Prospective study of combined use of bronchial aspirates and biopsy specimens in diagnosis and typing of centrally located lung tumours

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Abstract

Objective—To determine the diagnostic accuracy of examining bronchial secretions in pulmonary cytopathology and whether cytology and histopathology can complement each other in routine practice among lung specialists.

Design—A prospective study comparing 1225 cytological and biopsy results, conducted during 1987-93. Tumours were confirmed by histopathology, imaging techniques, or clinical outcome and imaging techniques combined.

Setting—11 lung or internal medicine units, France.

Subjects—1128 patients (874 men; 254 women) aged 65.3 (SD 13.7) years who underwent fiberoptic bronchoscopy for various pulmonary symptoms.

Results—Exact concordance between cytological and biopsy results was obtained in 1036/1187 (87.3%) satisfactory specimens. In all 574 lung tumours were diagnosed. One case (0.08%) was a false positive cytological diagnosis in a patient with tuberculosis. Patients with lung cancer were more likely to have positive cytological results than positive biopsy results ($P < 0.001$). Agreement in tumour typing was observed in 375/424 (88.4%) cases, when non-small cell carcinomas, small cell carcinomas and undifferentiated carcinomas were separated. In the 11 patients with squamous cell carcinomas in situ, eight (72.7%) of the carcinomas were diagnosed cytologically as squamous cell. Unsatisfactory material was obtained in only 20 (1.6%) and 19 (1.6%) cases by cytology and biopsy respectively. Examinations had to be repeated in 86 (7.6%) patients.

Conclusions—Examination of bronchial secretions complements histopathology in both diagnosing and typing lung tumours and could be performed more systematically in patients undergoing fiberoptic bronchoscopy.

Introduction

Lung tumours are the most common cause of death from cancer in men and according to recent studies are now emerging as one of the most common causes of death from cancer in women.¹ Usually for appropriate management of lung tumours a tissue diagnosis and typing must be obtained and a clear distinction between small cell carcinomas and non-small cell carcinomas must be made preoperatively or before any aggressive treatment. Histopathological investigation remains the mainstay of the diagnosis. Recent reports based on the recommendations of the pathology committee of the International Association for the Study of Lung Cancer show, however, that an approach that combines cytological examination and biopsy helps the search for mixed small cell and large cell carcinomas and may have important prognostic implications.² Moreover, bronchial biopsies cannot be performed in inaccessible sites or in patients at risk of haemorrhage, and alternative methods for obtaining tissue diagnosis (cytopathology) are sometimes needed.

Of the specimens that can be obtained by bronchoscopy, washings, brushing, and fine needle aspirations

may complement biopsies in the diagnosis of lung cancer.^{3,4} Traditionally, the bronchial secretions aspirated during fiberoptic bronchoscopy are considered to have little diagnostic value—as they are thought to contain mainly mucoid debris—and are discarded.⁴ Thus malignancy is often assessed by biopsies alone, whereas true carcinomatous cells can be readily recognised and typed with bronchial secretions.^{3,5} The aim of our study was to determine whether the simple aspiration of bronchial secretions can complement biopsies in the diagnosis and typing of lung tumours.

Patients and methods

During 1987-93 we obtained 1225 combined bronchial aspirates and biopsy specimens during fiberoptic bronchoscopy for various pulmonary symptoms in 1128 patients (874 men, 254 women; mean age 65.26 (SD 12.3-67) years). Patients were seen at 11 independent lung or internal medicine units. A questionnaire documenting clinical data was completed by the lung specialist at the first examination and at each control stage.

Bronchial secretions were usually obtained from the bronchial tree during bronchoscopy. In 98 cases additional material was obtained by instilling isotonic saline into a selected zone and reaspirating. All samples were preserved in 50% ethyl alcohol and posted to the cytopathology laboratory with clinical, radiological, and bronchoscopic information. Specimens were centrifuged (1500 rpm, 5 min). Three to five slides were prepared from the cell concentrate and stained according to a hypochromic Papanicolaou procedure.⁶ All slides were evaluated by the same pathologist. Malignant cells were typed at high power field and recorded as squamous cell carcinoma-type cells, adenocarcinomatous cells, poorly differentiated carcinomatous cells, large cell carcinoma-type cells, small cell carcinoma-type cells, or undifferentiated carcinomatous cells.

