Significant Cytologic Findings in Non-Malignant Pulmonary Disease

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In a relatively new field of study, such as pulmonary cytology, one is impressed by rapid changes in diagnostic acumen and confidence which occur during the space of a few years. During our years of investigative research in sputum cytologic studies for the detection of malignant cells, we have attempted to correlate appearances of the stained cells with any available histologic, roentgenologic and clinical findings, and we have been particularly interested in reviewing those cases in which our laboratory has made a false “positive” or false “suspicious” report of cancer.

In the course of this investigation it has become apparent that cytologic appearances of the cells in a large number of non-malignant pulmonary conditions may be quite characteristic of the specific disease. Equally important, they may show close resemblance to carcinoma cells. This was found to be true for such widely different conditions as bronchial squamous metaplasia, pulmonary infarction, asthma, and lipid pneumonia. We feel that careful evaluation of these cellular alterations will serve two purposes. In the first place, since many of these pathologic states produce cells which cytologically closely resemble cancer cells, cognizance of their possible presence should assist in reducing to an absolute minimum the hazard of false positive reports. Secondly, we have found that identification of these cellular alterations can often contribute materially to a differential diagnosis in a number of non-neoplastic pulmonary diseases.

Cytologic Criteria for Malignancy

The most reliable criteria for establishing malignancy of cells found in the sputum, as well as in other body fluids, pertain strictly to the concept of exfoliative cytology and, therefore, are based upon nuclear features. This is not to minimize the significance of cellular grouping, but in our experience the majority of false “positive” and false “suspicious” (or “inconclusive”) reports have resulted from undue emphasis being placed on

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the presence of cellular groupings, which to the unwary may suggest changes associated with cancer. While no single criterion can be relied upon by itself, we believe nuclear changes are much less likely to be erroneously interpreted.

Perhaps the most important single criterion for identification of malignant cells is increased nuclear size (Figure 1), with a corresponding increase in the nuclear-cytoplasmic ratio. Nuclear hyperchromatism also constitutes a strong presumptive evidence of malignancy, particularly when the chromatin is irregularly distributed and the nuclear rim is dense or "thick." Enlarged, multiple or jagged nucleoli are also of significance in leading to a diagnosis of malignancy, (Figure 2). Infrequently, nucleoli may be of such giant dimensions that the malignant nature of the cell is unquestionable.

The initial impression of malignancy in smears is usually suggested by the finding of single "suspicious" cells, and this impression is further strengthened when groups of such cells show relative variations in nuclear size, shape and structure as compared to normal cells. Accurate interpretation and evaluation, of course, is complicated by the fact that there is never a situation in which all the cells observed can be neatly separated into benign and malignant. Frequently, conclusively malignant cells are greatly outnumbered by atypical cells which, while not clearly malignant, offer supportive evidence of malignancy.

**Cytologic Findings in Non-Malignant Disease**

Production of sputum in sufficient quantity for cytologic study generally indicates the presence of some pulmonary abnormality. Therefore, the

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**FIGURE 1**

*Figure 1*: Malignant cells in sputum showing marked enlargement of nuclei and irregular distribution of chromatin. (× 760).

**FIGURE 2**

*Figure 2*: Malignant cells in sputum showing enlarged and multiple nucleoli. (× 760).
Figure 3: Stained sputum smear showing typical cells exfoliated from an area of bronchial metaplasia. (×760).

Figure 4: Typical cells in sputum from a bronchogenic squamous carcinoma. (×760).

Figure 5: Typical cells lacking. (×760).
cells "normally" found in sputum smears are usually the consequence of a pathologic process, and in this sense differ from cells found in normal vaginal secretions and normal gastric washings. Thus, they usually exhibit some degree of cellular abnormality. In many cases such abnormal or altered cells may show features which possess a cursory resemblance to those of malignancy; or, as in the case of lipid pneumonia, coccidioidomycosis and other conditions, the cytologic findings may suggest the specific disease process. It must be stressed that (a) differentiation of abnormal from malignant cells is possible primarily through the application of rigid nuclear criteria. Further, it must be emphasized that a cytologic diagnosis of a non-malignant pulmonary disease, like that of malignancy, is equivocal and demands confirmation by appropriate clinical studies.

