Early Detection of Lung Cancer With Laser-Induced Fluorescence Endoscopy and Spectrofluorometry*

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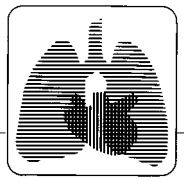

Early Detection of Lung Cancer With Laser-Induced Fluorescence Endoscopy and Spectrofluorometry*

Yoko Kusunoki, MD; Fumio Imamura, MD; Hiroshi Uda, MD; Masayuki Mano, MD; and Takeshi Horai, MD

Study objectives: **We performed a clinical trial of laser-induced fluorescence endoscopy (LIFE) for detection of precancerous lesions and cancer including carcinoma *in situ* (CIS), which are difficult to detect by white-light bronchoscopy.**
Design: **Results with LIFE were compared with the criterion standard, white-light bronchoscopy. The evaluation of these endoscopic results spectrofluorometrically was examined, and pixels of LIFE images composed of digital signals for the intensities of red and green were analyzed.**
Setting: **Tertiary-level hospital treating referrals and subjects with suspicious results in mass screening.**
Patients: **We examined 65 subjects with suspected lung cancer by both methods, and performed biopsy on 216 lesions.**
Results: **The accuracy of diagnosis by white-light bronchoscopy, with histopathologic results as the standard, was 48.6%. The accuracy by LIFE was 72.7%. The sensitivity of conventional bronchoscopy for detection of severe dysplasia (21 biopsy specimens) or cancer (28 biopsy specimens) was 61.2% and specificity was 85.0%. With results by LIFE added, these values were 89.8% and 78.4%, respectively. Of nine patients with CIS, only LIFE showed one lesion, and only LIFE showed the extent of seven of the lesions. The autofluorescence of eight lesions was measured spectrofluorometrically; normal bronchial tissue, severe dysplasia, and cancerous tissue had spectral differences. The red/green intensity of cancers on histograms of LIFE images generally was greater than the ratios for metaplasia or normal bronchial wall.**
Conclusions: **Use of both methods should facilitate early detection. Evaluation by spectrofluorometry and analysis of digital signal intensity of results by LIFE make results more objective.**
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Key words: autofluorescence; bronchoscopy; early detection; lung neoplasms; spectrofluorometry

Abbreviations: CIS = carcinoma *in situ*; LIFE = laser-induced fluorescence endoscopy

The annual mortality rate of lung cancer in Japan is increasing, and this disease became the most common cause of all deaths from cancer in 1998, when it first surpassed deaths from gastric cancer. Mortality can be decreased by smokers quitting this habit, but early detection and treatment also are needed. For peripheral lung cancer, conventional radiograph images generally do not show the disease in its curable early stage; spiral CT has been used for screening for several years. The cure rate for the central type of early-stage lung cancer has been improved by endobronchial treatment such as photodynamic therapy.[1–3] Unfortunately, the lesions are difficult even for experienced bronchoscopists to identify by conventional white-light bronchoscopy. Several authors[4–7] have reported the early detection of minute malignant lesions by methods involving

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the fluorescence of hematoporphyrin or a mixture of hematoporphyrin derivatives with affinity for neoplastic tissue. Side effects, particularly photosensitization, may occur. Small malignant lesions of the bronchus can be detected when stained with 5-aminolevulinic acid, which is inhaled.[8,9]

Laser-induced (originally, "lung imaging") fluorescence endoscopy (LIFE)[10–12] was developed for the detection of precancerous lesions and early-stage lung cancer. The method is based on differences in the autofluorescence of noncancerous and cancerous tissues. A photosensitizer is not used. Here, we evaluated the efficacy of this method for endobronchial detection of precancerous changes and cancer, including carcinoma *in situ* (CIS). We explored the use of autofluorescence spectroscopy and the calculation of digital signal intensity with analysis of the signal intensity of red and green for optical biopsy of tracheobronchial lesions.

