

Accuracy of morphological diagnosis of lung cancer in a department of respiratory medicine

MARTIN D CLEE, HELEN LD DUGUID,* DAVID JM SINCLAIR†

*From the Department of Respiratory Medicine, King's Cross Hospital, Dundee and the *Cytology Unit, Department of Pathology, Royal Infirmary, Dundee*

SUMMARY In view of the importance of determination of cell type in patients with bronchial carcinoma who are to be given chemotherapy a series of 140 histologically proved cases was analysed to assess the accuracy of pretreatment diagnosis by cytology and bronchial biopsy. Final histological diagnosis was made after thoracotomy (131 cases) or necropsy (9 cases).

Before treatment, malignancy was diagnosed in 71% of cases with 91% accuracy in cell typing. Diagnostic yield was greatest in squamous carcinoma (81%) and least with adenocarcinoma (50%). Large-celled and small-celled anaplastic carcinomas gave yields of 61% and 68% respectively. Accuracy of cell typing was high (90-97%) except for large-celled anaplastic carcinoma (71%).

All diagnostic methods were not employed in every case. In patients supplying samples the diagnosis was made by sputum cytology in 57%, by rigid bronchoscopy cytology in 57%, by fiberoptic bronchoscopy cytology in 71% and biopsy in 53%.

Causes of diagnostic failures and possible means of increasing accuracy of pretreatment diagnosis were discussed.

During recent years there have been significant advances in both the diagnosis and treatment of primary bronchial carcinoma. Fiberoptic bronchoscopy was developed in Japan in 1964 and introduced in America in 1969¹ and Britain in the 1970s.² It is a safe technique with few complications and can be performed quickly with minimal discomfort to the patient. Direct examination of the entire tracheo-bronchial tree to the level of the subsegmental bronchi is possible allowing biopsy of tumours inaccessible at rigid bronchoscopy.³ Beyond the range of the instrument a diagnosis may be made by brush or transbronchial biopsy under fluoroscopic control¹ and needle biopsy can be used for more peripheral tumours.⁴

Accurate morphological diagnosis of bronchial carcinoma before treatment is now possible in all cases and is increasingly important since it allows accurate evaluation of the various drug regimens of cytotoxic treatment in small-celled carcinoma and avoids subjecting patients with non-responsive, non-small-celled anaplastic carcinoma to drugs causing serious morbidity.

In this area the Department of Respiratory

Medicine undertakes the investigation of cases of possible bronchial carcinoma and the management of cytotoxic treatment. Cases requiring operation are referred elsewhere.

The purpose of this study is to review the yield of our pretreatment morphological diagnosis of malignancy and accuracy of cell typing and to investigate the problems involved in obtaining high diagnostic yield and accuracy.

Patients and methods

Case notes and laboratory reports of all patients admitted to the Chest Unit between 1976 and 1979 with a diagnosis of primary bronchial carcinoma confirmed histologically at thoracotomy (131) or necropsy (9) were studied.

Final histology was defined as the predominant cell type noted in the excised lung tumours or at necropsy. The tumours had been classified as epidermoid 73 (52%); small-celled anaplastic 22 (16%); large-celled anaplastic or poorly differentiated (tumour type not specified) 23 (16%); adenocarcinoma 18 (13%); and combined epidermoid and adenocarcinoma 4 (3%).

†Present address: Poole Hospital, Nunthorpe, Cleveland.

The diagnostic yield of each initial (pretreatment) investigation was defined as the percentage of patients submitting specimens in which malignant cells were identified.

The diagnostic accuracy was defined as the percentage agreement between the cell type obtained at initial investigation and the histological confirmation.

SPUTUM

A minimum of three specimens was obtained from each patient, those containing alveolar macrophages being accepted as satisfactory for examination. Special attention was directed to obtaining satisfactory specimens; all patients received physiotherapy and were issued with specific written instructions which were reinforced and supervised by the nursing staff. Since we have long recognised that better sputum specimens may be obtained after bronchoscopy the majority of the sputa submitted were first morning specimens on three consecutive days after bronchoscopy. The specimens were scrutinised at ward level. If obviously unsatisfactory they were discarded and further samples obtained unless the patient's cough was non-productive when all specimens obtained were sent for examination.

BRONCHOSCOPIC SPECIMENS

Distinction was made between patients who had rigid and fiberoptic bronchoscopy. A tumour was defined as visible only if endobronchial tumour tissue was seen and in these cases brushings were taken from the abnormal area; where there was no visible lesion, brushings were taken from the appropriate lobar or segmental bronchus indicated by the radiological positions on posteroanterior and lateral chest x-rays. Biopsies were taken only when tumour tissue was suspected at fiberoptic bronchoscopy. The rigid bronchoscope was employed during the earlier part of the study when only a limited number of biopsy specimens were obtained. Whenever possible the hospital MLSO was present at the bronchoscopy session. He prepared the specimens for histological and cytological examination and informed the bronchoscopist if samples were macroscopically adequate.

