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The Forum for Early Diagnosis and Treatment of Lung Cancer

Lung Cancer Frontiers

Comments may be submitted to:
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"The purpose of *Lung Cancer Frontiers* is to acquire and disseminate new knowledge about lung cancer and how it can be most quickly and effectively diagnosed and treated."

Alarming Report: Lung Cancer Called 'Indolent'

A recent report from the annual meeting of the American Society of Preventive Oncology, (Moon MA. Imaging reveals indolent cancers. *Intern Med News* 2000;33:1,5), by Dr. William Black of the Department of Radiology at Dartmouth Medical School in Hanover, New Hampshire stated, "...we do have definite proof that indolent cancers are abundant." Dr. Black cited an article by Takatoshi Aoki stating that "it would take about 8 years for them (lung cancers) to grow from 5 mm to 3 cm. If they hadn't been discovered on high-resolution CT and resected, they would have been around for at least 8 years before causing any problem."

Editor's (TLP) comment: Why lung cancer is considered indolent and innocent, is baffling. But, even if lung cancer is in fact indolent in its early evolutional stages, isn't this a strong argument for early identification of *in situ* and early stage invasive carcinoma? Besides, how does one really know that these tumors are indolent? At what point do they become aggressive and begin to metastasize? To compare with other common malignancies, it is a fact, that adenomatous polyps of the colon are precancerous lesions and they may be indolent for a time. Nonetheless, they are resected to prevent the transition into invasive cancers. Another example is actinic keratosis, the forerunner of invasive squamous carcinoma of the skin. This pre-cancerous lesion is also resected, to prevent more serious stages of disease. Uterine cervical carcinoma *in situ* may be present for years before invasive carcinoma results. This knowledge has led to aggressive use of PAP smear screening, cone biopsy, laser therapy, cryotherapy, or hysterectomy, for *in situ* or early invasive carcinoma. Breast carcinoma screening has altered the course of disease. And so it is, with prostate cancer. So, why is it any different with lung cancer? The paradox continues, the rhetoric increases, and rational action eludes us for reasons that are difficult to understand.

Correlation of Tumor Size and Survival in Patients with Stage IA Non-Small Cell Lung Cancer.

Patz EF, Jr., Rossi S, Harpole DH, Jr., et al. *Chest* 2000;117:1568-1571.

In 510 patients with stage IA non-small cell lung cancer, no significant correlation was found between tumor size and survival. The authors cautioned that improved small nodule detection with screening CT may not significantly improve lung cancer mortality. A prospective randomized trial appears warranted.

Editor's (TLP) comment: This article, hot off the press, is of great interest, not because of its cautions, but because of the documentation of excellent survival of the cohort, i.e., 88% with observations up to 16 years! Isn't this just the point of early identification and intervention? The authors admit that even small tumors may metastasize. Larger tumors may be more indolent. All lung cancers have a fatal potential!

Evolution of Peripheral Lung Adenocarcinomas: CT Findings Correlated with Histology and Tumor Doubling Time.

Aoki T, Nakata H, Watanabe H, et al. *Am J Roentgenol* 2000;174:763-768.

Objective: This study was performed to evaluate the evolution of peripheral lung adeno-

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The Editorial Board of *Lung Cancer Frontiers*, (*LCF*), welcomes Michael T. Johnston, Professor of Surgery at the University of Toronto, and a leader in lung cancer research and treatment, and Fred R. Hirsch, a vigorous cancer researcher and clinician from Copenhagen. We welcome their advice, selection of articles for *LCF*, and commentaries.

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carcinomas using CT findings and histologic classification related to tumor doubling time.

Materials and Methods: The subjects were 34 patients, each with an adenocarcinoma smaller than 3 cm. All patients underwent chest radiography and 10 of them had previously undergone CT more than 6 months before surgery. Tumor doubling time was estimated by examining sequential radiographs using the method originally described by Schwartz. Tumor growth was also observed by studying the changes on CT in the 10 patients who had previously undergone CT. The histologic classification, (types A-F) was evaluated according to the criteria of Noguchi et al.

Results: Five (83%) of the six adenocarcinomas with tumor types A or B showed localized ground-glass opacity on high-resolution CT. All six tumors had a tumor doubling time of more than 1 year. Fifteen (71%) of the 21 tumors with type C showed partial ground-glass opacity mixed with localized solid attenuation on high-resolution CT. Ten (48%) of these 21 type C tumors had a tumor doubling time of more than 1 year. In types B and C, the solid component or the development of pleural indentation and vascular convergence increased during observation before surgery. All seven tumors with types D, E, and F showed mostly solid attenuation, and the tumor doubling time was less than 1 year in six (87%) of the seven tumors.

Conclusion: Two main types of peripheral lung adenocarcinoma exist. The first type appears on CT as a localized ground-glass opacity with slow growth, and the other appears as a solid attenuation with rapid growth.

Editor's (SL) comment: In the U.S., many pathologists would consider Noguchi Type A as *in situ*

carcinoma. Recently, radiographic-pathology correlation showed that "ground-glass opacities" on spiral CT are due to atypical adenomatous hyperplasia which is a pre-malignant lesion (Vazquez MF, Flieder DB. Small peripheral glandular lesions detected by screening CT for lung cancer. *Radiol Clin North Am* 2000;38: 579-589). It is interesting that pre-invasive adenocarcinoma and invasive adenocarcinoma have two different roentgenographic patterns. The longer doubling time of pre-invasive lesions is similar to what has been described for squamous cell carcinoma (Saccomanno G. Carcinoma *in situ* of the lung: Its development, detection, and treatment. *Sem Respir Med* 1982;4:156-160). Further work needs to be done to standardize the classification of pre-invasive adenocarcinoma and to determine if the different radiographic patterns are predictive of the subsequent biological behavior. These are the small tumors that are considered "indolent" in some circles. But, who would wait to watch these small lesions grow with the knowledge that adenocarcinoma is present? In fairness, we certainly must learn more about the behavior of early lesions, particularly in older patients, or patients who are a poor risk for surgical resection or other therapeutic approaches.

