

Studies of Esophageal Balloon Cytology in Linxian, China

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Abstract

Esophageal cancer is the second leading cause of cancer death in China. Esophageal cancer has a very poor prognosis, principally because most tumors are asymptomatic until they are unresectable. Esophageal balloon cytology is an early detection method developed by Chinese scientists to identify resectable early cancers and precursor lesions. Previous studies have reported high sensitivities for detecting esophageal cancer in symptomatic patients. The current report describes several studies evaluating this diagnostic technique in asymptomatic individuals. A comparison of Chinese and U. S. cytological diagnoses of the same esophageal samples showed that the Chinese categories of precancerous neoplasia were more inclusive than the corresponding U. S. categories. Comparisons of both Chinese and U. S. cytological diagnoses with concurrent histological findings showed low (14–36%) sensitivities for the cytological detection of biopsy-proven cancers. Prospective follow-up studies of several screened cohorts showed a consistent progression of risk for developing esophageal cancer with increasing severity of initial cytological diagnosis. These preliminary studies suggest that esophageal balloon cytology is a useful technique that can benefit from additional research to improve its optimal performance.

Introduction

Esophageal cancer is a common malignancy with a very poor prognosis. In 1985, esophageal cancer was estimated to be the ninth most common cancer worldwide, with 300,000 new cases annually, and it was the fifth most common cancer in developing countries (1). In 1983–1990, the 5-year relative survival rate for all esophageal cancers reported to the Surveillance, Epidemiology and End Results cancer registry in the United

States was 9.2% (2). The principal reason for this poor survival is that most tumors are asymptomatic and do not reach medical attention until they have grown deep into the esophageal wall, have spread to lymph nodes, and are unresectable. In this setting, there is a clear need for clinical strategies that can successfully detect more esophageal cancers at an earlier, more curable, stage of disease.

Nearly 50% of the world's esophageal cancer occurs in China (1), where it is the second leading cause of cancer death (3). Since the late 1950s, Chinese scientists have performed many studies to investigate the epidemiology and etiology of esophageal cancer and to develop early detection strategies for this disease. Much of this research has been performed in Linxian, a county in northern Henan Province, which has some of the highest rates of esophageal cancer in China (3). The average annual incidence rates for esophageal cancer in Linxian from 1983 through 1987, age-adjusted to the population of China, were 149 per 10⁵ for males and 97 per 10⁵ for females (4). Because of similar symptomatology, both esophageal and gastric cardia tumors have traditionally been called "esophageal cancer" in Linxian; therefore, tumors from both sites were included in these rates.

The principal early detection technique developed for esophageal/gastric cardia cancer in Linxian has been esophageal balloon cytology (*lawang*) (5–8). In this technique, first developed by Dr. Qiong Shen of Henan Medical University, a deflated balloon covered by a cotton or silk net is swallowed into the stomach, inflated, and then withdrawn, collecting exfoliated cells and scraping the surface of the esophageal mucosa. At the upper esophageal sphincter, the balloon is deflated and removed, and the attached cells are smeared directly onto glass slides. The slides are then fixed in ethyl alcohol, stained with Papanicolaou's stain, and read for evidence of cellular abnormalities. EBC² is now commonly used in China for diagnosing patients with dysphagia and is also occasionally used for screening asymptomatic, high-risk populations.

In the years since the development of EBC, there has been little contact between Chinese and non-Chinese cytopathologists. There has been little chance to compare Chinese and Western esophageal cytological categories, to compare these cytological diagnoses with concurrent histological findings, or to estimate the predictive value of EBC results. During the past decade, Chinese-American collaboration in the conduct of two nutrition intervention trials in Linxian (9) has given us the opportunity to perform several such studies, leading to a better understanding of the meaning of each others' cytological criteria and the potential of EBC as an early detection method for esophageal cancer. The purpose of this report is to describe these preliminary studies.

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² The abbreviations used are: EBC, esophageal balloon cytology; CICAMS, the Cancer Institute of the Chinese Academy of Medical Sciences.

Materials and Methods

The Nutrition Intervention Trials in Linxian. The detailed methods and results of the Linxian trials have been reported previously (9–11). The Dysplasia Trial was a 2-arm, randomized, placebo-controlled intervention trial in which one-half of the participants received daily pills containing 26 vitamins and minerals and one-half received placebo pills. This trial enrolled 3318 men and women, 40–69 years of age, who had no previous history of cancer but had cytological evidence of esophageal dysplasia (a Chinese cytological diagnosis of dysplasia 1 or dysplasia 2, defined below) in a baseline EBC screening in 1983 (12). The Dysplasia Trial intervention began in May, 1985 and ended in May, 1991.

The General Population Trial was a randomized intervention trial with a fractional factorial design that allowed separate evaluations of four different combinations of nutrients. This trial enrolled 29,584 men and women, 40–69 years old, from the general population of Linxian. The General Population Trial intervention began in March, 1986 and ended in May, 1991.

In October through December, 1987, after 30 months of intervention, all living Dysplasia Trial participants were invited to have a repeat EBC procedure, and 2826 (88% of those invited) accepted. One month after these EBC exams, all individuals with a 1983 EBC diagnosis of dysplasia 2 and every fifth person with a 1983 EBC diagnosis of dysplasia 1 were also invited to endoscopy, and 833 (63% of those invited) accepted. 2.1% of those undergoing EBC and 3.0% of those undergoing endoscopy reported difficulty swallowing at the time of these exams. There were no cytological or endoscopic screenings before the end of intervention in the General Population Trial.

In March through May, 1991, at the end of the two trials, additional screening exams were performed in both trials. All living Dysplasia Trial participants were invited to have a repeat EBC procedure, and 1943 (70%) accepted. At the same time, all General Population Trial participants in selected villages were invited to undergo EBC, and 3033 (69%) accepted. One month after the EBC exams, all Dysplasia Trial and General Population Trial participants in selected villages were invited to undergo endoscopy, and 787 (80%) accepted. 0.9% of those undergoing EBC and 0.9% of those undergoing endoscopy reported difficulty swallowing at the time of these exams.

In both 1987 and 1991, the EBC exams were performed by the usual methods [Shu (6), summarized above]. Four direct smears were made from each balloon sample. The cytological diagnoses (a squamous cell diagnosis, a columnar cell diagnosis, and a worst overall case diagnosis) were made by teams of Chinese cytopathologists and cytotechnologists, the "Linxian cytology teams," under the supervision of Dr. Qiong Shen of Henan Medical University (in 1987) and Dr. Shu-Fan Liu of the CICAMS (in 1987 and 1991). The cytological categories and criteria that were used are given in the "Appendix." In both 1987 and 1991, the endoscopies were performed by Dr. Guo-Qing Wang of CICAMS. In both endoscopic surveys, biopsies were taken from all visible mucosal abnormalities in the esophagus and stomach and at standard sites in the mid-esophagus. In 1991, additional standard sites in the cardia and gastric angulus were also sampled. The biopsy diagnoses were consensus diagnoses made by three of the authors (S. M. D., K. J. L., and F-S. L.). The histological categories and criteria that were used are given in the "Appendix."

Comparison of Chinese and U. S. Cytological Diagnoses of EBC Samples. In 1988, one of the authors (Q. S.) picked 515 cases from the 1987 Dysplasia Trial EBC screening for this study. He personally reviewed all of these cases, recorded his

diagnoses, and sent one representative slide/case to a U. S. cytology team (S. M. D., R. K. N., and S. A. E.). The U. S. team recorded their diagnoses and then met with a Chinese cytology team (Q. S., J. C.) to review cytological criteria and findings. The cytological categories and criteria used by both teams are given in the "Appendix." The available slides (one/case) from cases with discordant diagnoses were jointly reviewed, to try to better understand differences in the criteria used, but the original diagnoses were not modified for the comparison analysis. Comparison of overall case diagnoses was performed on the 511 cases that both cytology teams considered satisfactory for evaluation.