BRONCHIAL BRUSH SPECIMENS

Smears from each brushing were spread over an area of 1 cm² on two previously labelled glass slides and immediately placed in cytological fixative (Carbowax 30 g, glacial acetic acid 2 ml, 95% alcohol to one litre). Bronchial aspirate was collected in a Vygon mucus extractor from which suitable portions were removed with an orange stick, spread between two slides and immediately fixed in cytological fixative.

BRONCHIAL BIOPSY

Usually several biopsies were taken. The biopsy material was washed from the biopsy forceps with sterile tissue culture fluid into a centrifuge tube containing formol saline. After the final biopsy the forceps were agitated in the fixative to remove small tissue particles.

METHOD OF REPORTING

In order to ensure uniformity in nomenclature of initial and final reports all specimens in which the original diagnosis had been undifferentiated or poorly differentiated carcinoma were reviewed without knowledge of the final tumour histology.

In 34 of 38 cytology specimens it was possible to make a more specific diagnosis of tumour type including eight cases of small-celled anaplastic carcinoma but in seven biopsy specimens due to the small amount of tissue and crush artefacts more precise specification was not possible. These specimens were therefore classified in the large-celled anaplastic group.

False-positive cytology reports in experienced hands should be less than 1% of the total positive cases.⁵ "Suspicious" reports are therefore given when the appearances do not conform to the recognised cellular patterns of the various types of malignancy or if necrosis or degeneration precludes a more accurate diagnosis. In this study reports "suspicious of malignancy" were not included in the analysis.

Results

Table 1 analyses the diagnostic accuracy of the various types of investigation. The percentage yield for sputum of 57% was similar to that of other series.^{5,6} With sputum there is a high degree of cell type accuracy (90%) and like others we have found that tumour cells which exfoliate in sputum are often more differentiated and therefore easier to classify.⁷ The cytological yield for fiberoptic bronchoscopy (71%) was greater than that for rigid bronchoscopy (57%) but accuracy of cell type diagnosis (91%) was greater in rigid bronchoscopy. This was probably due to the larger volume of specimens obtained. Both yield (53%) and cell type accuracy (76%) of biopsy specimens obtained at fiberoptic bronchoscopy were poor. However, as the forceps are very small, it is possible to miss tumour tissue, the sample being taken from overlying or adjacent normal or abnormal epithelium.⁸ Accurate tumour typing is difficult when the biopsy specimen is tiny and subject to crush artefact.^{8,9}

In Table 2 the accuracy of cell type diagnosis at the various initial investigations was compared with the final histological findings.

Table 1 Diagnostic accuracy of various types of investigation

	Sputum	Bronchial brushings and aspirations (rigid)	Bronchial brushings and aspirations (fiberoptic)	Bronchial biopsy (fiberoptic)
Patients supplying samples	107	58	70	55
Malignancy diagnosed	61	33	50	29
Percentage yield (%)	57	57	71	53
Accurate cell type diagnosed	55	30	42	22
Percentage accuracy (%)	90	91	84	76

Table 2 Preoperative diagnosis of cell type compared with tumour histology at thoracotomy or necropsy

Investigation	n	Tumour type (thoracotomy or necropsy)				
		Epidermoid	Small-celled anaplastic	Large-celled anaplastic	Adenocarcinoma	Epidermoid and adenocarcinoma
Preoperative cell type						
Sputum						
Epidermoid	44	39 (88%)	1	2		2 (5%)
Small-celled anaplastic	8		8 (100%)			
Large-celled anaplastic	4			4 (100%)		
Adenocarcinoma	5				5 (100%)	
Rigid bronchoscopy aspirations and brushings						
Epidermoid	23	21 (91%)		2		
Small-celled anaplastic	4		4 (100%)			
Large-celled anaplastic	5	1		4 (80%)		
Adenocarcinoma	1				1 (1/1)	
Fiberoptic bronchoscopy aspirations and brushings						
Epidermoid	26	24 (92%)	1	1		
Small-celled anaplastic	8		8 (100%)			
Large-celled anaplastic	9	3		5 (55%)	1	
Adenocarcinoma	7	1			5 (71%)	1 (14%)
Fiberoptic bronchial biopsy						
Epidermoid	17	17 (100%)				
Small-celled anaplastic	3		2 (67%)		1	
Large-celled anaplastic	7	3	2	2 (30%)		
Adenocarcinoma	2	1			1 (1/1)	

n = total number of patients producing positive samples.

