A Cytologic Evaluation of Sputum in Marijuana Smokers

Kevin Starr, MD, and Mark Renneker, MD
San Francisco, California

Background. There is growing evidence that smoking marijuana produces pulmonary effects similar to those of smoking tobacco. Cytologic analysis of sputum is readily available to practicing physicians and may be used in evaluating the pulmonary health of marijuana smokers. This study examined the use of sputum cytologic testing in young, athletic, marijuana-only smokers.

Methods. Sputum samples were collected from 25 marijuana-smoking members (surfers) who live in rural areas and do not smoke tobacco (mean age 27.5 years). The samples from the study group were compared with the sputum samples of 25 urban tobacco smokers and 25 nonsmokers of similar ages. Components of sputum were analyzed quantitatively and qualitatively. Subjects were educated and counseled as to the results.

Results. Compared with nonsmokers, marijuana smokers showed significantly higher levels of all pathologic components ($P<.05$), but lower mean levels of neutrophils (5.4 vs 6.4, $P=.005$) and pigmented macrophages (4.9 vs 6.1, $P<.001$) than those of tobacco smokers. Two cases of dysplasia were noted among the tobacco smokers and one among the marijuana smokers. Test-result counseling of a limited data set (6 subjects) at 6 months resulted in a 50% self-reported cessation rate.

Conclusions. In this pilot study, results of cytologic evaluations in marijuana smokers closely resembled those observed in tobacco smokers. Further studies are needed to determine longitudinal and dose-related effects of marijuana smoking on cytologic changes. As a noninvasive testing method, sputum cytologic analysis may be a useful tool for evaluating the pulmonary health of marijuana smokers and may present an opportunity to counsel them on the benefits of cessation.

Key words. Marijuana smoking; sputum; cytology; smoking; sports medicine. (*J Fam Pract 1994; 39:359-363*)

There is an emerging body of research suggesting that marijuana smoking may affect the lungs in a manner similar to that of cigarette smoking. With the exception of nicotine, marijuana smoke contains virtually all the lung irritants and cancer-causing compounds of tobacco smoke, in addition to 60 possibly harmful compounds not found in tobacco.[1] Smoking marijuana actually results in a greater respiratory burden of carbon monoxide and tar than does smoking a similar quantity of tobacco.[2] Lung airflow studies of heavy marijuana smokers found adverse effects on large airway function,[3,4] and marijuana smokers have been shown to have a higher prevalence of acute and chronic respiratory symptoms such as coughing, wheezing, and increased sputum production.[3]

Various studies have revealed pathologic microscopic changes in the airways of heavy marijuana smokers.[5,6] These changes are similar, though not identical, to those seen in tobacco smokers. The methods used in those studies, bronchoscopy and bronchoalveolar lavage, are invasive and involve some risk to subjects.

While the role of sputum cytologic analysis in lung cancer screening continues to be explored,[7] it is useful as a noninvasive research tool to investigate cytologic changes resulting from smoking. Sputum cytologic evaluation has been used extensively to assess the pulmonary health of cigarette smokers, and a simple objective and reproducible system has been developed to examine specimens.[8]

The succession of cytologic changes in the sputum of long-term cigarette smokers is well documented[9]: over a

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From the Department of Family and Community Medicine, University of California, San Francisco. Requests for reprints should be addressed to Kevin Starr, MD, 750 La Playa #623, San Francisco, CA 94121.

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period of 20 to 30 years, healthy cells gradually transform into abnormal cells that may become cancerous.

The lung's first response to cigarette smoke is proliferation and thickening of surface tissue in the airways (hyperplasia). As the irritation increases, small areas of delicate, glandular columnar cells transform into patches of scaly, toughened squamous cells (metaplasia). Further irritation may cause these cells to become precancerous (dysplasia). Dysplasia can evolve into cancer over a period of years. All these stages are accompanied by increased levels of inflammatory cells (macrophages, neutrophils) and other sputum components associated with chronic irritation (eg, mucus, Curschmann's spirals).