"Three postoperative cycles of chemotherapy were planned for patients undergoing complete resection."

Induction Chemotherapy Before Surgery for Early-Stage Lung Cancer: A Novel Approach.

Pisters KMW, Ginsberg RJ, Giroux DJ, et al. *J Thorac Cardiovasc Surg* 2000;119:429-439.

Objective: This phase II trial assessed the feasibility, as measured by response rate, toxicity, resectability rate, and surgical morbidity and mortality rates, of perioperative paclitaxel and carboplatin chemotherapy in patients with early-stage non-small cell lung carcinoma.

"Induction chemotherapy with paclitaxel and carboplatin is feasible and produces a high response rate with acceptable morbidity and mortality rates in early-stage non-small cell lung carcinoma."

Methods: All patients required negative mediastinoscopy results and adequate medical parameters to undergo induction chemotherapy and an operation. Superior sulcus patients were excluded. Chemotherapy consisted of paclitaxel 225 mg/m^2 over 3 hours and carboplatin (area under the curve=6) every 21 days for 2 cycles preoperatively. Three postoperative cycles of chemotherapy were planned for patients undergoing complete resection.

Results: Between June 1996 and July 1998, 94 patients were entered into the study. Sixty-five (69%) were men, and the median age was 64 years (range, 34–79 years). After induction chemotherapy, 53 of 94 (56%; 95% confidence interval, 46%–67%) had a major objective response, 88 (94%) underwent surgical exploration, and 81 (86%; 95% confidence interval, 78%–92%) underwent complete resection. Reasons for not undergoing an operation included disease progression (n=3), clinically unresectable status (n=1), death (n=1), and patient lost to follow-up (n=1). Two postoperative deaths occurred. Six (6%; 95% confidence interval, 0%–13%) pathologic complete responses were observed. Ninety (96%) patients received the planned preoperative chemotherapy versus 45% receiving postoperative chemotherapy. No unexpected chemotherapy or surgical morbidity occurred. The 1-year survival is currently estimated at 85%, and the median survival has not yet been reached.

Conclusions: Induction chemotherapy with paclitaxel and carboplatin is feasible and produces a high response rate with acceptable morbidity and mortality rates in early-stage non-small cell lung carcinoma. A prospective randomized trial comparing 3 cycles of induction chemotherapy and surgery with surgery alone in early-stage non-small cell lung carcinoma is planned.

Editor's (TLP) comment: This article was selected because it presents data on the tolerance and apparent effectiveness of chemotherapy for early stages of lung cancer. It forms the foundation for prospective randomized trials, comparing induction chemotherapy combined with surgery, with surgery alone in early stage tumors.

Synchronous Roentgenographically Occult Lung Cancer in Patients with Resectable Primary Lung Cancer.

Pierard P, Vermylen P, Bosschaerts T, et al. *Chest* 2000;117:779-785.

Objective: To assess the prevalence of synchronous roentgenographically occult lung carcinoma (ROLC) in patients with resectable roentgenographically visible lung cancer (RVLC).

Methods: Patients undergoing surgery for RVLC in the same University Hospital were prospectively evaluated before surgery by

fluorescence bronchoscopy under local anesthesia to detect synchronous ROLC. All abnormal areas, with the exception of the RVLC, had biopsies made.

Results: From June 1996 to January 1999, 43 patients (male/female ratio: 1.7/1.0) were evaluated before lobectomy (n=34) or pneumonectomy (n=10) for 44 primary RVLC. There were 10 T1N0, 19 T2N0, 1 T1N1, 9 T2N1, 1 T3N0, 3 T1N2, and 1 T3N1 lesions. The histologic type was mainly squamous carcinoma (n=21) and adenocarcinoma (n=14). All but two patients were smokers or ex-smokers (mean±SD, 48±28 pack-years). A total of 177 endobronchial biopsies were performed (4.1±2.5); 8 were too small to be informative, 43 showed non-preneoplastic alterations, and 50 were normal. There were 7 basal cell hyperplasias, 56 metaplasias, 9 dysplasias, and 4 carcinomas *in situ* (CIS). All the dysplasias and CIS lesions were observed in eight subjects. The synchronous CIS were treated by surgery (n=1) or localized therapeutic modalities (n=3).

"...both synchronous cancers and dysplastic changes are found in regions other than the primary lung cancer location..."

Conclusions: The high prevalence of synchronous early lung cancers (9.3%) as well as metaplasia and dysplasia in this series of patients with resectable RVLC suggests that fluorescence bronchoscopy may be a useful adjunct in the preoperative evaluation of lung cancer.

"Sputum cells can now be probed for altered gene expression to reveal more about the preneoplastic state of the airway."

Editor's (TLP) comment: The importance of this article is to underscore the possibility of multifocal lung cancers. That both synchronous cancers and dysplastic changes are found in regions other than the primary lung cancer location, is the reason for aggressive surveillance in the postoperative period, including sputum cytology, and by CT scanning.

Advances in Sputum Analysis for Screening and Early Detection of Lung Cancer.

Tockman MS. *Cancer Control; JMCC* 2000;7:19-24. (Reprinted with permission).

"We have found that MoAb 703D4 recognizes an epitope of hnRNP A2 and its splice variant, hnRNP B1."