Comparisons of EBC Diagnoses and Concurrent Histological Findings. The paired EBC and endoscopic examinations in 1987 and 1991 allowed comparisons of the cytological and histological findings in these surveys. In each analysis, the primary comparison was between the worst cytological diagnosis ("test") and the worst biopsy diagnosis ("truth") in each case. Because the esophageal balloon samples only the esophagus and gastric cardia, only the biopsies from these sites were included in the analyses.

In the 1987 study, three sets of cytological diagnoses, the original diagnoses of the 1987 Linxian cytology team ("total cohort"), the Chinese cytology team's diagnoses of the 515 cases selected for joint review ("selected cases—Chinese diagnoses"), and the U. S. cytology team's diagnoses of the same 515 cases ("selected cases—U. S. diagnoses"), were separately compared with the patients' worst biopsy diagnoses. In the 1991 study, the cytological diagnoses of the 1991 Linxian cytology team were compared with the patients' worst biopsy diagnoses. In all analyses, all cases were included that had satisfactory diagnoses by both cytological and histological examination methods.

Follow-Up Studies of EBC Screenings. We have now conducted prospective follow-up studies in three cohorts that were initially examined by EBC in 1974, 1983, and 1987. The methods of the 1974 follow-up study have been described previously (13). Briefly, 12,693 Linxian adults over 30 years of age were screened by EBC in 1974, using standard collection procedures and Chinese cytological diagnoses. Follow-up for vital status and cancer experience was performed by interviews and medical record abstracts in 1989, 15 years after the initial screening.

The methods of the 1983 follow-up study have also been reported previously (14). In this study, 10,066 Linxian adults 40–69 years of age were screened by EBC in 1983, using standard collection techniques and Chinese cytological diagnoses, as the baseline cytology screening for the Linxian Nutrition Intervention Trials (12). During the next 7.5 years, through the end of active intervention in May, 1991, these individuals were followed prospectively, with monthly visits by village doctors, cytological and/or endoscopic examinations of symptomatic subjects, and additional cytological and endoscopic screening examinations of selected subjects in 1987 and 1991 (described above). Case records and diagnostic materials (cytology slides, histology slides, and X-rays) were reviewed, and the cancer diagnoses were confirmed by an International End Points Review Committee of expert cytopathologists, pathologists, and radiologists from the U. S. and China.

The methods of the 1987 cytology exams are described above. During the next 3.5 years, through May, 1991, these individuals were followed prospectively by the same methods used in the 1983 follow-up study. In all of the follow-up studies, all individuals who were free of cancer at the initial

Table 1 Cytology results from the 1987 and 1991 EBC surveys in Linxian, China

	1987 Survey, Total cohort ^a						
	Normal	Hyperplasia	Dysplasia 1	Dysplasia 2	Near cancer	Cancer	Total
Chinese diagnoses	181 (6.8)	671 (25.0)	1005 (37.5)	467 (17.4)	242 (9.0)	113 (4.3)	2679 (100.0)
	1987 Survey, Selected cases ^a						
	Normal	Hyperplasia	Dysplasia 1	Dysplasia 2	Near cancer	Cancer	Total
Chinese diagnoses	11 (2.1)	54 (10.5)	177 (34.4)	92 (17.9)	118 (22.9)	63 (12.2)	515 (100.0)
	Normal	Reactive	Mild dysplasia	Moderate dysplasia	Severe dysplasia	Cancer	Total
	U. S. diagnoses	28 (5.5)	265 (51.9)	88 (17.2)	86 (16.8)	19 (3.7)	25 (4.9)
	1991 Survey, Total cohort ^a						
	Normal	Hyperplasia	Dysplasia 1	Dysplasia 2	Near cancer	Cancer	Total
Chinese diagnoses	1354 (27.5)	1621 (32.9)	1206 (24.5)	462 (9.4)	176 (3.6)	104 (2.1)	4932 (100.0)

^a Number of subjects (row percentage) by overall cytological diagnosis.

Table 2 Comparison of Chinese and U. S. cytological diagnoses in 511 EBC specimens from the 1987 EBC survey in Linxian, China

		Chinese diagnoses					Chinese diagnoses		
		CA ^a	No CA				DYS ⁺	No DYS	
U. S. diagnoses	CA	17	8	25	U.S. diagnoses	DYS ⁺	211	7	218
	No CA	45	441	486		No DYS	237	56	293
		62	449	511			448	63	511
		Concordance = 90%				Concordance = 52%			
		Kappa = 34%				Kappa = 14%			

^a CA, cancer; DYS, dysplasia; DYS⁺, dysplasia or worse diagnosis, including the categories of dysplasia, near cancer, and cancer.

screening and who had follow-up data available were included in the analysis.

Analysis. The current analyses were performed using the worst overall cytological and histological diagnoses in each case, which were derived by combining diagnoses from the esophagus and gastric cardia. This approach was taken because a previous study (14) and our personal experience suggest that squamous and columnar cell types are often difficult to distinguish in cytological smears of high-grade dysplasias and poorly differentiated cancers. In addition, the Linxian tradition of grouping esophageal and gastric cardia tumors as "esophageal cancer" makes these lesions impossible to separate in interview responses and medical record abstracts such as those used in the 1974 follow-up study. It should be recognized, however, that the great majority of the data used in these analyses came from observations in the esophagus. In most of the EBC smears, over 90% of the cells were squamous cells from the esophagus, and in the endoscopic surveys, most (61%) of the biopsies came from the esophagus. Thus, the analyses and results presented are primarily squamous, esophageal results.

The cytological and cytological-histological comparisons were performed at two diagnostic levels: cancer *versus* no cancer; and dysplasia or worse *versus* no dysplasia. In the latter analyses, "dysplasia or worse" included all diagnoses of dysplasia (all grades), near cancer, and cancer.

Concordance and kappa statistics (15) were calculated for the comparison of Chinese and U. S. cytological diagnoses. Sensitivity, specificity, and positive and negative predictive values were estimated for the comparisons of cytological and

histological diagnoses. The relative risks presented in the follow-up studies were calculated using proportional hazards models (16).

Results

Comparison of Chinese and U. S. Cytological Diagnoses of EBC Samples. The three sets of cytological diagnoses from the 1987 Dysplasia Trial EBC screening are given in Table 1, and comparisons of the Chinese and U. S. diagnoses of the 511 cases with satisfactory smears that were selected for joint review are shown in Table 2. The most common Chinese diagnoses were dysplasia and near cancer, whereas the most common U. S. diagnosis was reactive. Agreement was poor for both the cancer diagnoses (concordance, 90%; kappa, 34%) and the dysplasia or worse diagnoses (concordance, 53%; kappa, 14%). The most evident difference in the Chinese and U. S. diagnostic categorizations was that the Chinese categories of precancerous neoplasia (dysplasia 1 + dysplasia 2 + near cancer) were more inclusive than the corresponding U. S. categories (mild + moderate + severe dysplasia). Three hundred eighty-seven (75%) of the cases were diagnosed as dysplasia or near cancer by the Chinese team, whereas only 193 (38%) were diagnosed as dysplasia by the U. S. team. Only 152 cases were considered precancerous neoplasia by both teams (Fig. 1). During the joint review, many of the cells diagnosed as dysplasia by the Chinese team were thought to be reactive by the Americans. The Chinese diagnoses of dysplasia and near cancer were based primarily on the presence and size of nuclear

