Cytological features of non-small cell carcinomas of the lung in fine needle aspirates

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Abstract

Fifty eight lung tumours were typed according to the second World Health Organization histological classification and compared with the cytological appearances obtained by fine needle aspiration in a total of 47 primary non-small cell carcinomas. The presence of glands, cell balls, branching or papillary structures, cylindrical cells and nuclear grooving were major diagnostic indicators for adenocarcinoma. Cytoplasmic macrovacuoles were more common in adenocarcinomas (69%) than in squamous (37%) and large cell (50%) carcinomas. Two or more of these features were combined in all well and moderately differentiated adenocarcinomas and in 67% of poorly differentiated adenocarcinoma. The major cytological indicators for squamous carcinomas were the presence of keratin and eosinophilic spindle cells with glassy or laminated cytoplasm. Granular cytoplasm was not specific for any histological type. A combination of the major features for both adeno- and squamous carcinoma was present in 58% of adenosquamous carcinomas, including some poorly differentiated types.

Correct typing could be obtained in almost all the well and moderately differentiated carcinomas and in about two thirds of the poorly differentiated tumours using FNA, provided that combinations and not individual variables are considered.

Fine needle aspiration (FNA) cytology is usually a reliable and accurate method for the diagnosis of pulmonary malignancy, with a high specificity and sensitivity and low false positive and negative rates.¹⁻⁶ The accuracy of cytological typing of non-small cell carcinomas (NSCC), however, is poor,⁷ although FNA has been claimed to be more accurate.⁸ The aim of this study was to evaluate the cytological features of NSCC obtained by FNA using the histological type of the tumour,⁹ obtained by resection or at necropsy, to obtain more accurate typing using needle aspirates.

Methods

The cytohistological appearances of 58 lung tumours, obtained with fine needle aspirations over nine years were evaluated. Tumour tissue for histological examination was ob-

tained by wedge resection, lobectomy, pneumonectomy, or at necropsy. The specimens were fixed in buffered formalin, embedded in paraffin wax, and routinely stained with haematoxylin and eosin, as well as with periodic acid Schiff diastase, alcian blue (pH 2.5), and the World Health Organisation (WHO) stains.⁹ The sections were carefully screened by two pathologists (HRH and ARG) and tumour diagnosis agreed, using the second WHO histological classification of lung tumours.⁹ These comprised eight squamous cell carcinomas, 23 adenocarcinomas. Four large cell carcinomas, 12 adenosquamous carcinomas, three small cell carcinomas, two carcinoid tumours, one malignant fibrous histiocytoma, three secondary tumours (oesophagus, kidney, and thyroid), and two pulmonary hamartomas. Only the 47 primary non-small cell carcinomas were evaluated for this study (table 1). These were classified into well, moderately, and poorly differentiated. The adenocarcinomas were further subdivided into acinar or papillary according to the main architectural component; two tumours with equally predominant components were classified as papillary. Adenosquamous carcinomas were also subdivided according to their major component into predominantly squamous carcinoma or adenocarcinoma.

FNA was performed as described by Nordenstrom.^{10,11} One to three smears fixed in alcohol that yielded abundant material for cytological interpretation were available for each case; all were stained with haematoxylin and eosin. The smears from the primary non-small cell carcinomas were carefully scruti-

Table 1 Histological classification of 47 primary non-small cell lung carcinomas according to WHO system⁹

Squamous cell carcinoma (8 cases)	
Well (3) and moderately (1) differentiated	4
Poorly differentiated	4
Adenocarcinoma (23 cases)	
(a) Acinar adenocarcinoma	
Well (5) and moderately (3) differentiated	8
Poorly differentiated	3
(b) Papillary adenocarcinoma	
Well (7) and moderately (1) differentiated	8
(c) Bronchiolo-alveolar carcinoma	3
(d) Solid carcinoma with mucus formation	1
Large cell carcinoma (4 cases)	
(a) NOS	2
(b) Giant cell carcinoma	1
(c) Clear cell carcinoma	1
Adenosquamous carcinoma (12 cases)	
(a) Predominantly squamous cell carcinoma	
Well (2) and moderately (5) differentiated	7
Poorly differentiated	1
(b) Predominantly adenocarcinoma	
Moderately differentiated	2
Poorly differentiated	2