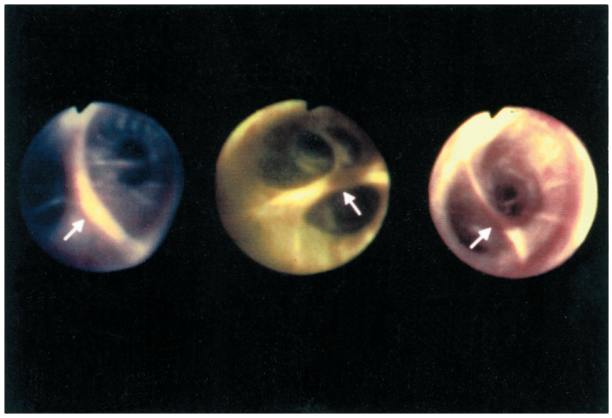

FIGURE 1. Classification by autofluorescence intensity in LIFE images. *Left*: class I, yellow-green; *middle*: class II, brown; *right*: class III, reddish brown.

MATERIALS AND METHODS

Subjects

A total of 65 subjects underwent bronchoscopy with LIFE. We performed a radiographic examination of heavy smokers identified as belonging to a high-risk group during mass screening for lung cancer, made available to individuals aged ≥ 40 years residing in any of seven cities in Osaka Prefecture, and examined sputum specimens cytologically. Results of the sputum examination called for further study of 26 patients, who were enrolled in our trial of bronchoscopy with LIFE. Another 21 patients presented with symptoms: cough, production of sputum or bloody sputum, or some combination of these. Fifteen other patients were being monitored after the treatment of lung cancer, and 3 patients were being monitored for other disease (esophageal cancer, idiopathic thrombocytopenia, and idiopathic neutropenia). Chest radiographs showed normal findings for 59 of the 65 patients, but other findings suggested lung cancer, and the remaining 6 patients had radiographic findings suggesting early lung cancer. Forty-three patients had findings from the cytologic examination of sputum that suggested or confirmed lung cancer, and the other 22 patients had negative findings. The median age was 65 years (range, 42 to 86 years); 63 patients were men and 2 patients were women.

LIFE

The LIFE apparatus (Xillix Technologies; Richmond, BC, Canada; and Olympus Optical; Olympus; Tokyo, Japan) was developed by Hung et al[10] and Lam et al[11] at the British Columbia Cancer Research Center. When LIFE is used as well as white-light bronchoscopy, examinations take 10 to 15 min longer. As a rule, the procedure could be carried out without additional use of anesthetics. A minor tranquilizer was used if coughing was severe or if the patient complained of dyspnea. The apparatus incorporates an image-intensified charge-coupled device with a red filter (wavelengths ≥ 630 nm) and green filter (wavelengths, 480 to 520 nm) under computer control. A beam of blue light with the wavelength of 442 nm produced by a helium-cadmium laser is delivered to the endobronchial wall via the illumination bundle of a fiberoptic bronchoscope, and the autofluorescence images are displayed on a red-green-blue image monitor.

Normal epithelial tissue gives off yellow-green fluorescence when excited by helium-cadmium laser light. Dysplastic tissue and CIS have less fluorescence than normal tissue.[12]

Spectrofluorometry

The spectrofluorometry system (optical multichannel analyzer IMUC-7000) was developed by Otsuka Electronics; Osaka, Japan; and Olympus cooperatively for collection of laser-induced fluorescence spectra of tissue during endoscopy. The apparatus includes an optical probe with 40 fibers for light delivery and collection. The spectrofluorometer sends data for processing and storage to the computer, and the spectrum is displayed. During the examination, the tips of the optical fibers are close to the surface of the tissue. The geometry of the laser illumination and fluorescence collection is exactly the same for both abnormal and normal sites, so the intensity of fluorescence spectra can be calibrated automatically. We examined autofluorescence measurements with this spectrofluorometer system in a preliminary study.

