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Cytologic Diagnosis of Oral, Esophageal, and Peripheral Lung Cancer

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Abstract Oral Cavity. Most carcinomas *in situ* of the oral cavity present as red or pink lesions that do not have a keratinized surface. Scrapings of such lesions readily disclose abnormal squamous cells diagnostic of cancer. Scrapings of the keratinized white lesions (so-called leukoplakia) are of no diagnostic value. Dentists, who are most likely to uncover precancerous lesions, are apparently not aware of the diagnostic options based on simple scrape smears. The method is also applicable to follow-up of patients with treated cancer of the oral cavity.

Esophagus. Cytologic evaluation of esophageal cancer, initially by washings and subsequently by brushings under endoscopic control, is an established method of diagnosis. The diagnostic results are very good in symptomatic cancer patients and have an accuracy reaching 85–90%. Unfortunately the results of treatment of advanced lesions are very poor, with 5-year survival of only about 5%. Serious efforts at detection of early esophageal cancer started in China in the 1960s, using an abrasive balloon technique which was applied to asymptomatic populations in high risk areas such as Linxian in the Henan province of Central China. The Chinese investigators reported the finding of numerous precancerous lesions of the esophagus classified as carcinoma *in situ* and as dysplasia. Surgical resection of some of the precursor lesions apparently resulted in a significant drop in the rate of invasive carcinoma, although the statistical results were not convincingly presented. The balloon technique has been tested by us and by others in South Africa and in Transkei, confirming its efficacy in the diagnosis of early esophageal cancer.

Peripheral Lung. Sputum and bronchial brush cytology may uncover bronchogenic carcinoma *in situ* and early invasive cancers located in the primary or secondary bronchi. Small, peripheral lung lesions usually do not shed cells in sputum or brushings, and their discovery is usually based on roentgenologic finding. The identity of such lesions can be confirmed in most cases by a transcutaneous aspiration. Most of the peripheral malignant lesions are small adenocarcinomas or epidermoid carcinomas, both resectable by routine surgical procedures. Less commonly, oat cell carcinomas may be observed and these lesions should not be treated by surgery. Benign lesions such as granulomatous inflammation and fungal infections may also be identified by aspiration techniques. The prognosis of the resectable carcinomas varies with their size and the presence or absence of regional lymph node metastases. Most importantly perhaps, many of these peripheral lesions occur in non-smokers or former smokers, slowly replacing in frequency the classical squamous bronchogenic carcinomas observed in smokers.

It is clear that the optimal targets for chemoprevention are the oral and esophageal precursor lesions.

Key words: Oral cavity carcinoma *in situ*, esophagus carcinoma *in situ*, lung peripheral carcinomas, cytologic detection, balloon technique, transcutaneous aspiration

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ORAL CAVITY

The most common malignant lesions of the oral cavity are squamous carcinomas, originating in the epithelium of the mouth, tongue, cheek, palate, tonsils, and pharynx.

The natural history of squamous carcinoma of the oral cavity is similar to that of carcinomas of other organs inasmuch as precancerous lesions precede invasive cancer [1]. Precancerous leukoplakia is a term frequently used in oral pathology to describe a lesion with nuclear abnormalities and a keratinizing surface that, in a certain percentage of cases, progresses to invasive squamous carcinoma. Nonkeratinizing epithelial lesions, classified as carcinomas *in situ*, are also well known in the oral cavity, both as primary lesions and as a marginal change accompanying invasive cancer. The issue of human papillomavirus (HPV) participation in the precancerous and cancerous events in the oral cavity has not been satisfactorily concluded [2]. Although HPV antigen could be documented by immunostaining in some precancerous lesions [3], as yet there is no evidence that the virus is present in invasive cancer. A newly described entity, "hairy" oral leukoplakia, observed in AIDS patients, was shown to be associated with Epstein-Barr virus (EBV) and not HPV [4].

The difficulty of clinical identification of precancerous leukoplakia and carcinoma *in situ*, both easily curable precursor stages of oral cancer, was not fully appreciated until an extensive cytologic study of mouth lesions was conducted by the Veterans Administration, under the chairmanship of Dr. Henry Sandler [5]. As a consultant, I was privileged to review a major portion of the material resulting from this study. There were 2,758 patients with visible mouth lesions screened by cytology, among them were 287 histologically documented cases of invasive carcinoma. Many of these lesions were very small (26 were less than 1 cm in diameter, and 69 were less than 2 cm in diameter); many were not ulcerated, indurated, or fixed to the underlying tissue. Seventy of the 287 lesions were not recognized clinically as cancers by the examining dentists.

There were also 28 patients with squamous or epidermoid carcinoma *in situ*. Only 11 of the lesions were suspected of being cancerous by the examining dentists; 17 were considered benign. Thirteen lesions were reddish in color, 6 were white, and the rest were of various colors; 6 were ulcerated and 5 indurated. Redness of circumscribed areas of oral epithelium is frequently characteristic of carcinoma *in situ* [5].

Of particular importance was the success achieved in the diagnosis of carcinoma *in situ*, since treatment of carcinoma of the oral cavity is significantly more successful if the lesion is superficial and has not metastasized to the regional lymph nodes.

The comparison of Sandler's data with observations on a non-screened population surveyed by Shafer is enlightening. Shafer [6] reviewed the clinical and histologic data on 66 oral carcinomas *in situ*, diagnosed by biopsy only [6]. While Sandler's survey found 28 carcinomas *in situ* in 2,758 patients with visible mouth lesions (1%), Shafer's survey found 66 oral carcinomas *in situ* in 45,702 histologic accessions (0.001%). It may be argued that the two populations of patients were unequal. Sandler's patients were men, mainly over 50 years of age, many among them drinkers and smokers, who were much more prone to oral cancer than Shafer's unselected population. Nevertheless, Sandler discovered carcinoma *in situ* at a rate 1,000 times higher than in Shafer's population. The comparison of clinical findings is also enlightening. Roughly one-half of the lesions Sandler observed were red; there were only 16% of such lesions in Shafer's survey, strongly suggesting that even the most competent observers consider red oral lesions as benign and do not perform biopsies. Such lesions are the prime target for cytologic screening. Sandler's, Shafer's, and Mashberg and Meyers' [5–7] surveys pointed out that the floor of the mouth was the most frequent site of oral cancer, followed by the tongue and soft palate.