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Table 2 "Adenocarcinoma" features in 47 carcinomas according to their histological type and degree of differentiation

Histological type (no)	Glands	Cell balls	Branching	Cylindrical cells	Cytoplasmic vacuoles	
					Micro-	Macro-
<i>Squamous cell carcinoma</i>						
Well/moderately (4)	0	0	1	1	2	1
Poor (4)	0	0	0	1	1	2
<i>Large cell carcinoma</i>						
Undifferentiated (4)	0	0	0	1	1	2
<i>Adenosquamous carcinoma</i>						
Well/moderately (9)	4	3	3	4	7	2
Poor (3)	2	0	1	0	2	1
<i>Adenocarcinomas</i>						
Well/moderately differentiated						
Acinar (8)	5	3	5	7	8	7
Papillary (8)	5	4	5	8	8	6
Bronchiol-alveolar (3)	1	3	2	3	2	2
Poorly differentiated						
Acinar (3)	0	1	0	1	3	0
Solid (1)	0	0	0	0	1	1

All the features were greater than grade 2.

nised by one of us (SBZH) without knowledge of the histological diagnosis of the tumours. The various features that may be observed in lung carcinomas were analysed according to current cytological criteria^{12 13} and the following features were assessed:

(a) *Cell type*: oval, round, cylindrical, spindle, small, large, pleomorphic, multinucleated, and ghost cells;

(b) *Cytoplasmic features*: scanty, abundant, microvacuolated or macrovacuolated, clear, eosinophilic granular, glassy or keratinised and laminated (due to cytoplasmic tonofilaments), well demarcated;

(c) *Nuclear characteristics*: size and shape as for cell type, hyperchromatic with coarse or finely granular chromatin, pale or vesicular, and pyknotic. The nucleoli, when present, were recorded as single or multiple, and small

or prominent. Special attention was paid to the presence of nuclear cytoplasmic inclusions and grooving which were only regarded as a feature in neoplastic cells showing little or no pleomorphism. These changes were semi-quantitatively graded from 1 (occasional) to 4 (frequent). Cellular arrangement was recorded as: single; clusters; sheets; glands; balls; branching or papillary structures; overlapping; monolayered; tight or loose. The presence of background keratin and mucus was also recorded.

Results

ADENOCARCINOMA

Morphological features did not clearly differentiate between well and moderately differentiated carcinomas. There was no difference between acinar, papillary and bronchiolo-alveolar carcinomas. Therefore the features are described together.

Well and moderately differentiated

In all cases the tumour cells tended to form tight sheets and overlapped. Single cells (16%) and loose clusters (5%) were observed in a few cases. Monolayered clusters occurred in 89% of cases, the cells being well demarcated in 40%. The neoplastic cells were usually round to oval in shape. Cylindrical cells occurred in 89% of cases. The cell cytoplasm was usually abundant and contained micro- and macrovacuoles (table 2). The nuclear chromatin was often finely or coarsely granular; pale (10%) and hyperchromatic (5%) nuclei occasionally occurred. Nucleoli were usually single and prominent but occasionally multiple (5%). Nuclear grooving and cytoplasmic inclusions were observed in 100% and 63% of the cases, respectively (figs 1 and 2) (table 3). Glands, cell balls, and papillary or branching structures were found in 58%, 53%, and 63% of the cases, respectively (fig 3) (table 2). One or more of these patterns were present in 87% of the smears. Large cells and pleomorphic cells with bizarre nuclei were observed in 53% and 69% of the cases, respectively. Multinucleated cells

Figure 1 Adenocarcinoma: clusters of well demarcated cells showing nuclear grooving.