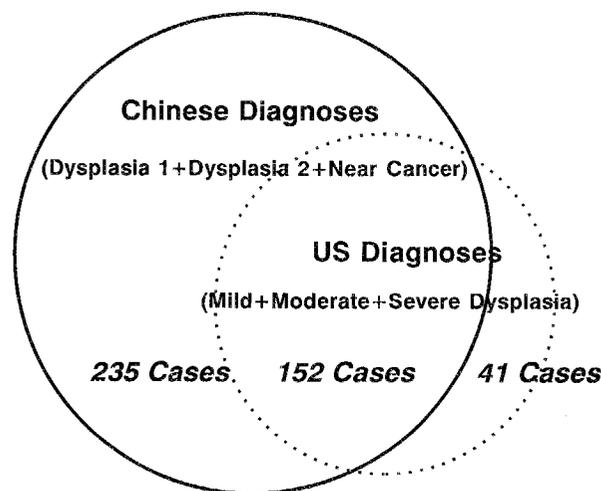


Fig. 1. Cases categorized as precancerous neoplasia by Chinese and U. S. readers of 515 esophageal balloon cytology smears from the 1987 EBC survey in Linxian, China.

enlargement, whereas the American diagnosis of dysplasia required definite abnormalities of nuclear chromatin and nuclear:cytoplasmic ratio as well.

Comparisons of EBC Diagnoses and Concurrent Histological Findings. The cytological and endoscopic biopsy results in the 1987 and 1991 studies are given in Tables 1 and 3. In Table 3, the higher prevalence of neoplastic (dysplasia or cancer) diagnoses in 1987 reflects the greater proportion of Dysplasia Trial participants in this study. The higher prevalence of inflammatory (esophagitis or gastritis) diagnoses in 1991 reflects the addition of standard biopsies from the gastric cardia in this survey. The comparisons of cytological and histological results from these studies are shown in Table 4. The sensitivity of EBC for detecting biopsy-proven esophageal or gastric cardia cancer was low in both studies, and the (combined) specificity of the Chinese cytological diagnoses of dysplasia 1, dysplasia 2, near cancer, and cancer for identifying biopsy-proven dysplasia or cancer was low in 1987. Comparisons of site-specific diagnoses (squamous cytological diagnoses *versus* worst esophageal biopsy diagnoses and columnar cytological diagnoses *versus* worst gastric biopsy diagnoses) showed similar sensitivities and specificities (data not shown).

Follow-Up Studies of EBC Screenings. The results of the 1974 and 1983 follow-up studies are shown in Table 5. Both studies showed an orderly progression of cumulative incidence and age- and gender-adjusted relative risk that paralleled the severity of the Chinese cytological diagnoses.

The results of the 1987 follow-up study are shown in Table 6, including the follow-up of all individuals screened (categorized by the original diagnoses of the Linxian cytology team) and the follow-up of the 515 selected cases (separately categorized by the Chinese and U. S. cytology teams). As in the earlier follow-up studies, there was a progression of cumulative incidence and relative risk with increasing severity of cytological categories. In the 515 selected cases, this trend was more pronounced when the cases were categorized by the U. S. cytological diagnoses.

Discussion

The single most important prognostic factor in esophageal cancer is the extent of disease at the time of diagnosis. Stage I tumors, confined to the mucosa or submucosa, without lymph node metastases, have a 90% 5-year survival after resection, but tumors with deeper invasion or nodal metastases have a much worse prognosis (17, 18). In most populations, however, early-stage esophageal cancers are rarely diagnosed (19, 20). The esophagus is a distensible organ, and most patients do not develop difficulty swallowing until a tumor is bulging into the lumen or is circumferentially constricting it. By then, the tumor has usually grown deep into the wall and has metastasized to lymph nodes. Significant reduction in esophageal cancer mortality will probably require successful strategies of primary prevention and/or early detection of precursor and early invasive lesions in asymptomatic people.

To date, efforts at early detection of esophageal cancer have concentrated on cytological or endoscopic screening of high-risk populations. Esophageal cytological screening studies have been reported from China (5–8), Iran (21), Italy (22, 23), Japan (24), South Africa (25–27), Thailand (28), and the United States (29–32). Recently, studies of primary endoscopic screening have been reported from France (33) and Japan (34). By far the greatest experience with cytological screening techniques has been accumulated in China, where investigators from Henan Medical University and CICAMS have worked for many years in Linxian and surrounding counties to develop practical and effective methods to screen large numbers of asymptomatic, high-risk individuals.

During and since the development of EBC in Linxian, there has been little interaction between Chinese and non-Chinese cytopathologists, and different experiences have led to different systems of esophageal cytological categorization. In Linxian during this period, nearly all cytological evaluations were made on EBC specimens, and diagnostic categories and criteria were developed *de novo* from observations in these esophageal samples. Because endoscopy and endoscopic biopsies were rarely available, there was little possibility to correlate cytological findings with same-site biopsies.

The development of Chinese esophageal cytological categories is summarized in Fig. 2. At first, cases were diagnosed only as "cancer" (ái) or "no cancer" (fēi ái). Then an intermediate category (zēng shēng) was introduced for cases that lacked typical malignant cells but contained cells with some abnormal nuclear features. Unfortunately, the Chinese term zēng shēng was translated several ways in English, including "atypia," "dyskaryosis," "hyperplasia," and "dysplasia," causing confusion among those trying to understand Chinese results in translation. Next, zēng shēng was divided into two grades, "light" zēng shēng (qīng dù zēng shēng) and "heavy" zēng shēng (zhòng dù zēng shēng), based primarily on the presence of abnormal cells with nuclei less than or greater than three times the size of normal nuclei. These two grades were translated as "mild" and "severe" hyperplasia, "hyperplasia" and "dysplasia", or (most commonly) "mild hyperplasia" and "severe dysplasia". Later, heavy zēng shēng was divided again, into "heavy zēng shēng, grade 1" (zhòng dù zēng shēng yī jí) and "heavy zēng shēng, grade 2" (zhòng dù zēng shēng èr jí), translated as "severe dysplasia group 1" and "severe dysplasia group 2." Finally, additional precancerous categories, such as "suspicious for cancer" (kě yí ái) or "near cancer" (jìn ái), were added between the three grades of zēng shēng and cancer.

In contrast to the three grades of zēng shēng, cytological evaluation of esophageal specimens has been uncommon in the

Table 3 Biopsy results from the 1987 and 1991 endoscopic surveys in Linxian, China

	Worst biopsy diagnosis ^a					Total
	Normal	Inflammation	Low-grade dysplasia	High-grade dysplasia	Cancer	
1987 Survey	470 (61.2)	65 (8.5)	81 (10.5)	96 (12.5)	56 (7.3)	768 (100.0)
1991 Survey	204 (26.0)	429 (54.6)	47 (6.0)	33 (4.2)	73 (9.3)	786 (100.0)

^a Number of subjects (row percentage) by worst biopsy diagnosis.

Table 4 Comparisons of EBC results ("test") and endoscopic biopsy diagnoses ("truth") from paired cytological and endoscopic surveys in Linxian, China

	Cancer/No Cancer			
	1987 Total cohort (731) [56] ^a	1987 Selected cases, Chinese diagnoses (462) [36]	1987 Selected cases, U. S. diagnoses (459) [36]	1991 Total cohort (772) [71]
Sensitivity	32%	36%	31%	14%
Specificity	92%	90%	97%	99%
Positive predictive value	25%	23%	48%	77%
Negative predictive value	94%	94%	94%	92%

	Dysplasia + ^b /No Dysplasia			
	1987 Total cohort (731) [149]	1987 Selected cases, Chinese diagnoses (462) [106]	1987 Selected cases, U. S. diagnoses (459) [105]	1991 Total cohort (772) [113]
Sensitivity	87%	92%	73%	56%
Specificity	21%	14%	64%	69%
Positive predictive value	22%	24%	37%	24%
Negative predictive value	86%	86%	89%	90%

^a (), number of patients in the cohort with satisfactory cytology and biopsy results; [], number of patients in the cohort with biopsy-proven cancer or dysplasia +.