**Bronchial Metaplasia:** In any chronic pulmonary disease, portions of the tall columnar epithelium which normally line the bronchi may be replaced by areas of stratified squamous epithelium whose superficial cells may or may not be keratinized. This condition, metaplasia of the bronchial mucous membrane, constitutes one of the most common problems encountered in pulmonary cytologic studies. In our earlier work it was responsible for a substantial number of erroneous diagnoses of epidermoid carcinoma. In both conditions the cells are hyperkeratinized and stain a brilliant orange by the Papanicolaou method. Metaplastic cells as well as many of the epidermoid carcinoma cells are generally spherical or slightly flattened and contain a single, centrally placed nucleus. The metaplastic nucleus is typically pyknotic, in contrast to the enlarged, hyperchromatic

![Figure 6](http://example.com/figure6.png)  
**Figure 6:** Tight, vacuolated cell groups sometimes seen in sputum from patients with bronchial asthma which may resemble acini of adenocarcinoma. Note cilia along periphery of group. (× 760).  

![Figure 7](http://example.com/figure7.png)  
**Figure 7:** Typical malignant acinus of adenocarcinoma seen in a stained sputum smear. Note prominent nucleoli and variation in nuclear size and a chromatin distribution. (× 760).
nucleus characteristic of epidermoid carcinoma. Thus, typical metaplastic cells (Figure 3) are readily distinguished from the typical malignant cells (Figure 4) of epidermoid carcinoma. This simplicity is complicated, however, by the fact that bizarre forms frequently are found in both conditions, so that cells are often encountered which are neither definitely metaplastic nor conclusively malignant (Figure 5). This situation of bizarre metaplasia emphasizes again the need to employ rigid nuclear criteria in order to eliminate as far as possible in suspected cases of epidermoid carcinoma cytologic error.

*Figure 8, A, B and C:* Characteristic pulmonary macrophages showing the empty, "Swiss cheese" vacuolation seen in cases of lipid pneumonia. (× 760).
Interestingly, sputum smears which show cells unequivocally of epidermoid carcinoma consistently contain numerous supporting "metaplastic" cells which may range from typical through bizarre to frankly suspicious forms. In view of the uncertainty of our knowledge concerning the role of metaplasia in carcinogenesis, this problem of cytologic differentiation is not surprising.

Asthma and Allergy: In our early work in pulmonary cytology, sputum from a number of patients with bronchial asthma was reported to contain malignant cells. In retrospect, when follow-up studies demonstrated that no malignancy was present, the smears were carefully re-evaluated. It was found that error had resulted from interpretation of tight, highly vacuolated groups of cells which are commonly present in the sputa of asthmatic patients (Figure 6) and confusing them with those which occur in the acini grouping of adenocarcinoma (Figure 7). Closer inspection showed that although both groups are somewhat similar in histioid cellular arrangement, the nuclei of the cells associated with asthma are uniformly small and show none of the nuclear features of malignancy. In addition, cilia are frequently seen along the periphery of benign cell groups. The associated findings of Curschmann spirals and eosinophiles in the sputum is further evidence that this is a benign atypia. However, since Curschmann spirals and eosinophiles are also frequently found in the sputum in cases of malignancy, careful evaluation of nuclear morphology as well as a search for ciliated cells within the groups are required for definitive identification.

Lipid pneumonia: Since organized lipid pneumonia may simulate neo-
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Figure 11: Coccioides seen in stained sputum smear. (× 700)

Figure 12: Stained sputum smear showing distinct endospores, (× 700). Note that they are hemispherical and contain an amorphous central material, resembling endospores of Figure 11. (× 700)

Figure 13: Lung section showing intra-alveolar collection of asbestos bodies. (× 700).
plasm both clinically and roentgenologically, we have occasionally been called upon to study the sputum of patients who were later shown to have had this disease. In some of the cases a diagnosis of lipid pneumonia had been suggested by the presence in the stained smears (Figure 8) of characteristic hypervacuolated macrophages and histiocytic syncitia. These enlarged cells of reticulo-endothelial origin contain tremendous, spherical and seemingly empty vacuoles within their cytoplasm which produce a "Swiss cheese" appearance. Normally, although histiocytic cells show vacuolations, the normal vacuoles are much smaller and interlace with one another, conferring a spongy appearance to the cytoplasm.

Frequently, lipid pneumonia may be overlooked in differential diagnosis because of its widely variable manifestations. It may follow aspiration of an oily substance in nasal sprays. It occurs perhaps as frequently in adults with no history of aspiration but who habitually take mineral oil or oily nose drops before retiring. In debilitated children it may also follow the use of cod liver oil. During sleep, the oil which coats the oral and pharyngeal mucosa may drain slowly into the bronchial tree. Progressive accumulation of this non-absorbable substance over a period of time finally results in foreign body reaction, secondary infection and extensive fibrosis. A pronounced reticuloendothelial response ensues as macrophages attempt to phagocytize and remove the foreign lipid material. It is the presence of these hypervacuolated cells in stained sputum smears which points to the diagnosis of lipid pneumonia. The lipid is removed from the cells in the process of alcoholic fixation and staining; however, the characteristic morphologic appearance remains. Further study of specimens specifically stained to demonstrate intracellular fat will aid in definitive diagnosis.

