Endobronchial Biomarkers of Lung Cancer

Emerging Strategies to Diagnose Early Stage Disease

John T. Battaile, M.D., M.Sc.

Internal Medicine Grand Rounds
University of Texas Southwestern Medical Center

February 10, 2012

Biographical Information

John T. Battaile, M.D., M.Sc.
Assistant Professor of Medicine
Fellowship Program Director
Division of Pulmonary and Critical Care Medicine
Department of Internal Medicine
University of Texas Southwestern Medical Center
Dallas VA Medical Center

Research Interests:

Endobronchial lung cancer biomarkers
Airway inflammation in COPD
Impact airway inflammation on clinical outcomes in COPD

I. Introduction

Lung cancer is the leading cause of cancer death in the United States and worldwide, and remains an urgent public health concern. In 2010 it is estimated that 28% of all cancer deaths were due to lung cancer, roughly equal to the number of deaths from cancers of the prostate, breast, colon, rectum, and pancreas combined [1] . Smoking is the principle risk factor for lung cancer, yet less than 15% of smokers will develop the disease while some who have never smoked will die from lung cancer [2]. For these reason and many others, public health initiatives targeting tobacco smoke avoidance to prevent lung cancer have not had sufficient impact on the lung cancer's imposing toll. The persistently high mortality rate from lung cancer is due in large measure to the fact that most lung cancer patients are diagnosed with advanced disease, which typically translates into a dismal prognosis [3]. Newer strategies to improve outcomes in individuals diagnosed with lung cancer are therefore desperately needed. Specifically, major advances in screening and early diagnosis are required to allow detection of the disease at a potentially curable stage.

Efforts to enhance early diagnosis of lung cancer have focused on effective screening of at risk individuals. However, despite being a priority since at least the 1970's, no lung cancer screening strategies have been widely implemented to reduce lung cancer mortality, and no consensus guidelines endorse a specific screening approach. Fortunately, a major change in lung cancer outcomes may be on the horizon. The National Lung Screening Trial [4], published within the last year, showed for the first time that screening has the potential to reduce lung cancer mortality. At the same time advances in biological markers of lung cancer, particularly alterations in gene expression that occur in the endobronchial mucosa in response to lung cancer, have raised hopes that an effective and reliable strategy for detecting early lung cancer can be implemented in the near future. In that context, the goals of this presentation are to:

1. Define the scope of the public health problem posed by lung cancer, including a description of the unacceptably high mortality rates associated with lung cancer and the reasons behind the lack of progress over the past 30 years to reduce lung cancer mortality.

2. Describe the efforts to develop effective lung cancer screening, and understand the reasons that most of those efforts have failed.

3. Detail the recent advances in the use biological markers of lung cancer, and understand how these techniques might be used in conjunction with newer screening modalities to achieve early lung cancer diagnosis, with a goal of reducing lung cancer mortality.

II. Death from Lung Cancer Remains an Urgent Public Health Concern

In 1971 Richard Nixon signed the National Cancer Act, which endowed the National Cancer Institute with special budgetary authority to, as Nixon put it in his 1971 State of the Union Address, "launch an intensive campaign to find a cure for cancer." The signing of the National Cancer Act is said to have started the "War on Cancer" which has seen remarkable

advances in the treatments and outcomes of many cancers. Between 1975 and 2005, 5-year survival rates from all cancers has increased from 50% to 68% [1]. Cancers that even today have relatively low 5-year survival rates, such as cancer of the esophagus, have seen significant improvements in survival during that time. Lung cancer is a tragic exception to this trend. While the overall death rate from lung cancer has decreased in males due to decreased prevalence of smoking, the 5-year mortality from lung cancer has not significantly changed in 30 years.

Lung cancer kills more people in the US and worldwide than any other cancer, killing an estimated 157,000 Americans in 2010. By comparison, breast cancer, which has annual incidence rates similar to that of lung cancer, killed nearly 40,000 Americans in the same year. While numerous factors contribute, the dramatic difference in outcomes between the two diseases can in part be explained by the stark differences in stage at diagnosis - over half of lung cancers are diagnosed at an advanced stage compared to fewer than 10% of breast cancers (figure 1). In 1975 the 5-year survival after a diagnosis of lung cancer was 13%. Today it is essentially unchanged at around 16%. However, the overall 5-year survival rates for lung cancer increase to 70% or higher when lung cancer is detected at its earliest stages. It is, therefore, fair to say that stage at diagnosis largely dictates survival after lung cancer diagnosis [5]. For that reason, efforts to improve screening and early diagnosis in patients at risk of lung cancer remain a very high priority.