^b Dysplasia +, dysplasia or worse diagnosis, including the categories of dysplasia, near cancer, and cancer.

low-risk populations of the West; therefore, the diagnostic categories and criteria used in esophageal cytology have largely been carried over from more widespread experience with cytological evaluation of the uterine cervix. The development of these cervical criteria, however, has been firmly grounded in extensive, careful correlation studies of same-site cytological and histological findings. Thus, both Chinese and Western cytopathologists have valuable experience to contribute to developing optimal criteria for diagnosing esophageal disease; Chinese cytopathologists have abundant experience studying esophageal samples, and their Western counterparts have extensive experience with cytological-histological correlation in another squamous organ. The desire to learn from each other and profit from each other's experience was the stimulus for the present studies.

Except for the 1974 and 1983 follow-up studies, all of the current studies are preliminary observations. Only these two initial follow-up studies were designed and carried out with formal protocols. The rest were observations that became available in the course of the Linxian Trials. Clearly, the comparison of Chinese and U. S. cytological diagnoses, the comparison of U. S. cytological and histological diagnoses, and the follow-up study of the U. S. cytology readings would have been more accurate if the U. S. team had been able to review all four EBC

slides in each case, and all of the comparisons of cytological and histological diagnoses would have benefited from a formal protocol in which all participants received both cytological and endoscopic examinations. Still, the current observations are worthwhile as a beginning and yield several potentially important insights.

The current comparison of cytological diagnoses is the first report directly comparing independent Chinese and Western cytological categorizations of the same esophageal samples. It is clear from this study that the Chinese precancerous categories of dysplasia and near cancer (zhōng dù zēng shēng and jìn ái) are considerably more inclusive than the corresponding U. S. diagnosis of dysplasia. This may be because of inclusion of reactive cases in the Chinese category and/or exclusion of early neoplastic lesions in the U. S. diagnoses.

In the comparisons of cytological and histological diagnoses, both the Chinese and U. S. diagnoses showed low sensitivity for identifying biopsy-proven invasive cancer, and the 1987 Chinese diagnoses showed low specificity for biopsy-proven dysplasia or cancer. Possible reasons for the low sensitivity (many false negatives) for detecting invasive cancer include inadequate sampler design, suboptimal sampling technique, incomplete processing of the cell sample, and undercalling malignant cells. All of these components may have contributed to the current results. The difficulty of completely sampling a large, nonvisualized organ like the esophagus, in contrast to sampling a small, visualized organ like the cervix, should not be underestimated.

The low specificity (many false positives) for histological dysplasia or cancer seen with the 1987 Chinese EBC diagnoses may reflect inclusion of reactive lesions in the cytological diagnosis of dysplasia and/or incomplete detection of histological dysplasia by the endoscopic biopsies. Incomplete detection of histological squamous dysplasia was probably not a major factor in the 1987 correlation results, however, because previous studies have shown that most histological squamous dysplasia in Linxian patients is associated with the endoscopically visible lesions that were targeted for biopsy in the 1987 endoscopic survey (35), and follow-up of these patients categorized by their worst biopsy diagnosis (36) showed that few patients who did not have squamous dysplasia in their 1987 biopsies developed invasive esophageal cancer within the next 3.5 years (Table 7). The specificity of the Chinese EBC diagnoses for histological dysplasia or cancer was considerably higher in 1991 than in 1987, implying a different application of the cytological criteria in the 1991 screening.

Nearly all previous Chinese studies of the accuracy of EBC have been evaluations of the sensitivity of this technique for detecting "esophageal cancer" (including *in situ* and invasive cancers of the esophagus and gastric cardia) in symptomatic patients. Seven such studies have reported sensitivities of 88%-99% (6, 8). Detailed methods of these studies, such as the

Table 5 Results of previously reported follow-up studies of EBC screenings

A. Esophageal and gastric cardia cancer incidence during 1974–1989, by worst 1974 cytology diagnosis ^a							
	1974 Cytology category						Total
	Normal	Esophagitis	Hyperplasia	Dysplasia 1	Dysplasia 2	Suspicious for cancer	
No. of subjects	6070	318	5411	338	496	60	12693
Cumulative incidence ^b	449 (7.4%)	37 (11.6%)	530 (9.8%)	43 (12.7%)	79 (15.9%)	24 (40.0%)	1162 (9.2%)
Age and sex-adjusted RR ^c	1.00	1.56 (1.12–2.18)	1.20 (1.05–1.36)	1.62 (1.18–2.21)	1.82 (1.43–2.32)	5.84 (3.87–8.80)	<i>P</i> for trend <0.001

B. Esophageal and gastric cardia cancer incidence during 1983–1991, by worst 1983 cytology diagnosis ^a							
	1983 Cytology category					Total	
	Normal	Hyperplasia	Dysplasia 1	Dysplasia 2	Near cancer		
No. of subjects	2949	3645	2634	800	38	10066	
Cumulative incidence ^b	117 (4.0%)	187 (5.1%)	279 (10.6%)	154 (19.3%)	10 (26.3%)	747 (7.4%)	
Age and sex-adjusted RR ^c	1.00	1.23 (0.98–1.55)	2.55 (2.05–3.17)	5.00 (3.92–6.37)	5.79 (3.03–11.07)	<i>P</i> for trend <0.001	

^a Modified from Dawsey *et al.* (13) for worst 1974 cytology diagnosis and from Liu *et al.* (14) for worst 1983 cytology diagnosis.

^b Number (percentage) of subjects in each cytology category who developed esophageal or gastric cardia cancer during the follow-up period.

^c Relative risk (95% confidence interval) of esophageal or gastric cardia cancer incidence during the follow-up period, adjusted for age and gender.

Table 6 Esophageal and gastric cardia cancer incidence during 1987–1991, by worst 1987 cytology diagnoses

A. Total cohort, Chinese diagnoses							
	1987 Cytology category					Total	
	Normal	Hyperplasia	Dysplasia 1	Dysplasia 2	Near cancer		
No. of subjects	181	671	1005	467	242	2566	
Cumulative incidence ^a	8 (4.4%)	41 (6.1%)	59 (5.9%)	37 (7.9%)	54 (22.3%)	199 (7.7%)	
Age and sex-adjusted RR ^b	1.00	1.41 (0.66–3.01)	1.38 (0.66–2.89)	1.87 (0.87–4.01)	5.86 (2.79–12.32)	<i>P</i> for trend <0.001	

B. Selected cases, Chinese diagnoses							
	1987 Cytology category					Total	
	Normal	Hyperplasia	Dysplasia 1	Dysplasia 2	Near cancer		
No. of subjects	11	54	177	92	118	452	
Cumulative incidence ^a	1 (9.1%)	4 (7.4%)	16 (9.0%)	15 (16.3%)	32 (27.1%)	68 (15.0%)	
Age and sex-adjusted RR ^b	1.00	0.68 (0.08–6.10)	0.85 (0.11–6.40)	1.69 (0.22–12.80)	2.82 (0.39–20.69)	<i>P</i> for trend <0.001	

C. Selected cases, U. S. diagnoses							
	1987 Cytology category					Total	
	Normal	Reactive	Mild dysplasia	Moderate dysplasia	Severe dysplasia		
No. of subjects	28	265	88	86	19	486	
Cumulative incidence ^a	1 (3.6%)	31 (11.7%)	22 (25.0%)	22 (25.6%)	12 (63.2%)	88 (18.1%)	
Age and sex-adjusted RR ^b	1.00	2.81 (0.38–20.62)	6.83 (0.92–50.78)	7.00 (0.94–52.06)	24.63 (3.18–190.60)	<i>P</i> for trend <0.001	

^a Number (percentage) of subjects in each cytology category who developed esophageal or gastric cardia cancer during the follow-up period.