Abnormal Protein (hnRNP A2/B1) Overexpression

Sputum cells can now be probed for altered gene expression to reveal more about the preneoplastic state of the airway. Morphologic changes in sputum epithelial cells and standard chest radiography, long the only preclinical lung cancer diagnostic evaluations available, had been carefully studied 20 years ago in the Collaborative Early Lung Cancer Detection studies of the National Cancer Institute (NCI) at Memorial Sloan-Kettering, Mayo Clinic, and Johns Hopkins.[1] These specific diagnostic tests, while useful for individual case finding, were found to be not sufficiently sensitive for lung cancer screening. Less than half (49%) of the lung cancer cases that arose during screening were detected by either standard sputum cytology or chest radiograph, and only 11% were detected by cytology alone.

During the Johns Hopkins Lung Project (JHLP), we developed an archive of sputum specimens and associated clinical data linking specimens to lung cancer outcome. To identify cancer-associated protein overexpression, we tested promising clinically available antibodies plus a series of murine monoclonal antibodies (MoAbs) raised by colleagues at the NCI. Differential display of two of these monoclonal antibodies (MoAbs 703D4 and 624H12) identified biomarkers of lung cancer in archived sputum specimens two years prior to clinical detection of lung cancer.[2] For the JHLP archived specimens, preserved for their cytologic atypia (moderate or grave atypical metaplasia), these antibodies together showed a sensitivity of 91% and a specificity of 88% for the diagnosis of lung cancer within two years.[2]

We have found that MoAb 703D4 recognizes an epitope of hnRNP A2 and its splice variant, hnRNP B1.[3] This target antigen for 703D4 was purified using Western blot detection after SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) separation. Purification steps included anion exchange chromatography, preparative isoelectric focusing, and polymer-based reverse-phase high-performance liquid chromatography (HPLC). After 25,000-fold to 50,000-fold purification, the principal immunostaining protein was more than 95% pure. Three sequences, including one across a site of alternate exon splicing, all identified a single protein, heterogeneous nuclear ribonucleoprotein-A2 (hnRNP-A2). The splice variant hnRNP-B1 was a minor copurifying immunoreactive protein. The hnRNPs are members of a family of ribonucleic acid proteins that are generally thought to regulate the shuttling of nascent RNA transcripts between the nucleus and cytoplasm. Interactions of these molecules are also thought to regulate mRNA splicing, capping, and polyadenylation. Recent information suggests other fundamental roles for this important nucleoprotein family.[4] The hnRNP A2/B1 family of antigens is frequently observed

in transformed bronchial epithelium,[3] and its increased expression is associated with a critical phase of fetal lung development for three mammalian systems, suggesting an oncofetal role for this protein.[5]

We began a clinical trial to evaluate the performance of the hnRNP A2/B1 protein as a biomarker for the early detection of second primary lung cancer (SPLC).[6] The patients at risk for SPLC have the highest annual incidence of lung cancer (2% to 5%) among asymptomatic populations.[7] The Lung Cancer Early Detection Working Group (LCEDWG), which is composed of thoracic surgeons and medical oncologists from leading medical centers throughout the United States and Canada, collaborate(d) in this ongoing trial. Accrual to this trial is now supported by a Collaborative Research and Development Agreement (CRADA) among the NCI, Moffitt Cancer Center, and Chiron/Bayer Diagnostics.

MoAb 703D4 binds hnRNP A2/B1 within selected epithelial cells exfoliated in the sputum.[8] In all cells correctly diagnosed by immunocytochemistry, we recognized at least a proplastic morphology. Proplasia consists of minimal cytologic changes that are usually regarded as normal epithelial responses to proliferative stimuli. To assure consistency in the selection of proplastic cells and reduce the possibility of false-positive diagnoses, we have agreed on a set of morphologic criteria based on the original description of these cells by Frost.[9] These morphologic criteria reflect proliferative changes in nuclear morphology and a level of cytoplasmic immaturity. When such cells bind MoAb, we consider them to be sentinel cells for preclinical lung cancer.[8]

Sentinel cells expressing upregulated levels of hnRNP A2/B1 are found infrequently (1 in 5,000) among cells that express normal low levels of this protein. By developing a cell-based diagnostic approach rather than a traditional mass extraction assay, we preserve in these isolated cells the natural upregulated signal compared with background noise.

Cellular distributions and concentrations of hnRPN A2/B1 indicative of lung cancer status were measured using semiautomated, quantitative image cytometry.[10] We programmed a commercially available workstation to perform feature extraction of digitally recorded transmission optical microscope video images of immunostained sentinel cells at 510 and 600 nm. Multidimensional cluster analysis led to the selection of features for a discriminant function evaluation. Differential light transmission of immunostained cells was scored against the gold standard of known histologic lung cancer, resulting in a diagnostic accuracy of 87%.[10] Development of hnRNP A2/B1 and its assay on a high-throughput platform are objectives in our CRADA with Chiron/Bayer Diagnostics.

Detection of hnRNP A2/B1 upregulation in morphologically 'normal-appearing' sentinel cells permits greater generalizability of results compared with the results from the earlier JHLP specimens with moderately or gravely atypical metaplastic appearance. After the first year of the LCEDWG trial, 13 SPLCs were identified.[11] The sensitivity and specificity of the hnRNP A2/B1 biomarker for later SPLC were 77% to 82% and 65% to 81%, respectively. Among the cases identified as positive by immunocytochemistry and image cytometry, 67% developed SPLC within one year. This diagnostic accuracy exceeds that commonly found in prostate-specific antigen (PSA) cancer screening tests.[12] Working independently, Sueoka and colleagues[13] recently published confirmation of this epitope to detect preclinical lung cancer in Japan. They propose to initiate lung cancer screening in that country. Detection of hnRNP A2/B1 overexpression in sputum epithelial cells with proplastic morphology appears to be the basis of a cytotest that could initiate a strategy of preclinical lung cancer diagnosis.

