Multitarget FISH Analysis in the Diagnosis of Lung Cancer

Lukas Bubendorf, MD,1 Phaedra Müller,1,2 Ladina Joos, MD,2 Bruno Grilli,1 Sandrine Vogel,1 Michelle Herzog,1 Audrey Barascud,1 Georg Feichter, MD,1 Peter Dalquen, MD,1 and Michael Tamm, MD2

Key Words: Lung cancer; Bronchial; Cytology; Fluorescence in situ hybridization; FISH; Chromosomes; Multiprobe; Field cancerization

Abstract

The aim of the present study was to explore the diagnostic usefulness of the multitarget fluorescence in situ hybridization (FISH) test, LA Vysion (Vysis, Downers Grove, IL), for the detection of lung cancer cells in cytologic specimens. Specimens from bronchial washings, bronchial brushings, and transbronchial fine-needle aspirates (TBNA) from 100 patients with suspected lung cancer and from a control group of 71 patients with nonneoplastic lung disorders were analyzed. FISH positivity was defined as more than 5 cells with gains of at least 2 chromosomes or gene loci. FISH significantly improved the sensitivity of bronchial brushings from 49% to 73%. The specificities of FISH and cytologic examination were 87% and 100%, respectively. In contrast, FISH provided no additional diagnostic information in TBNA and bronchial washings. There was no increased prevalence of genetic changes in contralateral bronchial washings from patients with lung cancer compared with the control group. The quantity of previous smoking had no effect on the prevalence of chromosomal changes.

Lung cancer is the most common cause of cancer death in developed countries,1,2 and its incidence continues increasing in females owing to changed smoking habits.3 In clinical practice, the workup of patients with a solitary pulmonary nodule found by chest radiography or chest computed tomography often represents a diagnostic dilemma.4 Bronchoscopy is the most commonly used diagnostic tool for obtaining a definite histologic or cytologic diagnosis of lung cancer.5 Forceps biopsy, brushing, washing, and transbronchial fine-needle aspiration (TBNA) are used routinely as complementary diagnostic tools, leading to a correct diagnosis in more than 85% of lung carcinomas.6,7 Peripheral lesions present the greatest diagnostic problem because they often are inaccessible by bronchoscopy,6 necessitating additional and repeated diagnostic procedures, including transthoracic biopsy and fine-needle aspiration or exploratory surgery with frozen section analysis.

Clinical lung cancer is preceded by a multistep process with gradually accrued genetic and cellular changes, driven by genetic damage through long-term exposure to tobacco carcinogens.8 Chromosomal alterations are a hallmark of cancer cells and have a high prevalence in small cell lung cancers and non–small cell lung cancers.9-11 Chromosome and gene copy number changes can be detected easily by fluorescence in situ hybridization (FISH) in diagnostic cytologic specimens.12 In a study of 48 selected patients, Sokolova et al13 found evidence suggesting that the multitarget FISH test LA Vysion (Vysis, Downers Grove, IL) for simultaneous analysis of chromosome 1 and the 5p15, 7p12 (EGFR [epidermal growth factor receptor] gene) and 8q24 (MYC gene) loci might improve sensitivity for lung cancer detection. The aim of the present study was to further study the diagnostic usefulness of this multitarget FISH test in a larger series of patients with clinical and radiologic findings suggesting lung cancer.
Materials and Methods

Cases and Specimens

A series of 171 cases was identified from the files of the Pneumology Department, University Hospital, Basel, Switzerland. Cytologic specimens that had been evaluated between July 2001 and February 2002 were retrieved from the archive of the Institute for Pathology, University Hospital. Three groups were selected specifically: (1) solitary peripheral lung lesions (n = 82; 35 women and 47 men; median age, 68 years; range, 40-89 years), (2) central lung lesions (n = 18; 5 women and 13 men; median age, 72 years; range, 35-87 years), and (3) lung disorders with no suspicion of cancer (negative control group, n = 71; 20 women and 51 men; median age, 61.6 years; range, 27-94 years).