^b Relative risk (95% confidence interval) of esophageal or gastric cardia cancer incidence during the follow-up period, adjusted for age and gender.

number of EBC examinations performed on each patient or the methods used to confirm the diagnosis of 52 cancer, were not included in these reports. In one study of 52 patients, however, it was noted that the sensitivity of EBC increased from 65% after a single examination to 90% after four examinations (8).

Only one previous report from China has tried to estimate the sensitivity of EBC for detecting esophageal cancer in a

population screening of asymptomatic people (8). In this screening, 5800 people were examined, and 121 cancers were found. The sensitivity of EBC was calculated by dividing the number of cancers found in the screening by that number plus the cancers found in the same population during the next 2 years. By this method, the sensitivity was estimated to be 93% (121 of 130). No previous studies from China have evaluated

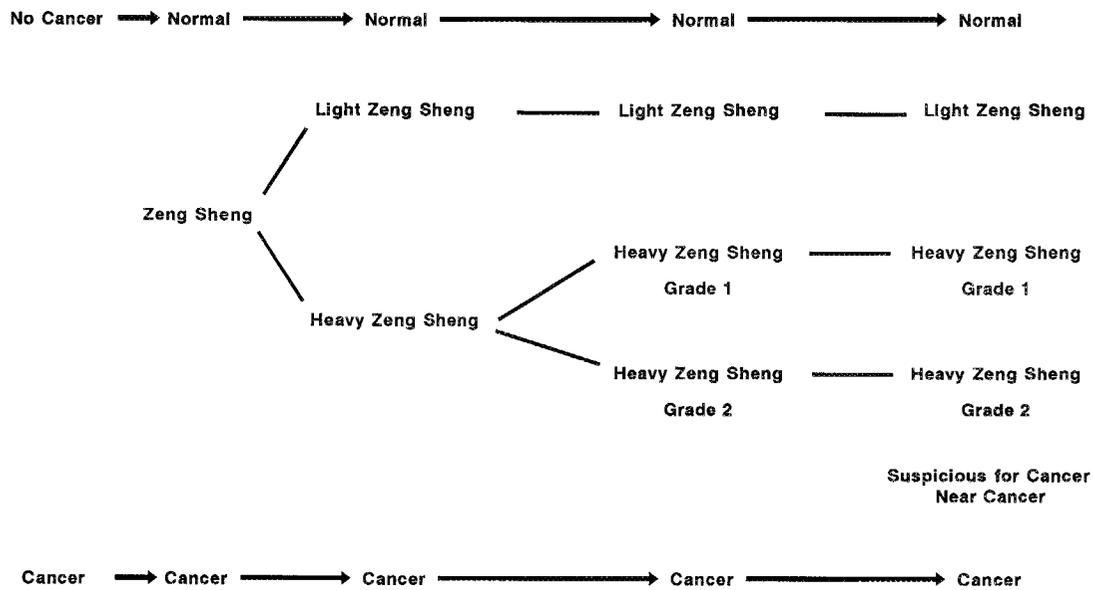


Fig. 2. The development of Chinese cytological categories.

Table 7 Esophageal cancer incidence during 1987–1991, by worst 1987 esophageal biopsy diagnosis^a

	Worst 1987 esophageal biopsy diagnosis									Total
	Normal	BCH ^b	Acanthosis	Esophagitis	mD	MD	SD	NOS	CIS	
No. of subjects	375	40	77	33	76	30	23	12	16	682
Cumulative incidence ^c	8 (2.1%)	2 (5.0%)	0 (0.0%)	0 (0.0%)	4 (5.3%)	8 (26.7%)	15 (65.2%)	4 (33.3%)	11 (68.8%)	52 (7.6%)
Age and sex-adjusted RR ^d	1.0	2.3 (0.5–10.8)			2.5 (0.7–8.2)	14.3 (5.3–38.2)	55.6 (23.4–132.1)	19.9 (5.9–67.2)	53.5 (21.0–136.6)	<i>P</i> for trend <0.001

^a Modified from Dawsey *et al.* (36).^b BCH, basal cell hyperplasia; mD, mild dysplasia; MD, moderate dysplasia; SD, severe dysplasia; NOS, dysplasia not otherwise specified; CIS, carcinoma *in situ*.^c Number (percentage) of subjects with each 1987 biopsy result who developed esophageal cancer during 1987–1991.^d Relative risk (95% confidence interval) of esophageal cancer incidence during 1987–1991, adjusted for age and gender.

the sensitivity, specificity, or predictive values of a single EBC examination for identifying concurrent, biopsy-proven dysplasia or cancer in asymptomatic individuals.

A few studies from other countries have evaluated the accuracy of EBC by comparing concurrent cytological and histological diagnoses. Berry *et al.* (25) found cancer cells in 48 (96%) of 50 patients known to have squamous esophageal cancer. They also screened 500 asymptomatic patients and made cytological diagnoses of cancer in 15, dysplasia in 26, and atypia requiring biopsy in 34. Subsequent unblinded endoscopy of these 75 patients confirmed all of the cancer and dysplasia diagnoses (although some cases required up to 40 biopsies for confirmation) and found esophagitis without dysplasia in all of the cases of cytological atypia. The other 425 screened patients were not endoscoped; therefore, the accuracy of these negative cytology diagnoses could not be determined.

Greenebaum *et al.* (29) screened 11 patients with known obstructive esophageal cancers, 74 patients with treated or untreated head and neck cancers, and 11 other patients thought to be at increased risk for esophageal cancer. Seven (64%) of the 11 patients with known cancer had suspicious or positive cells on their EBC smears, and the EBC examinations identified 7 additional cancers in the other 85 patients, all of which were later confirmed. The postscreening evaluation of the other 78

at-risk patients was not reported; therefore, the accuracy of their negative EBC examinations is unknown.

Tsang *et al.* (31) performed EBC on 76 patients with previous endoscopic biopsies showing cancer (11 patients), mild dysplasia (16 patients), esophagitis (23 patients), or normal mucosa (26 patients). Positive cytology (defined as an EBC diagnosis of severe dysplasia, suspicious for cancer, or cancer) was found in 10 of the 11 patients with biopsy-proven cancer and 4 of the 65 patients without cancer, yielding a sensitivity of 91%, a specificity of 94%, a positive predictive value of 71%, and a negative predictive value of 98% for the EBC procedure.

Chanvitan *et al.* (28) performed the evaluation most closely comparable to the current cytological-histological comparison studies. They screened 53 symptomatic and 73 asymptomatic patients by EBC and then, 1 week later, endoscoped all of them. Taking the endoscopic biopsy diagnoses as truth, they found the following correlation results; in the symptomatic patients, EBC had a sensitivity of 69% (20 of 29), a specificity of 100% (24 of 24), a positive predictive value of 100% (20 of 20), and a negative predictive value of 42% (14 of 33) for identifying biopsy-proven cancer. In the asymptomatic patients, only three of whom had cancer, the sensitivity, specificity, and both predictive values were all 100%.

All of the current follow-up studies showed definite pre-

dictive value for both Chinese and U. S. cytological diagnoses. Several aspects of these studies, however, are worthy of note. In both the 1974 and 1983 studies (Table 5), the cumulative incidence of invasive cancer associated with the Chinese diagnoses of dysplasia, suspicious for cancer, and near cancer after long-term follow-up was rather low. In the 1974 study, 60% of those with a diagnosis of suspicious for cancer and 85% of those with a diagnosis of dysplasia did not develop clinical cancer within the next 15 years. In the 1983 study, 74% of those with near cancer and 87% of those with dysplasia did not develop cancer within 7.5 years. These results suggest that these cytological diagnoses included significant numbers of reactive lesions and/or they included neoplastic lesions that regressed, remained stable, or progressed very slowly over many years.