Usually one to four biopsy specimens were taken from abnormal areas within bronchoscopic range. Tissue specimens were fixed in Bouin's solution, posted to the corresponding histopathology laboratories, and processed routinely for paraffin embedding. Independent pathologists examined the specimens using the World Health Organisation's classification.⁷

For both cytology and histopathology, only specimens with unequivocal malignant features were considered to be positive. No "suspicious" or "atypical" case was included in the cancer cases. The data were coded and computerised. The χ^2 test on paired series was used to compare categorical variables, and a probability level of 0.05 was regarded as significant.

Results

Twenty bronchial aspirate samples were unsatisfactory owing to poor cellularity or the presence of too much blood. Histological examination was impossible in 19 cases owing to poorly preserved or non-representative material.

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BMJ 1995;310:624-7

CYTOHISTOLOGICAL COMPARISON

Table I shows a comparison of cytological and biopsy results. If unsatisfactory specimens are ignored, exact concordance was obtained in 87.3% (1036/1187) of cases. Malignancy was diagnosed by cytology in 424 out of 479 (88.5%) positive results from biopsy specimens and in 519 out of 574 (90.4%) tumours proved by both histopathology and subsequent follow up. Patients with lung cancer were more likely to have positive cytological results than positive biopsy results ($\chi^2_{ps} = 11.13$; $P < 0.001$). All the tumours diagnosed by cytology or biopsy, or both, were confirmed by histopathology (repeated biopsy or surgery specimens, when patients could undergo an operation), computed tomography, or the combination of imaging techniques and clinical outcome, except in one case of a false positive cytological result in a patient with tuberculosis.

Table II shows the agreement between cytological and biopsy typing. Agreement occurred in specimens from 377 men and 47 women (mean age 65.21 (SD 11.11) years). When lung tumours were separated into non-small cell carcinomas, small cell carcinomas, and undifferentiated carcinomas, exact concordance in typing was obtained in 375 of the 424 (88.44%) cases. Eight of 11 (72.7%) carcinomas in situ were diagnosed by cytology as squamous cell carcinomas.

TABLE I—Cross classification of 1225 cytological and biopsy results in 1128 patients undergoing fiberoptic bronchoscopy. Figures are numbers (percentages) of procedures

Cytological results	Biopsy results			
	Positive	Negative	Unsatisfactory specimens	Total
Positive	424 (34.6)	96* (7.8)	11 (0.9)	531 (43.3)
Negative	55 (4.5)	612 (49.9)	7 (0.6)	674 (55.0)
Unsatisfactory specimens	7 (0.6)	12 (1.0)	1 (0.1)	20 (1.6)
Total	486 (39.7)	720 (58.8)	19 (1.6)	1225 (100.0)

*One case was false positive cytological result in patient with tuberculosis.

TABLE II—Cytohistological comparison of typing in 424 lung tumours diagnosed by both cytology and biopsy. Figures are numbers (percentages) of lung tumours

Cytological results	Biopsy results				Total
	Squamous cell carcinoma, adenocarcinoma, or poorly differentiated carcinoma	Large cell carcinoma	Small cell carcinoma	Undifferentiated carcinoma	
Squamous cell carcinoma, adenocarcinoma, or poorly differentiated carcinoma	295* (69.6)	19 (4.5)	4 (1.0)	10 (2.3)	328 (77.4)
Large cell carcinoma	3 (0.8)	1 (0.2)	0	0	4 (1.0)
Small cell carcinoma	2 (0.5)	0	51 (12.0)	6 (1.4)	59 (13.9)
Undifferentiated carcinoma	10 (2.3)	4 (1.0)	13 (3.1)	6 (1.4)	33 (7.7)
Total	310 (73.1)	24 (5.7)	68 (16.0)	22 (5.2)	424 (100.0)

*Eight cases were squamous cell carcinomas in situ.

TABLE III—Cytohistological comparison in 95 lung cancers diagnosed by cytology alone. Figures are numbers (percentages) of lung tumours

Cytological results	Biopsy results				Total
	Negative or non-specific	Suspicious for squamous cell carcinoma	Suspicious for adenocarcinoma	Atypia	
Squamous cell carcinoma, adenocarcinoma, or poorly differentiated carcinoma	69 (72.7)	3 (3.2)	3 (3.2)	6 (6.3)	81 (85.4)
Large cell carcinoma	1 (1.1)	0	0	0	1 (1.1)
Small cell carcinoma	5 (5.3)	0	0	1 (1.0)	6 (6.3)
Undifferentiated carcinoma	6 (6.3)	0	0	0	6 (6.3)
Other*	1 (1.0)	0	0	0	1 (1.1)
Total	82 (86.3)	3 (3.2)	3 (3.2)	7 (7.4)	95 (100.0)

*Lymphoid infiltrate of lung in one case of chronic lymphoid leukaemia.