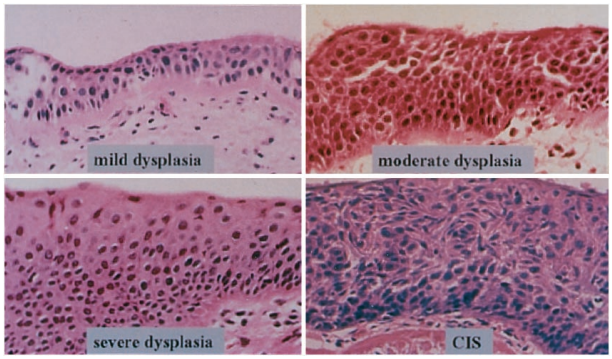

FIGURE 2. Grading from mild dysplasia to CIS.

Table 1—*Diagnosis by Histopathologic Examination, White-Light Bronchoscopy, or LIFE

Histopathologic Results	Biopsy Specimens (n = 216), No.	WLB			Class by LIFE		
		Normal (n = 65)	Mild-Mod, Inflam (n = 96)	Severe Dysplasia or Cancer (n = 55)	I (n = 49)	II (n = 106)	III (n = 61)
Normal	46	17	24	5	28	13	5
Mild-mod, inflam	121	43	58	20	19	88	14
Severe dysplasia	21	5	7	9	2	4	15
Cancer	28	0	7	21	0	1	27

*WLB = White-light bronchoscopy; Mild-mod, inflam = mild or moderate metaplasia, or inflammation. Accuracy by white-light bronchoscopy was 48.6%, and accuracy by LIFE was 73.1%.

Signal-Intensity Ratio of Red to Green

Pixels of LIFE images, composed of digital signals for the intensities of red and green, were analyzed. They were first saved in the tagged-image file format and then reconstructed on a computer display (Adobe Photoshop, version 5.0; Adobe Systems; Tokyo, Japan). Small areas with the typical appearance of normal bronchial tissue, metaplasia, or cancer (and verified histologically to be so) were selected, and the signal intensities of red and green were measured. The red signal intensity is affected by the total signal intensity, so we corrected for this effect by use of the red/green signal-intensity ratio.

General Methods

First, patients underwent conventional bronchoscopy done with a fiberoptic or video-assisted bronchoscope, or both. Then fluorescence bronchoscopy was done. We used a three-point scale for autofluorescence (Fig 1). Class I images had normal fluorescence intensity and were yellow-green, class II images had somewhat less fluorescence intensity than class I images and were brown, and class III images had much less intensity than class II images and were reddish brown. Biopsies were performed on eight lesions; these were lesions of all patients seen by us during a short time when the apparatus for autofluorescence measurements was on loan to us as part of its clinical evaluation. In addition, all abnormal areas discovered by either white-light or fluorescence examination underwent biopsy for histopathologic examination, and were graded as showing mild, moderate, or severe dysplasia, or as CIS (Fig 2).

Results

The mean cigarette index (packs of cigarettes per day times years at that consumption rate) for the 63 smokers was 55 packs (range, 5 to 140 packs), and two patients were nonsmokers. In all, 216 sites underwent biopsy. The histopathologic diagnoses of the specimens were as follows. Forty-six findings were normal. Of the 142 nonmalignant lesions, 28 were of inflammation or hyperplasia, 49 were of mild dysplasia, 44 were of moderate dysplasia, and 21 were of severe dysplasia (precancerous). Of the 23 lesions found to be lung cancer, 10 were radiographically occult, and 9 of these lesions were CIS. Five were cancers that originated in another organ.

With the histopathologic diagnosis as the standard, diagnostic accuracy by white-light bronchoscopy was 48.6% and that of LIFE was 73.1% (Table 1). The sensitivity of white-light bronchoscopy for detection of severe dysplasia and cancer as a single category was 61.2%, and the specificity was 85.0% (Table 2). With the inclusion of results from LIFE, the sensitivity and specificity were 89.8% and 78.4%, respectively.

