Quantitative Fluorescence Image Analysis of DNA Content and Nuclear Morphology on Esophageal Balloon Cytology Smears and Subsequent Development of Esophageal and Gastric Cardia Cancer in Linxian, China

Nan Hu,[1] Philip R. Taylor, Jian-Yu Rao, George P. Hemstreet, Shu-Fan Liu, Xiao-Nong Zou, Steven D. Mark, and Sanford M. Dawsey

National Cancer Institute, Bethesda, Maryland 20892 [N. H., P. R. T., S. D. M., S. M. D.]; University of Oklahoma, Oklahoma City, Oklahoma 73190 [J-Y. R., G. P. H.]; and Cancer Institute, Chinese Academy of Medical Sciences, Beijing, People's Republic of China [S-F. L., X-N. Z.]

Abstract

The highest incidences of esophageal and gastric cardia cancer in the world occur in northern China. Chinese scientists have developed esophageal balloon cytology screening to detect these cancers, but traditional cytology is sometimes inadequate to find some early, curable lesions. Several studies suggest that quantitative fluorescence image analysis (QFIA) of DNA ploidy and nuclear morphology may be able to improve upon traditional cytology results. In October 1987, esophageal balloon cytology was performed on 1331 adults in Linxian, China, and all samples were evaluated both by traditional cytology and QFIA. From 1987 to May 1991, 62 new squamous esophageal cancers and 44 new adenocarcinomas of the cardia were identified in this cohort. Proportional hazards models were used to evaluate the relationship of cytological diagnoses and six QFIA variables to subsequent cancer risk. These models showed significant trends for increasing esophageal cancer risk, with increasing values in five of the QFIA variables and with increasing severity of the traditional cytological diagnoses. A comparison of models with only cytology variables *versus* models with both cytology and QFIA variables indicated that the QFIA provided an important additional predictive value. Persons with both cytological dysplasia and high cellular DNA were 8 times more likely to develop esophageal cancer than were individuals with neither of these conditions. For cardia cancer, associations between QFIA variables or cytological diagnoses and later cancer were more limited. This study suggests that the QFIA variables evaluated here are independent predictors of squamous esophageal cancer and that combining QFIA with traditional cytology can improve prediction of esophageal cancer risk.

Introduction

The highest rates of esophageal and gastric cardia cancer in the world occur in north-central China. Because of their similar symptomatologies, both esophageal and gastric cardia tumors have traditionally been classified as "esophageal cancer" in this region. The highest rates of esophageal cancer within China are found in Linxian, a rural county in northern Henan Province. During the period 1973–1975, the annual age-adjusted mortality rates for esophageal cancer in Linxian were 161/100,000 for men and 103/100,000 for women, and by age 75, the cumulative mortality from these tumors was over 20% in both sexes (1, 2).

Symptomatic esophageal cancer is difficult to cure by surgery, radiotherapy, or chemotherapy, alone or in combination. Five-year survival rates are among the lowest for any cancer (3). Over the past 35 years, a research method, balloon cytology, has been developed and used for screening for esophageal cancer. The goal of balloon cytology screening is to detect surgically curable precancerous and early cancerous lesions. This method has been commonly used in parts of China for diagnosing symptomatic patients and for screening asymptomatic, high-risk populations (4–10). Recent studies suggest, however, that the sensitivity of balloon cytology for detecting asymptomatic early cancers may be lower than previously believed (11), and so ways to improve the accuracy of this technique are currently being sought.

The presence of excess DNA and enlarged nuclei are known characteristics of many kinds of malignant and premalignant neoplastic cells. QFIA[2] is a method that has the ability to quantitate biochemical, morphological, and molecular markers on a single-cell basis (12). Here, nuclear DNA ploidy level and nuclear size were analyzed by QFIA method to facilitate the identification of rare events, such as individual cancer cells in cytological samples. Several studies have shown that QFIA provides objective, reproducible, and accurate diagnoses of malignant tumor samples (13–18).

The focus of the present study was to evaluate the ability of QFIA to identify individuals at increased risk for developing esophageal or gastric cardia cancer within 3.5 years of initial assessment and to determine whether QFIA could improve the ability of routine cytopathology to identify these individuals.

Received 5/5/97; revised 10/9/97; accepted 10/30/97.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] To whom requests for reprints should be addressed, at Cancer Prevention Studies Branch, National Cancer Institute, Executive Plaza North, Room 211, 9000 Rockville Pike, Bethesda, MD 20892.