In the group of histologically proved small cell carcinomas exact typing was obtained by cytology in 51 out of 68 (75%) cases, and undifferentiated features were shown in 13 of the 68 (19.1%) cases. In six cases a small cell carcinoma component was shown by one method and a non-small cell carcinoma by the other; these cases were considered to be combined carcinomas. Undifferentiated components were found by biopsy in 10 out of 328 (3.0%) cytologically proved non-small cell carcinomas and by cytology in 10 out of 310 (3.2%) histologically proved non-small cell carcinomas. These cases were considered to be lung carcinomas showing anaplastic features.

DIAGNOSIS BY CYTOLOGY ALONE

In 95 cases lung cancer was diagnosed by cytology alone (81 men, 14 women; mean age 67.82 (10.61) years). Table III shows the comparison between the cytological and biopsy results. Biopsy specimens showed non-specific findings in 70 of the 82 (85.4%) cases. No significant differences occurred in the number of biopsies taken or in the bronchoscopic presentation of these cases. Except in one case (0.08%) of a false positive cytological result, malignancy was confirmed by repeated radiography and computed tomography.

DIAGNOSIS BY BIOPSY ALONE

In 55 cases lung cancer was diagnosed by biopsy alone (40 men, 15 women; mean age 64.30 (13.46) years). Table IV shows the comparison between the cytological and biopsy results. Non-specific cytological disorders such as inflammatory cells and metaplastic and hyperplastic changes of bronchial epithelial cells were found in 33 out of 55 (60.0%) cases, irrespective of the histopathological type of the tumours. Conversely, modifications of the squamous epithelium indicating potentially irreversible changes (keratinising metaplasia, moderate or severe nuclear atypias, cytological findings suggestive of squamous cell carcinoma) were found in nine out of 21 (42.9%) cases with squamous cell carcinoma on biopsy examination, and in two out of three (66.6%) cases with squamous cell carcinomas in situ.

REPEATED EXAMINATIONS

Examinations had to be repeated in 86 of the 1128 patients either to confirm the initial results or to resolve apparent discrepancies between results and clinical data. Examinations had to be repeated once in 76 patients, twice in nine patients, and three times in one patient. Table V shows the resulting 97 cytohistological comparisons.

Fifty three (54.6%) of the new paired results simply confirmed the initial data (42 pairs were concordant negative results, five were concordant positive results, four were negative cytology results with positive biopsy results and two were consecutively negative biopsy results in a patient with a positive cytological result). Of 57 concordant negative results initially recorded, 15 subsequent results (1.2% of all examinations) obtained in 10 patients were then found to be concordant positive. Concordant positive results were also obtained in four cases (three patients) after initial results that showed only positive cytology.

Overall, repeating the examinations confirmed the absence of abnormal cells in 42 cases, confirmed malignancy in 17, allowed documenting of malignancy in 29, and gave no additional information in nine.

Discussion

DIAGNOSTIC VALUE OF PULMONARY CYTOPATHOLOGY

Before the introduction of the flexible bronchoscope the diagnostic value of examining bronchial secre-

TABLE IV—Cytohistological comparison in 55 lung cancers diagnosed by biopsy alone. Figures are numbers (percentages) of lung cancers

Cytological results	Biopsy results							Total
	Squamous cell carcinoma, adenocarcinoma, or poorly differentiated carcinoma	Squamous cell carcinoma in situ	Large cell carcinoma	Small cell carcinoma	Malignant lymphangitis	Non-Hodgkin's lymphoma	Well differentiated neuroendocrine carcinoma	
Non-specific findings:								
Negative	6 (10.9)	0	2 (3.6)	3 (5.5)	1 (1.8)	2 (3.6)	1 (1.8)	15 (27.2)
Inflammation	7 (12.7)	0	1 (1.8)	3 (5.5)	2 (3.6)	0	1 (1.8)	14 (25.4)
Giant cells	0	0	1 (1.8)	0	0	0	0	1 (1.8)
Hyperplasia of bronchial mucosa	1 (1.8)	1 (1.8)	0	0	0	0	1 (1.8)	3 (5.5)
Features indicating potentially irreversible changes:								
Keratinising squamous metaplasia	—	1 (1.8)	0	0	0	0	0	1 (1.8)
Nuclear atypia (moderate and severe)	6 (10.9)*	0	1 (1.8)	0	2 (3.6)	0	0	9 (16.4)
Necrosis	1 (1.8)	0	0	0	0	0	0	1 (1.8)
Suspicious for squamous cell carcinoma	3 (5.5)**	1 (1.8)	1 (1.8)	0	0	0	0	5 (9.1)
Suspicious for adenocarcinoma	1 (1.8)***	0	0	0	0	2 (3.6)	0	3 (5.5)
Suspicious, not specified	3 (5.5)	0	0	0	0	0	0	3 (5.5)
Total	28 (50.9)	3 (5.5)	6 (10.9)	6 (10.9)	5 (9.1)	4 (7.3)	3 (5.5)	55 (100.0)