One of the nine lesions of CIS detected was seen only on LIFE images (Fig 3). The other eight lesions were detected in the white-light images also, but for seven of the lesions, the extent of the tumor was clear on the LIFE images alone. (All of these early lung cancers could be eradicated by photodynamic therapy.) For example, Figure 4 shows CIS at the bifurcation of the right lower-lobe bronchus. As seen by white light, the tumor seemed to occupy all of the area between the right basal bronchus and right B_6.[6] By LIFE, only part was reddish brown, and the rest looked like noncancerous tissue.

Spectrofluorometry of LIFE images of eight lesions suggested that four were invasive cancers and the remaining lesions were of CIS and of severe,

Table 2—*Sensitivity and Specificity of Methods Used Alone and Combined for Diagnosis of the Category 'Severe Dysplasia and Cancer'

Methods	Sensitivity, %	p Value†	Specificity, %
With WLB	61.2		85.0
With LIFE	83.7	0.016	88.6
With both	89.8	< 0.001	78.4

*See Table 1 for abbreviation.
†Compared with sensitivity with WLB alone, by χ^2 test.

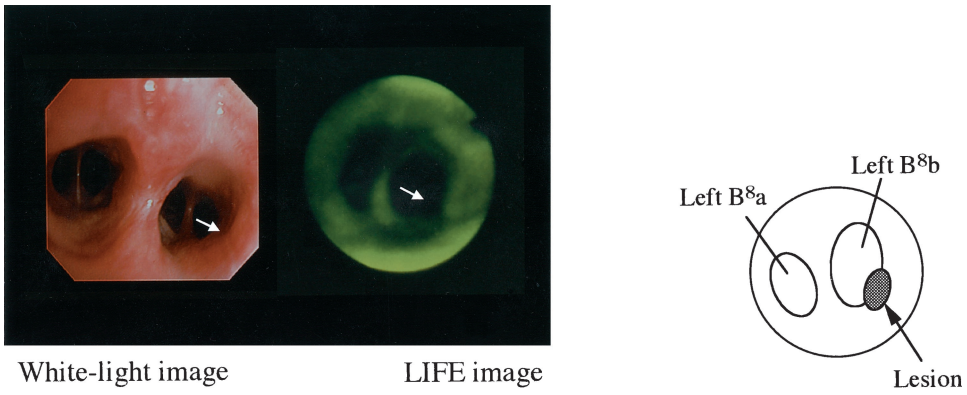

FIGURE 3. CIS lesion at left B_8b seen by LIFE image alone.

moderate, and mild dysplasia. Figure 5 shows LIFE spectra from three of the images; the lesions were of severe dysplasia, CIS, and an invasive tumor. The total fluorescence intensity from tumor sites was less than that from noncancerous sites. When the fluorescence intensity of the site of invasion was expressed as a percentage of the intensity from normal sites (Fig 5, *top*, *a*), the intensity for wavelengths from 600 to 680 nm (red fluorescence) was 190% of that for wavelengths from 500 to 550 nm (green fluorescence). For severe dysplasia and CIS, these values were 49% and 130%, respectively (Fig 5, *bottom*, *b*).

A LIFE image of an area of moderate dysplasia is shown in Figure 6. Pixels like the one in the Figure 6 were analyzed for red, green, and blue signal intensities (shown in the insets) and various calculations were made. In this example, the total signal intensity (± SD) in arbitrary units was 34.8 ± 2.6. The mean signal intensity was 24.7 ± 1.5 for red, and 46.4 ± 4.1 for green. The signal intensity for blue was zero. The red/green ratio was 0.53. We plotted the red/green signal-intensity ratio against the total signal intensity for 30 areas of normal bronchial tissue, 2 areas of moderate dysplasia, and 5 areas of CIS (Fig 7). The red/green ratios of areas of normal bronchial tissue were fairly uniform whatever the total signal intensity. The total signal intensities of areas of moderate dysplasia and CIS were low, without exception. The red/green ratios of moderate dysplasia were slightly higher than those of normal tissue. These ratios for CIS were higher than those of the normal tissue.