Up to the point of malignant cell development, this process is potentially reversible. In cigarette smokers, damaged lung tissue can revert to normal within 5 years of quitting smoking.[10]

Sputum cytologic testing can demonstrate irritative and precancerous changes in the lungs of cigarette smokers and is capable of detecting lung cancer at an early stage,[9,11,12] but it remains controversial whether sputum cytologic testing is sensitive enough to effectively screen for lung cancer. The use of biomarkers such as monoclonal antibodies may add greater sensitivity to sputum cytologic testing.[7]

Conducting valid research on the health effects of illegal drugs may best be facilitated by working with an organization trusted by the drug-using community. In this case, as members of the Surfer's Medical Association,* the authors had access to a group of frequent marijuana users: surfers. Surfers are among the fittest of athletes and are likely to be healthy.[13] Although few smoke tobacco, it has been estimated that 60% to 90% of surfers smoke marijuana.[14]

Methods

Subjects were volunteer participants drawn from the populations of surfers in the coastal areas of Mendocino and Humboldt counties in California, as well as the North Shore of Oahu, Hawaii. Criteria for inclusion in the study were: (1) smoking marijuana regularly (at least twice weekly) for more than 2 years; (2) no current or previous tobacco use; (3) no recent or chronic respiratory or systemic disease; (4) rural residency (ie, less air pollution); (5) no other known drug or toxic industrial exposure; and (6) surfing regularly (at least three times a week).

Local members of the Surfer's Medical Association volunteered to serve as the study coordinators in each location. The use of local coordinators known and trusted by subjects was considered essential to gaining participation in the study. Local coordinators were trained by the investigators to (1) alert surfers as to the existence of the study (by word of mouth); (2) administer questionnaires to potential subjects; (3) screen questionnaires for inclusion and exclusion criteria; (4) distribute sputum sample canisters; and (5) instruct eligible subjects in the proper method of collecting sputum specimens, and to inform, educate, and counsel subjects as to their individual results. To ensure complete confidentiality, the principal investigators and laboratory were blinded as to the subjects' identities. Local coordinators used coded lists matching subjects to specimens.

Each potential subject completed a 32-item screening questionnaire on smoking history (including passive smoking), respiratory health, marijuana and other drug use, and toxic exposures (including paraquat). This questionnaire was used by coordinators to determine which subjects met eligibility criteria.

Forty-four subjects were eligible for the study. Each subject was given a single, number-coded plastic canister containing ethylene glycol and rifampin as fixatives. They were carefully instructed in the proper means of producing and collecting specimens: to be done at home, in the morning upon first arising when sputum is easiest to produce; to place each successive specimen in the canister; and after collecting three specimens, to mail the preaddressed, prepostpaid canister to the laboratory (Cytosciences, Inc, Redwood City, Calif), a private firm specializing in sputum cytologic analysis.

Of the 44 potential subjects given canisters, 28 submitted specimens. Twenty-five were found suitable for analysis; unsuitable specimens contained saliva only, lacking pulmonary-origin cytologic evidence. The specimens were processed using the Saccomanno method[9] (material is mixed, centrifuged, and stained with a modified Papanicolaou technique). The microscopic components of the specimens were quantitatively analyzed and given a relative rating for each component (macrophages, pigmented macrophages, neutrophils, mucus, Curschmann's spirals, columnar cells, and metaplastic columnar cells) based on a scale of 0 to 10, with 0 = none present, 10 = maximum number.

Specimens were also examined for the presence or absence of dysplastic cells, eosinophils, reactive columnar cells, and benign bronchial hyperplasia.

The cytologic results from marijuana smokers were compared with those of age- and sex-matched male nonsmokers and tobacco smokers drawn from the laboratory's database of tested subjects. The nonsmoking group was composed of male Mormons from suburban Silicon

**The Surfer's Medical Association is an international sports medicine organization of over 700 surfing health care professionals and others interested in the health of surfers. Founded in 1986; its headquarters is in Santa Cruz, California.*

Table 1. Demographic Characteristics of Participants and Controls in a Sputum Cytology Study of Marijuana Smokers

Characteristic	Marijuana Smokers	Tobacco Smokers	Nonsmokers
Number	25	25	25
Mean age (±SD)	27.5 (7.9)	29.8 (4.8)	27.1 (3.5)
Male (%)	100	100	100
Environment	Rural	Urban	Urban
Tobacco use	None	28 cigarettes/day	None
Marijuana use	6 days*/week	None	None

**Mean of 25 long, deep inhalations from a joint (marijuana cigarette) per day.*
SD denotes standard deviation.

Valley, and the smoking group was composed of white men from throughout the San Francisco Bay area. Members of both of these comparison groups were recruited and paid by the laboratory to provide a broad database for the firm's sputum cytology research. They were healthy individuals with no other drug or industrial exposures. Specimens from all three groups were analyzed by the same cytologists, who were blinded to subjects' smoking status.

The association of nonquantified components of sputum with smoking status was assessed using chi-square analysis. Analysis of variance was used to compare mean values of quantifiable cytologic components of sputum among the three groups. Pairwise comparisons of means were performed using *t* tests.

