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The Pursuit of Noninvasive Diagnosis of Lung Cancer

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Abstract

The noninvasive diagnosis of lung cancer remains a formidable challenge. Although tissue diagnosis will remain the gold standard for the foreseeable future, questions pertaining to the risks and costs associated with invasive diagnostic procedures are of prime relevance. This review addresses new modalities for improving the noninvasive evaluation of suspicious lung nodules. Ultimately, the goal is to translate early diagnosis into early treatment. We discuss how biomarkers could assist in distinguishing benign from malignant nodules and aggressive from indolent tumors. The field of biomarkers is rapidly expanding and progressing, and efforts are well underway to apply molecular diagnostics to address the shortcomings of current lung cancer diagnostic tools.

Keywords

early detection; clinical utility; biomarkers; indeterminate pulmonary nodule

More than 200,000 people are diagnosed with lung cancer every year, and its incidence eclipsed that of prostate cancer in 2015, making it the most common cancer in men and second most common cancer overall.¹ It remains the most lethal cancer, with more than 160,000 annual deaths attributed to the disease. From 2005 to 2011, the 5-year survival rate of lung cancer was 17.4%, which was dismally lower than the survival rate for prostate cancer (98.9%), the most common cancer in men, and breast cancer (89.4%), the most common cancer in women.² When divided by stage, the 5-year survival for lung cancer is less than 5% for stage IV disease and 50% for stage I disease as of 2007.³ In light of these statistics and the growing success of chest computed tomographic (CT) screening for lung cancer in high-risk individuals, there is a strong imperative for early detection and meaningful diagnostics that can cause a stage shift and save lives.

Managing indeterminate pulmonary nodules is not only difficult and expensive but also it is becoming an epidemic. Although the number of cancers diagnosed annually remains relatively constant, the number of pulmonary nodules diagnosed every year is ever

increasing.⁴ This is largely driven by the increased use of chest CT in clinical practice and is magnified by the recent implementation of programs dedicated to low-dose chest CT lung cancer screening. Although results from the National Lung Screening Trial (NLST) in 2011 showed a significant earlier stage shift and decreased lung cancer-specific mortality, screening CT scans identified a pulmonary nodule at least 4 mm in diameter in 40% of patients screened, and 96.4% of these positive scans were false positives.⁵

High rates of pulmonary nodule detection and false positives exist in the incidental nodule population as well. In a recent retrospective survey of a health care database that includes hundreds of thousands of patients not stratified by risk, pulmonary nodules measuring 4 to 30 mm were detected in up to 29% of chest CT scans over 6 years, and the prevalence rose each year.⁴ Furthermore, 5% of those identified with a pulmonary nodule were diagnosed with lung cancer, meaning that the false-positive rate was 95%; extrapolation of these data shows that approximately 1.5 million people in America are diagnosed with a lung nodule on chest CT scan annually. There is significant additional morbidity and cost associated with such high false-positive rates. For example, rates of pneumothorax from percutaneous needle biopsy can be as high as 20%, with a smaller percentage of those requiring a chest tube.^{6,7} Even in a high-risk group of people who have been referred for surgical resection of a suspicious pulmonary nodule, up to 35% of these nodules are found to be benign.⁸ Noninvasive testing for indeterminate pulmonary nodules with positron emission tomography/CT scanning is also plagued by high false-positive rates, especially in areas of the country with endemic fungal pulmonary infections.^{9,10} Clearly, there is a need for tests with greater accuracy to reduce rates of unnecessary procedures as well as to control the duration and cost of work-up.

Biomarkers have emerged as noninvasive tools that can be derived from many different bodily sources and can give information about an underlying disease process. The assumption is that molecular biomarkers may improve the diagnostic accuracy of the clinical management of lung nodules. As more biomarkers become available in commercial laboratories, it becomes increasingly important to clarify their performance, clinical utility, and appropriate context for use. Here, we will summarize the state of the art of some of these biomarkers.

What Is a Diagnostic Biomarker?