DISCUSSION

The combination of conventional bronchoscopy and LIFE can improve detection of endobronchial lesions.[13,14] We found the sensitivity in detection of severe dysplasia and cancer, including CIS, by this combination to be 29 percentage points higher (improvement by slightly less than 50%) than by conventional bronchoscopy alone. The specificity with this combination was lower by about 7 percentage points. We concluded that all sites considered suspicious by either method should be examined.

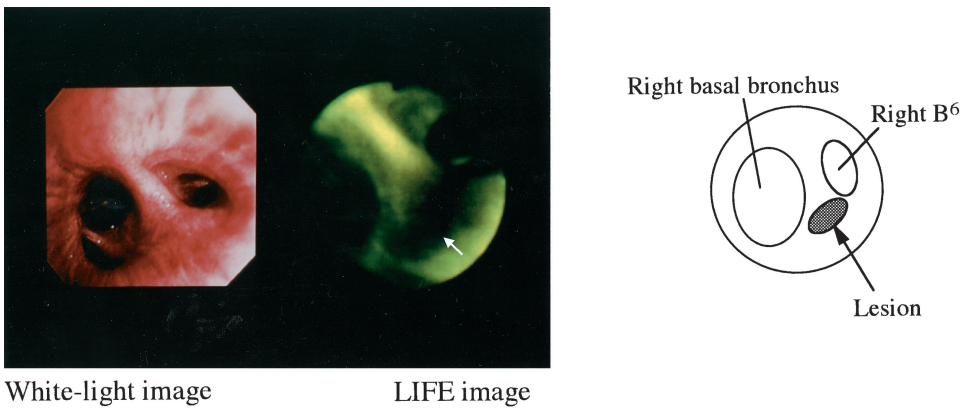

FIGURE 4. Extent of CIS lesion seen by LIFE image alone.