Other Cancers of the Oral Cavity

Although rare, malignant melanomas and malignant lymphomas or other sarcomas may occur in this location, more common are carcinomas of minor and major salivary gland origin. These are usually diagnosed by tissue biopsy or aspiration biopsy since they are not accessible to cytologic examination until ulceration has occurred [1,8]. The same applies to tumors origi-

nating in other deep structures of the oral cavity, such as the tenth anlage, bone, synovia, muscle, and nerve. For all practical purposes, squamous carcinoma remains the chief target of cytologic investigations.

Cytology of Oral Squamous Epithelium in the Absence of Disease

Squamous epithelial cells. The uniformity of the squamous epithelium lining the oral cavity renders the interpretation of a smear relatively simple. Normal squamous epithelium sheds cells resembling superficial and intermediate squamous cells of the vagina and cervix, except that nuclear pyknosis is not observed. Smaller, deeper, parabasal cells may be observed if the surface of the epithelium is vigorously scraped, or if an epithelial defect, such as an ulceration, is present. Fully keratinized squamous cells without visible nuclei are a common component of oral smears, especially from the palate, and do not necessarily reflect a significant abnormality. All stages of transition between nonkeratinized and keratinized cells may be observed.

A longitudinal condensation of the nuclear chromatin in the form of a nuclear bar, similar to that occurring in Anitschkow cells in the myocardium in rheumatic heart disease, has been recorded in squamous cells by Wood *et al.* [9]. Such changes were observed in smears from areas of the labial fold of the lower lip; most commonly in people with a history of aphthous ulcers. The significance of this change is unknown [9].

Buccal smears are extensively used for the study of sex chromatin to determine genetic sex. For all practical intents and purposes, the finding of 4% or more cells with a clear-cut sex chromatin body is diagnostic of the XX chromosomal constitution.

Other cells. Mucus-producing columnar cells originating in the nasopharynx or the salivary gland ducts may occasionally be observed. A vigorous scrape of the tonsillar area or the base of the tongue may result in shedding of lymphocytes, singly or in clusters.

Cytology of Benign Diseases of the Oral Mucosa

Acute and chronic inflammatory processes. Superficial erosion or ulceration of the squamous epithelium occurs frequently in the course of diffuse or localized inflammatory processes. As a result, the normal population of squamous cells in smears is partially or completely replaced by cells originating in the deeper epithelial layers. Such cells may vary in size and shape; their principal feature is relatively large, occasionally multiple, round or oval vesicular nuclei of similar size. As is common in nuclei of younger cells, chromocenters may be readily observed against a pale nuclear background; occasionally, small nucleoli may be noted. The cytoplasm is often poorly preserved. In the presence of a diffuse stomatitis or gingivitis, the preponderance of the deep cells may result in an initial impression of a significant epithelial abnormality; close attention to nuclear detail will prevent an erroneous diagnosis of cancer [1].

In chronic ulcerative processes, small squamous parabasal cells and mono- and multi-nucleated macrophages may be noted. Purulent exudate or leukocytes of various types are a common component of smears in these situations. Plasma cells are frequently observed. Except for diseases of viral etiology, especially *Herpes simplex*, identified by characteristic nuclear changes, specific diagnosis of an inflammatory disorder within the oral cavity is rarely possible. The organism of moniliasis (thrush) may be identified with ease [1]. This previously harmless infection, occurring mainly in debilitated patients and diabetics, has now been recognized as one of the first manifestations of the acquired immunodeficiency syndrome (AIDS).

Oral flora, especially in patients with poor oral hygiene, is rich in a variety of saprophytic fungi and bacteria. A protozoon, *Entamoeba gingivalis*, is fairly common. It is a multi-nucleated organism larger than *Amoeba histolytica*, from which it differs in that it does not phagocytize red blood cells. The presence of these organisms does not necessarily indicate an inflammatory process in the oral cavity.

Changes in oral squamous cells in deficiency diseases. Squamous cells of the oral mucosa may show significant enlargement of both the nucleus and the cytoplasm in pernicious anemia [10–12], megaloblastic anemia [13], and in tropical sprue [14]. These findings were documented by comparison with normal cell populations and

are statistically impressive. There is evidence that deficiencies in vitamin B_{12} and folic acid may be the cause of cell enlargement. If there is an insufficient supply of either factor, DNA synthesis becomes disturbed, resulting in cell enlargement. There is evidence that this change is not confined to the oral epithelium, but is a general one, affecting many epithelia. In my own experience, patients with a variety of disorders, who probably have malnutrition in common, may occasionally have populations of large squamous cells in oral smears. Such cells often have vesicular nuclei with numerous chromocenters. This is not infrequently observed in terminal patients.

Benign leukoplakia. Keratinization of oral epithelium is a common phenomenon occurring at the line of teeth occlusion, the palate, parts of the gingiva, and occasionally elsewhere. The milky white appearance of such areas is best classified clinically as leukoplakia and appears histologically as an epithelium topped with a layer of keratin. This anatomic variant or benign disorder must be differentiated histologically from precancerous leukoplakia discussed below. The clinical appearance of leukoplakia and precancerous leukoplakia may be similar. The cytology of benign leukoplakia is very simple: it is recognized by fully keratinized, yellow- or yellow-orange-stained cells without nuclei.

Hereditary disorders. A number of hereditary disorders affecting the oral squamous epithelium may result in cytologic abnormalities in the form of "cell-in-cell" or epithelial pearl arrangements. These are Darier-White disease (*keratosis follicularis*), hereditary benign intraepithelial dyskeratosis (Witkop) and white sponge nevus of Cannon [summarized in 1].