Biomarkers have a wide range of use including risk assessment, diagnosis, staging, prediction of response, and prognosis. It will help frame the scope of this review to define these different types of biomarkers. A risk assessment biomarker is one that predicts the future development of lung cancer in high-risk populations.¹¹ A predictive biomarker, on the other hand, informs treatment-dependent outcomes. Some of the most well-known predictive biomarkers include epidermal growth factor receptor and anaplastic lymphoma kinase tyrosine kinase receptor mutation. Testing in non-small cell lung cancer for these mutations can inform choice of treatment and thereby change clinical outcome.^{12–14} A prognostic biomarker is a measurable factor associated with an outcome that is independent of treatment. For example, KRAS mutation-positive tumors are associated with worse survival

among lung adenocarcinomas.^{12,15–17} The focus of this article will be the diagnostic biomarker, which noninvasively estimates the probability of disease.

Diagnostic biomarkers for lung cancer are measures that identify a disease state. In the clinic, most providers would use a diagnostic biomarker, as opposed to a biomarker of risk assessment, to assess the probability of lung cancer. This assessment could occur once the risk for lung cancer is established and significant (i.e., screening setting) or when the disease is suspected, regardless of whether the individual is symptomatic (i.e., diagnostic setting) (Fig. 1). The most common scenarios are screening tests or evaluations of an indeterminate pulmonary nodule, incidentally found or screening detected. To decrease false positives detected by chest CT, noninvasive tests such as diagnostic biomarkers could refine the probability of cancer and provide guidance as to whom should be offered invasive biopsy.

From Derivation to Validation of Candidates and Clinical Utility of a Diagnostic Biomarkers

The study design is critical in the discovery and validation process.¹⁸ Most impactful molecular biomarkers are derived in the clinical setting of intended use. As much as the analytical validation will be essential in addressing baseline characteristics of the test,^{19,20} clinical validation requires a careful plan of investigation and is lengthy and expensive. Independent validation of the biomarker requires confirmation of the prediction accuracy in cohorts from different institutions. This work requires concerted efforts between laboratories to reproduce the methods. To establish the clinical utility of any biomarker, the test should demonstrate improved diagnostic accuracy when measured against the “gold standard” used in practice, which, in this case, includes chest CT and demographic clinical predictors. The report/recommendations by the Institute of Medicine¹⁹ regarding clinical utility of a biomarker mirror the Evaluation of Genomic Applications in Practice and Prevention Working Group recommendations.²¹ The question at stake is whether the biomarker changes physician decision making or contributes to improvement in patient outcomes. This is very difficult to demonstrate because clinicians make decisions, not tests, and they do so based on multiple tests ordered in parallel rather than individual tests ordered serially. The metric for success of such biomarkers is in its ability to add value and reduce false-positive rates, ultimately saving cost and lives (Fig. 2).

Existing Noninvasive Diagnostic Tools to Predict Malignancy: Imaging-Based Biomarkers

Nodule Size

Although nodule size may not be thought of as a conventional biomarker, it is perhaps the oldest and most frequently used nontissue-based means of estimating likelihood of lung malignancy. Size of a pulmonary nodule and likelihood of malignancy are positively correlated, and 80% of benign incidental nodules are less than 2 cm in largest diameter.²² In application of Bayesian theory to observational studies of incidental nodules, one analysis calculated the likelihood ratio for malignancy to be 0.74 for nodules 1.1 to 2.0 cm in longest diameter compared with a likelihood ratio of 3.67 for nodules 2.1 to 3.0 cm in longest

diameter.^{23,24} The World Health Organization and Fleischner Society rely on nodule size in two dimensions to inform further investigation of potential cancer diagnosis.^{25–27} We have continued to refine our understanding of the accuracy of nodule size as a diagnostic biomarker since the institution of lung cancer screening programs across the country. In the 3 years of screening in the NLST, a total of 598 pulmonary nodules were diagnosed as lung cancer, and 70.5% of these were more than 2 cm, whereas only 15% were less than 1 cm.²⁸ In fact, analysis of the I-ELCAP (International Early Lung Cancer Action Program) screening data showed that moving the threshold for a positive result to 6 mm did not delay the diagnosis of cancer by 12 months for a single patient, and it reduced the positive rate from 16 to 10% (thereby decreasing further work-up by 36%).²⁹ These insights into nodule size as a diagnostic biomarker from screening cohorts are likely to change the guidelines for pulmonary nodule follow-up.