With epidermoid carcinoma there was a high degree of accuracy in all methods of investigation reaching 100% in biopsy specimens. Poorly differentiated epidermoid carcinoma was diagnosed cytologically in five cases (7 samples) where the final diagnosis was anaplastic carcinoma (small-celled 1 case, large-celled 4 cases). In the first case the most probable explanation was the occurrence of squamous differentiation in a predominately small-celled anaplastic carcinoma. The remaining four cases illustrate the difficulty in typing (both histologically and cytologically) of large-celled anaplastic and poorly differentiated carcinomas. Mennemeyer *et al*¹⁰ in a series of such cases verified the diagnosis by electron microscopy and found that cytology gave a more accurate diagnosis of cell type than histology.

The initial cytological and histological typing of small-celled anaplastic carcinoma and adenocarcinoma was also very accurate. Cytologically oat-celled carcinomas show characteristic cell groupings with moulding of adjacent cells.^{7 11} One adenocarcinoma

was diagnosed on biopsy as small-celled anaplastic carcinoma but on review crush artefact was noted. In most adenocarcinomas tumour acini or papillary clusters can be identified.¹² In two specimens (1 case) the original diagnosis was adenocarcinoma (with confirmed mucus secretion) but the final diagnosis was epidermoid carcinoma. Here the most likely diagnosis was combined epidermoid and adenocarcinoma.

Most discrepancies in tumour typing occurred when the initial diagnosis was large-celled anaplastic carcinoma and it is in this group that the accuracy of sputum (100%) and rigid bronchoscopy cytology (80%) was superior to fiberoptic bronchoscopy cytology (55%) and biopsy (30%). Tumour cells in bronchial brushings appear less mature^{6 7} than in sputum and the cells are often arranged in sheets losing their characteristic groupings.¹³ We have found that as fiberoptic bronchoscopy cytology specimens are scanty they must be spread and fixed very rapidly; if drying occurs before fixation, the cells flatten and appear larger, the characteristic chromatin pattern of wet-fixed preparations is lost

and the cell type cannot be diagnosed. Accurate diagnosis in biopsy specimens was only possible in two of seven cases.

In Table 3 the yield and accuracy of diagnosis in practice are analysed. Of the 99 cases providing positive samples, 83 had positive bronchoscopic cytology either alone or with positive biopsy or sputum. In 10 cases sputum provided the only morphological evidence of malignancy and in four cases the biopsy alone was positive. Yield was highest for epidermoid carcinoma (81%) as here tumour breakdown and exfoliation occurs readily. With small-celled carcinoma the yield was smaller (68%). Large-celled anaplastic tumours gave a yield of 61% and the lowest yield (50%) was obtained with adenocarcinomas which are often peripheral. In assessing the predominant cell type, the results of all initial investigations were compared and where there was a discrepancy (18 cases) the final diagnosis was made in the light of our experience. If epidermoid or adenocarcinoma was noted in an adequate biopsy, this was taken as correct but if the biopsy was inadequate or reported as containing anaplastic or poorly differentiated carcinoma, the findings in sputum, bronchial brushings and aspirations were taken as being more accurate. Taking these factors into consideration a high rate of diagnostic accuracy was achieved except in the large-celled anaplastic carcinoma group where classification by tumour histology is also difficult.¹⁰

Discussion

In view of the intensive investigation into the best treatment for small-celled anaplastic carcinoma, accurate pretreatment diagnosis is essential. The small differences likely to be detected in therapeutic trials for non-operable small-celled anaplastic carcinomas, demands a minimum of inaccurate diagnoses for inclusion of non-responsive tumours would

invalidate the results. Chemotherapy has many side effects, notably nausea, vomiting, and debility. Accurate cell typing would prevent patients with non-responsive tumours entering such trials and receiving inappropriate therapy. In this study, using three types of primary investigation (sputum, bronchoscopic cytology and biopsy) 14 of 22 cases (64%) of small-celled carcinomas were diagnosed accurately; one case was diagnosed as squamous carcinoma.

The results of fiberoptic bronchial biopsy in small-celled anaplastic carcinomas were disappointing. Eight biopsies were carried out, tumour tissue was identified in four and in only two (25%) was the correct diagnosis made.

In experienced hands pulmonary cytology is a very reliable technique which contributes significantly to the accurate diagnosis of bronchial carcinoma.⁶ Although the highest percentage of positive results are obtained by a combination of biopsy and cytology using fiberoptic bronchoscopy,^{8 15-17} all diagnostic methods should be employed if maximum yield and accuracy of diagnosis is to be obtained.^{2 4 6 14 16 18}

We intend to use several additional techniques which will improve the accuracy of pretreatment investigation.

SPUTUM

A high yield from sputum examination depends on careful instruction and supervision of the patient to ensure that material from a "deep cough" only is submitted and in the laboratory care in selecting suitable blood-stained areas or tissue fragments for spreading.