We have discussed an example of successful translation of a lung cancer biomarker (hnRNP A2/B1) from the laboratory through platform development and to application in a clinical trial. More than 6,000 individuals have been screened with this biomarker in ongoing clinical trials in North America, the United Kingdom, China, and Japan. Other less well-studied biomarkers have also been applied to exfoliated sputum epithelial cells.

Specific Oncogene Activation *(ras)* and Tumor Suppressor Gene Deletion (*p*53, 3p, 9p)

Tumor development progresses through a series of specific genetic changes in protooncogenes and tumor suppressor genes.[14-16] Changes that cannot be repaired and do not trigger a program of cell death (apoptosis) may lead to a cellular growth advantage. Many of these genetic changes are acquired prior to and during the earliest stages of clonal expansion and are retained by daughter cells through the course of carcinogenesis and malignancy. If detected during the premalignant period, these specific genetic changes could serve as cancer markers.

(continued)

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"Tumors not containing K-*ras* mutations were sequenced for *p*53 mutations to detect tumor- specific markers."

Three closely related genes, H-*ras*, N-*ras*, and K-*ras*, comprise the *ras* family of oncogenes. The highly conserved 21 kDa protein products of these genes are important signal transduction elements that participate in cell cycle regulation by controlling proliferation. Mutation of the K-*ras*-2 oncogene is one of the most commonly occurring genetic lesions in colorectal cancer[17] and is frequently mutated in lung cancer.[18] The JHLP archive of preclinical sputum linked to tumor outcome allowed us to demonstrate that specific mutations could be detected in non-malignant sputum specimens prior to clinical lung cancer.[19]

In this pilot study, we selected 15 participants in the JHLP with no malignancy in sputum cytology who went on to develop adeno-carcinoma or large-cell carcinoma of the lung. These histologic cell types were selected because they have a higher incidence of K-*ras* mutations (30%) than other lung tumors.[20] We also looked for *p*53 gene mutations because these are among the most common genetic alterations found in lung cancers (and other cancers)[21,22] The first exon of K-*ras* or exons 5–8 of the *p*53 gene were amplified by polymerase chain reaction (PCR) from DNA extracted from the paraffin-embedded primary lung tumor. After cloning, the K-*ras* gene was sequenced to detect mutations.

Tumors not containing K-*ras* mutations were sequenced for *p*53 mutations to detect tumor-specific markers. Once mutations specific for each tumor were identified, oligonucleotide probes were prepared, specific for the wild-type sequence or individual mutant K-*ras* and *p*53. These probes were hybridized to sputum DNA that had been amplified by PCR, cloned into a phage vector, and transferred to nylon membranes. Ten of the 15 patients had primary tumors that contained either a K-*ras* or *p*53 gene mutation. Identical mutations were detected in nonmalignant sputum cells from 8 of 10 patients who had tumors containing oncogene mutations. Patients whose tumors did not contain mutations as well as control patients without cancer were negative for sputum mutations by this assay.

"Chromosomal alterations have been extensively documented in lung cancer."

This study demonstrated that 8 (53%) of 15 patients with adenocarcinoma or large cell carcinoma of the lung could be detected by mutations in sputum cells from 1 to 13 months prior to clinical diagnosis. Less sensitive than the protein marker described above, the identification of specific gene abnormalities is further limited by the need to know the specific mutation sequence with which to probe the sputum specimens. In this pilot study, the mutation sequence was determined from the resected tumor. Presently, this approach is obviously not practical for screening undiagnosed individuals. Perhaps with future advances in gene chip technology, it might become feasible to probe for all possible mutations of common oncogenes and tumor suppressor genes in sputum specimens of asymptomatic individuals.

Genomic Instability (Loss of Heterozygosity, Microsatellite Alterations)

Chromosomal alterations have been extensively documented in lung cancer.[23] In 63 non-small cell lung carcinomas, Tesa et al[24] found loss of chromosomes 13 (71%) and 9 (65%) to be the most frequent changes, while a gain on chromosome 7 was seen in 41%. The chromosomal arms with most frequent loss were 9p (79%), 3p, 6q, 8p, 9q, 13q, 17p, 18q, 19p, 21q, 22q, and the short arm of the acrocentric chromosomes. Regions of chromosomal loss are suspected to encode tumor suppressor genes, the loss of which confers a cellular growth advantage. For each chromosomal locus, individuals will have two alleles, one contributed by each parent. While occasionally parents may provide identical genetic contributions at a given locus (homozygosity), often slight differences are observed among alleles (heterozygosity). It has been suggested that allelic imbalance (generated by loss of heterozygosity, LOH) on the short arms of chromosomes 3, 9, and 17 indicates the location of tumor suppressor genes associated with the early stages of lung cancer development.[25-28]

Microsatellite markers are small repeating DNA sequences found in the introns (noncoding regions) of a gene. PCR amplification of these repeat sequences provides a rapid method for assessment of LOH and facilitates mapping of tumor suppressor genes.[29,30] Yet microsatellites can provide additional information. Expansion and deletion of these repeating elements are called microsatellite alterations. These microsatellite alterations, acquired during division of a single transformed cell, are passed onto daughter cells during clonal expansion. Since they are not transcribed, microsatellite alterations provide no growth advantage to the cell. However, detection of microsatellite alterations in histologic specimens is equivalent to the detection of neoplastic (clonal) cell populations. Although the detection of microsatellite alterations does not indicate the specific genetic change in the tumor, detection of clonal cell populations might serve as a cancer screening marker.[31]