In the 1987 follow-up studies, the cumulative incidences and relative risks associated with the Chinese cytological diagnoses of dysplasia and near cancer (Table 6, A and B) were considerably lower than those associated with the histological diagnoses of dysplasia (especially moderate and severe dysplasia) in the similar follow-up study of a subset of the same patients categorized by their endoscopic biopsy diagnoses (Table 7). It is possible that in some of these cases, the cytological diagnoses were identifying neoplastic lesions that would take longer than 3.5 years to develop into cancer, similar to the histological lesions diagnosed as mild dysplasia.

On the other hand, in the same follow-up studies, the cumulative incidences and relative risks associated with some of the U. S. cytological diagnoses (Table 6C) were higher than the figures for similar biopsy diagnoses (Table 7), implying that the U. S. cervix-based criteria may well have been undercalling lesions that were clinically significant in the esophagus. It seems quite unlikely that 25% of lesions that were in fact only mildly dysplastic would progress to invasive cancer within 3.5 years. This was certainly not the case among the 1987 patients with biopsy diagnoses of mild dysplasia. Perhaps the squamous epithelium of the esophagus does not respond to carcinogenic stimuli in quite the same way as the squamous epithelium of the cervix, and the cytological morphology associated with mild dysplasia in the cervix is more indicative of moderate dysplasia in the esophagus (at least in this high-risk population). If so, additional cytological criteria may have to be developed to identify the earlier neoplastic lesions.

Thus, optimal esophageal cytological criteria may lie somewhere between the Chinese and U. S. criteria used in these studies. The Chinese may need to add a reactive category to include cases with enlarged nuclei but minimal or no chromatin abnormalities, and the U. S. criteria may need to be liberalized to include more "borderline" lesions as dysplasia. The development of more accurate criteria will probably require multiple, well-designed, cytological-histological correlation studies of esophageal lesions. Such studies should become more possible as endoscopy becomes increasingly available in high-risk populations.

The current preliminary studies suggest several areas in which modifications may improve the accuracy and usefulness of EBC, including sampler design, sampling technique, processing of the cell samples, and cytological criteria. The imperfect conditions of several of the current studies also suggest the need for more controlled investigations to evaluate current (baseline) and improved techniques.

There remains a great need for successful strategies for the early detection of esophageal cancer. Chinese balloon cytology has shown that it can detect asymptomatic, curable, early esophageal cancers and that it can identify individuals without

current cancer who are at increased risk for developing esophageal cancer in the future. Our preliminary studies suggest that esophageal balloon cytology is a promising early detection technique that can benefit from additional research to improve its optimal performance.

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Appendix

I. Cytological categories and criteria

A. Chinese categories

The Chinese cytological categories and criteria used in 1983 (the 1983 follow-up study), 1987 (the 1987 survey, total cohort), 1988 (the 1987 survey, selected cases), and 1991 (the 1991 survey, total cohort) were as follows (12):

Normal (zhèng cháng). Most cells are normal intermediate cells, with 10–15% normal superficial cells. Rare parabasal cells may be present.

Hyperplasia (qīng dù zēng shēng). The nuclei are mildly hyperchromatic and enlarged, being two or more but less than three times the size of nuclei in normal intermediate cells.

Dysplasia 1 (zhòng dù zēng shēng yī jí). The nuclei are hyperchromatic, with finely granular and evenly distributed chromatin. The nuclei are three or more but less than four times the size of the nuclei of normal intermediate cells. When hyperplasia cells are present in the smear, finding a single cell meeting the criteria for dysplasia 1 is sufficient for this diagnosis.

Dysplasia 2 (zhòng dù zēng shēng èr jí). The pattern is similar to dysplasia 1, except that the abnormal nuclei are four or more but less than five times the size of the nuclei of normal intermediate cells. When hyperplasia or dysplasia 1 cells are present in the smear, finding a single dysplasia 2 cell is sufficient for this diagnosis.

Near Cancer (jìn ái). The pattern is similar to dysplasia 2, except that the abnormal nuclei are five or more times the size of the nuclei of normal intermediate cells. Finding a single near cancer cell is sufficient for this diagnosis. Typical cancer cells are absent.

Cancer (ái; lín ái; xiàn ái). Typical cancer cells are present. Typical cancer cells have coarse chromatin granules that vary in size and are irregularly distributed. They may have irregularly thickened and irregularly contoured nuclear membranes. The nuclear:cytoplasmic ratio is increased. Nucleoli may be present (nucleoli are only occasionally seen in squamous cancer; they are always seen in adenocarcinoma).

The same Chinese categories and criteria were also used in 1974 (the 1974 follow-up study), except that there was an additional category of esophagitis (*shí guǎn yán*), and suspicious for cancer (*kě yí ái*) was used instead of near cancer (*jìn ái*). These additional categories were defined as follows (13):

Esophagitis (shí guǎn yán). Normal cells are found in association with a significant number of inflammatory cells. Inflammatory cells infiltrating sheets of epithelial cells may be seen.

Suspicious for cancer (kě yí ái). The cytopathologist is suspicious but not certain that cancer is present. This usually occurs when there are many dysplasia 2 cells or spindle-shaped squamous cells, without definite malignant cells, or when rare cells are found with some atypical nuclear features but more abundant cytoplasm than is usually seen in malignant cells.

In all surveys, the category names and the defining nuclear

criteria were the same for both squamous and columnar cells; the identification of cell type was based on cytoplasmic characteristics. The overall case diagnosis was the worst squamous or columnar diagnosis made.

B. U. S. categories

The U. S. cytological categories and criteria used in 1988 (the 1987 survey, selected cases) were as follows:

Squamous cell categories:

Normal. The majority of the cells are single intermediate cells. No nuclear abnormalities are seen.

Reactive. The cells have degenerative and/or regenerative features. In degenerating cells, the nuclei are of normal size or enlarged, without increased chromatin. The chromatin is evenly distributed or may be clumped along the nuclear membrane. The nuclear membranes are smooth, and nucleoli are not seen. A special type of degenerative change is parakeratosis, in which the cells have small, pyknotic nuclei and eosinophilic cytoplasm. Regenerating cells are usually found in flat, cohesive sheets. The nuclei are enlarged and may vary in size. The chromatin is evenly distributed, and the nuclear membranes are smooth. Nucleoli are prominent, and cytoplasm is abundant.

Dysplasia. The cells are usually single. The nuclei are enlarged and vary in size. There is increased chromatin, which is finely to coarsely granular and evenly distributed. The nuclear membranes are smooth or mildly irregular, and nucleoli are not seen. The nuclear:cytoplasmic ratio is increased. Increasing grades of dysplasia (mild, moderate, and severe) are characterized by increasing nuclear size, increasing amounts and coarseness of chromatin, and increasing nuclear:cytoplasmic ratio.

Cancer. The cells are usually single and may be quite pleomorphic. The nuclei are enlarged and vary in size and shape. There is increased chromatin, which is coarsely granular and irregularly distributed. The nuclear membranes are irregular in thickness and contour. Nucleoli are commonly seen. There is scant to abundant cytoplasm, which may be keratinized. The nuclear:cytoplasmic ratio is increased. There is often a background tumor diathesis of blood, fibrin, and necrotic debris.

Columnar cell categories:

Normal. The cells are in flat cohesive sheets and have regular round nuclei, small nucleoli, and distinct cell borders.