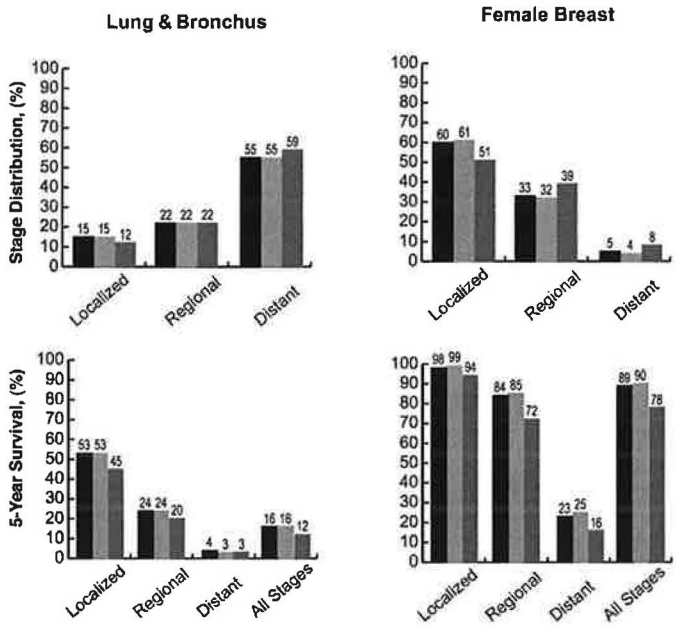


Figure 1. Clinical stage at diagnosis and 5-year survival in cancers of the lung and the breast. Over 70% of lung cancers have spread to regional lymph nodes or beyond by the time of diagnosis; whereas, most breast cancers are diagnosed at a localized stage. 5-year survival after a diagnosis of lung cancer drops precipitously if regional or distant spread of the disease has occurred at the time of diagnosis. Shaded bars within each stage represent racial differences. Modified from reference (1).

III. Lung Cancer Screening

A. Chest Radiography

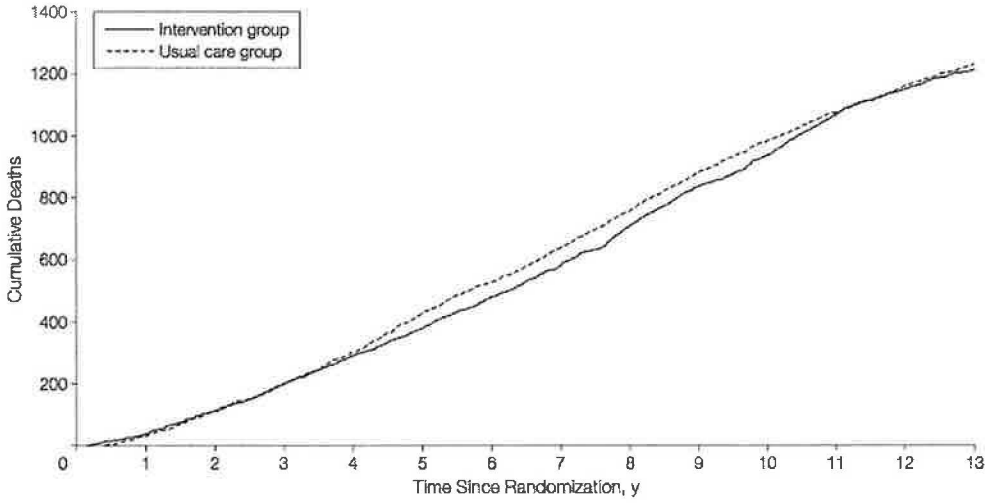
Three landmark studies of chest radiography for lung cancer screening were conducted in the 1970's and published in the 1980's as part of a cooperative study funded by the National Cancer Institute [6-9]. There were important difference among the three studies, but the conclusions were the same: regular screening with chest radiography, with or without sputum cytology, does not reduce lung cancer mortality. This conclusion remained conventional wisdom despite important limitations of the studies: the exclusion of women, relatively few subject for a cancer screening study, ensuing advances in surgical and non-surgical cancer treatment that might augment observed survival differences, and the fact that only one of the three studies meaningfully compared screening chest radiography to a control group without chest radiography screening (Table 1).

Study	Year	Design	Subjects	Screen	Mortality Benefit
Mayo Lung Project	1986	RCT	male smokers age > 45 *n* > 9000	CXR, sputum cytology q 4mo x 6 years vs "Normal care"	No
Johns Hopkins Lung Project	1985	RCT	male smokers age > 45 *n* > 10,000	Yearly CXR plus sputum cytology q 4mo vs Yearly CXR	No
Sloan Kettering Study	1984	RCT	male smokers age > 45 *n* > 10,000	Yearly CXR plus sputum cytology q 4mo vs Yearly CXR	No
Czech Study	1990	RCT	male smokers 40-64 *n* > 6000	CXR, sputum cytology q 6mo x 3 years vs "No asymptomatic investigation"	No

Table 1. Summary of previous randomized trials evaluating lung cancer screening with chest radiography.

Because of these limitations and to definitively resolve the role of chest radiography the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial randomized over 155,000 men and women to receive either annual chest radiography screening for lung cancer or "usual care" for a period of 4 years with a subsequent follow-up period of up to 13 years total [10, 11]. Because PLCO did not deal exclusively with lung cancer, the risk profiles of the participants varied, with each group comprised of approximately 45% never smokers. As expected a similar number of cancers were diagnosed in the two groups after the follow-up period. However, the stage at diagnosis among non-small cell cancers was similar across the two groups suggesting that screening with chest radiography did not result in a substantial increase in the detection of early stage cancers. Not surprisingly, then, lung cancer mortality was not

different between the intervention and usual care groups (figure 2), leading most to conclude that "[t]he PLCO lung cancer study result provides convincing evidence that lung cancer screening with chest radiography is not effective [12]."