In all cases of suspected lung cancer, bronchial wash (BW) specimens, bronchial brush (BB) specimens, and TBNAs were obtained from the affected side Table 1. In addition, BW specimens were obtained from the nonaffected lung in 33 patients with suspected lung cancer. One patient had metastases of a gallbladder adenocarcinoma; data were excluded from the analysis of field effect. Control specimens consisted of 68 BW and 3 BB specimens. They were obtained from patients with pulmonary infections (n = 21), chronic bronchitis (n = 17), interstitial lung disease (n = 7), sarcoidosis (n = 4), or cryptogenic organizing pneumonia (n = 1) and from 9 patients who had been evaluated for hemoptysis with no subsequent evidence for malignancy. In 12 patients, no specific diagnosis was made. In addition, a separate series of sputum specimens from 10 patients with suspected lung cancer were analyzed.

Cytologic Examination

Cytologic smears were prepared at the cytology laboratory according to routine procedures, using Delaunay solution as a fixative (500 mL of absolute ethanol and acetone, with 0.5 mL of a 1-mol/L concentration of trichloroacetic acid). Of 10 sputum specimens, 6 were prepared using ThinPrep slides after fixation in PreservCyt (Cytyc, Boxborough, MA) and removal of mucus by using Sputolysin Reagent (Calbiochem-Novabiochem, La Jolla, CA). The specimens first were stained according to the Papanicolaou (Pap) method and evaluated by a cytopathologist (L.B., P.D, or G.F.) in a routine diagnostic setting as negative, equivocal, or positive. Equivocal cytologic findings were considered negative. A hybridization target area of 22 × 22 mm was selected based on high cellularity. This area was marked with a waterproof pen. Before uncovering and hybridization, the target area was marked permanently on the slide with a diamond pen.

FISH Assay

The commercially available multitarget FISH test, LA Vysion, was provided by Vysis at no charge. It includes directly labeled DNA FISH probes for the EGFR gene (7p12, SpectrumRed), the MYC gene (8q24, SpectrumGold), chromosome 5 (5p15.2, SpectrumGreen), and chromosome 6 (centromeric at 6p11.1-q11, SpectrumAqua). In a previous study, the probe mixture included a probe for chromosome 1.14 In the present assay, a probe for chromosome 6 replaced this probe because it has superior hybridization properties, resulting in better signals at an equally high sensitivity (I.A. Sokolova, Vysis, oral communication, October 2004).

FISH was performed according to the recommendations of the manufacturer with minor modifications. The slides processed for FISH had been stained previously by the Pap method. A separate procedure for decolorization was not required.

Briefly, the archived slides were soaked in xylene until the coverslips could be removed and then were washed in fresh xylene twice, 5 minutes per wash. The slides subsequently were immersed twice in 100% ethanol for 5 minutes and soaked in 2× saline sodium citrate (SSC) buffer for 1 minute at room temperature. The slides were incubated in 0.5 mg/mL of pepsin in solution in a 10-mmol/L concentration of hydrochloric acid for 10 minutes at 37°C, followed by a phosphate-buffered saline wash for 5 minutes. The slides were fixed in a freshly prepared solution of 1% neutral buffered formalin for 5 minutes at room temperature, followed by soaking in phosphate-buffered saline for 5 minutes. The slides then were washed twice in Carnoy fixative for 5 minutes each time at room temperature and dehydrated by immersing in 70% ethanol solution for 5 minutes at room temperature followed by immersion in 80% ethanol and 100% ethanol. The washed slides were denatured for 10 minutes in 70% formamide/2× SSC at 73°C; dehydrated in a series of 70%, 85%, and 100% ethanol, 2 minutes per solution; and put on a slide warmer at 37°C to 45°C for 2 minutes to dry.