Vesicle- or bullae-forming conditions of the oral mucosa. These conditions may be divided into oral manifestations of generalized skin disorders and diseases confined to the oral cavity and immediately adjacent organs. Most of the latter disorders are associated with acute, painful, clinical episodes and are due to *Herpes simplex* Type I infection. Changes in squamous cells due to herpetic infection include multinucleated cells with "ground glass" opaque, tightly packed nuclei and large, eosinophilic intranuclear inclusions.

The most important dermatologic disorders affecting the oral cavity are *erythema multiforme* and the group of diseases known as pemphigus. The most important of the latter group is the nearly always fatal *pemphigus vulgaris*, which may have its primary manifestations in the oral cavity.

Pemphigus vulgaris. The group of skin diseases known as pemphigus is characterized by formation of vesicles and bullae, either by a split between the epidermis and the dermis or within the epidermis.

Recent investigations documented that lesions of the pemphigus family are due to the action of self-produced antibodies against the surface of squamous epithelial cells (keratinocytes), destroying adhesions among these cells. The resulting lack of cohesion among the epithelial cells leads to vesicle formation [summarized in 15]. As a consequence of these events, immunoglobulins (IgG) can be demonstrated on the surfaces of the affected squamous cells.

Of importance in the present context is the involvement of the oral mucosa in acute or chronic *pemphigus vulgaris*. Ruptured vesicles shed abundant, highly abnormal, small squamous cells with somewhat frayed, scanty cytoplasm, singly and in loose clusters (Tsanck's cells). The atypia is characterized primarily by large, prominent, usually spherical single or multiple nucleoli, located centrally within pale nuclei. "Cell-in-cell" arrangements, similar to those in epithelial "pearls," may be observed. Nuclear hyperchromasia is generally negligible. Similar abnormalities of squamous cells may be observed in ulcerative esophagitis.

As a consequence of autoimmune events, the cells shed from pemphigus are coated with immunoglobulins. Faravelli *et al.* [16], using IgG and the peroxidase-antiperoxidase reaction, provided a permanent record of the presence of an immunoglobulin coat on the surfaces of acantholitic cells in smears of oral pemphigus. Harris and Mihm [17] summarized the immunologic differential diagnoses between pemphigus and other bullous lesions of the oral cavity.

Changes caused by therapy. Changes in oral epithelial cells caused by radiation therapy were studied by Zimmer (unpublished) in my laboratory and by Umiker [18]. Radiotherapy produces

cellular enlargement with synchronous enlargement of pale nuclei. Vacuolization of the cytoplasm and the nucleus is common.

Changes caused by chemotherapy were also observed. Nuclear degeneration and "cell-within-cell" structure occur following treatment with methotrexate and other anticancer chemotherapeutic agents. Excessive shedding and ulceration of the buccal epithelium is a common complication of chemotherapeutic agents, especially the folic acid antagonists such as methotrexate.

Cytologic Presentation of Squamous Carcinoma of the Oral Cavity

Invasive squamous carcinoma. The majority of patients with invasive squamous carcinomas show ulcerative lesions with indurated borders that are easily identified as cancer on clinical grounds. Rarely, inflammatory processes may imitate ulcerative oral cancer. However, there is also a group of oral carcinomas that, when first observed, are not ulcerated. Some of these lesions may have wartlike configurations (verrucous carcinomas) and others may present as white patches with irregular borders (precancerous leukoplakia)—somewhat similar in appearance to benign leukoplakia. The cytologic diagnosis of ulcerated invasive lesions is relatively simple if care has been taken to remove the layer of necrotic surface material prior to cytologic sampling. These lesions show an abundance of cancer cells, with their customary variation in sizes and shapes. Cytologic preparations closely reflect the degree of keratinization of the lesion. In heavily keratinized squamous cancer, cells with orange- and yellow-staining cytoplasm and large, sometimes pyknotic, dark-staining irregular nuclei will be noted. "Ghost" cells, with heavily keratinized cytoplasm and virtually no residual nuclear material, are frequent. "Pearls" of malignant cells are frequently observed. In nonulcerated, but invasive keratinizing carcinomas, the cytologic diagnosis of cancer may be obscured by abundant, fully keratinized "ghost" cells without perceptible nuclear abnormalities. In such cases, close attention must be paid to relatively minor nuclear abnormalities, which may occur in only a few cells. Nuclear enlargement and irregularity of outline, with or without nuclear hyperchromasia, are also of diagnostic significance. In case of doubt, a biopsy is recommended. In less well-differentiated squamous carcinoma, the cytoplasmic keratinization is not nearly so prominent, but the nuclear abnormalities, such as large nucleoli and a coarse pattern of chromatin distribution, are evident. In the latter form of oral cancer there is usually a reversal of the nucleocytoplasmic ratio, a feature not always observed in cells of the keratinizing variety. Regardless of the type of tumor, the smear background nearly always shows necrotic material, blood, and numerous leukocytes. To anyone familiar with the principles of cytologic diagnosis of cancer, diagnosing invasive squamous carcinoma of the oral cavity will cause little difficulty if the potential pitfalls discussed above are taken into consideration.

Carcinoma *in situ*. The term "oral carcinoma *in situ*," in the context of cytologic diagnosis, is based on malignant epithelial lesions without significant keratin formation on their surfaces. Many of these lesions present clinically as areas of redness.

Scrape smears from such lesions are characterized by a mixture of well-differentiated parabasal or intermediate squamous cells, with translucent cytoplasm and significant nuclear enlargement and hyperchromasia. It is not uncommon to observe a few squamous cancer cells with the same type of nuclear abnormality in such smears.

The accurate cytologic diagnosis of keratinizing carcinoma *in situ* or of precancerous leukoplakia may prove difficult if the abnormal cells in smears are overshadowed by keratinized benign cells. However, after thorough screening of cytologic material, it is rare not to find at least a few cells suggesting a well-differentiated squamous cancer. In these situations, knowledge of the clinical presentation of the lesion is invaluable and should lead to a confirmatory biopsy, even though the cytologic evidence may be very scanty.