Intervention group													
Cumulative deaths	36	113	196	292	378	480	582	711	838	937	1070	1150	1213
Cumulative person-years	77268	154053	230270	305833	380691	454773	527937	600004	670274	735098	789540	832441	864227
Usual care group													
Cumulative deaths	30	111	198	301	426	527	639	761	884	987	1076	1162	1230
Cumulative person-years	77286	154116	230348	305902	380725	454719	527804	599790	669955	734523	788854	831678	863330

Figure 2. Lung cancer mortality by year in the PLCO randomized trial of lung cancer screening with chest radiography. Taken from reference (10).

B. Screening with Low Dose Computed Tomography

Diagnostic computed tomography offers several potential advantages over chest radiography for lung cancer screening. Enhanced contrast between the lung parenchyma and tumor nodules allows visualization of more subtle lesions, while the cross-sectional nature of CT can reveal lesions that might be obscured by overlying structures on plain films [13]. However, the increased sensitivity of CT can lead to a series of potential problems such as an increase in false positive results that might initiate invasive evaluations associated with significant cost and risk. These issues plus concerns about the relatively high radiation doses with diagnostic CT have limited its appeal as a screening modality. Beginning in the late 1990's interest began to focus instead on low-dose CT for lung cancer screening as several studies confirmed its ability to detect early cancerous lesions while limiting radiation doses to around 20-25% that of diagnostic CT [14-20].

Starting in 2002 the National Cancer Institute (NCI) funded the National Lung Screening Trial (NLST) to determine whether screening with low dose CT leads to a reduction in lung

cancer mortality when compared to screening with chest radiographs. Participants, consisting of more than 53,000 current or former smokers between the ages of 55 and 74, were randomized to screening with either low dose CT or chest radiography [13]. Screening was performed at the time of enrollment then yearly for the subsequent two years followed by a period of follow-up without screening. The primary endpoint was death from lung cancer. Secondary endpoints included lung cancer incidence, stage at diagnosis, and all-cause mortality. NLST investigators chose to compare low dose CT to screening chest radiography instead of routine care because of the ongoing PLCO trial described above. They reasoned that if the PLCO trial showed a difference between chest radiography and routine care, then any benefit of low dose CT over routine care would be difficult to interpret.

Results from NLST, first reported by NCI in November 2010 and then published in full in August 2011, seemed promising [4]. More cancers were diagnosed in the CT screening group (figure 3A), and these cancers were more likely to be stage IA or IB and less likely to be stage IV than those diagnosed in the control group. This translated into a 20% relative reduction in mortality (figure 3B) with low dose CT screening, marking the first time that a randomized controlled trial of lung cancer screening has demonstrated a mortality benefit. However, screening with low dose CT has not been widely adopted or recommended by any national society including the National Cancer Institute because of unresolved concerns about the economic and health costs associated with positive screens [21], among other concerns. Approximately one quarter of screening CTs were screen positive in NLST. Of those, 96% were falsely positive and 7% of the false positive screens led to an invasive work-up. Data from NLST suggest that the invasive evaluations were relatively safe, with complications occurring at a rate of less than 2%, yet the risk of complications and the cost associated with additional diagnostic evaluation, invasive or not, raises a major concern. Add to that the cost associated with performing and interpreting the CT itself, and it is easy to understand why no consensus has yet emerged regarding the cost effectiveness of low-dose CT for lung cancer screening.

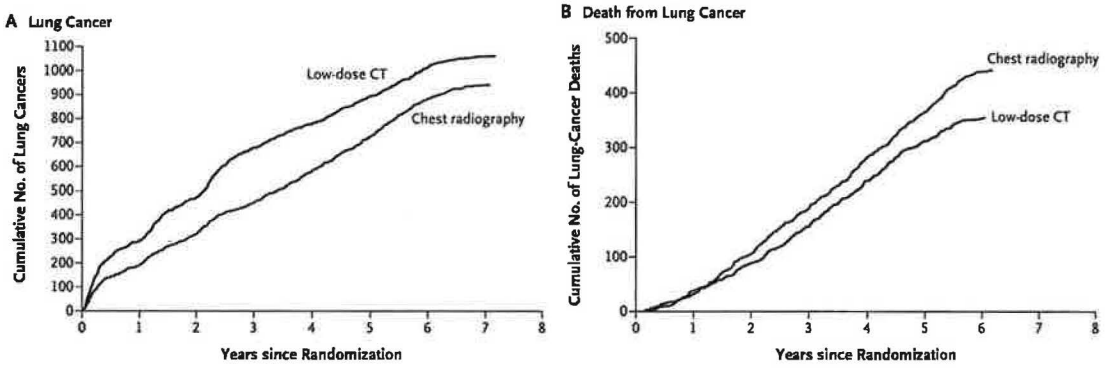


Figure 3. **A)** Cumulative number of lung cancers diagnosed in subjects enrolled in NLST screened by low-dose CT and chest radiography. **B)** Fewer lung cancer deaths in NLST participants screened by low-dose CT compared to those screened using chest radiography. Taken from reference (4).