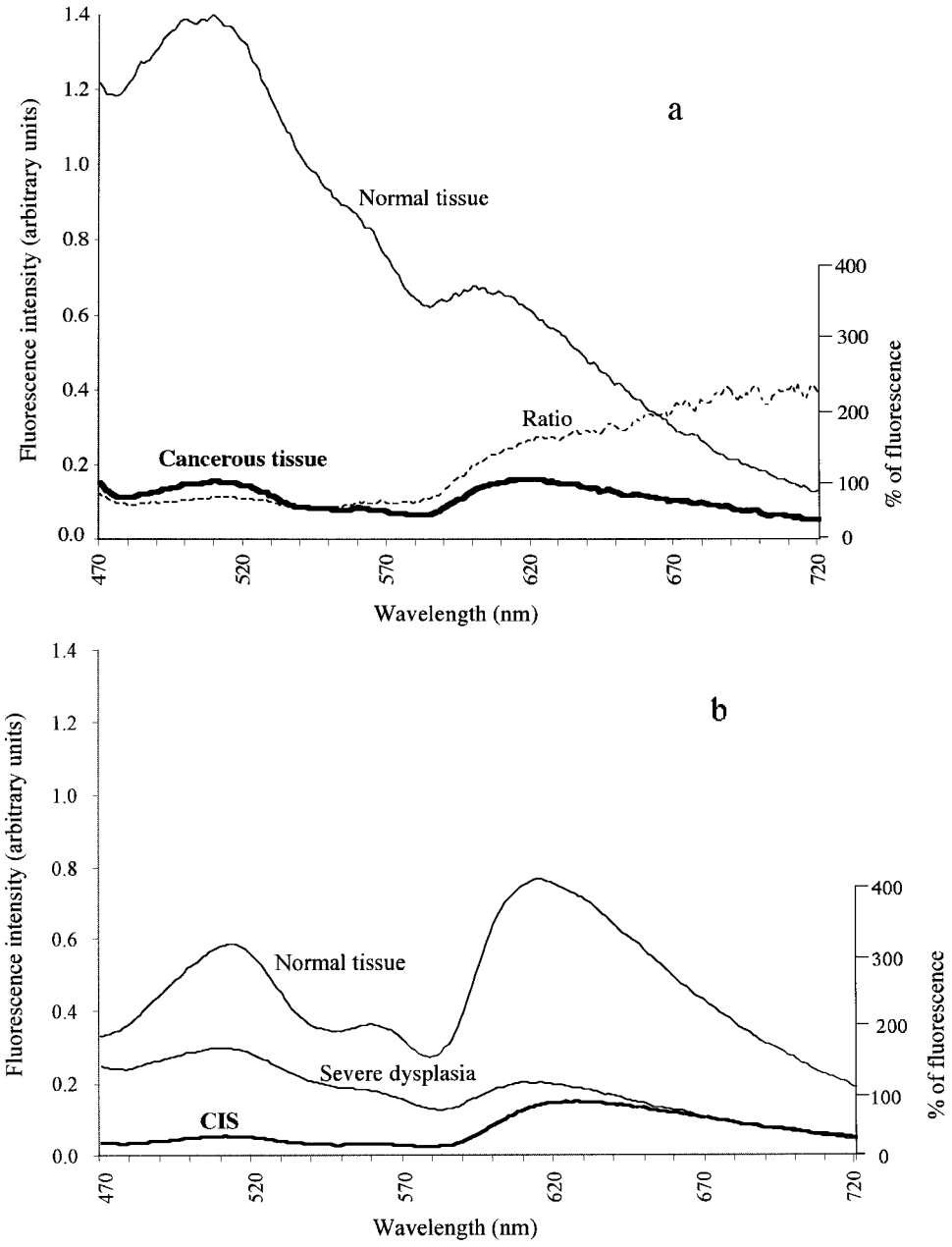

FIGURE 5. Fluorescence spectra. *Top*, *a*: Invasive cancer in a 64-year-old man; cigarette index, 70; positive findings by sputum cytologic examination. *Bottom*, *b*: CIS and severe dysplasia in a 68-year-old man; cigarette index, 69; positive findings by sputum cytologic examination. LIFE images in *bottom*, *b* show CIS and severe dysplasia (class III).

The extent of the tumor in patients with CIS was not apparent by the conventional method, but in general could be identified by LIFE.

Autofluorescence of tissues can change if the epithelial layer thickens, or if the concentration in the tissue of certain substances, including flavins, collagen, and nicotinamide adenine dinucleotide (NADH), changes. For example, red autofluorescence (at wavelength of 600 to 680 nm) may be greater in tumor tissues because the amount of porphyrin increases as a result of tumor hypervascularity.[15] Reasons for the differences in autofluorescence of tumor tissue compared with normal tissue are not understood in detail.

Vo-Dinh et al[16] suggest division of the amplitude of fluorescence intensity at each wavelength by the integrated area under the spectrum from 430 to 720 nm to eliminate error arising during *in vivo* measure-

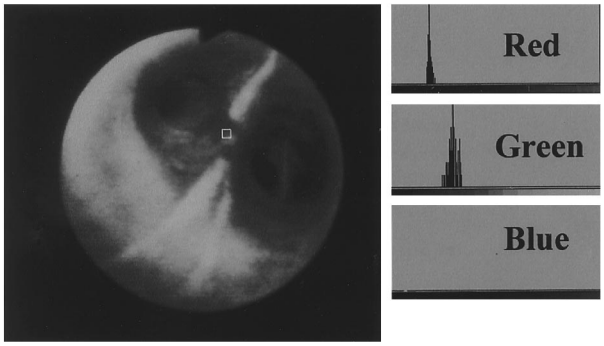

FIGURE 6. Example of LIFE image of area of moderate dysplasia shown with red, green, and blue signal intensities of the single pixel outlined in white. Mean (± SD) signal intensity: red, 24.7 ± 1.5 (± SD); green, 46.4 ± 4.1 (± SD); blue, 0. Total intensity = 34.8 ± 2.6 (± SD) (0.30 R + 0.59 G + 0.11 B), where R = red; G = green, and B = blue. Red/green ratio = 0.532.

ments and also to amplify differences in the low-intensity spectra of altered tissues. However, we found during comparison of fluorescence spectra of normal bronchial tissue and tissues with severe dysplasia or cancer that even without such correction, differences in the fluorescence intensity were obvious. The LIFE images showed both severe dysplasia and CIS as class III by our classification.