*One case had moderate nuclear atypia on cytological material and was correlated with a mixed squamous cell carcinoma and small cell carcinoma on biopsy material.

**The three cases with features suspicious for squamous cell carcinoma on cytological material were correlated with squamous cell carcinomas on biopsy material.

***The case with features suspicious for adenocarcinoma on cytological material was correlated with adenocarcinoma on biopsy material.

TABLE V—Cross classification of 97 results from follow up examinations

Initial result	Follow up results						
	Unsatisfactory cytology and biopsy	Unsatisfactory cytology and negative biopsy result	Negative cytological results and unsatisfactory biopsy	Negative cytological and biopsy results	Negative cytological result and positive biopsy result	Positive cytological result and unsatisfactory biopsy	Positive cytological result and negative biopsy result
Negative cytological results and unsatisfactory biopsy	0	0	0	0	1	0	0
Negative cytological and biopsy results	0	0	4	42*	5	0	6
Unsatisfactory cytology and positive biopsy result	0	0	0	0	0	0	0
Negative cytological result and positive biopsy result	1	0	0	1	4	0	0
Positive cytological result and unsatisfactory biopsy	0	0	0	0	0	0	0
Positive cytological result and negative biopsy result	0	0	0	0	0	0	2
Positive cytological and biopsy results	0	0	0	1	0	0	2
Total	1	0	4	44	10	0	10

Among the patients whose initial examination yielded negative cytological and biopsy results and in whom follow up confirmed this, one patient had a further (consecutive) examination that yielded negative cytological result and positive biopsy result and another had a further (consecutive) examination that yielded positive cytological results and negative biopsy result (included in table). Among the patients whose initial examination yielded negative cytological result and positive biopsy result and in whom follow up confirmed this, one patient had a further (consecutive) examination that yielded positive cytological and biopsy results (included in table).

*Four were in two patients (two sets of results in each).

**Six were in three patients (two sets of results in each); three were in one patient who had twice previously had negative cytological and biopsy results.

***Two were in one patient who had twice had positive cytological and biopsy results.

tions in detecting lung tumours was often lower than 50%: in 1964 Koss reviewed 560 patients with primary bronchial carcinomas in which examination of 288 bronchial aspirates (51.4%) yielded positive results.⁸ In 1975 Atay and Brandt reported that in a study of 885 patients 79% of centrally located lung tumours could be diagnosed cytologically, whereas only 45% of peripheral tumours could be detected.⁹ In 1979 Payne *et al* reported that the diagnostic accuracy of examining bronchial aspirates was 46% (50 of 109 primary bronchial carcinomas).¹⁰

Since fibreoptic bronchoscopy was introduced as a diagnostic procedure in 1968, several methods for obtaining satisfactory material have become available. Though examination of sputum can provide evidence of malignancy in many cases of cancer,^{2-4 11 12} specimens collected from fibreoptic bronchoscopy yield a higher rate of positive results. In 1980 Taft *et al* reported that the sensitivity of bronchial aspirates in diagnosing lung cancers was 75% and could reach 88.7% when the examination was performed by two pathologists.¹³ In 1991 DiBonito *et al* reported a sensitivity of 81% with no false positive results.⁵ The present series shows a sensitivity value of 88.3% if initial biopsy results are considered as the diagnostic reference, and 90.4% if both histopathology and follow up (the gold standard) are taken into account. Data about the cytopathology of bronchial secretions remain rare, and many authors have had difficulties in obtaining representative material from the bronchial tree, thus leading them to

discontinue examination of bronchial secretions in favour of other cytological methods.^{4 14}

Bronchial brushings are favoured by most groups for the cytological investigation of proximal lung cancers, and samples that arrive at the laboratory as prepared slides may seem much easier to handle. Standardisation is very difficult to obtain, however, especially in terms of quality of fixation. Many brushings are not satisfactory when they arrive at the laboratory from regional hospitals.