Figure 2 Adenocarcinoma: clusters of cells, some showing macrovacuoles.

(10%) were rare. There was an almost twofold decrease of these cell types when grade 3 or greater was considered. Background mucus occurred in a quarter of the smears. The only solid carcinoma with mucus formation showed large cells which were round to oval in shape, often with micro- and macrovacuolated cytoplasm lying singly or arranged loosely in sheets. Pleomorphic cells were also present. The nuclei usually showed coarse chromatin and had small nucleoli. Nuclear grooving occurred in neoplastic cells but not in nuclear cytoplasmic inclusions.

Poorly differentiated

Neoplastic cells occurred in tight sheets and overlapped. Monolayered clusters were present in one case. Most cells were round to oval in shape. The nuclei usually showed coarse chromatin and had prominent nucleoli (fig 4). Features of adenocarcinoma were identified in two cases. One of these showed cylindrical cells and nuclear grooving, and the other cell balls. Both cases had cells with cytoplasmic microvacuoles. The third case

showed only cells with nuclear grooving as a distinctive feature (tables 2 and 3). Pleomorphic cells and large cells were present in one and two cases, respectively.

SQUAMOUS CELL CARCINOMA

Well and moderately differentiated

Tumour cells were generally arranged in tight sheets and overlapped. Single cells and monolayered clusters, sometimes with cells loosely packed or well demarcated, were also present. The neoplastic cells were oval to elongated in shape with abundant, eosinophilic, granular and sometimes laminated cytoplasm (table 4). Spindle cells with similar cytoplasmic features were present in half of the cases. The nuclei, usually hyperchromatic, showed finely granular chromatin and often small nucleoli. Ghost cells as well as cells showing cytoplasm with a glassy appearance and pyknotic nuclei also occurred. These elements are usually related to keratinisation which was present in variable amounts in all four cases (figs 5 and 6). Large and pleomorphic cells with bizarre nuclei were always present and, in one case, multinucleated cells occurred. Nuclear grooving was present in one case (table 3). Branching structures were seen in one case; microvacuolated and macrovacuolated cells were seen in about one third of the cases (table 2).

Poorly differentiated

Neoplastic cells were arranged in tight sheets, often overlapping. They were elongated in shape and had eosinophilic and, in three cases, granular cytoplasm. Cells with glassy cytoplasm occurred in two cases, spindle-shaped cells were present in one. The nuclei showed coarse chromatin and prominent nucleoli. Keratin was present in minimal amounts in three of the four cases. One case had eosinophilic granular cells and nuclear grooving as distinctive features. Nuclear grooving was evident in another case that also showed nuclear cytoplasmic inclusions.

Figure 3 Adenocarcinoma: cell balls and branching structures.

Table 3 Lung carcinomas with nuclear grooving and cytoplasmic inclusions according to histological type and degree of differentiation

Histological types (no of cases)	Grooving		Cytoplasmic inclusions	
	Grade 2	Grade 3	Grade 2	Grade 3
Well/moderately differentiated:				
Adenocarcinomas (19)	3 (16%)	16* (84%)	3 (16%)	9 (47%)
Squamous cell (4)		1 (25%)		
Adenosquamous (9)	3 (33%)	3 (33%)	3 (33%)	
Poorly differentiated:				
Adenocarcinoma (3)		2 (67%)		
Squamous cell (4)	1 (25%)	1 (25%)	1 (25%)	
Adenosquamous (3)		1 (33%)		1 (33%)
Large cell undifferentiated (4)		3 (75%)	1 (25%)	2 (50%)

The solid adenocarcinoma with mucus formation is not included.
*This group showed the only one case with 4+ from the whole series.