Hong *et al.* [19] reported that oral administration of 13-*cis*-retinoic acid had a beneficial effect on the size and degree of cellular abnormalities in the oral precancerous leukoplakias of some patients, despite fairly severe toxic effects.

TABLE I. Results of Cytologic Follow-up on Patients With Treated Cancer of the Oropharynx Without Visible Lesions at the Site of Prior Surgery

Patients examined			**177**	**(100%)**
Positive smears	12			
Suspicious smears	2			
			14	(9%)
Carcinoma confirmed		8		
Died *		3		
Still being followed without clinical evidence of lesions (smears still positive)		3		
		14		

*2 with evidence of cancer

(Hutter RVP, Gerold FP: Cytodiagnosis of clinically inapparent oral cancer in patients considered to be high risks. A preliminary report. Am J Surg 112:541–546, 1966). Reproduced from Koss LG: "Diagnostic Cytology and its Histopathologic Bases." (Ed) 4 Philadelphia: J.B. Lippincott, 1992. ©L.G. Koss.

The Diagnosis of Recurrent Oral Cancer After Treatment

Local recurrence of oral cancer after treatment by surgery, radiation, or a combination of these two techniques, is sufficiently common to warrant a close follow-up of all patients. The possibility of a second primary cancer within the same anatomic area is very high in patients with treated cancer of the oral cavity. There is excellent evidence that the addition of cytologic techniques to the follow-up examination may result in the diagnosis of a recurrent or new cancer before it is suspected clinically.

Hutter and Gerold [20] applied cytologic techniques in the follow-up of patients previously treated by surgery. Limiting the application of cytology to patients without a visible lesion, they uncovered clinically unsuspected recurrent cancer in 10 of 177 patients investigated (6%). These authors were using material scraped from the general area of prior surgery by an endometrial curette. The results are summarized in Table I.

An interesting aspect of Hutter and Gerold's work concerns the time that elapsed between the cytologic evidence of recurrent (or new) carcinoma and the appearance of a clinical abnormality, however slight, amenable to biopsy. In several of the cases, 4 to 6 months of follow-up by a very experienced observer were required to identify a lesion, usually an area of redness or a whitish patch. In six of the eight patients with cytologic diagnosis of recurrent carcinoma, the major histologic component of the lesion was a carcinoma *in situ*, either with or without superficial infiltration. In this work the cytological identification of carcinoma *in situ* was extremely accurate and is summarized in Table II.

This work, as well as the results of the Veterans Administration study reviewed in the opening paragraph of this article [5] suggests very strongly that a silent stage of carcinoma *in situ*, not readily identified clinically, precedes

TABLE II. Comparison of Cytologic Diagnosis With Histologic Findings in Eight Cases of Recurrent Oral Epidermoid Carcinoma

Number of Patients	Cytologic Diagnosis	Histologic Findings
4	Epidermoid carcinoma	2 invasive carcinoma
		2 *in situ* and infiltrating carcinoma
3	Carcinoma *in situ*	3 carcinoma *in situ* with foci of very superficial invasion
1	Suspect carcinoma *in situ*	1 carcinoma *in situ*

(Modified from Hutter RVP, Gerold FP: Cytodiagnosis of clinically inapparent oral cancer in patients considered to be high risks. A preliminary report. Am J Surg 112:541–546, 1966). Reproduced from Koss LG: "Diagnostic Cytology and Its Histopathologic Bases." Philadelphia: J.B. Lippincott, 1992. ©L.G. Koss.

invasive squamous carcinoma of the oral cavity. This stage of cancer may last for several months, and possibly much longer, before producing a visible lesion. Carcinoma *in situ*, whether primary or recurrent, is often accurately identifiable by cytology.

Mass Screening for Oral Cancer

Cancers of the oropharynx account for approximately 5% of all cancer in the United States and are found preponderantly in men. One of 1,000 adult men is estimated to develop a cancer of the oropharynx. Is a mass cytologic screening of asymptomatic persons justified?

A survey by Stahl *et al.* [21] comprising the area of New York City demonstrated the feasibility of such an endeavor. Practicing dentists were instructed to obtain smears from all visible abnormalities of the oropharynx. Forty-seven cancers were found in 2,297 patients examined; 11 cancers (24%) were clinically unsuspected, confirming the results of the Veterans Administration study [5]. It does not appear feasible or reasonable to cytologically screen all dental patients. However, a scrape smear of an oral lesion may well be lifesaving. In situations where an exceptionally high risk of oral cancer exists, more extensive surveys may be justified. Wahi, from Agra, India, (personal communication, 1966) demonstrated the value of cytologic techniques among betel-nut chewers who have a very high incidence of oral carcinoma. His results are summarized in Table III. It is probable that tobacco chewers and heavy pipe smokers could be profitably screened for oral cancer by cytologic techniques.

Opportunities for Chemoprevention

The search for precancerous lesions such as carcinoma *in situ*, offers the best opportunity for chemoprevention. Cytologic scraping of all visible oral lesions, even if they are not considered clinically suspicious, is a screening method *par excellence* that in itself may alter the clinical picture of invasive squamous carcinoma.

THE ESOPHAGUS

Histology and Normal Cytology

The esophagus is lined by nonhornifying squamous epithelium. Islands of gastric epithelium may be found in the areas immediately adjacent to the cardia and, rarely, elsewhere

TABLE III. Cytologtic Diagnosis of Oral Carcinoma Among Betel-nut Chewers

Total cases of oral cancer studied	812
Clinically unsuspected	69*
Clinical diagnoses	
Leukoplakia	26
Ulceration	27
Trismus	9
Dysphagia	4
Tonsillar enlargement	3
Cytologic diagnoses	
Malignant cells	39
Cells suggestive of cancer	21
Dyskaryotic cells, possibly malignant	9

*(66 squamous carcinoma, 2 reticulum cell sarcoma, 1 adenocarcinoma)

(Prof. P.N. Wahi, Agra, India, personal communication). Reproduced from Koss, LG: "Diagnostic Cytology and Its Histopathologic Bases." Philadelphia: J.B. Lippincott, 1992. © L.G. Koss.

within the esophagus. Small mucus-producing glands are found in the submucosa.