The change in the volume of a pulmonary nodule over a discrete period of time can also significantly improve diagnostic accuracy. Doubling time and its inverse, growth rate, are common measurements to quantify changes over time. Studies that have assessed growth rate by serial CT-based volumetric analysis have used thin-section imaging, with slice thicknesses typically less than 2.5 mm. Interpretation of these values can vary considerably, however, based on the method used for derivation of nodule volume.³⁰ In a retrospective analysis of benign nodules, lymphoma, and various histologic types of lung cancer, the growth rate of nodules varied by whether volume measurements were performed by software automation, by modeling a sphere based on area via perimeter delineation, or by modeling a sphere based on short and long axes of the section with largest diameter. In a high-risk population that underwent chest CT screening for lung cancer in the Netherlands and Belgium, volumetric growth was used as a marker of malignancy between initial screening CT and follow-up CT scan at 3 months for solid nodules < 10 mm.³¹ There was a negative predictive value (NPV) of 99.9% if any of the following occurred: nodule volume of < 50 mm³ at initial screen, lack of growth between the screening and second CT scan, or volume doubling time greater than 400 days.

Our group tested the reproducibility of volumetric CT (VCT) analysis of lung nodules in the clinical setting. We used VCAR (GE Healthcare, Little Chalfont, United Kingdom) VCT software to analyze stable calcified nodules in patients who underwent two to four repeat VCT. Using a 400-day doubling time for differentiation of benign and malignant nodules, the shortest interval between measurements, for 4, 6, 8, and 10 mm nodules, the optimal patient follow-up scan interval was calculated to be 81 days, independent of initial nodule volume.³²

Computer-assisted volumetric analysis and quantitative imaging are expected to assist in clinical decision making. Automated volumetric nodule measurement may improve intra- and interobserver variabilities, though there are practical implementation barriers that have prevented its transition into the clinical realm. Namely, variability between software and instruments for image acquisition complicates integration of computer-assisted diagnostic technology into picture archiving and communication system (PACS).

Clinical Models

There are also diagnostic tools immediately available to the clinician to enhance the diagnostic accuracy for lung cancer. Clinical prediction models utilize radiographic biomarkers in addition to demographic data to estimate the likelihood of malignancy for a pulmonary nodule measuring between 8 and 30 mm (Table 1). One of the earliest is colloquially known as the “Mayo Clinic” model, which will be the only model presented here that uses chest X-ray instead of CT. The investigators used logistic regression analysis to determine the radiographic and demographic independent predictors of malignancy in a retrospective cohort of 629 men and women.³³ This cohort was based in the northern United States at a single center with a lung malignancy rate of 23%. Three demographic characteristics (age, smoking history, and history of cancer) and three radiographic characteristics (nodule longest diameter, spiculation, and upper lobe location) were independent predictors of lung cancer, and the derived clinical model had an area under the curve (AUC) of 0.83. The Veterans Affairs SNAP Cooperative study group developed a separate clinical prediction model that is more generalizable to the veteran population, who had been observed to have a higher rate of malignancy than reported in the Mayo Clinic cohort.³⁴ Furthermore, the imaging modality used for obtaining radiographic characteristics was the more technologically advanced CT. In addition to the longest diameter of the nodule, they identified three other independent predictors of malignancy via multivariate logistic regression analysis: age, positive smoking history, and time since quit smoking. The AUC for this model was 0.79. Compared with the Mayo Clinic model, this cohort was almost entirely made up of male veterans, and the rate of malignancy was 54%.

A recent model for clinical prediction of lung malignancy was developed by McWilliams et al.³⁵ This model is unique in that it estimates the risk of malignancy for incidental pulmonary nodules found at the time of lung cancer screening by CT chest. The discriminatory power was > 90% for both prospectively enrolled Canadian-based cohorts that were studied. Eligibility for inclusion mirrored that for lung cancer screening (50–75 years old, current smoker, or former smoker who has quit within past 15 years), and the rate of malignancy was approximately 5%. Our group established a lung cancer model (Thoracic Research Evaluation and Treatment [TREAT] model³⁶) using all clinical parameters available at the time of preoperative evaluation. This model is more intensive in data requirements but achieves accuracy of 0.89 in a population of nodules with high lung cancer prevalence (72%).

There is no one clinical prediction model that is superior to all others, and none is one size fits all. The limitations of these models are that they were derived in different populations, they do not always include the value of stability or growth of a nodule over time, and their accuracy largely depends on the size of the nodules. There is still considerable room for interpretation of the results from these models, as they only give a probability of cancer. Therefore, clinical judgment and patient values are necessary adjuncts to these diagnostic biomarkers for choosing the next best step. These clinical models are currently used by some in clinical practice to provide an estimate of baseline probability of cancer, but there seems to be current lack of consensus about their use in recommendations from professional societies. Current American College of Chest Physicians guidelines for evaluation of a

solitary pulmonary nodule recommend with a 2C evidence level that physicians use either clinical judgment or one of the validated clinical models.