Widespread microsatellite instability was first reported in colorectal tumors.[32] In hereditary nonpolyposis colorectal carcinoma (HNPCC), mutations of mismatch repair genes are probably responsible for microsatellite alterations at multiple locations in the genome.[33] However, in non-HNPCC-associated tumors, including lung cancer, there is not a similar widespread loss of mismatch repair, indicating that another as yet unknown mechanism is responsible for somatic alterations of repeat sequences.[34]

The pattern of microsatellite alterations and LOH may be specific for different types of cancer. The high incidence of these changes on chromosomes 3,5,8,9,10,11,17, and 20 have been described in lung cancer specimens,[34,35] although the role of these changes in carcinogenesis is not yet known. Perhaps it is the cumulative effect of these genetic injuries that is important. Wistuba et al[36] reported a progressive increase of overall LOH frequency within clones with increasing severity of histopathologic changes in lung squamous carcinoma. We have already shown that microsatellite alterations are clonal markers for the detection of human lung cancer. Also, we and others have shown microsatellite alterations at selected loci can be recognized in sputum cells prior to clinical lung cancer.[31] More recently, we found that when microsatellite alterations occurred at more than one locus, there was a significant association with hnRNP A2/B1 overexpression.[37] We tested 41 paired tumor and normal DNA specimens from non-small cell lung cancer patients surgically resected at Moffitt Cancer Center. Eleven dinucleotide and tetranucleotide repeat markers were selected for their high frequency of loss (LOH) or alteration in lung cancer. In 41 paired tumor/normal samples, we found 19 patients (46%) had more than two loci of microsatellite instability (loss or alteration), while 13 patients (32%) had only one locus of microsatellite instability. The total frequency for microsatellite alteration was 78%, which suggests that this panel of markers may have a sensitivity comparable to hnRNP protein markers for detection of lung cancer. We are now evaluating whether this performance is maintained in sputum specimens of high-risk individuals.

Abnormal Methylation

The *p16* gene is located on the short arm of chromosome 9 (at 9p21) and is frequently mutated or inactivated in tumors and cells lines derived from lung cancer.[38,39] This gene codes for a protein that binds to the cyclin-dependent kinases 4 or 6 (cdk4 or cdk6) and prevents the kinase from phosphorylating (activating) cyclin D1. When phosphorylated, the activated cyclin D1 phosphorylates the retinoblastoma protein to allow the release of E_2F transactivator and progression through the cell cycle.[40] Therefore, *p16* acts as a tumor suppressor gene, inhibiting mitosis and cell proliferation. Downregulation or loss of *p16* expression could contribute to the loss of cell cycle control and provide a cellular growth advantage. Mao and colleagues[41] have shown that nearly two thirds of former smokers show some genetic alteration in their bronchial cells. These investigators show that the frequency of LOH at the tumor suppressor sites 9p21 (*p16*) and 17p13 (*p53*) is nearly the same for smokers and for former smokers. In contrast, lifetime nonsmokers show no LOH at 9p21, suggesting that loss of 9p21 sequences might be an early event for the development of lung cancer.

Established causes of loss of tumor suppressor function include the loss of *p16* expression through gene deletion and expression of an altered protein through gene mutation.[38] More recently, Merlo and colleagues[42] described inhibition of *p16* gene transcription by promoter region hypermethylation, and Herman et al[43] described a novel PCR assay for detection of this condition. Briefly, the addition of methyl groups to a sequence motif (CpG islands) in the gene promoter region results in gene transcription failure.[44] For *p16*, these sequence motifs start at the promoter and extend into exon (transcribed region) 1-alpha.[42] Myohanen and colleagues[45] showed that CpG island methylation-induced transcriptional repression could be at least partly reversed by cell culture treatment with the demethylating agent 5-aza-2y-deoxycytidine.

Belinsky et al[46] recently measured hypermethylation of the CpG islands of the *p16* gene in the sputum of lung cancer patients and demonstrated a high correlation with the early stages of non-small cell lung cancer. These investigators suggest that detection of *p16* CpG island hypermethylation might be useful in the prediction of individuals who might develop lung cancer. As yet, however, no prospective studies have been conducted to assess the performance of the hypermethylation assay on samples from individuals at risk for developing lung cancer.

Conclusions

These sputum tests offer great promise in determining a molecular diagnosis of lung cancer far in advance of clinical presentation. Any or all

"We tested 41 paired tumor and normal DNA specimens from non-small cell lung cancer patients surgically resected at Moffitt Cancer Center."

"Downregulation or loss of *p16* expression could contribute to the loss of cell cycle control and provide a cellular growth advantage."

"These sputum tests offer great promise in determining a molecular diagnosis of lung cancer far in advance of clinical presentation."

of these tests could be incorporated into the routine management of individuals at risk of developing primary or second primary lung cancer. However, several issues must be considered before these tests are ready for clinical application. First, test performance characteristics must be confirmed in prospective trials. For several of these tests, those trials are currently underway. Second, we must develop a management and intervention strategy appropriate to the state at which lung cancer is diagnosed. The ability to detect lung cancer at the stage of clonal expansion, well in advance of malignant invasion of the basement membrane, suggests that noninvasive chemoprevention might be appropriate in such cases. Preliminary studies of chemopreventive agents are now underway at the NCI. Several of these agents could be delivered by inhaler to place a maximum dose directly on the transformed epithelium. We must now begin to plan for clinical trials that evaluate combined diagnostic and therapeutic approaches to assess their impact on the incidence of clinical lung cancer. Finally, the larger public health issues of cost and accessibility of lung cancer screening must be considered before these advances in sputum screening can reach their potential.