The cytology of esophageal aspirates, brushings, and washings in the absence of disease is extremely simple. The smears are composed essentially of superficial squamous cells identical to those observed in smears of the oral cavity. Smaller, deeper squamous cells with relatively large nuclei and occasionally squamous "pearls" may be less commonly noted. It is not unusual to find swallowed cells of respiratory origin, such as dust-containing macrophages and ciliated bronchial cells [1]. Gastric epithelial cells, singly or in clusters, may also occur. Foreign material, especially plant cells, may be present if there is an obstruction of the esophageal lumen.

Noncancerous Diseases

Acute and Chronic Esophagitis. This group of diseases of varying etiology is very important from the point of view of diagnostic cytology, since it produces cells that may be readily confused with cancer. Primary ulcerative esophagitis may be due to trauma or *Herpes*; secondary ulcerative esophagitis may be due to such diseases as cardiospasm of long standing, Plummer-Vinson syndrome, some forms of avitaminosis, scleroderma, and hiatal hernia. Chronic erosive esophagitis is a rare, sometimes fatal, disorder of unknown etiology.

The histologic lesion in esophagitis is mucosal ulceration of varying depth and configuration. There is moderate infiltration of the stroma with inflammatory cells. The surface of the lesion may be covered with fibrin. Chronic erosive esophagitis is characterized by loss of superficial epithelial layers (mucosal erosions). Squamous metaplasia of the submucosal glands may occur [22].

Cells desquamating from the eroded epithelium are essentially the same size as small squamous or parabasal cells. The most outstanding features of the comparatively large nuclei are large isolated clumps of chromatin and occasionally, large nucleoli. However, there is no significant hyperchromasia. The cytoplasm is evenly distributed, and the cells do not vary much in size. In clusters, there is good adherence of the cells to each other. Careful attention to cellular detail helps in the correct interpretation of the cytologic findings. The cells seen in erosive esophagitis are similar to those observed in pemphigus as discussed above.

Herpetic esophagitis. Herpetic esophagitis produces extensive though superficial ulcerations of the esophageal mucosa. Using cytology resulted in the primary clinical diagnosis of several such cases. Most patients observed had vague complaints referable to the esophagus, such as retrosternal pain or mild dysphagia. Simultaneous involvement of the esophagus and the bronchial tree has been noted. The disease may be diagnosed in sputum or in material obtained from the esophagus by aspiration, washings, or brushing. Multi-nucleated cells with molded "ground-glass," opaque nuclei, and cells

with intranuclear eosinophilic inclusions, are characteristic of this disorder [1].

Esophageal infections in acquired immunodeficiency syndrome. Esophagitis is a common early manifestation of AIDS. Candidiasis (moniliasis), cytomegalovirus infection, herpetic esophagitis, and other infectious agents may be identified in cytologic samples obtained during esophagoscopy [1].

Barrett's Syndrome. First described by Barrett in 1950 [23], this syndrome consists of an extensive replacement of the esophageal squamous epithelium by columnar epithelium of gastric or intestinal type, associated with hiatal hernia and, quite often, esophageal stricture.

Cytologic examination of esophageal aspirates may contribute in a major way to accurate diagnosis. The smears contain goblet cells and mucus-producing benign columnar cells, usually in clusters, which are characteristic of mucus-producing epithelium.

Because adenocarcinoma may occur in Barrett's esophagus (although the frequency of this event is disputed) and because the esophagus can be closely monitored by endoscopic techniques, this disorder has come under intense scientific scrutiny [summarized in 24]. The sequence of morphologic events in the genesis of adenocarcinoma became the subject of numerous scientific communications. Briefly summarized, morphologic precancerous abnormalities in columnar epithelium (named dysplasia, rather than carcinoma *in situ*) precede carcinoma [25,26]. Dysplasia consists of nuclear enlargement and hyperchromasia, occasionally with branching of the affected glands and a marked increase in abnormal mitoses [27]. The lesions are very similar to precancerous abnormalities and carcinoma *in situ* of the gastric epithelium. Nuclear abnormalities in smears of columnar cells were reported in a case of dysplasia [28]. Prospective studies of patients with Barrett's dysplasia indicate a very high level of progression to carcinoma [24,26]. Carcinomas occurring in Barrett's mucosa are usually adenocarcinomas of the gastrointestinal type [25]. Other variants of gastrointestinal cancers may also occur (adenosquamous carcinoma, *etc.*).

Patients with Barrett's syndrome can be monitored by endoscopic biopsies and, sometimes, by cytologic studies.

Cancer of the Esophagus

Carcinoma of the esophagus has an interesting geographic distribution. The disease is quite common in Northeastern Iran, in Central China, in the Chinese population in Singapore, among Africans in parts of Southern Africa, and in Brittany, France. The disease is more common in males than in females. Epidemiologic data suggest that intake of hot beverages, cigarette smoking, or alcohol intake may, in part, account for esophageal carcinoma, although a recent Chinese study failed to reveal any risk factors [29].

Epidermoid carcinoma is by far the most common type of esophageal cancer. The disease may affect any part of the esophagus, but occurs most frequently in areas where the esophagus is slightly narrowed: at the level of the thyroid cartilage, the bifurcation of the trachea, and the diaphragm. The degree of differentiation may vary from highly keratinized (verrucous) types to poorly differentiated small cell carcinomas [30].

Invariably, as with all squamous cancers, the question of human papillomavirus (HPV) as a factor in the genesis of this tumor was raised. Except for squamous papilloma, the evidence of HPV presence in esophageal cancer was initially limited to one paper [31] in which the presence of HPV DNA was observed in five invasive cancers. More recently, work on invasive esophageal carcinoma based on biopsy material from 51 Chinese patients revealed the presence of HPV in 25 cases (49%). In 16 of the 25 specimens, HPV types 16 and 18 were documented by *in situ* hybridization. Other types of HPV were observed in the remaining 7 patients [39]. In the same study of 80 cytologic preparations from Chinese patients from a high-risk area, 53 samples were positive for HPV by filter *in situ* hybridization. It was of note that HPV was detected in 2 of 9 patients without cytologic abnormalities, in 3 of 6 patients with mild dysplasia, in 25 of 31 patients with moderate dysplasia, in 19 of 28 patients with severe dysplasia, and in 4 of 6 patients with invasive carcinoma. Since a person-to-person transmission of HPV is unlikely in these patients, an activation of the latent viral infection is a more likely explanation of these findings.