[2] The abbreviations used are: QFIA, quantitative fluorescence image analysis; ppu, phosphor particle unit; RR, relative risk; CI, confidence interval.

Table 1 Mean values of QFIA variables by descriptive strata

Descriptive strata	QFIA variable					
	NUC_MAX (μm^2)	DNA_MAX (ppu)	INT_MAX (ppu/μm^2) × 100	NUC_MEAN (μm^2)	DNA_MEAN (ppu)	INT_MEAN (ppu/μm^2) × 100
Total (n = 1331)	183.4 (39.8)[a]	0.620 (0.186)	0.369 (0.063)	148.7 (14.9)	0.476 (0.083)	0.319 (0.043)
Age (years)						
<50 (n = 439)	181.9 (40.9)	0.610 (0.179)	0.366 (0.062)	148.5 (16.0)	0.473 (0.083)	0.318 (0.043)
50–59 (n = 589)	181.7 (36.7)	0.617 (0.172)	0.367 (0.065)	148.8 (14.2)	0.477 (0.082)	0.320 (0.043)
>59 (n = 303)	189.0 (43.5)	0.640 (0.216)	0.372 (0.062)	149.1 (14.5)	0.478 (0.084)	0.320 (0.044)
Sex						
Female (n = 793)	184.3 (39.5)	0.629 (0.190)	0.374 (0.066)	149.0 (14.1)	0.481 (0.082)	0.322 (0.044)
Male (n = 538)	182.2 (40.3)	0.606 (0.177)	0.362 (0.059)	148.3 (15.9)	0.469 (0.082)	0.315 (0.042)
Smoking						
No (n = 969)	184.3 (39.9)	0.626 (0.189)	0.372 (0.065)	148.9 (14.0)	0.479 (0.082)	0.321 (0.044)
Yes (n = 359)	181.4 (39.6)	0.604 (0.175)	0.360 (0.059)	148.3 (17.1)	0.469 (0.083)	0.316 (0.040)
Alcohol use[b]						
No (n = 1067)	184.1 (40.2)	0.626 (0.190)	0.371 (0.064)	149.1 (15.1)	0.479 (0.084)	0.321 (0.043)
Yes (n = 261)	181.0 (38.2)	0.596 (0.165)	0.361 (0.061)	147.3 (13.9)	0.464 (0.078)	0.315 (0.042)
Education[b]						
None (n = 571)	184.2 (39.1)	0.625 (0.195)	0.372 (0.065)	149.1 (13.7)	0.480 (0.085)	0.321 (0.045)
Some (n = 757)	183.0 (40.4)	0.616 (0.179)	0.367 (0.062)	148.5 (15.7)	0.473 (0.081)	0.318 (0.042)
Family history[b]						
No (n = 809)	182.9 (38.2)	0.617 (0.187)	0.369 (0.063)	148.8 (14.4)	0.476 (0.081)	0.319 (0.043)
Yes (n = 519)	184.5 (42.2)	0.625 (0.183)	0.370 (0.065)	148.7 (15.6)	0.477 (0.085)	0.320 (0.045)
Treatment						
Placebo (n = 661)	183.3 (38.9)	0.615 (0.166)	0.370 (0.064)	148.8 (14.6)	0.475 (0.081)	0.319 (0.044)
Active (n = 670)	183.5 (40.8)	0.624 (0.203)	0.368 (0.062)	148.7 (15.1)	0.477 (0.084)	0.320 (0.042)

[a] Values in parentheses are SDs.
[b] Three cases are missing.

Patients and Methods

Study Population. In 1983, Chinese scientists conducted a population-based esophageal balloon cytology screening in Yaocun, Rencun, and Donggang communes in Linxian, Henan Province (8). In the fall of 1984, persons with a cytological diagnosis of severe dysplasia in the 1983 screening were given a baseline evaluation that included an interview and a physical examination. In May 1985, 3318 screenees with dysplasia were randomized in a two-arm, double-blind, placebo-controlled nutrition intervention trial to receive either multiple vitamin and mineral supplements or placebo pills (19). In October 1987, at the midpoint of the intervention, a series of examinations, including balloon cytology, were conducted. Specimens for QFIA analysis were collected in 1404 of the 2826 participants who underwent a balloon cytology examination.