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Editor's (SL) Comment: The prospect of using molecular biomarkers to detect early lung cancer is exciting. However, it is important to recognize that in early detection programs, specificity is more important than sensitivity. False-positive tests have important implications in terms of psychological trauma and morbidity from subsequent diagnostic tests or treatment. The cost of "downstream" investigations and treatment from a false-positive test has to be considered, as well. In developing tests for early detection, the aim is to achieve a specificity of at least 90%, and preferably 95%.

National Lung Health Education Program Spirometry Statement Published.

"A consensus statement on office spirometry by the National Lung Health Education Program, (NLHEP), has been published in *Chest*..."

A consensus statement on office spirometry by the National Lung Health Education Program, (NLHEP), has been published in *Chest* (Ferguson GT, Enright PL, Buist AS, Higgins MW. Office spirometry for lung health assessment in adults. A consensus statement from the National Lung Health Education Program. *Chest* 2000; 117:1146-1161). This same statement has also been published in the May issue of *Respiratory Care*. The NLHEP recommends spirometric screening of all smokers over the age of 45 and anyone with cough, dyspnea, mucus hypersecretion, or wheeze. This recommendation is directed primarily at the 210,000 primary care practitioners who see the great majority of smokers in their offices each day. It is estimated that approximately 70% of all smokers are seen once a year for some problem, often a smoking-related disorder. The NLHEP believes that the early identification and intervention in COPD can change the course and prognosis of this nation's most rapidly growing health problem, now the fourth most common cause of death.

"...industry has responded to the NLHEP's call for simple office screening spirometers."

Heavy smokers, i.e., greater than 30 pack-years with COPD, are also identified as a population appropriate for lung cancer screening with sputum cytology and CT scanning. Thus, the spirometer becomes a key instrument for the identification of both COPD and related lung cancer, in both current and former smokers.

Fortunately, industry has responded to the NLHEP's call for simple office screening spirometers. At least three such devices are now on the market. They preserve the high accuracy of research instruments at a much lower cost, and are much easier to use in primary care physicians' offices.

Angiogenic Squamous Dysplasia in Bronchi of Individuals at High Risk for Lung Cancer.

"This new lesion may be a precursor of bronchogenic, squamous carcinoma, thus suggesting anti-angiogenesis therapy."

Keith RL, Miller YE, Gemmill RM, et al. *Clin Can Res* 2000;6:1616-1625.

Abstract: Lung carcinogenesis is assumed to be a multistep process, but detailed understanding of the sequential morphological and molecular changes preceeding invasive lung cancer remains elusive. To better understand early lung carcinogenesis, we initiated a program of fluorescence bronchoscopy in smokers at high

risk for lung cancer. In the bronchial biopsies from these subjects, we observed a unique lesion consisting of capillary blood vessels closely juxtaposed to and projecting into metaplastic or dysplastic squamous bronchial epithelium, angiogenic squamous dysplasia, (ASD). Serial sections of the capillary projections confirmed that they represent intramucosal capillary loops. Microvessel density in ASD was elevated in comparison to normal mucosa (P=0.0003) but not in comparison to other forms of hyperplasia or dysplasia. ASD thus represents a qualitatively distinct form of angiogenesis in which there is architectural rearrangement of the capillary microvasculature. Genetic analysis of surface epithelium in a random subset of lesions revealed loss of heterozygosity at chromosome 3p in 53% of ASD lesions. No confirmed *p53* mutations were identified. Compared with normal epithelium, proliferative activity was markedly elevated in ASD lesions. ASD occurred in 54 of 158 (34%) high-risk smokers without carcinoma and in 6 of 10 patients with squamous carcinoma who underwent fluorescence bronchoscopy. One early-stage invasive carcinoma was noteworthy for the occurrence of ASD juxtaposed to invasive tumor. Seventy-seven (59%) of the ASD lesions were detected by abnormal fluorescence alone. Twenty bronchial sites (11 patients), were rebiopsied 1 year after the initial diagnosis. At nine (45%) of these sites, the lesion was found to persist. The lesion was not present in biopsies from 16 normal nonsmoker control subjects. The presence of this lesion in high-risk smokers suggests that aberrant patterns of microvascularization may occur at an early stage of bronchial carcinogenesis.

Editor's (TLP) comment: This new lesion may be a precursor of bronchogenic, squamous carcinoma, thus suggesting anti-angiogenesis therapy. But, it may not relate directly to the evolutionary pathogenesis of the progressive dysplastic changes that result in carcinoma *in situ,* and finally, invasive carcinoma. Thus, more study of its connection with the evolutionary stages in the pathogenesis of squamous carcinoma will be needed. The clinical and pathological significance of this lesion was discussed in an accompanying editorial by A. F. Gazdar and J. D. Minna (Angiogenesis and the multistage development of lung cancers. *Clin Can Res* 2000;6:1611-1612), pointing out this lesion was not found in any nonsmoker. This only suggests that this lesion is associated with the products of tobacco smoke. That it can regress on smoking cessation, is probably important both in the understanding of the pathogenesis of squamous carcinoma and in its prevention. Thus far, there is no evidence this lesion is a precursor of the more common adenocarcinoma, which follows the development of atypical adenomatous hyperplasia (Carey FA. Pulmonary adenocarcinoma: Classification and molecular biology. *J Pathol* 1998;184:229-230).

Observer Variability in Histopathological Reporting of Bronchial Biopsy Specimens.