Quantitative Structural Image Analysis

A variety of image analysis techniques provide both qualitative as well as quantitative methods to relate lung lesions structure with clinical outcome. Of particular importance are automated methods that allow analysis of a broad spectrum of anatomical measures including volume, texture, density, and shape. Conventional imaging methods fail to differentiate malignant from benign nodules or indolent from aggressive cancers in many cases. Recent advances have brought a series of structural imaging analytical approaches^{37,38} including a computer-aided nodule assessment and risk yield approach to categorize lung adenocarcinomas into categories tightly associated with survival,^{39,40} including in patients enrolled from the NLST.⁴¹ Such radiomics approaches are a burgeoning focus within the field of radiographic biomarkers. The premise is to expand the extractable information from CT imaging well beyond three dimensions by way of automated algorithms that can quantify a multitude of pulmonary nodule imaging features.^{42–44} Examples of features include quantification of previously qualitative traits (contour, convexity, and spiculation) and granular detail such as voxel-to-voxel difference in gray level. Heat mapping of radiomic features with biopsy-proven lung cancer and head and neck cancer showed significant association between radiomic features and T stage of TNM staging, but not N or M stage.⁴² It also showed a strong correlation between radiomic features and tumor histologic type. These findings were validated in three independent datasets and suggest the value of radiomics as a diagnostic biomarker for identifying malignant lesions as well as for obtaining histologic information prior to biopsy.

The ultimate goal of radiomics is to infer the underlying genomic pattern by discovering its radiographic signature. The degree of detail provided by conventional imaging analysis is not fine enough to create a signature that can consistently differentiate benign from malignant pulmonary nodules, but the hope is that radiomics can extract enough radiographic characteristics to make gene expression patterns distinguishable.⁴³ Segal et al showed a direct correlation between certain radiomic features and gene expression patterns in hepatocellular carcinoma.⁴⁵ Currently, there is not enough prospective data to know which of the > 200 radiomic features hold the greatest diagnostic value for malignancy. Obviously, it would be of great benefit to use imaging analysis techniques as a surrogate for gene expression profiling. To expand radiomics' role in the diagnostic biomarker realm, there is a need for large registries of high fidelity imaging as well as available datasets for external validation.⁴³ Moreover, there is a need for standardized methods for CT image acquisition and processing to reliably and accurately measure quantitative imaging features. The Quantitative Imaging Biomarker Alliance is one such group that is working to create uniform techniques for CT scanning that will reduce the variability in measurements derived from images, thereby enhancing the accuracy of quantitative structural image-based biomarkers.⁴⁶

Commercially Available Biomarkers

There are several commercially available tissue and serum biomarkers that can be processed by specialty laboratories, and they are in various stages of preclinical and clinical validation (Table 2). Some are illustrated later. In regard to blood biomarkers, the challenges have been many, including: (1) the abundance of the biomarkers at a concentration several logs below detection limits,⁴⁷ which begs for novel technologies; (2) the variability in sample collection, which would be best addressed by following standard operating procedures⁴⁸; and (3) the relative lack of availability of biospecimens saved and annotated in the clinical context of interest which could be addressed by multiple national and international efforts. Most of the biomarkers described later have managed to take these issues on with promising results (Table 3).

Bronchial Genomic Signature

The genomic signature of bronchial brushings has the power to rule out disease and add value to current noninvasive diagnosis of lung cancer in patients undergoing bronchoscopy for a suspicious lung nodule. The appropriate patient to apply this test to would be a current or former smoker who has an incidentally found lung nodule with an intermediate probability of lung cancer as determined by best practices, defined as a pretest probability of 10 to 60%. While gene expression analysis of epithelial cells from a main bronchus alone had a sensitivity for detecting lung cancer of < 90%, when bronchoscopy results were combined with gene expression analysis, the sensitivity went up to 98% for all patients with lung cancer.⁴⁹ For the specific population that includes intermediate pretest probability with a nondiagnostic bronchoscopy, the addition of gene expression analysis gave a NPV of 92%. This is a clinically important subgroup due to the high rate of nondiagnostic bronchoscopies (81%) in this study. This test provides an opportunity to change the pretest probability and to influence the clinical management of the nodule. The primary diagnostic utility of the Percepta test offered by Veracyte (South San Francisco, CA) is in the setting of a negative test, where a high NPV can help prevent unnecessary invasive procedures for likely benign disease.