Overdiagnosis is an important concept in cancer screening. Overdiagnosis occurs when cancers that would otherwise remain clinically occult, either through slow growth or regression of the tumor, are diagnosed through screening. In the absence of overdiagnosis, one would expect that the number of cancers diagnosed after a period of follow-up would be similar in the CT and chest radiography groups in NLST. In other words, cancers missed through screening in the chest radiography group should become clinically evident during the follow up period so that the numbers of cancers in the two groups would be similar by the end of the study. Otherwise, one might reasonably suspect that some of the cancers diagnosed by CT would never have become clinically evident, indicating overdiagnosis by CT. Overdiagnosis probably did occur in NLST, but the extent of overdiagnosis and its relevance to the decision to adopt low dose CT screening is not yet clear, adding further uncertainty to the cost-effectiveness of low-dose CT screening.

IV. Endobronchial Biomarkers of Lung Cancer

NLST succeeded in proving the concept that lung cancer screening can improve survival by detecting disease at an early, more treatable stage. However, the cost and risk associated with CT's high false positive rate and the potential for overdiagnosis of lung cancer have prevented its widespread adoption in clinical practice. If CT screening is to be implemented, strategies will need to be developed to better predict which screen-detected lesions represent cancer and which of those are likely to progress. Biological markers of lung cancer are actively being studied to do just that [22].

In its most general sense a biological marker, or biomarker, is any substance that can be detected or measured as an indicator of a disease state or the risk for development of a disease. The term biomarker originally referred to proteins, but increasingly other metrics including alterations in gene expression have been used as biomarkers. A desirable biomarker is one that is accurate, can be easily measured, and provides information to the clinician beyond what is available through existing tests [23]. A marker of lung cancer that fulfils those criteria would be a major breakthrough, especially if it allowed detection of cancer at its earliest stages. Potential lung cancer biomarkers have been identified in the sputum, blood, and exhaled breath and will be discussed briefly. However, endobronchial biomarkers of lung cancer may allow the most accurate means of detecting early stage or even pre-cancerous disease, and will be discussed in detail.

A. Lung Cancer Biomarkers in the Blood, Sputum, and Breath

Various serum markers of lung cancer have been investigated since the 1990's. Early studies focused on correlating markers from the tumors themselves with serum levels of the same proteins. Carcinoembryonic antigen (CEA), squamous cell carcinoma antigen (SCC) [24-26], cytokeratin-19 fragment, and neuron-specific enolase [25] have been evaluated, among others. None of the serum tests demonstrated sufficient sensitivity and specificity for clinical use. For example, the sensitivity of CEA is around 30% at a specificity of 95%. Others focused on detecting genetic alterations in DNA isolated from the blood as indicator of cancer status in the

lung [27] in addition to other markers [28]. Subsequent advances in proteomics and mass spectrometry techniques have allowed for a more sophisticated and potentially more accurate approach to detecting lung cancer through serum biomarkers [29-31]. One group has recently reported that a panel of seven serum peptides measured by matrix-assisted laser desorption ionization mass spectrometry (MALDI MS) is capable of distinguishing lung cancer from matched controls with an accuracy of 72.6% [32]. While not ready for clinical application, with further refinement serum biomarkers may soon be useful tools in the early detection of lung cancer.

Sputum biomarkers have also proved capable of discriminating between individuals with and without lung cancer, albeit with insufficient accuracy for clinical use to this point [33, 34]. In a cohort of patients with a diagnosis of squamous cell carcinoma from whom sputum samples had been previously collected, methylation in the promoter region of at least one of two genes was present in the sputum of all patients. However, many high-risk patients without lung cancer also demonstrated promoter methylation in the same genes, diminishing the utility of promoter methylation as a clinical marker. Others have shown that microRNAs (miRNAs), small non-coding RNA sequences that regulate gene expression, are differentially expressed in certain cancers including cancer of the lung and can be detected in sputum as a marker of lung cancer [35]. The clinical utility of miRNAs is limited by, among other issues, poor sensitivity on par with sputum cytology. Finally, volatile organic compounds in exhaled breath samples have also shown promise as markers for lung cancer, but have not been adopted in clinical practice [36, 37].

B. Endobronchial Biomarkers of Lung Cancer

The term endobronchium refers to the mucosal lining of the large, cartilagenous conducting airways, or bronchi. These airways are lined predominantly with a ciliated, columnar, psuedostratified epithelium that also includes mucus-producing goblet and other cells. The endobronchium functions as much more than a simple conduit for air. The bronchial epithelium serves numerous known functions and likely many as yet unknown functions. For example, in addition to its role in mucociliary clearance, the bronchial mucosa is capable of initiating an epithelial immune/inflammatory response to various noxious stimuli. In some cases, this response is adaptive such as in the body's response to airway infections. In other instances this response can become problematic such as in patients with mucous hypersecretion in asthma and COPD. Recent evidence suggests that otherwise normal endobronchial mucosa also undergoes profound alterations in response to lung processes that are occurring at site far removed from the bronchi, including in patients with lung cancer. This understanding has led to the appreciation of "airway-wide fields of injury," a concept central to the development of endobronchial biomarkers for lung cancer.