Approximately 3% of esophageal cancers are

adenocarcinomas. Most adenocarcinomas of the esophagus occur in the area of the cardia and are histologically indistinguishable from gastric cancers.

Among the rare neoplastic disorders of the esophagus that may become targets of cytologic examination are squamous papillomas, small cell (oat cell) carcinoma, and primary melanomas [summarized in 1].

Precursor Lesions of Epidermoid Carcinoma and Their Cytologic Detection

Epidermoid carcinoma of the esophagus is preceded by precancerous epithelial changes, such as carcinoma *in situ* and related abnormalities. Support for this concept was provided by extensive cytologic and histologic studies of esophageal cancer conducted by Chinese investigators. The stimulus for these studies was the very high prevalence of esophageal cancer in certain areas of Central and Northern China. Incidentally, in the same areas of China, chickens are susceptible to a cancer-like tumor of the gullet [32].

The purpose of the Chinese studies was to detect precancerous states, such as carcinoma *in situ*, in asymptomatic, high-risk populations by cytologic means, with the hope that early surgical intervention would prevent invasive esophageal cancer with its very high mortality rate. The instrument used in these investigations was a small, inflatable plastic balloon with an abrasive surface devised by Chinese scholars. The balloon was attached to a narrow-caliber tube with colored markers to indicate the position of the balloon in the esophagus. The balloon could be easily swallowed in the deflated state, moved by peristalsis to the cardia, inflated, and slowly withdrawn to the level of the cricoid cartilage. At this point, the balloon was deflated and withdrawn. The abrasive surfaces of the balloon contained cells scraped from the esophageal epithelium, which were then examined in the form of smears. The method causes trivial discomfort to the patients and was well accepted.

Based on cytologic and histologic criteria, the Chinese investigators divided the precancerous lesions into two groups; dysplasia and carcinoma *in situ* [33,34]. The criteria were derived from the now-obsolete classification of precancerous lesions of the uterine cervix. Lesions with more orderly epithelial growth, surface differentiation, and relatively minor nuclear abnormalities were classified as dysplasia; lesions with more significant atypia were classed as carcinoma *in situ*.

The dysplasias were further subdivided into mild, moderate, and severe, based mainly on cytologic criteria. The true significance of dysplasia is not clear, but there is no doubt that, in a substantial number of untreated patients with this disorder, invasive cancer of the esophagus developed subsequently. It appears though, that in some patients, the lesions either failed to progress or even regressed [32,34]. In any event, precancerous lesions of the esophagus and subsequent events appear to have a remarkable similarity to lesions of the uterine cervix.

The accuracy of balloon sampling was tested on several large hospital populations, totaling 1,861 patients, with overt esophageal cancer documented by biopsy. The accuracy varied in a number of studies from 87.2% to 99.0%, averaging 94.9% [summarized in 32,34]. For diagnosing carcinoma *in situ* and early invasive cancer, cytologic sampling proved to be much superior to either endoscopy or radiologic examination [33].

The accomplishments of the Chinese scholars soon found several imitators. Berry *et al.* [35] attempted a similar project in South Africa (where the rate of esophageal cancer is very high among some black populations), resulting in the discovery of 15 occult invasive carcinomas and carcinomas *in situ* in 500 patients. Dysplasia was illustrated, but the clinical significance of the lesion was not discussed. Greenebaum *et al.* [36] used the balloon technique in 96 high-risk Montefiore Hospital patients in New York City, unexpectedly finding 3 occult recurrent oropharyngeal cancers and one carcinoma *in situ* of the esophagus.

Cytology

Precursor lesions. Much of the current knowledge about the cytology of precursor lesions comes from Chinese sources [32,34]. There is remarkable similarity between the cytologic presentation of carcinoma *in situ* and related lesions of the esophagus and the uterine cervix.

Lower grade lesions of the esophagus (dysplasia) are characterized by well-differentiated, superficial and intermediate squamous cells with marked nuclear enlargement and hyperchromasia.

Squamous cancer cells derived from such high grade lesions as carcinoma *in situ* are of the smaller, parabasal variety. Although the nuclear abnormalities are approximately the same as in low grade lesions, the cytoplasm is scanty, reflecting a lesser degree of surface maturation. Shu [34] illustrated several examples where dysplasias progressed to carcinoma *in situ* over a period of 2 to 4 years and, in some cases, progressed to invasive carcinomas.

Results of Screening

There is no doubt that mass screening for esophageal carcinoma in high-risk areas of China had a major beneficial effect. Before screening was instituted, a diagnosis of carcinoma *in situ* or early invasive carcinoma was 2 per 1000 (0.2%) in low-risk areas, and 10 per 1000 (1.0%) in high-risk areas where physicians and surgeons were alerted to this possibility. Screening of 81,187 asymptomatic people over the age of 30 in the high-risk Henan Province resulted in the discovery of 880 esophageal cancers (1.1%), of which 649 (73.7%) were early and treatable by surgery [33]. Little is known about survival of these patients, but Dr. Shu assured me that most of the treated patients survived 5 years or longer with a good quality of life. This information must be compared with a survival of 5% or less commonly observed in the United States for patients with invasive cancer of the esophagus.

The question of screening of patients in Western countries by the esophageal balloon technique must remain open. The experience from this laboratory [36] suggests that in high risk patients (those with prior cancers of the larynx and pharynx, alcoholics who are also heavy cigarette smokers, *etc.*), a larger survey may be worthwhile. Clearly, in geographic areas at high risk for esophageal cancer, a major effort at balloon screening may be indicated.