We have shown that, provided that conditions are adequate, the diagnostic accuracy of bronchial aspirates may exceed 80%. Moreover, cases that are difficult to manage—that is, contraindications for biopsy, positive fibreoptic bronchoscopy findings but positive cytological and negative biopsy results, mucosal abnormalities but negative cytological and biopsy results—are clearly resolved by repeating both examinations. Repeating examinations also led to the absence of false negative results for both diagnostic techniques together.

TYPING OF LUNG TUMOURS

According to the WHO's classification⁷ malignant epithelial tumours are separated into squamous cell carcinomas (30-60% of histopathology cases), small cell carcinomas (about 25%), adenocarcinomas (15-25%), and large cell carcinomas (10-20%). From a therapeutic point of view, lung tumours are better separated into small cell carcinomas, which require

Key messages

- Bronchial secretions aspirated during fiberoptic bronchoscopy have been thought to have little diagnostic value and are often discarded
- In this study an accuracy of 88.4% was achieved in typing lung tumours by examining bronchial secretions
- Repeated examinations for confirmation or to resolve apparent discrepancies led to confirmation of previous findings in two thirds of cases and to the recognition of malignancy in a third of cases
- Simple aspiration of bronchial secretions is of value in diagnosing and typing lung tumours

intensive chemotherapy and sometimes radiation therapy, and non-small cell carcinomas, which are better treated surgically.¹⁵

As far back as 1952 Foot had shown that typing could be correctly obtained by studying sputum in 89.9%, 78.4%, and 83.5% of squamous cell carcinomas, adenocarcinomas, and small cell carcinomas respectively.¹⁶ In 1972 Lange and Hoeg showed comparable results.¹⁷ In 1985 Truong *et al* showed that typing of squamous cell and small cell carcinomas could be correctly obtained in more than 80% of cases regardless of the cytological method used (sputum, washings, or brushings).¹⁸ In a recent series of 100 cases with confirmation at necropsy, the diagnostic yield of examining bronchial aspirates reached 80%.⁵ The present series shows that exact concordance can be achieved in 88.4% of cases.

A correct, if not definitive, diagnosis can be obtained in almost all cases of centrally located lung tumours by examining bronchial secretions. Moreover, when a definitive histopathological diagnosis cannot be obtained because of risk of haemorrhage, severe respiratory failure, or inconclusive sampling diag-

nostic procedures such as cytological examinations of bronchial aspirates become necessary.

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(Accepted 30 December 1994)

Apolipoprotein E genotype and association between smoking and early onset Alzheimer's disease

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Abstract

Objective—To investigate the hypothesis that differential survival between smokers and non-smokers leading to a decrease in the frequency of the e4 allele of the apolipoprotein E gene may explain the inverse relation between smoking history and early onset Alzheimer's disease.

Design—A population based case-control study.

Setting—The four northern provinces of the Netherlands and metropolitan Rotterdam.

Subjects—175 patients with early onset Alzheimer's disease and two independent control groups of 159 and 457 subjects.

Main outcome measures—Frequencies of the apolipoprotein e4 allele and relative risk of early onset Alzheimer's disease.

Results—The inverse association between smoking history and early onset Alzheimer's disease could not be explained by a decrease in the frequency of the apolipoprotein e4 allele. Among carriers of this allele with a family history of dementia subjects with a history of smoking had a strongly reduced risk of early onset Alzheimer's disease (odds ratio 0.10 (95% confidence interval 0.01 to 0.87)).

Conclusions—The results suggest that the inverse relation between smoking history and early onset Alzheimer's disease cannot be explained by an increased mortality in carriers of the apolipoprotein e4 allele who smoke. The association is strongly modified by the presence of the apolipoprotein e4 allele as well as by a family history of dementia.

Introduction

In several case-control studies an inverse association between smoking and Alzheimer's disease has been observed.¹⁻³ This observation has led to debate about whether smoking exerts a neuroprotective effect or whether the relation can be explained by bias related to reduced survival among smokers.¹⁻³ Riggs has argued that differential survival between smokers and non-smokers leading to a shift in gene pools may underlie the inverse relation between smoking and Alzheimer's disease.⁴ This hypothesis assumes that among smokers mortality is higher in those who are susceptible to Alzheimer's disease than in those who are not and therefore predicts a decreased frequency of a susceptibility gene among smokers.

Apolipoprotein E is a polymorphic protein that plays

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BMJ 1995;310:627-31