1. Airway-Wide Fields of Injury

Recognition of airway-wide fields of injury came with the discovery that various molecular changes in the bronchial mucosa occur diffusely in response to localized injury elsewhere within the lung. The airways of smokers, for example, exhibit a variety of genetic changes despite the absence of observable histological abnormality. In one report from investigators at this institution, researchers obtained endobronchial biopsies from a group of smokers and non-smokers. They found that smokers, whether current or former, had a high frequency of genetic changes including loss of heterozygosity similar to genetic alterations found in lung cancer tissue [38]. These changes were present even in histologically normal areas and persisted after smoking cessation. A separate group demonstrated a similar finding in patients with lung cancer. Specifically, loss of heterozygosity was present even in uninvolved airways in the majority of subjects with lung cancer as well as in current and former smokers without cancer [39]. Other abnormalities including telomerase expression [40], promoter hypermethylation [41], and oncogene mutations [42] have also been described as field effects involving normal airways in patients with lung cancer. Airway-wide fields of injury have also been described in other diseases of the lung, including COPD. Together, these findings confirm that abnormal processes in one part of the lung can affect changes in remote, otherwise normal areas of the lung. This phenomenon is the basis for the development of endobronchial biomarkers for lung cancer.

More recent work has furthered our understanding of airway-wide fields of injury and has refined the tools by which these changes can be detected. For example microRNAs (miRNAs) - small noncoding RNAs that regulate gene expression with a role in many processes including carcinogenesis [43, 44] - have been shown to participate in the regulation of the bronchial epithelial response to cigarette smoke [45]. One miRNA in particular was down-regulated four-fold in the airways of smokers, and knockdown of that miRNA *in vivo* abrogated the usual epithelial response to cigarette smoke *in vitro*. In other work evaluating genome-wide mRNA expression in healthy individuals, investigators identified nearly 100 genes that are differentially expressed in the airways of current smokers compared to never smokers [46, 47]. A similar pattern was observed in former smokers who had quit smoking within the prior two years, indicating that the epithelial response to cigarette smoke is persistent [48]. These findings suggest that alterations in gene expression occur as part of an airway wide field of injury and raise the possibility that specific gene expression signatures within the bronchial mucosa could be developed to detect remote pathology in the lung, including lung cancer.

2. Endobronchial Gene Expression Signatures Can Detect Lung Cancer

Flexible bronchoscopy is a minimally invasive technique commonly used in the evaluation of patients with suspected lung cancer, but it has certain limitations. For centrally positioned lesions the sensitivity of bronchoscopy is 80% or higher, especially for more advanced disease. But for the evaluation of peripheral lung lesions, the sensitivity drops to 30% or less [49], and many peripheral lung lesions especially those less than 1 cm are typically not considered accessible by bronchoscopy at all. For that reason patients with suspected early stage lung cancer on CT often cannot be definitively diagnosed bronchoscopically and typically must

endure more invasive testing that could delay treatment and pose additional risks. In contrast, access to the mainstem bronchus can be easily and safely achieved with a bronchoscope with few risks to the patients.

Exploiting the principle of airway-wide fields of injury, investigators have begun to explore the possibility that gene expression changes in normal cells from the mainstem bronchus might serve as a surrogate for detecting distant lung cancer, even in its earliest stages. The rationale for this approach rests on the hypothesis that alterations in gene expression in the bronchial epithelium in response to lung cancer occur early, perhaps even before the development of clinically detectable cancer. In addition to occurring earlier, gene expression changes within the lung may also be a more accurate surrogate for lung pathology than changes detected in the blood or other tissues. Otherwise, biological markers that could be obtained less invasively might be more desirable targets.

One particularly well-executed study of endobronchial lung cancer biomarkers has yielded promising results [50]. Investigators developed a panel of 80 genes to reliably discriminate between individuals with and without lung cancer by first performing gene expression profiling of bronchial epithelial cells obtained from the right mainstem bronchus in a subset of patients undergoing bronchoscopy for suspicion of lung cancer. They identified the 40 most up-regulated and the 40 most down-regulated genes in patients who were ultimately diagnosed with lung cancer compared to those without cancer. This panel of 80 genes was then tested to assess its accuracy in a separate group of patients with suspected lung cancer. The accuracy of the gene panel in discriminating between patients with and without lung cancer was 83%, with a sensitivity of 80% and specificity of 84%. In contrast bronchoscopy alone had a sensitivity of only 53% in diagnosing lung cancer. Importantly, the ability of the gene panel to predict lung cancer was not influenced by the location of the cancer relative to the right mainstem bronchus, where the samples were obtained.

The biomarker panel was then validated prospectively in a group of 35 smokers with suspected lung cancer. Of these, 18 were ultimately diagnosed with lung cancer. Lung cancer was excluded in the remaining 17. Combining bronchoscopy with the gene expression biomarker resulted in a sensitivity of 94%, specificity of 76%, negative predictive value of 93%, and positive predictive value of 81% in the diagnosis of lung cancer (figure 4). The strength of these findings rests in the negative predictive value of combing bronchoscopy with gene expression analysis. In clinical practice, except when an alternate diagnosis is established, a negative bronchoscopy does not exclude the presence of lung cancer especially when targeting a small peripheral lesion. Many patients in this situation are referred for surgical biopsy with considerable attendant risk [51]. Others are not candidates for surgical biopsy due to coexisting lung disease. This study suggests that by combining a gene expression biomarker with conventional, minimally invasive bronchoscopy, it may be possible to safely rule out cancer and avoid additional invasive testing.