Venmans BJ, Linden HC, Elbers HR, et al. *J Bronchology* 2000; (in press).

Ben Venmans and colleagues draw attention to a vexing problem in the growing body of literature on autofluorescence bronchoscopy: observer variability in histopathological reporting. Fifty-nine patients were examined by conventional and lung intensified fluorescent endoscopy, (LIFE), for preneoplasias or early cancers. Among 343 biopsies, the site pathologist reported 45 lesions. A review panel concurred on only 22 lesions. One preinvasive lesion was upgraded; 35 lesions were downgraded. Kappa values, a statistical measure of the strength of interobserver concurrence, for the borderline lesions of moderate and severe dysplasia were 0.02 and 0.46, representing, respectively, poor and fair agreement only. The pathological distinction between normal mucosa and cancerous tissues is rarely a matter of dispute. In the present study, the Kappa value of 0.85 for carcinoma was very satisfactory. The proportion of specimens regarded as unsatisfactory in the opinion of the review panel was double that of the site pathologist. Most of the rejected biopsies originally had been classified as normal or inflammatory, however.

"The pathological distinction between normal mucosa and cancerous tissues is rarely a matter of dispute."

How Golden is the Gold Standard? The Elusive Pathology of Early Lung Cancer.

Khanavkar B. *J Bronchology* 2000; (in press).

Commenting on her paper in an editorial, Barbara Khanavkar noted that in the context of early lung cancer diagnosis, very subtle differences between severe dysplasia and carcinoma *in situ* will more likely be assessed differently by individual pathologists, reflecting

"Variability in histological assessment of preneoplastic and early cancer lesions is just one factor making life for the endoscopist more difficult."

"^{99m}Tc depreotide scintigraphy is a safe and useful method for the noninvasive evaluation of SPN with a sensitivity and accuracy comparable to that reported for fluorine-18 fluorodeoxyglucose positron emission tomography."

a lack of agreed standards. She also stressed the importance of providing generous mucosal samples for the pathologist. Of similar relevance for observed outcome, is evaluation of representative slices only rather than viewing all material submitted. Variability in experience, training, and special interests of the pathologists naturally also leads to discrepancies. For the clinician, there is an as yet arbitrary cut-off point between lesions that warrant treatment from those that may remain under close observation only. The need for an unequivocal diagnosis therefore remains critical. Unnecessary investigations and therapy will weigh heavily against the advantages of lives saved through early lung cancer diagnosis. Khanavkar underscored the need to initiate a cooperative effort between chest physicians and pathologists interested in early lung cancer, to polish the gold standards.

Editor's (JAN) comment: Variability in histological assessment of preneoplastic and early cancer lesions is just one factor making life for the endoscopist more difficult. At present, three autofluorescence systems (Xillix, Pentax, and Storz) which may be identifying differing nuances in oncogenetic pathways, are in use, with more probably to come. This too, underscores the importance of endoscopists, pathologists, and cytologists collaborating to establish firm standards for early lung cancer diagnosis.

A Multicenter Trial with a Somatostatin Analog ^{99m}Tc Depreotide in the Evaluation of Solitary Pulmonary Nodules.

Blum J, Handmaker H, Lister-James J, et al. *Chest* 2000;117:1232-1238.

"At least for detecting cancer among solitary lung lesions, the ^{99m}TC depreotide scan promises to be as accurate as PET."

Objective: The affinity of various malignant neoplasms including small cell and non-small cell lung cancer for peptide analogs of somatostatin has been well documented. Depreotide is such an analog and can be complexed with technetium-99m, (^{99m}Tc depreotide), for optimal imaging properties. Using this radiopharmaceutical, solitary pulmonary nodules, (SPN), were previously evaluated in a successful phase II/III trial. The results of the larger multicenter phase III study using ^{99m}Tc depreotide to differentiate malignant and benign etiologies in SPN are now presented.

Methods: Patients with SPN ≤6 cm on chest radiograph were referred to evaluation. One hundred fourteen individuals who had an absence of a benign pattern of calcification on CT scan, age >30 years, and no demonstratable radiographic stability for the prior 2 years were studied. All underwent single-photon emission CT, (SPECT), with ^{99m}Tc depreotide and subsequent tissue histologic examination. Three nuclear medicine specialists blinded to histologic findings examined the SPECT images and scored them as positive or negative based on the presence or absence of activity in the radiographic region of the SPN. The final result was determined by the majority score, which was then compared with the histologic result.

Results: Of the 114 individuals studied, 88 had a histologic result compatible with malignant neoplasm. ^{99m}Tc depreotide scintigraphy correctly identified 85 in this group, with three false-negative determinations compared with histology. There were seven false-positive determinations, including six granulomas and one hamartoma. ^{99m}Tc depreotide scintigraphy correctly excluded malignancy in 19 of 26 patients with benign histologic findings. The sensitivity of this method was 96.6% with a specificity of 73.1%.

Conclusion: ^{99m}Tc depreotide scintigraphy is a safe and useful method for the noninvasive evaluation of SPN with a sensitivity and accuracy comparable to that reported for fluorine-18 fluorodeoxyglucose positron emission tomography.