**FIGURE 14A**

*Figure 14A:* Cytologic preparation made at bronchoscopy showing cords of cells aspirated from a bronchial adenoma. (× 480).

**FIGURE 14B**

*Figure 14B:* Higher magnification of cells from a bronchial adenoma. (× 760).
Incidentally, since sputum in cases of lipid pneumonia may contain free oily material, it may be difficult to make the specimen adhere to the glass slide. This gross finding alone may suggest the possibility of lipid pneumonia.

**Pulmonary infarction:** Abnormal cells in the sputum may result from pulmonary infarction, although in our experience this is rare. Extremely bizarre mesothelial cells may, however, predominate in cytologic smears of the pleural fluid sediments from pulmonary infarction cases. Many of the morphologic changes which are generally associated with malignant cells may be exhibited by these cells. This finding has been reported by others.⁴ We believe this cytologic finding may be transitory, occurring as an initial reaction of pleural mesothelium to infarction of the lung. This observation is based upon the disappearance of abnormal cells in subsequent studies from patients who initially showed the reaction.

We have observed these cells to occur singly in the smears, although occasionally, small clusters of rosette formations of abnormal cells have been seen. Such grouping lacks the tight formation and smooth periphery which is seen in true acini of adenocarcinoma. Some groups, as well as single cells, contain cytoplasmic vacuolization; however, these do not include the extremely large ones which are associated with malignant cells. The nuclei of “infarction cells” are markedly enlarged and contain a fine distribution of chromatin. Occasionally smaller nuclei are seen with condensation of chromatin resulting in nuclear pyknosis, but not hyperchromatism. The nuclear membrane is relatively thin and the nuclear contour reveals a scalloped irregularity, but is seldom jagged. The nucleoli of “infarction cells” closely resemble those of malignant nuclei and are multiple (usually three or more), enlarged and irregular. Mitotic cells may be abundant in cases of pulmonary infarction.

The strong possibility of cytologic error in cases of pulmonary infarction has increased our conviction that demonstration of both nuclear features of malignancy and grouping consistent with malignancy are needed to justify a positive cytologic report for pleural fluid sediment. It is essential to obtain pertinent clinical information when examining pleural fluid sediments for the detection of malignant cells. A more complete investigation of this source of cytologic error is needed in a series of clinically known cases of pulmonary infarction, obtained during the acute and healing phases of this condition.

**Coccidioidomycosis:** Although this fungus disease is endemic in southwestern United States, and particularly in central and southern California, it occurs sporadically in other sections of the country. Two forms of the disease are seen: a relatively benign disease of short duration which may simulate pneumonia, and a prolonged process, usually granulomatous, which may produce multiple pulmonary lesions almost from the beginning. The latter form is apt to suggest lung cancer, particularly metastatic tumor, and we have been called upon to study a few of these cases. The presence of spherules containing endospores (Figure 11) in the stained sputum has
led to the diagnosis of coccidioidomycosis in several of these cases. In vivo, the fungus, *Coccidioides immitis*, multiplies by endosporulation and characteristically is productive of refractile, double-walled spherules which range from 10 to 300 micra in diameter. In the larger forms, well-defined endospores may be recognized, thus permitting cytologic identification of the fungus.

Some care must be exercised to distinguish the spores of coccidioidomycosis from contaminants, especially pollen (Figure 12). Pollen bodies are generally hemispherical, rather than spherical, and may contain round granules which resemble, but are less distinct than, the well-defined endospores of *Coccidioides*. In addition, pollen bodies usually manifest a central stellate nucleus, whereas Coccidioides has no nucleus. Pollen bodies may represent air-borne contamination of the specimen, or they may have reached the bronchial tract by inhalation.

Although cytologic demonstration of spores has not been consistently accomplished in cases of known coccidioidomycosis, the possibility of this disease should be kept in mind during cytologic studies, particularly when the patient is known to have lived in an endemic area. Definitive diagnosis, of course, should be established by sputum cultures and serum antibody studies.

**Pneumoconiosis:** We have been unable, so far, to associate specific cytologic findings with pneumoconiosis. Perhaps the only consistent finding we have observed has been the abundance of macrophages filled with amorphous and particulate material. We have been unable either to identify the specific inclusions, or to distinguish them from the intracellular inclusions of carbon which are "normally" present in pulmonary macrophages. One might expect to identify asbestos bodies (Figure 13) in cases of asbestosis. Although we rarely see this disease in the San Francisco area, we anticipate such cases as we may have the opportunity to study.