After denaturation at 73°C for 5 minutes, the probe hybridization mixtures were applied to the specimens, covered with a coverslip (22 × 22 mm), and sealed with rubber cement.
After overnight incubation of the slides in a humid chamber at 37°C, they were washed in 0.4× SSC/0.3% NP-40 at 73°C for 2 minutes. The slides were placed twice in 2× SSC/0.1% NP-40 for 2 minutes. After air drying of the slides, 4',6-diamidino-2-phenylindole (DAPI) II was applied to the target areas.

Enumeration of FISH Signals

A Zeiss Axioplan 2 fluorescence microscope (Zeiss, Jena, Germany) with a filter set including DAPI single bandpass (DAPI counterstain), aqua single bandpass (chromosome 6), yellow single bandpass (8q24 locus), red single bandpass (7p12 locus), and green single bandpass (5p15.2 locus) was used. As in previous multitarget FISH studies of urinary and lung cytologic specimens, the scanning method was used for evaluation of the FISH slides. This method is based on a higher likelihood of chromosomal aberrations in cells with morphologically abnormal nuclei as opposed to randomly selected cells. The advantages of the scanning method as opposed to the counting of random cells has been demonstrated. Enumeration of the FISH signals was done at a magnification of ×400 on selected cells that appeared morphologically abnormal in the DAPI stain. In case of a low number of morphologically abnormal nuclei, cells with the largest nuclei were chosen. At least 25 such cells were analyzed, or the entire sample was screened.

A normal cell contains 2 red, 2 gold, 2 green, and 2 aqua signals. A copy number of more than 2 signals of a probe indicates gene, locus, or chromosome gain. Based on a previous study, a test result was defined as positive when at least 6 cells showed a gain of at least 2 of the 4 signals, also referred to as multiple gains.

Statistics

Contingency table analysis was used to calculate the association of cytologic findings, tumor type, and FISH results.

Results

A final diagnosis of cancer was based on histologic findings in 65 cases. In another 7 cases, the cancer diagnosis was established by cytologic examination and an unequivocal clinical course (eg, growing lung mass and distant metastases). The 72 cancers in this study included 69 primary lung cancers (adenocarcinoma, 33; large cell carcinoma, 7; non–small cell carcinoma, not otherwise specified, 6; squamous cell carcinoma, 13; small cell carcinoma, 10) and 3 pulmonary metastases from nonpulmonary carcinomas (colon, endometrium, and gallbladder). In 28 cases of “suspicious” lesions with no evidence of malignancy, specific diagnoses after workup included pulmonary infection (n = 19), interstitial lung disease (n = 2), Wegener disease (n = 1), benign hamartoma (n = 1), tuberculous scar (n = 2), and rheumatic nodule (n = 1). In 1 case, there were several lung opacities but no change of size during a period of 11 years. In another case in which no specific diagnosis was made, there was no evidence of malignancy after clinical follow-up of 324 days.

In BW specimens, the multitarget FISH assay did not significantly enhance sensitivity for the detection of cancer cells. Similarly, FISH did not add information beyond that from cytologic examination in the 25 TBNAs. Cytologic examination and FISH detected 63% and 50%, respectively, of the 16 peripheral carcinomas, whereas both methods were

| Table 21 | Multitarget FISH and Cytologic Results for the Detection of Lung Cancer Cells in Bronchial Wash and Bronchial Brush Specimens |
|----------|-------------------------------------------------|--|----------|-------------------------------------------------|--|
|          | Bronchial Washing                                |                       |          | Bronchial Brushing                               |                       |
|          | Total No. | Cytology† | FISH‡ | Combined§ | Total No. | Cytology† | FISH‡ | Combined§ |
| Carcinoma | All       | 66        | 36 (55) | 34 (52) | 43 (66)† | 37        | 18 (49) | 27 (73) | 27 (73)‡ |
|          | Central   | 17        | 12 (71) | 12 (71) | 12 (71)  | 6         | 6 (100) | 6 (100) | 6 (100) |
|          | Peripheral| 49        | 24 (49) | 18 (37) | 29 (59) ‡ | 31        | 12 (39) | 21 (68) | 21 (68) * |
|          | Benign lesion** | 24        | 0 (0)   | 4 (17)  | 4 (17)   | 13        | 0 (0)   | 2 (15)  | 2 (15)  |
|          | Control samples†† | 68        | 0 (0)   | 3 (4)   | 3 (4)    | 3         | 0 (0)   | 0 (0)   | 0 (0)   |