The local coordinators gave each subject his results, educating and counseling each as appropriate. The pulmonary cytology reports from the laboratory included graphic displays of each sputum component analyzed, a composite graph indicating where each subject's composite results lay on a continuum of normal through class IV, a written summary, and color photomicrographs of notably abnormal cells. Local coordinators were instructed to advise subjects with abnormal results that stopping marijuana smoking may result in a trend toward cytologic normalization in future sputum sampling, and that continued marijuana smoking would be unlikely to show that normalization trend. The coordinators ensured that subjects had physician follow-up, if needed.

Results

Table 1 provides a description of the study population. In the study group of 25 marijuana-smokers, the average age was 27.5 years (range, 15 to 38 years). All the subjects were men, reflecting the male predominance in the sport of surfing. They had smoked marijuana an average of 5.75 days per week with a mean of 25 "hits" per day smoked. A "hit" is a long, deep inhalation from a "joint"; 10 to 15 hits can be obtained from each joint. The average ages of the matched nonsmokers and smokers were 27.1 and 29.8, respectively. The tobacco smoking group had consumed an average of 28 cigarettes a day over a span of 13.5 years.

Results of sputum cytologic tests for the three groups are summarized in Tables 2 and 3. Marijuana smokers showed significantly higher levels of all pathologic components than did nonsmokers. Marijuana smokers had significantly lower levels of neutrophils (5.4 vs 6.4;

Table 2. Average Quantitative Values of Cytologic Components of Sputum in Marijuana Smokers, Tobacco Smokers, and Nonsmokers

Cytologic Components	Marijuana Smokers* n=25 Mean (SD)	Tobacco Smokers* n=25 Mean (SD)	Nonsmokers* n=25 Mean (SD)	*P* Value†	*P* Value‡
Macrophages	5.4 (1.04)	5.8 (0.94)	4.0 (1.02)	.000	NS
Pigmented macrophages	4.9 (1.18)	6.1 (0.90)	3.8 (0.95)	.000	.000
Neutrophils	5.4 (1.08)	6.4 (1.32)	3.8 (1.58)	.000	.005
Mucus	4.4 (1.82)	5.2 (1.31)	3.1 (1.81)	.002	NS
Curschmann's spirals	1.0 (1.89)	1.3 (2.05)	0 (0)	.016	NS
Columnar cells	6.0 (3.06)	5.6 (2.58)	2.2 (3.14)	.000	NS
Metaplasia	4.4 (2.90)	5.1 (2.21)	1.2 (2.09)	.000	NS

**Relative levels of sputum components are given on a scale of 0 to 10: 0 = none present, 10 = maximum number present.*
†Values derived by analysis of variance.
‡Values derived by pairwise t test: marijuana vs tobacco.
SD denotes standard deviation; NS, not significant.

Table 3. A Comparison of Marijuana Smokers, Tobacco Smokers, and Nonsmokers Regarding the Presence of Nonquantified Components of Sputum

Component	Marijuana Smokers n=25 No. (%)	Tobacco Smokers n=25 No. (%)	Nonsmokers n=25 No. (%)	*P* Value*	*P* Value†
Dysplasia	1 (4)	3 (12)	0	.157	.297
Eosinophils	14 (56)	9 (36)	6 (24)	.020	.156
Reactive columnar cells	5 (20)	3 (12)	0	.070	.440
Bronchial hyperplasia	6 (24)	2 (8)	3 (12)	.250	.123

**Values derived by chi-square.*
†Values derived by pairwise t test: marijuana vs tobacco.

P=.005) and pigmented macrophages (4.9 vs 6.1; *P*=.0004) than did tobacco smokers.

There were two cases of dysplasia among the 25 cigarette smokers and one among the 25 marijuana smokers (in an 18-year-old). In all three cases, the degree of dysplasia was rated as mild in a continuum of mild–moderate–severe, with carcinoma in situ as the next stage beyond severe dysplasia. Dysplasia was not seen in the nonsmokers.

Discussion

In this group of 25 relatively young, healthy, athletic marijuana smokers living in a rural, presumably clean-air environment, cytologic changes in the lungs similar to those observed in a group of urban cigarette smokers were seen. In every component analyzed except bronchial hyperplasia, marijuana smokers resembled cigarette smokers more closely than they did nonsmokers.