Table 4 "Squamous" features in 47 carcinomas according to histological type and degree of differentiation

Tumour type	Differentiation (no)	Spindle cells	Glassy cells	Granulocyte cells	Keratin
Squamous					
Well/moderate	(4)	2	2	2	3
Poorly differentiated	(4)	1	2	1	3
Large cell					
Undifferentiated	(4)	0	1	3	0
Adenocarcinoma					
Well/moderate					
Acinar	(8)	0	0	1	0
Papillary	(8)	0	0	1	0
Bronchiolo-alveolar	(3)	0	0	1	0
Poorly differentiated					
Acinar	(3)	0	0	1	0
Solid	(1)	0	0	1	0
Adenosquamous					
Well/moderate	(9)	3	1	3	6
Poor	(3)	0	1	2	0

ADENOSQUAMOUS CARCINOMA
Well and moderately differentiated

Sixty seven per cent of the tumours (six cases) showed cytological features of both adenocarcinoma and squamous cell carcinoma (tables 2–4); the other tumours (two cases and one case, respectively) had features of either a glandular or squamous component, respectively. The pattern of cytological presentation did not always correlate with the predominant histological type. Nuclear grooving and cytoplasmic inclusions were present in 67% and 33% of the cases, respectively (table 3).

Poorly differentiated
Neoplastic cells occurred in tight sheets and overlapped. Monolayered clusters were present in two cases and a branching pattern in one. Most cells were round to oval in shape often with abundant vacuolated cytoplasm containing microvacuoles or macrovacuoles. The

Figure 4 Poorly differentiated adenocarcinoma showing macrovacuoles.

Figure 5 Squamous carcinoma showing single spindle and rounded cells. There is a cell with a dense pyknotic nucleus.

nuclei usually showed coarse chromatin and had prominent nucleoli. Nuclear grooving and cytoplasmic inclusions occurred in one case each, respectively (table 3). Pleomorphic and large cells were present in two cases. One tumour had the distinct cytological features of adenosquamous carcinoma: the other two cases showed characteristics of one component each.

LARGE CELL UNDIFFERENTIATED CARCINOMA
The neoplastic cells occurred singly or were arranged in tight or, more frequently, loose sheets. Most of the cells were large and had abundant cytoplasm with large hyperchromatic, oval, or bizarre pleomorphic nuclei with prominent, sometimes multiple, nucleoli. Cytoplasmic vacuoles were present in three of the four cases. Multinucleated giant cells occurred in three cases.

Nuclear grooving and cytoplasmic inclusions, only present in large and pleomorphic cells with irregular nuclei, occurred in three cases (table 3).

Discussion
In previous studies there was a wide range of cytohistological agreement in non-small cell carcinomas of the lung, from 72% to 100% for squamous cell carcinoma, 29% to 100% for adenocarcinoma, and 19% to 74% for large cell undifferentiated carcinoma.^{4 5 12 14–18} Even in biopsy specimens a wide intra- and interobserver variability has been shown.^{19 20} Immunohistochemistry does not seem to be of great value in the subclassification of non-small cell carcinomas.²¹ In general, pulmonary cytology has been more accurate in the diagnosis of well to moderately differentiated carcinomas, than in poorly differentiated and undifferentiated carcinomas.^{22 23} One reason has been the lack of rigid cytological and histological criteria used for the various tumour types.²⁴ Therefore, we decided to evaluate certain cytological features and correlate them with a series of lung carcinomas classified according to the WHO system.⁹ Our findings indicate that no cytological distinction between the subtypes of adenocar-

Figure 6 Squamous carcinoma showing laminated keratinised cells.