FISH, fluorescence in situ hybridization.
† Data are given as number (percentage).
‡ Criteria for FISH positive specimens: 6 or more cells with gain of more than 1 chromosome or chromosomal locus.
§ Positive by FISH or by cytologic examination.
\[ P = .0321. \]
\[ P = .022. \]
** Radiologically “suspicious” lesions, but no evidence of malignancy by histologic examination and clinical follow-up.
†† Nonneoplastic lung disorders with no suspicion of malignancy.
negative in the 7 benign peripheral lesions. In contrast, sensitivity was significantly improved by FISH in BB specimens (Table 2). Subgroup analysis showed that the improved sensitivity was particularly evident in the 31 peripheral carcinomas, in which FISH increased the sensitivity from 39% (12/31) to 68% (21/31; \( P = .022 \)). This difference between cytology and FISH remained significant when the distant metastasis of a colorectal adenocarcinoma diagnosed by brush cytology was excluded from the analysis (\( P = .0384 \)). FISH and cytologic examination were equally effective in the small subgroup of central carcinomas (n = 6). There was no significant association between the histologic subtype of cancer and the sensitivity of cytologic examination or FISH (data not shown). Separate analysis of the 3 metastases from nonpulmonary adenocarcinomas revealed a positive FISH test in 2 of 3 cases. Representative images of FISH findings are illustrated in [Image 2](#).

### Specificity

There were no false-positive results of cytologic examination. A false-positive FISH result was found in 7 (8%) of 92 BW specimens and 2 (13%) of 16 BB specimens with no evidence of cancer. The final diagnoses for these 9 cases were common pneumonia (n = 1), aspergillosis (n = 2), cytomegalovirus pneumonia (n = 1), tuberculosis (n = 2), siliccosis (n = 1), and methotrexate lung injury (n = 1). In another patient with a peripheral lesion but no definitive diagnosis, clinical and radiologic follow-up provided no evidence of malignancy after a period of 324 days.

### Field Effect

To study the prevalence of a field cancerization in high-risk patients, we specifically analyzed the impact of smoking history and the chromosomal alterations in the bronchial tree contralateral to the bronchial tree with confirmed cancer. There was no significant association between the quantity of previous smoking (measured as pack-years) and the prevalence of a positive FISH result in 47 control cases with no history or clinical suspicion of malignancy [Table 3](#). This suggests that smoking by itself is not a relevant cause of false-positive results by LAVysion FISH. FISH was positive in only 2 of 26 contralateral specimens from patients with cancer [Table 4](#). This low prevalence does not exceed the false-positive rate observed in control cases (Table 2).

### Cases With Equivocal Cytologic Findings

Cytologic findings were equivocal in 9 specimens from 8 patients, including 2 BB specimens, 6 BW specimens, and 1 TBNA. The final diagnosis was adenocarcinoma in 4 cases, including 1 metastasis from colorectal adenocarcinoma, and small cell lung cancer in 2 patients. FISH was positive in 2 of 4 adenocarcinomas but negative in both small cell carcinomas. FISH gave a false-positive result in 1 of 2 cases with no evidence of lung carcinoma.

### Sputum Analysis

To estimate the diagnostic potential of the LAVysion test in sputum, specimens from 10 cases were analyzed. Nine specimens were from lung cancer cases, including 4 adenocarcinomas, 3 squamous cell carcinomas, 1 large cell lung cancer, and 1 small cell lung cancer. Five of these were positive by cytologic examination, 3 of which were positive by FISH. A specimen in a case of chronic lung congestion and atypical cytologic findings but no evidence of lung cancer was negative by FISH.