In our study, cytologic findings of marijuana smokers were similar to those of tobacco smokers, but there were some important differences. There was no obvious explanation for the lower levels of neutrophils in the marijuana smokers. Earlier bronchoalveolar lavage studies have shown higher levels of neutrophils.[5] The lower levels of pigmented macrophages is consistent with a study showing that macrophages of marijuana smokers release decreased amounts of destructive oxidants, compared with those of tobacco smokers.[15] In our study, the levels of neutrophils and macrophages in marijuana smokers are closer to those of cigarette smokers than to those of nonsmokers.

Marijuana smokers showed the highest levels of bronchial hyperplasia, with cigarette smokers demonstrating lower levels than nonsmokers. While this result was not statistically significant (*P*=.25), it may suggest that in this group of long-time cigarette smokers, much of the susceptible airway cell layer has progressed beyond the initial stages of hyperplasia. This hypothesis is supported by the finding that the highest levels of metaplasia occurred in the tobacco group.

Our study shows similar cytologic changes in marijuana smokers averaging 25 hits per day (roughly equivalent to two joints) and cigarette smokers with a mean of 28 cigarettes daily. This finding concurs with earlier studies estimating that, in terms of lung damage, one joint equals about one pack of 20 cigarettes.[16]

There are a number of reasons why marijuana smoke appears to be more harmful than tobacco smoke. Marijuana smoke is unfiltered and contains more tar than cigarette smoke. In a joint, resins are concentrated and the smoke is inhaled more deeply and also held in the lungs for a longer time. Marijuana also contains a greater amount of carcinogenic substances such as benzopyrene than tobacco does.[16]

Our study does not demonstrate a causal relationship between marijuana and cancer. Our results do suggest, however, that smoking marijuana can lead to a cellular progression similar to that observed in cigarette smokers. Because tobacco is a known cause of lung cancer, it appears likely that the cellular abnormalities seen in marijuana smokers may progress to cancer as well. It would require 15- to 20-year longitudinal studies of marijuana smokers to demonstrate a causal relationship between marijuana and cancer.

Although the potential for cancer is worthy of concern, it is a relatively less common outcome of cigarette smoking when compared with other respiratory illnesses, such as infection and bronchitis. Similarly, a recent study by Kaiser Permanente in San Francisco showed that frequent marijuana smokers who do not smoke tobacco have significantly elevated rates of health care service utilization for respiratory and nonrespiratory illnesses.[17]

The present study was designed to examine whether cytologic analysis of sputum would demonstrate cytologic changes in marijuana smokers. However, as family physi-

cians we were also interested in sputum cytologic testing as a tool for health promotion. Given the peripatetic nature of surfers, long-term follow-up of the subjects in the study was difficult, but informal follow up surveys indicate that many of the subjects subsequently decreased their use of marijuana. Six months after testing, the local coordinator for the Mendocino group followed up on six subjects: three reported having quit smoking marijuana, two had cut down by about 50%, and one had made no change.

Our study is limited by small sample size and by the vagaries of self-reported smoking and drug-use histories. Although other studies have used terms such as "joint-years," there is no standardization of marijuana dosage. Additionally, variability of subject recall can be significant. For these reasons, no attempt was made to correlate dosages with cytologic findings.

Our conclusions would have been strengthened had we used more closely matched controls, ie, nonsmoking and tobacco-smoking surfers from the same rural areas. However, time and budget constraints did not allow us to recruit and test the necessary number of controls. Furthermore, we believe it is unlikely that we could have found the requisite number of tobacco-smoking surfers in the study areas. Because the principal difference between the subjects and the nonsmoking controls was that of rural vs urban air quality, our study may underestimate the differences between cytologic changes in marijuana smokers as compared with nonsmokers.

Although validity of specimen collection is often a concern in studies involving illicit drugs, we saw no reasons for local coordinators not to follow study guidelines or for subjects to alter or send in specimens other than their own. All the subjects we studied were volunteers, there was no incentive system for coordinators, and subjects were motivated solely to receive an assessment, albeit experimental, of the health of their own lungs.

Larger scale studies are needed to confirm the findings of this pilot study and to attempt a correlation of dosages over time with cytologic findings. Virtually all studies on marijuana smokers, as in this one, have focused on heavy rather than light users. On the basis of early studies, episodic use of marijuana has been assessed as being "not obviously damaging to the lungs,"[18] but further studies are needed to investigate the pulmonary effects of rare or occasional use of marijuana.

This study demonstrated evidence of cytologic changes in sputum resulting from marijuana smoking similar to those of tobacco smokers, and suggests that sputum cytologic analysis may be a useful tool for health education and promotion among marijuana smokers.

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