cinomas of the lung could be distinguished from FNA. Previous studies suggested that adenocarcinomas could be differentiated from bronchioloalveolar carcinomas on exfoliative material.²⁵ This discrepancy may be related to the smaller number of bronchioloalveolar tumours contained in this study, or, more likely, to the morphological variables used for their typing. Another factor to consider is the diagnosis of bronchio-alveolar carcinoma which relies on architecture, and it is not surprising, therefore, that it can only reliably be diagnosed in histological sections. The presence of glands, cell balls, branching or papillary structures, cylindrical neoplastic cells, and cytoplasmic macrovacuoles were considered here as major diagnostic indicators for adenocarcinomas regardless of their histological type.^{5 12 13} Cytoplasmic macrovacuoles, however, were observed in three out of the eight squamous carcinomas; this makes it unreliable on its own as a specific feature of adenocarcinoma. When two or more of these variables were present in combination diagnostic accuracy was 100% for well and moderately differentiated adenocarcinomas, as well as for two of the three poorly differentiated tumours where either cylindrical cells or cell balls, together with cytoplasmic vacuoles, were present. Nuclear grooving and cytoplasmic inclusions in round to oval and cylindrical cells were present in 96% and 52%, respectively, of the total series of adenocarcinomas studied. These characteristics, combined, seem to be useful as a complementary criterion for detecting or suspecting these lung tumours. They also occurred frequently in large cell undifferentiated carcinomas, but in cells with large pleomorphic nuclei. Nuclear cytoplasmic inclusions have been noted in bronchiolo-

alveolar and papillary adenocarcinomas of the lung,^{5 26} but not nuclear grooving to our knowledge. These two features were found to be important cytological criteria for diagnosing thyroid papillary carcinoma,²⁷ even in its smallest variants,²⁸ though we found no special predilection for neoplastic cells of papillary carcinoma or any other type of lung adenocarcinomas in this survey. Nuclear grooves also occurred in two poorly differentiated adenocarcinomas. The other case, the only solid adenocarcinoma with mucus formation studied, showed nuclear grooving without any of the other major cytological criteria for diagnosing adenocarcinoma.

In agreement with previous observations,^{5 12 13} we found that the major distinctive cytological features for bronchial squamous cell carcinomas were the presence of keratin and eosinophilic, spindle, or elongated cells with glassy, granular, or laminated cytoplasm. Seven of the eight tumours studied, including three poorly differentiated, contained minimal to large amounts of keratin in the smears in combination with at least one of the above mentioned cell types. One poorly differentiated squamous carcinoma only showed elongated cells with eosinophilic granular cytoplasm and nuclear grooving. In this case the possibility of adenosquamous carcinoma cannot be excluded. Granular cytoplasm, however, was seen in a small proportion of the other histological types. Previous multidisciplinary studies have suggested that cytology can be more accurate for typing lung tumours than conventional histology.^{24 29}

Our findings show that a combination of the major features that may be observed in squamous cell carcinomas, and adenocarcinomas can be cytologically diagnostic for adenosquamous carcinoma in 58% of the cases, including some poorly differentiated types. The other cases could have been diagnosed solely as either adenocarcinoma or squamous cell carcinoma, regardless of their degree of differentiation. This should be expected as sampling of cytological material might include one or both tumour components. Similar results have been shown by other authors.^{22 29} Early studies have emphasised the difficulties of differentiating cytologically large cell undifferentiated carcinomas from other carcinomas of the lung, mainly poorly differentiated tumours.^{4 22-24 29} Our results suggest that diagnosis of large cell undifferentiated carcinomas should be suspected both by the presence of tight and loose clusters of large cells accompanied by pleomorphic, bizarre, and multinucleated neoplastic cells, and by the absence of glands, cell balls, branching structures, spindle and keratinised cells. These observations should be tested on a larger series of large cell undifferentiated carcinomas.

According to our observations correct typing of poorly differentiated carcinomas could be achieved in about two thirds of the cases, regardless of their histological type, when following the main diagnostic variables used for the more differentiated counterparts. These observations need to be tested in a prospective

survey to establish their true utility. We are still left with a small proportion of poorly differentiated tumours, however, where a precise cytological diagnosis cannot be made on light microscopy. Perhaps ultrastructural studies might have an important role.^{29 30}

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