Autoantibodies

Autoantibodies are biomarkers that are directed against tumor antigens, and their value as a biomarker has been explored recently in the context of screening.⁵⁰ Tumor-associated antigens (TAAs) represent nonself-targets for recognition and antibody formation by the immune system. This phenomenon of autoantibodies to TAAs was explored first in other cancers such as breast, but the generalizable concept has allowed for testing in lung cancer.⁵¹ The current commercially available assay is marketed as EarlyCDT-Lung by Oncimmune (Nottingham, UK) and includes seven TAAs (CAGE, GBU4-5, HuD, MAGE A4, NY-ESO-1, p53, and SOX-2) to test against serum for presence of autoantibodies.⁵² The sensitivity of the seven-antigen panel for detecting lung cancer was calculated across 655 cases of lung cancer with matched controls. The investigators set the optimum specificity at 90% and found a sensitivity of approximately 40% for detection of all lung cancer types.⁵³ Lung cancer detection rates were examined for the first 1,613 patients for whom the EarlyCDT-Lung assay was ordered, and sensitivity was very similar when compared with

the retrospective case-control studies.⁵⁰ The current seven-antigen panel had a sensitivity of 37%, specificity of 91%, and positive predictive value of 16%. The investigators also noted that 57% of the NSCLC detected by a positive serum test was stage I or II. The overall positive likelihood ratio of the seven-antigen EarlyCDT-Lung assay was 4.11.

MicroRNA

Aberrant levels of specific circulating microRNAs (miRNAs) have been associated with several malignancies, including lung cancer. Signatures of these small molecules have been found to be independent predictors of disease and to reduce false-positive rates when used in conjunction with other diagnostic tools, such as low-dose CT (LDCT).⁵⁴ miRNAs are endogenous, noncoding RNAs that regulate gene expression after transcription by inhibiting the translation of messenger RNA. These small molecules are measurable in serum, plasma, and sputum using quantitative reverse-transcription polymerase chain reaction. They are also stable in blood despite high levels of circulating RNases, making them prime candidates to serve as biomarkers of disease.^{55,56}

Over the past several years, researchers have identified panels of miRNAs from serum, plasma, and sputum for use in diagnosing lung cancer. Boeri et al profiled miRNA in lung tumor tissue and normal lung tissue, as well as in plasma, at the time of lung cancer diagnosis and found that a signature of 15 specific miRNAs could predict patients at high risk of developing lung cancer with 80% sensitivity and 90% specificity.⁵⁷ Similarly, Xing et al developed a panel of three miRNAs from sputum that can distinguish between early NSCLC and benign lung nodules in smokers with 82.93% sensitivity and 87.84% specificity.⁵⁸ A systematic literature review of all studies pertaining to the use of miRNA as a biomarker of NSCLC found that miRNAs in body fluids, including blood and sputum, have high accuracy in identifying NSCLC; the AUC of the summary receiver operator curve was 0.915⁵⁹ but only few of the miRNAs overlapped between studies.

Researchers are now working to validate miRNA signatures in larger, independent cohorts. Wang et al tested a signature of five miRNAs (miR-483-5p, miR-193a-3p, miR-25, miR-214, and miR-7) in a single cohort and found it to be diagnostic of lung cancer. The signature was then validated in four multicenter, multiethnic cohorts, suggesting that the panel of biomarkers is highly sensitive and specific for NSCLC in patients of different ethnicities.⁶⁰ Several research groups have validated other miRNAs panels with comparable results, and several have been validated within existing lung screening studies. For example, plasma samples collected prospectively for the Multicenter Italian Lung Detection clinic trial were analyzed for miRNA signatures associated with lung cancer and then assigned a “miRNA signature classifier” (MSC) risk score using a predesigned algorithm. The diagnostic performance of the MSC in detecting lung cancer was 87% sensitivity and 81% specificity, compared with 88% sensitivity and 80% specificity for LDCT. Using the MSC in combination with LDCT for a subgroup of noncalcified nodules > 5 mm resulted in a fivefold decrease in the LDCT false-positive rate to 3.7%, from 19.4% for LDCT alone.⁵⁴ Taken together, these results suggest that miRNA may have the potential to distinguish benign from malignant nodules and identify those who deserve surveillance.