Invasive Epidermoid Carcinoma

These tumors occur in a variety of grades and degrees of differentiation, ranging from keratinizing, pearl-forming squamous cancer to anaplastic small cell epidermoid carcinoma. Cytologic findings from esophageal washings or brushings closely reflect these structural varieties.

Squamous carcinoma produces highly keratinized abnormal cells with either completely pyknotic, hyperchromatic nuclei, or with nuclear shadows. Epidermoid cancers are characterized by smaller and less well-differentiated cells, frequently with very scanty basophilic cytoplasm. The most anaplastic varieties of epidermoid cancer produce cells that often are very small, with abnormally large nuclei and very scanty cytoplasm. All of the epidermoid cancers are characterized by marked nuclear abnormalities, especially hyperchromasia and frequently large, prominent nucleoli. Horai *et al.* [37] described several examples of a small cell anaplastic carcinoma.

Carcinomas of the distal end of the esophagus may extend into the cardia and fail to produce radiographic abnormalities of cancer. In such situations, cytologic examination may be of critical diagnostic importance.

Other Cancers of the Esophagus

Adenocarcinoma. These tumors occur most often in the lowest portion of the esophagus. Adenocarcinomas of the esophagus usually resemble gastric adenocarcinomas. Occasionally, papillary adenocarcinomas composed of large, columnar cancer cells may be observed. These uncommon lesions may be related to Barrett's esophagus, wherein precancerous lesions of a similar histologic and cytologic type have been observed.

Rare primary tumors include malignant melanomas, several of which have been described in the literature [1].

Chemoprevention

Several dietetic regimens have been tried in China to prevent the occurrence of esophageal cancer. The effects were either nil or poorly documented [33], but the very high rate of this disease in parts of rural China would make a serious prevention effort very worthwhile.

TABLE IV. Space-occupying Peripheral Lung Lesions That May Become the Target of Aspiration Biopsies

Benign nonneoplastic lesions	Malignant tumors	Tumor-like lesions
Focal fibrosis	Adenocarcinoma	Hamartoma
Lipoid pneumonia	Squamous (epidermoid) carcinoma	Inflammatory pseudotumors
Infarct	Large cell carcinoma	
Abscess	Small cell carcinoma Intermediate type Oat cell type	
Specific bacterial or fungal infections: Tuberculosis Actinomycosis Aspergillosis Other fungal diseases	Rare types of lung cancer Carcinoid tumors Well-differentiated Neuroendocrine carinoma	
Pneumocystis carinii	Malignant lymphoma	
Sarcoidosis	Metastatic tumors	

From Koss LG, Woyke S, Olszweski W: "Aspiration Biopsy; Cytologic Interpretation and Histologic Bases." New York: Igaku-Shoin, 1992. ©Koss, Woyke, and Olszewski.

PERIPHERAL LUNG LESIONS*

Diagnosis by Thin Needle Aspiration

Transthoracic aspiration biopsy is the method of choice to identify small, peripheral lung lesions that cannot be diagnosed by bronchoscopy or sputum cytology. The procedure is performed under fluoroscopic or computer tomography guidance and requires significant manual skills to be successful. The material obtained during the aspiration is examined in the form of smears and cell blocks. In experienced hands, it is quite accurate in determining the nature of the lesion. Peripheral pulmonary lesions that may be encountered are listed in Table IV.

Because many of the peripheral lung nodules are cancerous, and may be curable, a rapid identification of the nature of any peripheral lung nodules is of major consequence to the patient. If sputum cytology and fiberoptic bronchoscopy are noncontributory or negative, the remaining diagnostic options are either observation, exploratory thoracotomy with frozen section, or the transthoracic thin needle aspirate. Observation alone would not be considered an acceptable option unless the lesion is judged to be unequivocally benign. For debatable or suspicious lesions, the choice between exploratory thoracotomy and thin needle aspiration is more difficult. Still, it must be pointed out that at least 20% of all peripheral lung lesions are inflammatory and many not require surgical treatment. A better approach to treatment can be planned for lesions proved malignant by aspiration before surgery. Speed, convenience, and cost make a preoperative aspiration a better way to approach the problem of diagnosing pulmonary nodules. With experience, the diagnostic reliability of the transcutaneous thin

*This summary was based on the text of Chapter 12 in [8]: "Aspiration Biopsy; Cytologic Interpretation and Histologic Bases." by Koss, L.G., Woyke, S. and Olszewski, W., 2nd Ed., New York, Igaku-Shoin, 1992.

needle aspirates may equal that of frozen sections. It has the added potential benefit of preventing unnecessary thoracotomies.

Although some institutions perform aspirates only after negative sputum examination and negative fiberoptic bronchoscopies, we do not hesitate to recommend thin needle aspiration as a primary diagnostic procedure and as the method of choice to identify peripheral lung lesions.

Contraindications. The contraindications for transthoracic aspiration are (1) hemorrhagic diathesis, (2) anticoagulant therapy, (3) severe pulmonary hypertension, (4) uncontrolled cough, (5) advanced emphysema, (6) suspected arteriovenous malformation, (7) uncooperative patient, and (8) suspicion of a pulmonary hydatid cyst.

Method of procedure. This is described in detail elsewhere and the reader is referred to [8].

Complications. The principal complication of using transcutaneous thin needle aspiration to biopsy pulmonary nodules is pneumothorax. In our experience, after more than 1,000 lung aspirations using needles with an external diameter of 0.9 mm, we observed pneumothorax in about 25% of the patients. In most cases, small pneumothorax required no treatment and disappeared spontaneously. Only six patients required intercostal tubular drainage.

Other complications include hemoptysis, which is usually transient. Transient and brief symptoms of central nervous system ischemia, possibly secondary to air embolism, may sometimes be observed. Subcutaneous implantation of tumor in the needle track is very rare but has been recorded [8].

