phage-display
		102	V, NSCLC, SC	13[j]/68	15[j]	13[j]	4[j]	-	80[j]/83	88	NA	
Yagihashi et al.[92]	Survivin, Livin	38	D, LC, HC	11	5	30	54	-	71	100[h]	NA	ELISA

[a]Note: Data are listed by most recent publication first (2016–2005). [b]I, stage I; II, stage II; III, stage III; IV, stage IV; AABs, tumor-associated autoantibodies; AAH, atypical adenomatous hyperplasia (preneoplastic adenocarcinoma); AUC, area under the curve; BNs, benign pulmonary nodules; D, discovery set; ELISA, enzyme-linked immunosorbent assay; ES, early stage: NSCLC I, II and limited SCLC; FMAT, fluorometric microvolume assay technology; HC, healthy controls; LC, lung cancer (NSCLC and SCLC); LLC-LIMS, *Early*CDT-Lung test based on ELISA; LS, late stage: NSCLC III, IV and extensive SCLC; *N*, patient number; NA, not applicable; SC, smoking controls; SCD, squamous cell carcinoma dysplasia (preneoplastic squamous cell carcinoma); scFv, single chain fragment variable antibodies; TAAs, tumor-associated antigens; Tx, tumor stage unknown (or not described), V, validation set. [c]5 AABs panel to TTC14, BRAF, ACTL6B, MORC2, and CTAG1B. [d]5 AABs panel to keratin 8 (type II), TTC14, Kruppel-like factor 8, BRAF, and tousled like kinase 1. [e]Measurement of antigen panel: CEA, CA125, CYFRA 21-1. [f]A positive test result for the 6 AABs panel was defined as a positive autoantibody response to at least one of the 6 TAAs in the panel: p53, NY-ESO-1, CAGE, GBU4–5, Annexin I, and SOX2 (*Early*CDT-Lung). [g]7 AABs panel: p53, NY-ESO-1, CAGE, GBU4–5, SOX2, HuD, and MAGE A4 (new *Early*CDT-Lung). [h]Preneoplastic samples. [i]Preclinical samples within 1 year before diagnosis. [j]Preclinical samples 1–5 years before diagnosis.

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of 96 and 84% and specificities of 100 and 90% in a discovery set and a validation set, respectively, at baseline CT screening.[98] This work indicates that specific antibodies are present at an early disease stage and that a panel of antibodies may be able to detect lung cancer at an earlier stage than CT screening. Autoantibody profiling has the potential to be a tool for early detection when incorporated into a comprehensive screening strategy, provided that technical challenges of de novo sequencing of CDRs based on high-resolution MS/MS spectra can be overcome.

CONCLUSIONS AND FUTURE PERSPECTIVES

There is a medical need for blood-derived biomarkers that, in combination with CT imaging, can detect lung cancer earlier in individuals at high risk. In a complex and heterogeneous disease like lung cancer, it is likely that only a panel of biomarkers will achieve the necessary sensitivity and specificity. A number of studies have attempted to discover and in certain cases validate such biomarker panels (see Table 2). Panels have been derived from common plasma/serum proteins such as AAT, CRP, or SAA that are related to the acute-phase response or from well-known tumor markers like CEA or SCCA. None of these panels reached sufficient sensitivity or specificity for clinical use, and most have not been validated in large independent cohorts. There is thus a need for new approaches.

Autoantibodies against TAAs are of interest as a potential source of biomarkers because they allow monitoring the immunological response at a very early stage of cancer development. In addition, autoantibodies are more stable in serum than most other proteins and tumor-derived DNA. Table 3 gives an overview of autoantibody-based approaches toward biomarkers for lung cancer. While promising, it is fair to say that none of these approaches has delivered better biomarker panels. It is thus necessary to move beyond quantifying autoantibodies using immunoassays or display techniques to methods that focus directly on the regions that are related to the recognition of tumor-associated antigen, the CDRs. The combination of top-down (protein-based) and bottom-up (peptide-based) proteomics using high-resolution mass spectrometry allows for identification of antigen-binding sites in antibodies and thereby facilitates the de novo sequencing of CDRs.[99,100] The combination of proteomics and genomics (proteogenomics) further facilitates this approach by opening the opportunity to identify CDR-derived peptides in patient-derived genomic databases.[101−104] Whether such combined, personalized approaches will be successful in diagnozing lung cancer at an earlier stage and in prognosticating tumor development and response to therapy remains to be seen and is the focus of ongoing large-scale studies.

AUTHOR INFORMATION

Corresponding Author

*E-mail: r.p.h.bischoff@rug.nl. Phone: +31-(0)6-52 000 506.

Notes

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