**Bronchial adenoma:** If pathologic studies were any guide, this abnormality ought to be readily identified cytologically since the cellular structure is usually remarkably uniform and the cells show an ample nuclear-cytoplasmic ratio. However, since these tumors are separated from the bronchial surface either by normal bronchial mucosa or by an arcade of squamous metaplasia, one would not expect ordinarily to find exfoliated tumor cells in specimens of bronchial secretions. One of us (BP), however, recently studied a case in which numerous typical cells from a bronchial adenoma were found in smears prepared from bronchial aspiration obtained during bronchoscopy. In our experience and knowledge this is the first reported case in which correct cytologic diagnosis of this entity was made. In its study, only the first and second of three bronchial aspirations resulted in the cytologic findings of tumor cells. It is assumed that this was possible because the fragile and congested mucosa was traumatized by the bronchoscopy with the result that tumor cells were detached into the aspirated material. The cells were found singly, as well as in fairly large groups arranged in cords and trabeculae (Figure 14). The cuboidal cells had poorly defined cytoplasmic borders and the nuclei were uniformly
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round, ranging from 10-15 micra in diameter (Figure 15). Although we feel that this is an interesting cytologic observation, we emphasize that success in applying cytologic methods to the detection of bronchial adenomas is unlikely, since the epithelium overlying the tumor is usually intact and prevents the exfoliation of tumor cells into the bronchial lumen.

SUMMARY

While the cytologic examination of sputum, in the hands of experienced pathologists and their co-workers, can be an accurate and useful method, it must be pointed out that the morphologic identification of cells is often difficult. This problem is more frequently associated with examination of sputum than cytologic examination of other body secretions, since sputum, in sufficient volume for study, generally indicates some type of pulmonary disease and consequent alteration of morphology.

Although the cellular changes observed in most non-malignant pulmonary diseases are minimal, some non-malignant pulmonary diseases produce cellular alterations which closely resemble those of malignancy, while still others result in cytologic findings indicative of a specific disease. Among the chest diseases and pathologic entities in the latter category are asthma, pulmonary infarction, lipid pneumonia, bronchial metaplasia, pneumoconiosis, coccidioidomycosis and bronchial adenoma.

It is hoped that greater attention to the cytologic findings in sputum smears from non-malignant pulmonary diseases will add ultimately a new dimension to the usefulness of exfoliative cytology. Acquaintance with the cytologic patterns attendant thereto should contribute materially to improvement in accuracy of diagnosis by providing a better basis of distinction between malignant cells and benign cellular abnormalities. In addition, the result of such a comparative study is of equal value as an aid toward definitive diagnosis of a number of non-malignant pulmonary diseases which clinically may be confused with cancer. The significance of employing rigid nuclear criteria for identifying malignant cells is emphasized, since similarities in cellular grouping rather than in nuclear features are the most common source of cytologic error.

RESUMEN

Si bien el examen citológico del esputo en manos de anatomopatólogos experimentados y de sus colaboradores puede ser un método preciso y útil, debe señalarse que identificación morfológica de las celdillas es a menudo difícil. Este problema es más frecuente cuando se examinan los esputos que cuando se trata de otras secreciones puesto que el esputo en suficiente volumen para su estudio generalmente indica alguna enfermedad pulmonar y ya hay en él la consecuente alteración de la morfología.

Aunque los cambios celulares observados en la mayoría de las enfermedades pulmonares no malignas son mínimos, algunas enfermedades no malignas producen alteraciones celulares que se parecen mucho a las de la malignidad y hay otras que indican una enfermedad específica. Entre las afecciones torácicas de la última categoría se encuentran el asma, el infarto pulmonar, neumonía lipídica, metaplasia bronquial, pneumoconiosis, coci-
dioidomicosis y el adenoma bronquial.

Es de esperarse que una mayor atención a los hallazgos citológicos en los esputos de afeciones pulmonares no malignas, termine por agregar un campo nuevo en la utilización de citología exfoliativa.

El conocer los aspectos citológicos hasta aquí asentados, contribuirá materialmente a mejorar la exactitud del diagnóstico al proveer una base más segura en el diagnóstico diferencial entre las células malignas y no malignas. Además, el resultado de tal estudio comparativo es de igual valor como un auxiliar que lleve al diagnóstico definitivo de ciertas enfermedades no malignas que pueden confundirse con el cáncer. La significación de usar un criterio rígido nuclear para identificar las células se recalca, puesto que las similaridades del agrupamiento celular más que las características nucleares son la fuente de error citológico común.