### Discussion

Cytologic examination, in concert with radiologic and clinical modalities, is an established tool for the evaluation of lung lesions that are suggestive of cancer. Although a firm diagnosis can be made by examining both cytologic and histologic biopsy specimens in more than 80% of the lung carcinomas, a proportion of cases remains unresolved, leaving patients and physicians with uncertainty. Because chromosomal aberrations are a hallmark of cancer but uncommon or absent in normal tissues, molecular cytogenetic analyses have high potential as diagnostic adjuncts when morphologic findings alone are not sufficiently reliable. Furthermore, chromosomal aberrations can precede the morphologic appearance of neoplastic transformation and identify patients at an increased risk for developing clinical cancer.

We used the recently developed 4-target FISH test, LAVysion, for the simultaneous analysis of chromosome 6 and the 5p15, 8q24 (MYC gene) and 7p12 (EGFR gene) loci in cytologic lung specimens from patients with suspected lung cancer. We found that this 4-target FISH test can improve the sensitivity of lung cytologic examination for the detection of lung carcinomas in selected groups of lesions and types of samples.

A high prevalence of chromosomal aberrations in lung cancer has been demonstrated by comparative genomic hybridization. A more detailed view on selected chromosomes has been provided by FISH to interphase nuclei in several studies. In a pilot study by Schenk et al using FISH with DNA probes specific for chromosomes 3, 8, 11, 12, 17, and 18, aneusomy was present in all 10 primary tumors and 10 malignant effusions from 18 patients with lung cancer. They also found FISH for chromosomes 7, 11, 17, and 18 to be positive in BB specimens from 5 patients with lung cancer. Because 2 of these cases seemed normal or reactive by cytologic examination, an implication of FISH for early detection of lung cancer was suggested.
The possible diagnostic usefulness of the present 4-target FISH test has been demonstrated on touch preparations from non–small cell lung cancers, in which it showed a sensitivity of 100%. Sokolova et al found that FISH can effectively detect lung cancer cells in BW specimens. In the present study, however, FISH did not increase the detection rate in several specimen subgroups, including centrally located carcinomas and TBNAs and BW specimens from peripheral tumors. Our inability to detect additional neoplastic cells by visualizing chromosomal aberrations in TBNAs and BW specimens from peripheral tumors suggests that the limited sensitivity of lung cytology is primarily due to incomplete sampling of tumor cells. The difference in the performance of FISH in BW specimens in the present study compared with an earlier study might be due to differences in patient selection and the size of the hybridization target area. The previous series was highly selected and consisted of equal numbers of correctly positive and false-negative cytologic specimens from 48 patients with lung cancer. In addition, the whole cytologic slide was hybridized in the previous study, whereas a restricted hybridization target area of 22 × 22 mm on each slide was selected in the present study. A significantly improved sensitivity of 4-target FISH was found in BB cytologic specimens from 31 peripherally located carcinomas, for which the sensitivity increased from 39% to 68%. This increased sensitivity might be due to the inability of cytologists to correctly recognize cancer cells in some cases. In such specimens, cancer cells could be mistaken for reactive cells in the Pap stain or be covered by mucus and cellular debris but correctly be identified as neoplastic based on abnormality by FISH. More likely, however, the increased sensitivity of FISH could be explained by regional cytogenetic field cancerization in the bronchial mucosa immediately adjacent to the invasive carcinoma. Such a mucosal cancer field in the peritumoral mucosa, which contains chromosomal aberrations but not yet overt neoplastic atypia, would facilitate the sampling of abnormal cells even in cases in which the invasive and overtly malignant components of the peripheral carcinoma cannot be reached by the brush. The presence of a peritumoral cancerization field also is supported by previous reports showing aneusomy or allelic losses in morphologically normal bronchial mucosa adjacent to lung carcinoma.