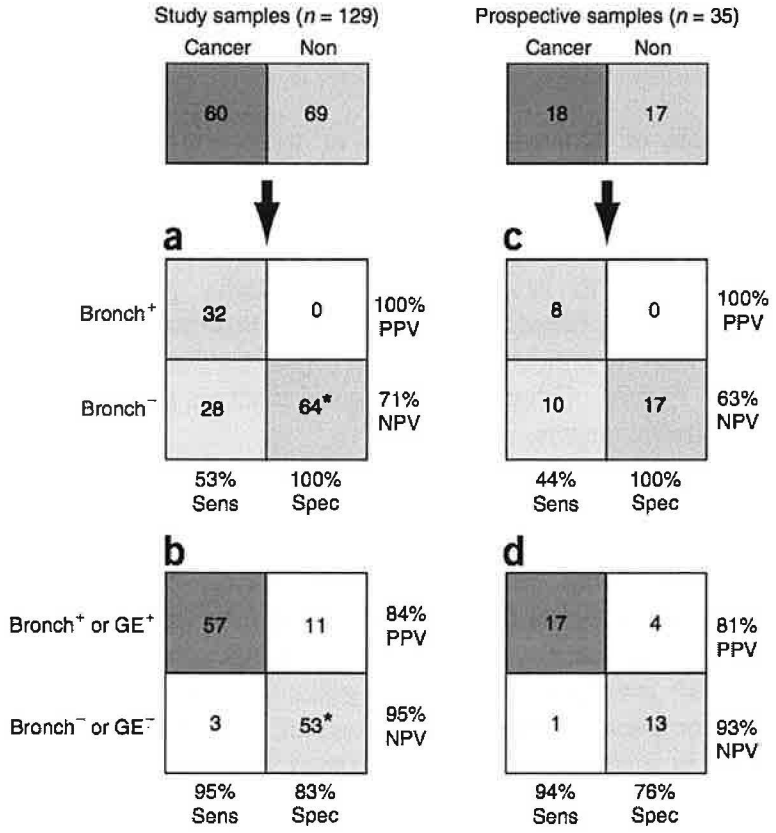


Figure 4. Performance of an endobronchial gene expression biomarker panel in predicting lung cancer among patients undergoing bronchoscopy for suspected lung cancer.

Another advantage of this approach is its potential to be incorporated into a low-dose CT screening strategy to address the principle limitation uncovered by NLST - that the overwhelming majority of positive screening CT's are false positives, and there is not a reliable way to determine radiographically which lesions represent cancer. If a gene expression signature can be refined to discern early stage lung cancer from benign lesions among patients with suspicious pulmonary nodules, then the cost effectiveness of large-scale low-dose CT screening for lung cancer might become feasible by drastically reducing the number of screen positive patients who must undergo additional invasive work-up. Likewise, the same gene signature might be capable of identifying which high-risk patients without lesions on CT are likely to develop lung cancer later.

3. Detection of Early Lung Cancer Among Military Personnel (DECAMP)

As with the general population smoking-related lung diseases, especially lung cancer, are a serious problem among military personnel and veterans. The smoking rate among military personnel is over 30% according to a 2005 Department of Defense report, significantly higher than that of the general population. Other inhaled exposures to lung carcinogens such as radon, asbestos, and depleted uranium are also more common among military personnel. It is not unexpected, then, that the cancer incidence rates among United States Veterans is 25-75% higher than that of the general population [52, 53], with prevalence rates as much as twice as high as that of the general population by some estimates [54].

In response to these statistics, the Department of Defense established the Lung Cancer Research Program (LCRP) in 2009 to fund research aimed at "the development of integrated components to identify, treat, and manage early curable lung cancer" (http://cdmrp/army.mil/lcrp/). One project funded through the LCRP, Detection of Early Lung Cancer Among Military Personnel (DECAMP), aims to develop and validate endobronchial and other biomarkers for the early detection of lung cancer among US Veterans and military personnel. DECAMP is a multi-center effort coordinated by the Translational Bioinformatics Program at Boston University in conjunction with the American College of Radiology Imaging Network (ACRIN) [55]. Study participants will be recruited from seven VA Hospitals - in Boston, Los Angeles, Philadelphia, Denver, Pittsburgh, Nashville, and Dallas - and 4 military hospitals in an effort to refine and validate a panel of endobronchial and blood biomarkers capable of distinguishing which high risk patients will go on to develop lung cancer. One arm of the study will enroll former or current smokers with a solitary pulmonary nodule on CT, allowing an assessment of the added diagnostic value of the biomarker to CT. With that information an integrated model of clinical, radiographic, and molecular data to reliably detect early stage lung cancer can be developed. A separate arm of the study will validate the ability of endobronchial and serum biomarkers to detect preclinical lung cancer among at-risk individuals without radiographic findings concerning for lung cancer. Ultimately, these biomarkers could be incorporated into other modalities, such as low-dose CT screening, to detect early lung cancer and reduce lung cancer mortality.

IV. Conclusions: The Future of Early Lung Cancer Detection

Recent advances in lung cancer screening have provided renewed hope that detecting early lung cancer through screening can save lives. However, low-dose CT screening has not yet been widely adopted in part because imaging alone cannot distinguish between benign and malignant lesions, leading to frequent false positive screens, potentially risky diagnostic evaluation, and excessive cost. Efforts like DECAMP and other studies of lung cancer biomarkers are an important step toward developing a strategy to complement CT screening to detect early lung cancer in a safe and cost-effective manner. In one potential scenario we could soon see annual screening of high-risk individuals with low-dose CT, followed by endobronchial and/or serum biomarker profiling to determine who will require more thorough evaluation and who can be followed conservatively. Whatever form screening for lung cancer ultimately takes, it is likely to involve an integrated approach incorporating chest imaging with biological markers with the potential to save tens of thousands lives each year in the US alone.