RESUME

Bien que l'examen cytologique de l'expectoration, entre les mains d'ana-to-mopathologistes expérimentés et de leurs collaborateurs puisse être une méthode précise et efficace, on doit insister sur le fait que l'identification des cellules est souvent difficile. Ce problème est beaucoup plus le fait de l'expectoration des autres sécrétions. En effet, l'expectoration en volume suffisant pour être l'objet d'examen suppose déjà à elle seule une atteinte pathologique des poumons qui est susceptible d'entraîner des altérations morphologiques des cellules.

Bien que les altérations cellulaires observées dans la plupart des affectionspulmonaires non-malignes soient discrètes, certaines de ces affections peuvent entraîner des aspects qui ressemblent de près au cancer, tandis que d'autres permettent d'avoid la preuve cytologique d'une affection spécifique. Parmi les affections du thorax de cette catégorie, figurent l'asthme, l'infarctus pulmonaire, la pneumonie lipoïdique, la métaplasie bronchique, la pneumoconiose, la coccidioidomycose et l'adénome bronchique.

Les auteurs espèrent qu'une plus grande attention accordée aux constatactions cytologiques dans les frottis d'expectoration provenant d'affections pulmonaires non-malignes permettra ultérieurement d'augmenter la valeur de la cytologie. En perfectionnant nos connaissances des aspects cytologiques, on devrait arriver à faciliter le diagnostic grâce à une meilleure discrimination entre cellules malignes et anomalies bénignes. De plus, le résultat d'une telle étude comporte un autre intérêt : c'est de permettre un diagnostic précis de certaines affections pulmonaires non-malignes, qui peuvent être cliniquement confondues avec le cancer. Les auteurs insistent sur l'importance d'utiliser des critères irréfutables de l'aspect du noyau pour identifier les cellules malignes. Ce sont en effet plus les similitudes du groupement cellulaire que les caractéristiques nucléaires qui sont le plus souvent à l'origine des erreurs de diagnostic cytologique.

ZUSAMMENFASSUNG

Während die cytologische Untersuchung von Sputum in den Händen erfahrener Pathologen und ihrer Mitarbeiter eine genaue und nützliche
Methode sein kann, muss darauf hingewiesen werden, dass die morphologische Identifizierung von Zellen oft schwierig ist. Dieses Problem tritt häufiger auf in Verbindung mit der Auswurfuntersuchung als die cyto-
logische Untersuchung anderer Körperausscheidungen, weil der Auswurf
in genügender Menge für die Untersuchung im allgemeinen irgendwelche
Formen von Lungenkrankheit anzeigt und eine entsprechende Abwandlung
der Morphologie.

Obwohl die zellulären Veränderungen, die bei den meist nicht bösartigen
Lungenkrankheiten beobachtet werden, minimal sind, führen einige nicht
bösartige Lungenkrankheiten zu zellulären Abwandlungen, sehr ähnlich
denen bei bösartigkeit, während wieder andere cytologische Befunde zur
Folge haben, die eine spezifische Krankheit anzeigen. Unter den Thorax-
krankheiten und pathologischen Einheiten der letztgenannten Kategorie
befinden sich Asthma, Lungeninfarkt, Lipoid-Pneumonie, bronchiale Meta-
plasie, Pneumonoconiose, Coccidioidomycose und Bronchial-Adenom.

Es ist zu hoffen, dass grössere Aufmerksamkeit auf die cytologischen
Befunde bei Sputumausstrichen von nicht bösartigen Lungenkrankheiten
der Brauchbarkeit der Cytologie abgestossener Zellen schliesslich ein
neues Gebiet hinzufügen wird. Vertrautheit mit den damit zusammen-
hängenden cytologischen Strukturen muss wesentlich zur Verbesserung
in der Genauigkeit der Diagnose beitragen, da sie eine besser Grundlage
schaft für die Unterscheidung zwischen bösartigen Zellen und gutartigen
zellulären Abnormalitäten. Zusätzlich ist das Ergebnis einer solchen ver-
gleichenden Untersuchung von gleichem Wert, als eine Hilfe hinsichtlich
einer definitiven Diagnose einer Anzahl von nicht bösartigen Lungenkran-
keiten, die klinisch mit Krebs verwechselt werden können. Die Bedeutung
der Verwendung eines strengen, auf den Kern bezogenen Kriteriums zur
Identifizierung maliger Zellen wird betont, weil Ähnlichkeiten in der
zellulären Gruppenbestimmung öfter als in den Kernstrukturen die häu-
figste Quelle cytologischer Fehler sind.

REFERENCES