To assess the prevalence and possible extent of molecular field effects, we studied bronchial cells from the lung contralateral to the lung affected by cancer. There was no increased prevalence of chromosomal abnormalities in BW specimens from the nonaffected side of the lungs in our lung cancer patients. Therefore, a molecular field cancerization in the contralateral bronchial tree might only rarely reach the level of chromosomal instability detectable by the LA Vysion FISH test. Previous reports suggest that other molecular abnormalities such as altered microsatellites and promoter hypermethylation might be more frequent in noncancerous bronchial mucosa of patients at risk. For example, microsatellite alterations have been found in 35% of histologically normal lung tissue specimens of patients with lung cancer and also in 23% of bronchial lavage specimens from 47 patients with no clinical evidence of lung cancer. Similarly, promoter hypermethylation of p16INK4a occurs frequently in smokers, in whom it can precede the diagnosis of non–small cell lung cancer. It is possible that the probe set in the LA Vysion FISH test is not optimal for the detection of early chromosomal changes in noninvasive lesions because it has been designed to identify invasive lung cancers. Previous reports suggest that loss of DNA sequences is more prevalent in preinvasive lesions than gain of chromosomes and chromosomal loci. For example, loss of 3p14 and 9p21 shown by FISH on normal-appearing bronchial biopsy specimens was reported in 5 of 13 patients with concurrent lung cancer but in none of 9 control subjects without lung cancer. A high prevalence of loss of heterozygosity at 3p14, 9p21, and 17p13 (sites of the tumor-suppressor genes FHIT, CDKN2, and TP53) has been demonstrated in the lungs of smokers with no clinical evidence of lung cancer. Also, there was no influence of the smoking history on the prevalence of a positive FISH test in our control patients because the prevalence of chromosomal abnormalities did not occur, even in heavy smokers. Whereas other molecular changes such as aberrant promoter methylation

### Table 3

<table>
<thead>
<tr>
<th>Final Diagnosis</th>
<th>Cytology</th>
<th>FISH</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral (n = 21)†</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Benign lesion (n = 7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridization.

† Three cases with equivocal cytologic findings were considered negative. One case with lung metastasis from gallbladder adenocarcinoma was excluded.

### Table 4

<table>
<thead>
<tr>
<th>Final Diagnosis</th>
<th>Positive Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytology</td>
</tr>
<tr>
<td>Carcinoma</td>
<td></td>
</tr>
<tr>
<td>Peripheral</td>
<td></td>
</tr>
<tr>
<td>Benign lesion</td>
<td></td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridization.

**Chromosomal Aberrations in Bronchial Wash Specimens and Smoking History in 47 Control Patients Without Lung Cancer**

<table>
<thead>
<tr>
<th></th>
<th>0-9</th>
<th>10-29</th>
<th>30-150</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH negative</td>
<td>20</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>FISH positive</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>10</td>
<td>15</td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridization.

**Multitarget FISH on Specimens From Contralateral Side in 32 Patients With “Suspicious” Lesions**
or loss of heterozygosity often are found in long-term smokers,\textsuperscript{28,31,32} the result of the LA Vysion FISH test, therefore, is not confounded by smoking history.

Our data are discordant with those of a recent study in which significant chromosomal abnormalities were found in 26\% of cultured bronchial epithelial cells from high-risk smokers using the same 4-color FISH test as in our study.\textsuperscript{19} However, this series is not entirely comparable to ours. Nine of the cell cultures were from the lungs affected by cancer. The other 33 cases were selected because of dysplastic cells in sputum cytologic specimens. All positive FISH results were from patients with histologically diagnosed bronchial dysplasia.\textsuperscript{19} In contrast, the 5 patients without dysplasia and with histologic specimens available from the culture site were negative by FISH.\textsuperscript{19} Furthermore, it cannot be excluded that chromosomal abnormalities developed or were selected for during the culturing process in some cell lines.