Proteomic Biomarkers

Nonantibody protein biomarkers that are overexpressed or uniquely expressed in lung cancer tissue and serum represent an emerging diagnostic tool. There have been several proteins identified from lung tissue postresection or biopsy that are differentially expressed between benign nodules and lung cancer, such as downregulation of selenium-binding protein 1⁶¹ and upregulation of peroxiredoxins 1 and 3.⁶² These lung tissue-based proteins are important for exploring cellular mechanisms of tumorigenesis and guiding further biomarker development. Blood-based proteomic signatures are valuable because they can serve as noninvasive diagnostic biomarkers. One of the better validated plasma-based panels of proteins is marketed as Xpresys Lung from Integrated Diagnostics, Inc. (Seattle, WA), and it represents multiple reaction monitoring assays for 13 proteins that have been identified via mass spectrometry as having differential expression in malignant and benign tissue.⁶³ These proteins are LRP1, BGH3, COIA1, TETN, TSP1, ALDOA, GRP78, ISLR, FRIL, LG3BP, PRDX1, FIBA, and GSLG1. The primary intention with this panel is to assist in the diagnosis of benign lesions via robust NPVs. In a retrospective case-control ($n = 104$) validation of the protein classifier panel, the test performed with a NPV of 90% and a specificity of 44% at a cancer prevalence of 15%. This panel was clinically validated using a prospectively enrolled cohort ($n = 353$) with plasma samples available prior to bronchoscopic or surgical diagnosis.⁶⁴ The rate of benign disease among the various invasive diagnostic modalities ranged from 15 to 21%, with a total of 66 benign lesions found among the 353 who underwent biopsy. The protein classifier panel predicted a “likely benign” status in 21 of the 66 benign lesions (31.8%), representing the amount who could have potentially avoided unnecessary invasive procedures by triaging to CT surveillance. This test is best utilized after CT screening for indeterminate size nodules (8–30 mm), and a result of “likely benign” indicates a lower likelihood of finding malignant disease on invasive biopsy and that CT surveillance could be favored.

Other Selected Upcoming Candidate Diagnostic Biomarkers

Volatile Organic Compounds

Volatile organic compounds (VOCs) found in exhaled breath are being investigated as noninvasive biomarkers of lung cancer. VOCs are emitted from cancer cells or from the surrounding microenvironment into the bloodstream and are then excreted into the lungs and exhaled by patients.^{65–67} Because VOCs interact so closely with the respiratory tract and can be sampled noninvasively, they hold great promise as biomarkers of lung disease. Researchers have applied a variety of chemical analytical-based and pattern-based methods of analysis to better understand the diagnostic potential of VOCs.⁶⁸ In a pilot study from 2012, Peled et al used gas chromatography–mass spectrometry (GC-MS) to study the breath of 72 patients with benign or malignant solitary pulmonary nodules and found that 1-octene appeared in higher concentrations in patients with lung cancer. With this information, the nanoarray was able to distinguish between benign and malignant pulmonary nodules, types of lung cancer, and disease stage.⁶⁶ Recently, Mazzone et al used a colorimetric sensor array, or a cross-reactive chemical sensor, to identify differences in the breath biosignatures of subjects with untreated, biopsy-proven lung cancer, and cancer-free control subjects. A

series of breath biosignature models were tested and found to accurately distinguish between subjects with lung cancer and without.^{67,69}

If VOCs are potential biomarkers of histologic subtypes of lung cancer, perhaps they also reflect mutations known to be associated with lung cancer. Davies et al used thermal desorption-GC-MS to analyze the VOCs in derived human bronchial epithelial cell (HBEC) lines and one parental HBEC line. The group was able to identify combinations of 20 VOCs that could detect differences in cell lines with specific, minimal genetic differences, such as the presence of the KRAS mutation. This suggests that analysis of VOCs in exhaled breath may be able to detect cancers in early stages with only minimal genetic changes.⁷⁰ Although these studies suggest the potential of VOCs as noninvasive diagnostic biomarkers of lung cancer, much work remains to be done to validate their findings. A systematic review by Krilaviciute et al in 2015 identified 73 studies that have used exhaled breath analysis to identify cancer.⁷¹ Two-thirds of these focused on lung cancer. Among these studies, different methods of breath sample collection, storage, and handling were used.⁶⁹ These differences prevent pooling of data and validation of VOCs as novel biomarkers. Further investigations are clearly needed to determine the value of VOCs as candidate biomarkers of lung cancer.