References:

1. Jemal, A., et al., *Cancer Statistics, 2010.* CA Cancer J Clin, 2010. 60(5): p. 277-300.
2. Jemal, A., et al., *Annual report to the nation on the status of cancer, 1975-2005, featuring trends in lung cancer, tobacco use, and tobacco control.* J Natl Cancer Inst, 2008. 100(23): p. 1672-94.
3. Jemal, A., et al., *Global patterns of cancer incidence and mortality rates and trends.* Cancer Epidemiol Biomarkers Prev, 2010. 19(8): p. 1893-907.
4. Aberle, D.R., et al., *Reduced lung-cancer mortality with low-dose computed tomographic screening.* N Engl J Med, 2011. 365(5): p. 395-409.
5. Salomaa, E.R., et al., *Delays in the diagnosis and treatment of lung cancer.* Chest, 2005. 128(4): p. 2282-8.
6. Fontana, R.S., et al., *Lung cancer screening: the Mayo program.* J Occup Med, 1986. 28(8): p. 746-50.
7. Frost, J.K., et al., *Early lung cancer detection: results of the initial (prevalence) radiologic and cytologic screening in the Johns Hopkins study.* Am Rev Respir Dis, 1984. 130(4): p. 549-54.
8. Melamed, M.R., et al., *Screening for early lung cancer. Results of the Memorial Sloan-Kettering study in New York.* Chest, 1984. 86(1): p. 44-53.
9. Berlin, N.I., et al., *The National Cancer Institute Cooperative Early Lung Cancer Detection Program. Results of the initial screen (prevalence). Early lung cancer detection: Introduction.* Am Rev Respir Dis, 1984. 130(4): p. 545-9.
10. Oken, M.M., et al., *Screening by chest radiograph and lung cancer mortality: the Prostate, Lung, Colorectal, and Ovarian (PLCO) randomized trial.* JAMA, 2011. 306(17): p. 1865-73.
11. Hocking, W.G., et al., *Lung cancer screening in the randomized Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial.* J Natl Cancer Inst, 2010. 102(10): p. 722-31.
12. Sox, H.C., *Screening for lung cancer with chest radiographs.* JAMA, 2011. 306(17): p. 1916-8.
13. Aberle, D.R., et al., *The National Lung Screening Trial: overview and study design.* Radiology, 2011. 258(1): p. 243-53.
14. Henschke, C.I., et al., *Early Lung Cancer Action Project: overall design and findings from baseline screening.* Lancet, 1999. 354(9173): p. 99-105.
15. Henschke, C.I., et al., *Early lung cancer action project: initial findings on repeat screenings.* Cancer, 2001. 92(1): p. 153-9.
16. Sone, S., et al., *Results of three-year mass screening programme for lung cancer using mobile low-dose spiral computed tomography scanner.* Br J Cancer, 2001. 84(1): p. 25-32.
17. Sobue, T., et al., *Screening for lung cancer with low-dose helical computed tomography: anti-lung cancer association project.* J Clin Oncol, 2002. 20(4): p. 911-20.
18. Swensen, S.J., et al., *Screening for lung cancer with low-dose spiral computed tomography.* Am J Respir Crit Care Med, 2002. 165(4): p. 508-13.
19. Diederich, S., et al., *Screening for early lung cancer with low-dose spiral CT: prevalence in 817 asymptomatic smokers.* Radiology, 2002. 222(3): p. 773-81.