Reactive. The cells are usually in flat cohesive sheets but may also be seen as single cells or bare nuclei. The nuclei are enlarged and vary in size but retain their normal polarity within the sheet. Large nucleoli are common, but there is no increase in chromatin or nuclear membrane irregularity. With degeneration, the cells become discohesive and may lose their cytoplasm.

Dysplasia. The cells are seen in small fragments or as single cells. The nuclei are enlarged and vary in size. There is increased chromatin, which is evenly distributed, and an increased nuclear:cytoplasmic ratio. Nuclear membranes are smooth, and nucleoli are not prominent.

Cancer. The cells are present in dysplastic three-dimensional fragments or as single cells with intact cytoplasm. The nuclei are eccentric and enlarged and vary in size and shape. There is increased chromatin and parachromatin. There are sharp irregularities in nuclear membranes and prominent large nucleoli. There are variable amounts of cytoplasm, which may be vacuolated. The nuclear:cytoplasmic ratio is increased. A background tumor diathesis may be present.

The overall case diagnosis was the worst squamous or columnar diagnosis made.

II. Histological categories and criteria

The histological categories and criteria used in 1987 and 1991 were as follows (37, 38):

Esophageal categories:

Normal. A stratified squamous epithelium was present that showed no features diagnostic of acanthosis, esophagitis, squamous dysplasia, or squamous cancer, as defined below.

Acanthosis. An otherwise normal epithelium was ≥ 0.5 mm thick.

Esophagitis. One or more of the following three criteria were present: elongation of lamina propria papillae into the upper third of the epithelium together with basal cell hyperplasia, defined as a basal zone thickness $>15\%$ of total epithelial thickness; epithelial infiltration by neutrophils or eosinophils; or a dense non-follicular infiltrate of mononuclear inflammatory cells or neutrophils in the lamina propria.

Squamous dysplasia. Nuclear atypia (enlargement, pleomorphism, and hyperchromasia), loss of normal cell polarity, and abnormal tissue maturation were present in the lower third (mild), in the lower two-thirds (moderate), or in all thirds (severe) of the epithelium, without invasion. Biopsies containing dysplastic cells that could not be graded because of biopsy size or orientation were categorized as squamous dysplasia, not otherwise specified.

Squamous cancer. Neoplastic squamous cells were present, which had invaded through the basement membrane.

Gastric categories:

Normal. A gastric mucosa was present, which showed no features diagnostic of gastritis, gastric dysplasia or adenocarcinoma, as defined below. No inflammatory infiltrate was allowed in normal biopsies from the gastric fundus or body, but a mild lymphoplasmacytic infiltrate was permitted in normal biopsies from the cardia or antrum.

Gastritis without atrophy. Any inflammation other than a mild lymphoplasmacytic infiltrate in biopsies from the cardia or antrum was called gastritis. For the purposes of these studies, we did not separate superficial from full-thickness involvement or chronic from chronic active inflammation. No atrophy (loss of glands) or metaplasia was identified.

Atrophic gastritis. There was variable inflammation and loss of normal glands, with or without intestinal or pyloric metaplasia.

Gastric dysplasia. Neoplastic features, including nuclear atypia and/or architectural abnormalities, were present but confined to the gastric epithelium, without invasion. Dysplasia was categorized as low-grade or high-grade based on the severity of the neoplastic features (39).

Adenocarcinoma. Neoplastic gastric epithelial cells were present, which had invaded through the basement membrane. For each patient, a worst biopsy diagnosis was derived as follows:

Normal. A worst biopsy diagnosis of normal or acanthotic esophageal mucosa or normal gastric mucosa.

Inflammation. Esophagitis, gastritis without atrophy, or atrophic gastritis.

Low-grade dysplasia. Mild squamous dysplasia or low-grade gastric dysplasia.

High-grade dysplasia. Moderate or severe squamous dysplasia or high-grade gastric dysplasia.

Cancer. squamous cancer or adenocarcinoma.

References

1. Parkin, D. M., Pisani, P. and Ferlay, J. Estimates of the worldwide incidence of eighteen major cancers in 1985. *Int. J. Cancer*, 54: 594-606, 1993.