Editor's (RAM) comment: During the past decade, whole-body positron emission tomography, (PET), utilizing $^{[18F]}$ fluoro-deoxy-2-D-glucose, (FDG), has been shown to be an excellent noninvasive imaging technique for lung cancer diagnosis, intrathoracic staging, extrathoracic staging, and assessment of tumor persistence or recurrence after treatment (Scott WJ, Dewan NA. Use of positron emission tomography to diagnose and stage lung cancer. *Clin Pulm Med* 1999;6:198-204). PET is greater than 90% accurate when used to determine whether a lung nodule is malignant or benign. Slow growing bronchioalveolar lung carcinomas may produce false-negative results, and PET studies have been false-positive in patients with pneumonia, tuberculosis, aspergillus lung disease, and acute necrotizing inflammatory granulomas. Compared to chest CT alone, CT plus PET is significantly more accurate for staging the

mediastinum (Vansteenkiste JF, et al. Lymph node staging in non-small-cell lung cancer with FDG-PET scan: A prospective study on 690 lymph node stations from 68 patients. *J Clin Oncol* 1998;16:2142-2149). Moreover, whole-body PET imaging detects clinically occult extra-thoracic metastases in such organs as brain, bones, liver, or the adrenal glands.

Largely due to the high cost of PET scans and the scarcity of PET centers, investigators have been seeking to reproduce PET's excellent results in detecting lung cancers utilizing less expensive, more readily available equipment at a lower cost to the health care system. In this phase III study by Blum and colleagues, depreotide, an analog of somatostatin, was complexed with technetium-99m. Images of the chest with this radio-pharmaceutical proved to be comparable to PET in accuracy, correctly identifying 85 of 88 lung cancers, (96%) among 114 individuals with solitary pulmonary nodules, (≤6 cm). Three small adenocarcinomas, (all ≤2 cm) were not detected by ^{99m}Tc depreotide. Similar to PET, the specificity of ^{99m}Tc depreotide scintigraphy, (73%) was less than its sensitivity, (96.6%). Both granulomas and a benign tumor, a hamartoma, yielded false-positive results. The authors state that this new imaging method should be readily available and more cost-effective than PET in the scintigraphic differentiation of benign and malignant lesions. Unclear from this report, however, are: 1) What will each ^{99m}Tc depreotide scintigram cost the health care system? (Currently, a PET scan costs up to $2,000 and the typical cost for a chest CT is $800 to $1,000), 2) How readily available will this new technique be? 3) Can the ^{99m}Tc depreotide scan be adapted to stage the mediastinum and accomplish whole-body images? Clinicians and their patients may benefit markedly if this new technique becomes widely available and is substantially less expensive than PET. At least for detecting cancer among solitary lung lesions, the ^{99m}TC depreotide scan promises to be as accurate as PET.

First Successful Pneumonectomy for Lung Cancer Performed in 1933.

In April, 1933, Evarts A. Graham performed the first successful pneumonectomy for lung cancer in a 48-year-old physician at Barnes University in St. Louis. The patient had reported cough and chest pain for seven months before the surgery. Chest x-rays showed left upper atelectasis with a pneumothorax. Bronchoscopic diagnosis of squamous cell carcinoma was made. The 1.0 cm tumor was at the bifurcation between the left upper lobe and the left lower lobe. Large hilar nodes were present. All were removed en bloc. The first and second ribs were resected in a manner that had been established for thoracoplasty, with the purpose of controlling space problems. This later was shown to be unnecessary. In this article, (Graham EA, Singer JJ. Successful removal of an entire lung for carcinoma of the bronchus. *JAMA* 1933;101:1371-1374), Graham commented that the prevalence of lung cancer was increasing. He estimated that at that time it represented 5% to 10% of all cancers.

In his review of the current status of pneumonectomy in 1950, (Graham EA. The problem of bronchiogenic carcinoma. *Surg Clin N Am* Oct. 1950;1259-1277), Graham commented that his patient was still alive. By this time, lung cancer had exceeded stomach cancer as the most prevalent malignancy in men. Graham was convinced that smoking was the cause of lung cancer. This statement antedated the formal report that he and Ernst L. Wynder made that same year (Wynder EL, Graham EA. Tobacco smoking as a possible etiologic factor in bronchiogenic carcinoma. *JAMA* 1950;143:329-336). In this review, Graham commented on sputum cytologic diagnosis of lung cancer, which was first introduced in the 1930's. The Papanicolaou stain for sputum cytology was added in the 1940's, well in advance of Geno Saccomanno's reawakening of interest in sputum cytology (Concentration of carcinoma or atypical cells in sputum. *Acta Cytol* 1963;7:305-310; Sputum cytology: Collection fixation and concentration of sputum, bronchial aspirates and bronchial brushings. *Lab Med* 1979;10:523-527). Graham lamented the fact that lung cancer was diagnosed at such late stages of disease. Alas, the same is true today.

Graham's patient actually outlived Graham, who died of lung cancer due to smoking. What irony!

"In April, 1933, Evarts A. Graham performed the first successful pneumonectomy for lung cancer in a 48-year-old physician at Barnes University in St. Louis."

"Graham's patient actually outlived Graham, who died of lung cancer due to smoking. What irony!"

Second International Conference on COPD Held in Birmingham, UK, June 14-17, 2000.

Conference organizers, Robert Stockley, Sue Hill, and Carol Llewellyn-Jones, welcomed more than 600 physicians, nurses, therapists, and others to a unique program which was packed with varied and interesting topics designed to shed new light on the growing worldwide problem of COPD. The basic inflammatory processes resulting in the disease spectrum, COPD were vigorously discussed in plenary and smaller breakout sessions. These presentations, augmented by scientific poster presentations helped to created the foundation for new approaches to management. Oxygen and pulmonary rehabilitation and the effect of these treatments in improving both the length and quality of life were featured. The need for early identification and new strategies for treatment of mild to moderate stages of disease are immediate challenges. The link between COPD and lung cancer creates a new frontier for the future. At the close of the meeting, Professor Stockley announced that COPD 3 is already in its early planning stages for June, 2002.

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