20. Nawa, T., et al., *Lung cancer screening using low-dose spiral CT: results of baseline and 1-year follow-up studies.* Chest, 2002. 122(1): p. 15-20.
21. Sox, H.C., *Better evidence about screening for lung cancer.* N Engl J Med, 2011. 365(5): p. 455-7.
22. Hirsch, F.R., D.T. Merrick, and W.A. Franklin, *Role of biomarkers for early detection of lung cancer and chemoprevention.* Eur Respir J, 2002. 19(6): p. 1151-8.
23. Morrow, D.A. and J.A. de Lemos, *Benchmarks for the assessment of novel cardiovascular biomarkers.* Circulation, 2007. 115(8): p. 949-52.
24. Pastor, A., et al., *Diagnostic value of SCC, CEA and CYFRA 21.1 in lung cancer: a Bayesian analysis.* Eur Respir J, 1997. 10(3): p. 603-9.
25. Seemann, M.D., et al., *An evaluation of the tumour markers, carcinoembryonic antigen (CEA), cytokeratin marker (CYFRA 21-1) and neuron-specific enolase (NSE) in the differentiation of malignant from benign solitary pulmonary lesions.* Lung Cancer, 1999. 26(3): p. 149-55.
26. Kulpa, J., et al., *Carcinoembryonic antigen, squamous cell carcinoma antigen, CYFRA 21-1, and neuron-specific enolase in squamous cell lung cancer patients.* Clin Chem, 2002. 48(11): p. 1931-7.
27. Andriani, F., et al., *Detecting lung cancer in plasma with the use of multiple genetic markers.* Int J Cancer, 2004. 108(1): p. 91-6.
28. Yee, J., et al., *Connective tissue-activating peptide III: a novel blood biomarker for early lung cancer detection.* J Clin Oncol, 2009. 27(17): p. 2787-92.
29. Chaurand, P., M. Stoeckli, and R.M. Caprioli, *Direct profiling of proteins in biological tissue sections by MALDI mass spectrometry.* Anal Chem, 1999. 71(23): p. 5263-70.
30. Rahman, S.M., et al., *Lung cancer diagnosis from proteomic analysis of preinvasive lesions.* Cancer Res, 2011. 71(8): p. 3009-17.
31. Hassanein, M., et al., *Advances in proteomic strategies toward the early detection of lung cancer.* Proc Am Thorac Soc, 2011. 8(2): p. 183-8.
32. Yildiz, P.B., et al., *Diagnostic accuracy of MALDI mass spectrometric analysis of unfractionated serum in lung cancer.* J Thorac Oncol, 2007. 2(10): p. 893-901.
33. Belinsky, S.A., et al., *Promoter hypermethylation of multiple genes in sputum precedes lung cancer incidence in a high-risk cohort.* Cancer Res, 2006. 66(6): p. 3338-44.
34. Palmisano, W.A., et al., *Predicting lung cancer by detecting aberrant promoter methylation in sputum.* Cancer Res, 2000. 60(21): p. 5954-8.
35. Yu, L., et al., *Early detection of lung adenocarcinoma in sputum by a panel of microRNA markers.* Int J Cancer, 2010.
36. Phillips, M., et al., *Volatile organic compounds in breath as markers of lung cancer: a cross-sectional study.* Lancet, 1999. 353(9168): p. 1930-3.
37. Mazzone, P.J., et al., *Diagnosis of lung cancer by the analysis of exhaled breath with a colorimetric sensor array.* Thorax, 2007. 62(7): p. 565-8.
38. Wistuba, II, et al., *Molecular damage in the bronchial epithelium of current and former smokers.* J Natl Cancer Inst, 1997. 89(18): p. 1366-73.
39. Powell, C.A., et al., *Loss of heterozygosity in epithelial cells obtained by bronchial brushing: clinical utility in lung cancer.* Clin Cancer Res, 1999. 5(8): p. 2025-34.
40. Miyazu, Y.M., et al., *Telomerase expression in noncancerous bronchial epithelia is a possible marker of early development of lung cancer.* Cancer Res, 2005. 65(21): p. 9623-7.

41. Guo, M., et al., *Promoter hypermethylation of resected bronchial margins: a field defect of changes?* Clin Cancer Res, 2004. 10(15): p. 5131-6.
42. Franklin, W.A., et al., *Widely dispersed p53 mutation in respiratory epithelium. A novel mechanism for field carcinogenesis.* J Clin Invest, 1997. 100(8): p. 2133-7.
43. He, L., et al., *A microRNA component of the p53 tumour suppressor network.* Nature, 2007. 447(7148): p. 1130-4.
44. Kumar, M.S., et al., *Impaired microRNA processing enhances cellular transformation and tumorigenesis.* Nat Genet, 2007. 39(5): p. 673-7.
45. Schembri, F., et al., *MicroRNAs as modulators of smoking-induced gene expression changes in human airway epithelium.* Proc Natl Acad Sci U S A, 2009. 106(7): p. 2319-24.
46. Beane, J., et al., *Reversible and permanent effects of tobacco smoke exposure on airway epithelial gene expression.* Genome Biol, 2007. 8(9): p. R201.
47. Spira, A., et al., *Effects of cigarette smoke on the human airway epithelial cell transcriptome.* Proc Natl Acad Sci U S A, 2004. 101(27): p. 10143-8.
48. Spira, A., et al., *Impact of cigarette smoke on the normal airway transcriptome.* Chest, 2004. 125(5 Suppl): p. 115S.
49. Schreiber, G. and D.C. McCrory, *Performance characteristics of different modalities for diagnosis of suspected lung cancer: summary of published evidence.* Chest, 2003. 123(1 Suppl): p. 115S-128S.
50. Spira, A., et al., *Airway epithelial gene expression in the diagnostic evaluation of smokers with suspect lung cancer.* Nat Med, 2007. 13(3): p. 361-6.
51. Smith, M.A., et al., *Prevalence of benign disease in patients undergoing resection for suspected lung cancer.* Ann Thorac Surg, 2006. 81(5): p. 1824-8; discussion 1828-9.
52. Harris, R.E., J.R. Hebert, and E.L. Wynder, *Cancer risk in male veterans utilizing the Veterans Administration medical system.* Cancer, 1989. 64(5): p. 1160-8.
53. Campling, B.G., et al., *A population-based study of lung carcinoma in Pennsylvania: comparison of Veterans Administration and civilian populations.* Cancer, 2005. 104(4): p. 833-40.
54. Crawford, R.S., 3rd, et al., *A study of cancer in the military beneficiary population.* Mil Med, 2007. 172(10): p. 1084-8.
55. Patz, E.F., Jr., et al., *National Lung Cancer Screening Trial American College of Radiology Imaging Network Specimen Biorepository originating from the Contemporary Screening for the Detection of Lung Cancer Trial (NLST, ACRIN 6654): design, intent, and availability of specimens for validation of lung cancer biomarkers.* J Thorac Oncol, 2010. 5(10): p. 1502-6.