Cytology of Benign Lesions

Most of the benign lesions, such as lipoid pneumonia, abscesses, fungal infections, and *Pneumocystic carinii*, can be accurately identified in cytologic preparations. The reader is referred to other sources for descriptive details [1,8]. Significant diagnostic problems may be caused by the difficulty of differentiating between chronic pneumonitis with proliferating

TABLE V. Differentiation of Very Well-differentiated Adenocarcinoma and Atypical Bronchial Cells

Cytologic features	Atypical bronchial cells	Adenocarcinoma
Number of cell sheets	Few	Many
Configuration of sheets	Compact, occasional cilia[a]	Compact and loose, no cilia
Nuclear size	Normal	Enlarged
Nuclear crowding	Very rare	Common
Hyperchromasia	Slight	Slight, rarely marked
Nucleoli	Single, very small, difficult to see	Single or multiple, usually large, sometimes small but readily identified
Detached single cells or small cell clusters with nuclear abnormalities	Rare	Common
Mitoses	Exceptional	Fairly common

[a]The diagnosis of cancer should never be rendered if the atypical cells are ciliated.

From Koss LG, Woyke S, and Olszewski W: "Aspiration Biopsy; Cytologic Interpretation and Histologic Bases." (Ed) 2 New York: Igaku-Shoin, 1992. ©Koss, Woyke and Olszewski.

TABLE VI. Dominant Features of the Four Most Common Types of Lung Cancer in Aspirates

Feature	Adenocarcinoma	Squamous cell carcinoma	Large cell carcinoma	Small cell carcinoma
Cell clusters	Abundant, cohesive, often papillary or monolayer	Frequent, less cohesive, no definite configuration	Infrequent, small, loosely arranged	Rare, very small, with molding of cells
Cell configuration	Columnar or cuboidal with eccentric nuclei	Oddly shaped, nuclei, often central	Round or oval	Round, oval, or spindly
Cytoplasm	Abundant, pale, sometimes vacuolated	Sharply demarcated, often keratinized; ghost cells and pearls	Fairly abundant, pale, fragile	Very scanty, fragile
Nuclei	Large, pale, round, or oval, rarely hyperchromatic	Often hyperchromatic, oddly shaped and pyknotic	Large, hyperchromatic	Two types: (1) larger vesicular, coarsely granular and (2) small pyknotic
Nucleoli	Prominent, often multiple	Prominent in nonpyknotic nuclei	Prominent and multiple	Small, difficult to see
Special features	Mucus formation	Keratin formation	Can have features of adeno- or squamous carcinoma	Crush artifacts
Principal points of differential diagnosis	Large cell carcinoma, metastatic tumors	Metastatic tumors	Adenocarcinoma (poorly differentiated), metastatic tumors	Atypical carcinoid, malignant lymphoma

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bronchiolar epithelium and well-differentiated adenocarcinoma. The main points of differential diagnosis are listed in Table V.

Malignant Tumors

The 3 main types of malignant tumors observed in the aspirated material from periphery of the lung are adenocarcinoma, squamous or epidermoid carcinoma, and small cell carcinoma.

Adenocarcinoma. Adenocarcinomas are by far the most common tumors. They vary greatly in histologic patterns and degree of differentiation. Some of these tumors also have an epidermoid component. Regardless of the histologic presen-

tation, all of the peripheral adenocarcinomas are capable of metastases. The prognosis depends on the size of the lesion; very small carcinomas (<2 cm in diameter) have a significantly better prognosis than larger tumors.

Peripheral adenocarcinomas may be classified into several subgroups. However, for all practical purposes, the following simple classification is adequate: (1) well-differentiated adenocarcinomas (including bronchioloalveolar carcinomas); (2) moderately and poorly differentiated adenocarcinomas; and (3) carcinomas of mixed types, combining elements of adenocarcinomas and epidermoid carcinomas.

Squamous cell carcinoma (epidermoid carcinoma). Squamous cell tumors, with varying degrees of differentiation, constitute the second largest group of peripheral bronchogenic cancers. About 25% of peripheral lung cancers presenting as small roentgenologic abnormalities are epidermoid carcinomas.

Keratin-forming, well-differentiated cancers occur mainly in the principal bronchi. Peripheral epidermoid carcinomas are usually, but not always, poorly differentiated.

Small cell carcinoma. Although small cell bronchogenic carcinomas originate predominantly in the central bronchi, peripheral tumors of this type have been shown to occur. Some of these tumors are thought to derive from bronchial Kulchitsky cells and belong to a group of tumors capable of producing a variety of polypeptide hormones and related substances, which may cause endocrine symptoms.

The WHO classification distinguishes two subtypes of this group of tumors: *intermediate cell type* of carcinoma and *oat cell carcinoma*. Both types of small cell carcinoma have a poor prognosis, regardless of size, but may respond to chemotherapy.

Large cell carcinoma. Large cell carcinoma is a poorly differentiated tumor that may be either of epidermoid or glandular derivation. Such tumors are rarely seen as peripheral lesions.

The principal cytologic features of the four principal types of lung cancer are summarized in Table VI.

Other uncommon primary tumors. Peripheral space-occupying lesions include hematomas, inflammatory pseudotumors, carcinoids, lymphomas, and other uncommon tumors [8].

Metastatic tumors. Metastatic tumors of various origin may occasionally appear as solitary peripheral lung nodules that can be aspirated.

Comment

It does not appear likely that peripheral lung tumors could benefit from chemoprevention. Bronchogenic carcinomas *in situ* and related abnormalities appear to be a much better target for such endeavors, but cannot be diagnosed by aspiration.

CONCLUSION

Cytologic techniques, applied to the oral cavity and the esophagus, may uncover early primary cancerous lesions, such as carcinoma *in situ*, that may become targets for chemoprevention. Cytologic techniques may also be used to monitor the effects of chemoprevention. Recurrent cancers of these organs after surgical treatment may also be detected early by cytology. Although experience using cytologic detection of carcinoma *in situ* of the larynx is still very modest, such lesions have been observed and reported [1]. On the other hand, it does not appear likely at this time that peripheral pulmonary cancers could become a target for chemoprevention.

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