Circulating Tumor Cells and Circulating Tumor DNA

Circulating tumor cell (CTC) and circulating tumor DNA (ctDNA) are not commercially available yet in a clinically validated form for early detection but have great potential as diagnostic biomarkers.

CTCs are rare in peripheral blood compared with the density of other circulating cells, and the utility of CTCs as a diagnostic tool is tied to the evolving technology and methodology of the assay. CTC identification thus far has used a ligand specific to or overexpressed by cancer cells, such as epithelial cell adhesion molecule (EpCAM), that binds with a high enough affinity to give a signal despite extremely low concentrations of CTCs in whole blood samples.^{72–75} The bulk of the CTC literature reports their use as a prognostic biomarker for surveillance of carcinoma recurrence after treatment, and there are few prospectively enrolled clinical validations of CTCs as a diagnostic biomarker. There is evidence from Ilie et al that a high-risk cohort of patients with chronic obstructive pulmonary disease had CTCs detectable on average 3.2 years prior to development of a CT-detected lung nodule.⁷⁶ Moreover, of the five patients with detectable CTCs, they all went on to develop early stage (1A) lung cancers (four adenocarcinomas and one squamous cell) that were resectable, and there were no cancer recurrences by the date of publication. This biomarker is promising, but it requires more robust prospective clinical validation as a diagnostic tool before it is ready for widespread use.

ctDNA is also proposed as a diagnostic biomarker for lung cancer. Cell-free DNA can be found in the serum from both host and tumor cells,^{77–79} and the hallmark of tumor DNA is somatic mutations that are not present in host DNA. The low prevalence of tumor DNA compared with normal host DNA makes it difficult to develop an assay with sufficient sensitivity to reliably detect tumor DNA in blood.^{80–82} The most significant limiting factor for use as a diagnostic biomarker for early detection of lung cancer is that ctDNA quantities correlate with tumor burden.^{81,83,84} Larger tumors release more tumor DNA into circulation,

and the sensitivity of assays declines for earlier stage tumors. Even advanced methods that are 100% sensitive for identifying ctDNA in stages II to IV NSCLC have 50% sensitivity for stage I tumors.^{85,86} There is a commercially available ctDNA assay for use as a predictive and prognostic biomarker that is marketed by Guardant Health (Redwood Shores, CA), and it has been technically and clinically validated.⁸⁷ It is a promising field and will likely enter clinics soon, but using ctDNA as a diagnostic biomarker still requires further study to overcome the hurdle of early detection.

Summary

Tremendous progress is being made in the pursuit of the noninvasive diagnosis of lung cancer. Lung cancer screening efforts are putting pressure on the scientific community to solve these difficult diagnostic dilemmas. Tools will soon be available to providers to assess risk and recommend screening; they will allow for diagnostic assessment of nodules that may represent life-threatening tumors. We will be able to provide, based on a combination of biomarkers, a more accurate, noninvasive diagnosis of lung cancer. The biomarker research community will increase the specificity of diagnosis in the face of a suspicious lung nodule. From analytes found in peripheral circulation, exhaled breath, or other surrogate biospecimens, to more accurate molecular imaging techniques, we will soon inform patients and providers about individualized risk of disease and its progression. We expect that the integration of clinical, imaging, and molecular variables will translate into an accurate and rapid estimation of disease probability. These advances will translate into tools that should outperform current diagnostic algorithms, and this will lead to improved decision making with patients in the clinic. This research should reduce invasive procedures for suspicious nodules, reduce overdiagnosis and overtreatment of indolent tumors, save health care costs, and reduce patient anxiety.

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Fig. 1.
Early detection biomarker tests in the continuum of care.

Fig. 2.

Metric for evaluation of clinical use of candidate biomarkers. Proposed metric for success is suggested for the level at or above the red arrows.