Cancer cells contain a higher concentration of protoporphyrin derivatives than normal cells because of impaired heme metabolism.[15] For that reason, the emission of red fluorescence on excitation of cancerous tissue by blue light increases more than the emission of green fluorescence. Our results suggested that the detection of cancer and metaplasia by LIFE images may be improved by the plotting of red/green ratios.

LIFE is useful for the early detection of minute malignant lesions that could be overlooked by conventional bronchoscopy. It might be used in the screening of high-risk subjects with suspected occult lung cancer. Results obtained by analysis of spectrofluorescence and the red/green signal intensity ratio give a more objective basis for the diagnosis of precancerous and cancerous lesions than evaluation by eye.

For the endobronchial treatment of early-stage lung cancers of the central type by photodynamic therapy, LIFE can be done to identify the extent of any dysplasia or cancer found. Endoscopic ultrasonography done in addition to LIFE can show deep invasion of the bronchial wall,[17] so that correct decisions about treatment can be made.

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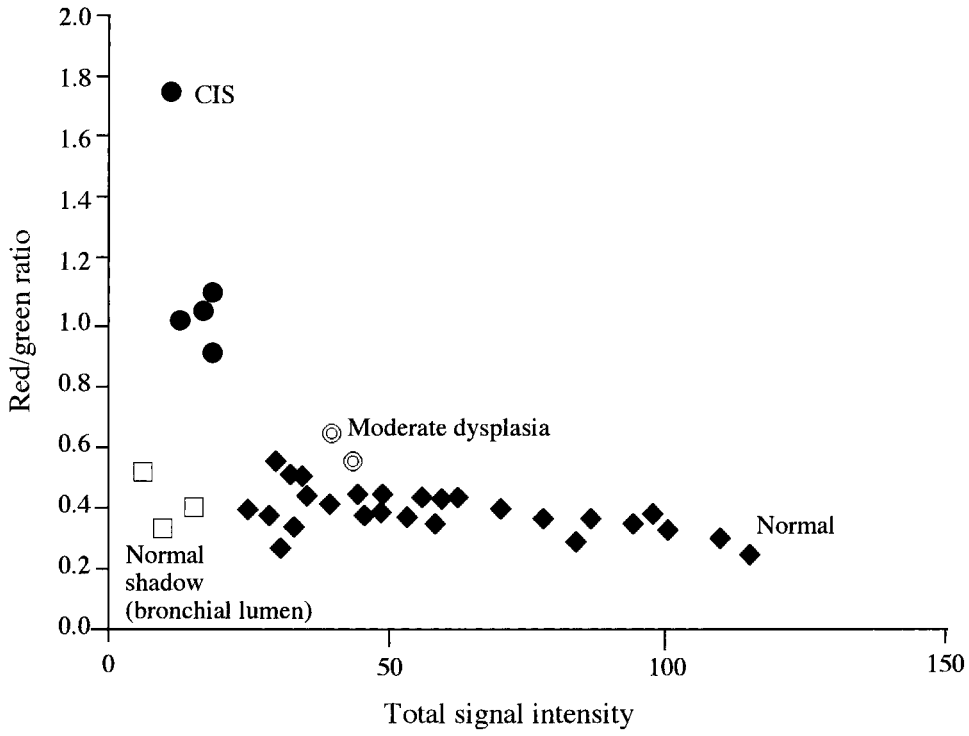

FIGURE 7. Red/green ratios and total signal intensities for samples with different histologic findings.

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