The technique of the collection and preparation of specimens can be monitored by recording the percentage of unsatisfactory samples (containing no alveolar macrophages). In 1976 at the beginning of this investigation our unsatisfactory rate was 48% but this has now been reduced to 28% which is in

Table 3 Combined yield and accuracy for all preoperative diagnostic methods

	Tumour type (thoracotomy or necropsy)					Total
	Epidermoid	Small-celled anaplastic	Large-celled anaplastic	Adenocarcinoma	Epidermoid and adenocarcinoma	
	73	22	23	18	4	140
Yield of positive findings						
Sputum alone	5	1	2	1	1	10
Biopsy alone	3	1	—	—	—	4
Sputum + biopsy	1	—	—	1	—	2
Aspiration and brushings alone	11	4	6	3	—	24
+sputum	22	6	4	3	1	36
+biopsy	6	2	1	1	—	10
+sputum and biopsy	11	1	1	—	—	13
Combined yield	59 (81%)	15 (68%)	14 (61%)	9 (50%)	2 (50%)	99 (71%)
Accuracy						
(Predominant tumour type)	57 (97%)	14 (93%)	10 (71%)	8 (90%)	1 (1/2)	90 (91%)

keeping with figures quoted from a general hospital⁵ but lower figures (9%) from a chest unit have been obtained.⁵

The preparation of smears of sputum is a time-consuming and meticulous procedure and should not be delegated to a junior member of the laboratory staff;¹⁴ in a busy laboratory, however, this may be unavoidable. Improved results have been quoted using a homogenisation and concentration technique⁶ but we have no experience of this method.

In practice sputum cytology can be expected to diagnose between 50 and 60% of an unselected series of lung cancer patients.^{5, 6} Although increasing the number of satisfactory samples examined from each patient will improve the yield, the additional work makes heavy demands on highly trained staff. An additional series of sputa, therefore, should only be submitted when there is a strong clinical suspicion of malignancy and the diagnosis cannot be made by other methods.^{5, 14}

FIBROPTIC BRONCHOSCOPIC CYTOLOGY

Samples obtained at fiberoptic bronchoscopy are the most rewarding of all methods of diagnosis. The bronchoscope is passed nasally and is not withdrawn until the procedure is completed. Aspirations and brush material must therefore be drawn along the whole length of the bronchoscope and it is possible that much of the material is lost in the interior.¹⁶ Some withdraw the bronchoscope with brush in situ after each brushing¹⁶ but this necessitates the insertion of the bronchoscope through an endotracheal tube which causes considerable discomfort to the patient. An alternative is to submit to the laboratory material obtained when cleaning the bronchoscope after use, mixed with an equal part of cytological fixative. When tumour tissue is not seen it has been our practice to take brushings from abnormal areas indicated by posteroanterior and lateral x-ray examination but an improved yield is quoted if brushings are performed under fluoroscopic control.^{1, 8}

BRONCHIAL BIOPSY

With biopsy, tiny specimens and crush artefacts preclude accurate diagnosis. Toothed or fenestrated forceps may give a larger biopsy and multiple biopsies at one point, thereby obtaining tissue from deeper within the tumour, may also improve the results. It has been our practice only to take biopsy specimens when there was visible evidence of tumour but in almost 50% of cases indirect evidence of tumour only is present.² Improved results will be expected if in these cases transbronchial biopsy under fluoroscopic control is employed.^{9, 15}

In the histological laboratory great care is required

in the processing of tiny portions of often friable tissue. Identification of such particles in embedding wax may be difficult and it has been suggested that colouring the material by the addition of saturated aqueous solution of acid fuchsin (acid violet 19) to give a definite pink colour to the fixing fluid is extremely valuable.¹⁹ Alternatively processing may be carried out in resin and sections (1 μ m) cut. Precise identification of tumour type using electron microscopy is not practicable outwith research centres.

FINE NEEDLE ASPIRATION BIOPSY

We have used fine needle aspiration successfully in a few peripheral carcinomas. Here a yield of 90% has been quoted.⁴ With this technique there is a risk of pneumothorax, but it is rarely a serious problem.

Conclusion

A high yield and a high degree of accuracy in pre-operative lung cancer diagnosis is possible outwith highly specialised units but considerable experience and meticulous attention to technique is required both clinically and in the laboratory. When malignant cells show poor differentiation it is often possible, by paying careful attention to cellular arrangement, chromatin pattern, size, number and arrangement of nucleoli and cytoplasmic detail, to give an accurate cell type diagnosis. In this way it becomes possible to improve the recruitment rate to treatment trials particularly for small-celled carcinoma of the bronchus.

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Requests for reprints to: Dr Helen LD Duguid, Department of Pathology, Cytology Unit, Royal Infirmary, Dundee DD1 9ND, Scotland.