One might speculate that FISH analysis of sputum specimens could serve as a tool for early, noninvasive identification of lung carcinomas in high-risk patients. However, the lack of improved sensitivity of FISH in BW specimens for the detection of lung carcinomas in the present study and the missing evidence for extended field cancerization in the lung with gross chromosomal aberrations could raise doubts about LA Vysion FISH as an effective screening tool for sputum specimens. Nevertheless, encouraging data from a recent study by Varella-Garcia et al\textsuperscript{20} suggest that the diagnostic yield of LA Vysion FISH in sputum specimens might be increased by improving the sampling method. By combining cytologic examination and LA Vysion FISH on pooled 3-day samples, more frequent abnormalities were found in 18 specimens sampled within 12 months before lung cancer diagnosis than in 15 control cases (83\% vs 20\%; $P = .0004$).\textsuperscript{20} Analysis of earlier changes in the tumorigenesis of lung cancer such as promoter hypermethylation or deletion at 9p and 3p might be more promising for the identification of patients at high risk. For example, hypermethylation of several genes has been identified up to 3 years before clinical detection of lung cancer.\textsuperscript{28} Further studies are required to explore whether FISH in sputum samples might be a valuable adjunct to other screening modalities such as high-resolution computed tomography.\textsuperscript{33}

FISH was helpful for clarifying equivocal cytologic findings in 2 of 6 cases in which cancer subsequently was diagnosed. Because atypia typically was restricted to rare cells or cell groups on the cytologic smears, it is possible that these atypical cells in question might not have been found again after hybridization for FISH analysis in the remaining 4 cases in which FISH did not support malignancy in the atypical cells. Further studies with automated relocation tools are needed to explore the usefulness of multitarget FISH for the analysis of rare atypical cells in cytologic specimens.

The increased sensitivity of FISH in BB specimens was at the cost of decreased specificity, which questions the practical usefulness of this test. The reasons for the false-positive rates of up to 17\% in the BB specimens and bronchial secretions from patients with benign pulmonary lesions are unclear. It cannot be excluded that transient chromosomal abnormalities can occur rarely under nonneoplastic conditions, including chemotherapy or inflammatory changes. However, further studies are needed to explore whether the subset of patients with false-positive FISH results might be at increased risk of subsequent cancer, as suggested by a recent study on sputum specimens.\textsuperscript{20} In clinical practice for patients with clinically suspicious lung lesions, we suggest that identification of a chromosomal abnormality in a lung cytologic specimen should be a reason to support the diagnosis by repetitive biopsies and other diagnostic means or at least to closely follow up the patient, clinically and radiologically. In contrast, a negative FISH result by itself cannot exclude malignancy with sufficient certainty.

The multitarget FISH test, LA Vysion, can improve the sensitivity of cytologic examination for the detection of peripheral lung cancer in BB specimens and is not affected by smoking history. There is no compelling evidence of an extended cancer field with gross chromosomal aberrations across the whole bronchial tree.

From the \textsuperscript{1}Institute for Pathology and \textsuperscript{2}Department of Pneumology, University Hospital, Basel, Switzerland.

Supported by Vysis, Downers Grove, IL.

Address reprint requests to Dr Bubendorf: Institute for Pathology, University Hospital, Schönbeinstrasse 40, 4003 Basel, Switzerland.

Acknowledgments: We thank the staff of the cytology division of the Institute for Pathology and of the Pneumology Department, University of Basel, for excellent technical and logistic support. We are grateful to Dr Sauter for valuable comments on the manuscript. We also acknowledge the support from Albrecht Breitenbücher, MD, Kantonsspital Bruderholz, for providing sputum specimens.

References


23. Sanz-Ortega J, Saez MC, Sierra E, et al. 3p21, 5q21, and 9p21 allelic deletions are frequently found in normal bronchial cells adjacent to non–small–cell lung cancer, while they are unusual in patients with no evidence of malignancy. J Pathol. 2001;195:429-434.