Table 1

Prediction models for the noninvasive diagnosis of suspicious lung nodules

	Mayo Clinic model (Swensen)	VA model (Gould)	McWilliams–Tammemagi model	Treat model
Cohort characteristics	Males and females from single center in the United States; lung malignancy prevalence of 23%	Male veterans; lung malignancy prevalence of 54%	Pan-Canadian Early Detection of Lung Cancer Study (PanCan), validation dataset from chemoprevention trials at the British Columbia Cancer Agency; prevalence of cancer of 5.5 and 3.7%, respectively	Development and internal validation at VUMC. External validation in the Nashville VAMC cohort. Prevalence of cancer 72%
Independent predictors of lung cancer	Age, smoking history, history of cancer, nodule size, spiculation, upper lobe location	Age, smoking history, time since quit smoking, nodule size	Age, female sex, family history of lung cancer, emphysema, nodule size, upper lobe location, part-solid nodule type, lower nodule count, spiculation	Age, gender, BMI, COPD, PKY of smoking, hemoptysis, previous cancer, nodule size, nodule growth, shape, location, and FDG PET avidity
AUC in external validation	0.83	0.79	0.94; for lung nodules < 10 mm, AUC remained > 0.90	0.89
Source	Swensen et al (1997) ³³	Gould et al (2007) ³⁴	McWilliams et al (2013) ³⁵	Deppen et al (2014) ³⁶

Abbreviations: AUC, area under the curve; BMI, body mass index; COPD, chronic obstructive pulmonary disease; FDG, fluorodeoxyglucose; PET, positron emission tomography; PKY, pack years; VAMC, Veterans Affairs Medical Center; VUMC, Vanderbilt University Medical Center.

Table 2

Commercially available biomarker for early detection of lung cancer

Year	Company	Test name	Biomarkers	FDA approval	Source
2010	Oncimmune	EarlyCDT-Lung	Panel of seven autoantibodies	CLIA-certified laboratory	Chapman et al (2012). EarlyCDT@-Lung test: improved clinical utility through additional autoantibody assays. <i>Tumor Biol</i> 33(5):1319–1326 Jett et al (2014) ⁵⁰
2012	Genesys Biolab	PAULA test (Protein Assays Utilizing Lung Cancer Analytes)	Panel of three autoantibodies and one tumor antigen	CLIA-certified laboratory	Doseeva et al (2015). Performance of a multiplexed dual analyte immunoassay for the early detection of non-small cell lung cancer. <i>J Transl Med</i> 13:55
2014	Integrated Diagnostics, Inc.	Xpresys Lung	Panel of 13 nonantibody proteins		Vachani (2015). Validation of a multi-protein plasma classifier to identify benign lung nodules. <i>J Thorac Oncol</i> 10 (4):629–637 Vachani et al (2015) ⁶⁴
2015	Global Cancer Diagnostics, Inc.	The Lung Cancer Test		CLIA-certified laboratory	
2014	Veracyte	Percepta	Panel of 23 bronchial epithelial cell sRNA	CLIA-certified laboratory	Silvestri et al (2015) ⁴⁹ Vachani et al (2016). Clinical utility of a bronchial genomic classifier in patients with suspected lung cancer. <i>Chest</i> 150:210–218

Abbreviations: CLIA, Clinical Laboratory Improvement Amendments; FDA, Food and Drug Administration.

Table 3
Candidate biomarkers along the Early Detection Research Network proposed development guidelines

Phase of biomarker development	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
	Preclinical exploration and discovery: identify possible biomarkers	Assay validation: study biomarkers in tumor and nontumor tissues	Retrolongitudinal: use biomarkers to detect preclinical disease	Prospective screening: apply biomarkers as screening tool to detect disease	Cancer control: do biomarkers reduce cancer mortality?
Serum/plasma					
MALDI TOF MS profiling	X	X	X		
Autoantibodies	X	X	X	X	
Specific antigens, proteins	X	X	X		
miRNA	X	X	X		
DNA methylation	X	X			
Circulating tumor cells	X	X			
Tumor/airway epithelium					
Preinvasive histopathology/cytology	X	X	X		
DNA methylation	X	X	X		
RNA airway signature	X	X	X	X	
Proteomic profiling	X	X	X	X	
Chromosome aberrations	X	X			
Sputum/EBC					
DNA methylation	X	X	X		
DNA CN—FISH	X	X			
VOCs	X	X			

Abbreviations: CN, copy number; EBC, exhaled breath condensate; FISH, fluorescence in situ hybridization; MALDI TOF MS, matrix-assisted laser desorption/ionization/time-of-flight mass spectrometry profiling; miRNA, microRNA; MS, mass spectrometry; VOCs, volatile organic compounds.