2. Ries, L. A. G., Miller, B. A., Hankey, B. F., Kosary, C. L., Harras, A., and Edwards, B. K. SEER cancer statistics review, 1973-1991, p. 175. Bethesda, MD: National Cancer Institute (NIH Pub. 94-2789), 1994.
3. Li, M. X., Li, B., and Li, B. R. Recent progress in research on esophageal cancer in China. *Adv. Cancer Res.*, 33: 173-249, 1980.
4. Zhang, Z. X., Li, B. Y., and Jin, S. S. Epidemiologic trends of esophageal cancer in Linxian. Research on Esophageal Cancer Prevention and Treatment (Shi Guan Ai Fang Zhi Yan Jiu—Linxian), 1: 1-14, 1990. (In Chinese).
5. Shen, Q., and Shu, Y. J. Cytology as a screening method for esophageal carcinoma in the People's Republic of China. In: C. J. Pfeiffer (ed.), *Cancer of the Esophagus*, Vol. II, pp. 3-15. Boca Raton, FL: CRC Press, 1982.
6. Shu, Y. J. Cytopathology of the esophagus: an overview of esophageal cytopathology in China. *Acta Cytol.*, 27: 7-16, 1983.
7. Shu, Y. J. *The Cytopathology of Esophageal Carcinoma*. New York: Masson, 1985.
8. Shen, Q. Diagnostic cytology and early detection. In: G. J. Huang and W. Y. Kai (eds.), *Carcinoma of the Esophagus and Gastric Cardia*, pp. 155-190. Berlin: Springer-Verlag, 1984.
9. Li, B., Taylor, P. R., Li, J. Y., Dawsey, S. M., Wang, W., Tangrea, J. A., Liu, B. Q., Ershow, A. G., Zheng, S. F., Fraumeni, J. F., Jr., Yang, Q. P., Yu, Y., Sun, Y. H., Zhang, D. H., Greenwald, P., Lian, G. T., Yang, C. S., and Blot, W. J. Linxian nutrition intervention trials: design, methods, participant characteristics, and compliance. *Ann. Epidemiol.*, 3: 577-585, 1993.
10. Blot, W. J., Li, J. Y., Taylor, P. R., Guo, W., Dawsey, S., Wang, G. Q., Yang, C. S., Zheng, S. F., Gail, M. H., Li, G. Y., Yu, Y., Liu, B. Q., Tangrea, J. A., Sun, Y. H., Liu, F. S., Fraumeni, J. F., Jr., Zhang, Y. H., and Li, B. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J. Natl. Cancer Inst.*, 85: 1483-1492, 1993.
11. Li, J. Y., Taylor, P. R., Li, B., Dawsey, S., Wang, G. Q., Ershow, A. G., Guo, W., Liu, S. F., Yang, C. S., Shen, Q., Wang, W., Mark, S. D., Zou, X. N., Greenwald, P., Wu, Y. P., and Blot, W. J. Nutrition intervention trials in Linxian, China: multiple vitamin/mineral supplementation, cancer incidence, and disease-specific mortality among adults with esophageal dysplasia. *J. Natl. Cancer Inst.*, 85: 1492-1498, 1993.
12. Shen, Q., Liu, S. F., Dawsey, S. M., Cao, J., Zhou, B., Wang, D. Y., Cao, S. G., Zhao, H. Z., Li, G. Y., Taylor, P. R., Guo, W. D., Liu, F. S., Blot, W. J., Li, J. Y., and Li, B. Cytologic screening for esophageal cancer: results from 12,877 subjects from a high-risk population in China. *Int. J. Cancer*, 54: 185-188, 1993.
13. Dawsey, S. M., Yu, Y., Taylor, P. R., Li, J. Y., Shen, Q., Shu, Y. J., Liu, S. F., Zhao, H. Z., Cao, S. G., Wang, G. Q., Liu, F. S., Blot, W. J., and Li, B. Esophageal cytology and subsequent risk of esophageal cancer: a prospective follow-up from Linxian. *China Acta Cytol.*, 38: 183-192, 1994.
14. Liu, S. F., Shen, Q., Dawsey, S. M., Wang, G. Q., Nieberg, R. K., Wang, Z. Y., Weiner, M., Zhou, B., Cao, J., Yu, Y., Guo, W. D., Li, J. Y., Blot, W. J., Li, B., and Taylor, P. R. Esophageal balloon cytology and subsequent risk of esophageal and gastric cardia cancer in a high-risk Chinese population. *Int. J. Cancer*, 57: 775-780, 1994.
15. Fleiss, J. L. *Statistical Methods for Rates and Proportions*, pp. 212-236. New York: John Wiley and Sons, 1981.
16. SAS Institute. *SAS User's Guide: Statistics*. Cary, NC: SAS Institute, Inc., 1985.
17. Wu, Y. K., Huang, G. J., Shao, L. F., Zhang, Y. D., and Lin, X. S. Progress in the study and surgical treatment of cancer of the esophagus in China, 1940-1980. *J. Thorac. Cardiovasc. Surg.*, 84: 325-333, 1982.
18. Yoshinaka, H., Shimazu, H., Fukumoto, T., and Baba, M. Superficial esophageal carcinoma: a clinicopathological review of 59 cases. *Am. J. Gastroenterol.*, 86: 1413-1418, 1991.
19. Earlam, R., and Cunha-Melo, J. R. Oesophageal squamous cell carcinoma. I. A critical review of surgery. *Br. J. Surg.*, 67: 381-390, 1980.
20. Katlic, M. R., Wilkins, E. W., and Grillo, H. C. Three decades of treatment of esophageal squamous carcinoma at the Massachusetts General Hospital. *J. Thorac. Cardiovasc. Surg.*, 99: 929-938, 1990.
21. Dowlatshahi, K., Daneshbod, A., and Mobarhan, S. Early detection of cancer of oesophagus along Caspian littoral: report of a pilot project. *Lancet*, 1: 125-126, 1978.
22. Aste, H., Saccomanno, S., and Munizzi, F. Blind pan-esophageal brush cytology: diagnostic accuracy. *Endoscopy*, 16: 165-167, 1984.
23. Costantini, M., Tremolada, C., Tiozzi, M., Nosadini, A., Zaninotto, G., Norberto, L., and Peracchia, A. La cytologie exfoliative oesophagienne a l'aveugle par capsule abrasive dans la surveillance des patients a haut risque de cancer oesophagien. *Acta Endoscopica*, 15: 319-325, 1985.
24. Nabeya, K. Markers of cancer risk in the esophagus and surveillance of high-risk groups. In: P. Sherlock, B. C. Morson, L. Barbara, and U. Veronesi (eds.), *Precancerous Lesions of the Gastrointestinal Tract*, pp. 71-86. New York: Raven Press, 1983.
25. Berry, A. V., Baskind, A. F., and Hamilton, D. G. Cytologic screening for esophageal cancer. *Acta Cytol.*, 25: 135-141, 1981.
26. Tim, L. O., Leiman, G., Segal, I., Hamilton, D. G., and Mannell, A. A suction-abrasive cytology tube for the diagnosis of esophageal carcinoma. *Cancer (Phila.)*, 50: 782-784, 1982.
27. Jaskiewicz, K., Venter, F. S., and Marasas, W. F. Cytopathology of the esophagus in Transkei. *J. Natl. Cancer Inst.*, 79: 961-967, 1987.
28. Chanvitan, A., Geater, A. F., Ubolcholket, S., and Huang, G. J. Early detection of oesophageal carcinoma in southern Thailand. *J. Med. Assoc. Thai.*, 73: 565-570, 1990.
29. Greenebaum, E., Schreiber, K., Shu, Y. J., and Koss, L. G. Use of the esophageal balloon in the diagnosis of carcinomas of the head, neck and upper gastrointestinal tract. *Acta Cytol.*, 28: 9-15, 1984.
30. Dowlatshahi, K., Skinner, D. B., DeMeester, T. R., Zachary, L., Bibbo, M., and Wied, G. L. Evaluation of brush cytology as an independent technique for detection of esophageal carcinoma. *J. Thorac. Cardiovasc. Surg.*, 89: 848-851, 1985.
31. Tsang, T. K., Hidvegi, D., Horth, K., and Ostrow, J. D. Reliability of balloon-mesh cytology in detecting esophageal carcinoma in a population of US veterans. *Cancer (Phila.)*, 59: 556-559, 1987.
32. Jacob, P., Kahrilas, P. J., Desai, T., Hidvegi, D., Walloch, J., Yokoo, H., Gurley, A. M., and Ostrow, J. D. Natural history and significance of esophageal squamous cell dysplasia. *Cancer (Phila.)*, 65: 2731-2739, 1990.
33. Meyer, V., Burtin, P., Bour, B., Blancaf, A., Oberti, F., Person, B., Croue, A., Cales, P., Dohin, S., Benoit, R., and Boyer, J. Endoscopic detection of early esophageal cancer in a high risk population. Does the lugol coloration improve the results of video-endoscopic examination? *Gastrointest. Endosc.*, 41: 357, 1995.
34. Yokoyama, A., Ohmori, T., Makuuchi, H., Maruyama, K., Okuyama, K., Takahashi, H., Yokoyama, T., Yoshino, K., Hayashida, M., and Ishii, H. Successful screening for early esophageal cancer in alcoholics using endoscopy and mucosa iodine staining. *Cancer (Phila.)*, 76: 928-934, 1995.
35. Dawsey, S. M., Wang, G. Q., Weinstein, W. M., Lewin, K. L., Liu, F. S., Wiggert, S., Nieberg, R. K., Li, J. Y., and Taylor, P. R. Squamous dysplasia and early esophageal cancer in the Linxian region of China: distinctive endoscopic lesions. *Gastroenterology*, 105: 1333-1340, 1993.
36. Dawsey, S. M., Lewin, K. J., Wang, G. Q., Liu, F. S., Nieberg, R. K., Yu, Y., Li, J. Y., Blot, W. J., Li, B., and Taylor, P. R. Squamous esophageal histology and subsequent risk of squamous cell carcinoma of the esophagus: a prospective follow-up study from Linxian, China. *Cancer (Phila.)*, 74: 1686-1692, 1994.
37. Dawsey, S. M., Lewin, K. J., Liu, F. S., Wang, G. Q., and Shen, Q. Esophageal morphology from Linxian, China: squamous histologic findings in 754 patients. *Cancer (Phila.)*, 73: 2027-2037, 1994.
38. Wang, G. Q., Dawsey, S. M., Li, J. Y., Taylor, P. R., Li, B., Blot, W. J., Weinstein, W. M., Liu, F. S., Lewin, K. J., Wang, H., Wiggert, S., Gail, M. H., and Yang, C. S. Effects of vitamin/mineral supplementation on the prevalence of histologic dysplasia and early cancer of the esophagus and stomach: results from the general population trial in Linxian, China. *Cancer Epidemiol., Biomarkers & Prev.*, 3: 161-166, 1994.
39. Reid, B. J., Haggitt, R. C., Rubin, C. E., Roth, G., Surawicz, C. M., Van Belle, G., Lewin, K., Weinstein, W. M., Antonioli, D. A., Goldman, H., MacDonald, W., and Owen, D. Observer variation in the diagnosis of dysplasia in Barrett's esophagus. *Hum. Pathol.*, 19: 166-178, 1988.