Information on age, sex, smoking habits, alcohol intake, education, and family history of cancer was obtained from the 1984 baseline interview data, collected from study participants.

From the time of the balloon examinations in 1987 through the end of the intervention trial in May 1991, the participants were followed prospectively for evidence of cancer, with monthly visits by village doctors and cytological and/or endoscopic examinations of symptomatic individuals. Case records and diagnostic materials (cytology slides, histology slides, and X-rays) were reviewed, and the cancer diagnoses were confirmed by an International Endpoints Review Committee of international experts (19).

Cytological Evaluation. All esophageal balloon cytology samples were interpreted by doctors from the Cancer Institute of the Chinese Academy of Medical Science in Beijing and Henan Medical University in Zhengzhou, China. Subjects were classified into one of six cytological categories, based on the worst squamous or columnar cells found in their 1987 smears. The cytological diagnoses, listed in order of increasing severity, were: normal, hyperplasia, dysplasia 1, dysplasia 2, near cancer, and cancer (8).

QFIA Evaluation. QFIA DNA and morphometric analyses were performed at the Department of Urology, University of Oklahoma Health Sciences Center. The method of sample collection for QFIA has been described previously (14). Briefly, after slides for routine cytology reading had been smeared, the balloon was immersed and agitated in a Saccomanno collection vial containing equal volumes of azide-free blood bank saline and 50% ethanol. The vials were refrigerated (but not frozen) and transported cold to the United States.

The cells were smeared and then stained with Hoechst 33258 dye, a stain that emits fluorescence that is linearly proportional to nuclear DNA content (14). A computer-controlled stage and a fluorescence microscope were used to automatically scan and image the nucleus of each cell on each slide (CTAS-Plus, E. Leitz, Rockleigh, NJ). The amount of fluorescence emitted from each nucleus was quantified, the nuclear area of each nucleus was measured, and the slide coordinates of each cell that was recognized as abnormal were recorded (13–18, 20, 21). A cytotechnologist located and reviewed each abnormal cell that was identified during the automated analysis for morphological evaluation and elimination of artifacts (overlapping nuclei or "junk"). A total of six QFIA variables were calculated for each esophageal balloon cytology specimen. The six QFIA variables were as follows: NUC_MAX (μm^2), largest nuclear area found in the specimen; DNA_MAX (ppu), highest DNA content for a single cell in the specimen; INT_MAX (ppu/area), highest DNA intensity (DNA content/nuclear area) reading for a single cell in the specimen; NUC_MEAN (μm^2), mean of the nuclear areas of all "non-junk alarms" in the specimen; DNA_MEAN (ppu), mean of the DNA content readings for all non-junk alarms in the specimen; and INT_MEAN (ppu/area), mean of the DNA intensity readings for all non-junk alarms in the specimen. The ppu is an

Table 2 Median values of QFIA variables in cases and noncases for esophageal and cardia cancer

QFIA variable	Esophageal cancer			Cardia cancer		
	Case (n = 62)	Noncase (n = 1269)	P^b	Case (n = 44)	Noncase (n = 1287)	P
NUC_MAX (μm^2)	205.1 (125.0–389.0)[a]	182.4 (106.0–393.0)	0.0009	189.0 (127.0–335.0)	183.2 (106.0–390.0)	0.4799
DNA_MAX (ppu)	0.7573 (0.4220–2.3660)	0.6132 (0.2310–1.7120)	0.0001	0.6511 (0.3310–1.3620)	0.6188 (0.2310–2.3660)	0.5700
INT_MAX (ppu/μm^2) × 100	0.3989 (0.2795–0.7136)	0.3677 (0.1893–0.7104)	0.0037	0.3763 (0.1893–0.5537)	0.3690 (0.1909–0.7136)	0.6045
NUC_MEAN (μm^2)	154.9 (125.0–192.1)	148.4 (80.4–270.0)	0.0001	154.9 (80.4–203.0)	148.5 (86.7–270.0)	0.0057
DNA_MEAN (ppu)	0.5097 (0.3720–0.7820)	0.4745 (0.2150–0.9220)	0.0129	0.4981 (0.2245–0.7760)	0.4754 (0.2150–0.9220)	0.1558
INT_MEAN (ppu/μm^2) × 100	0.3262 (0.2478–0.4551)	0.3191 (0.1668–0.5588)	0.4439	0.3196 (0.1861–0.4068)	0.3194 (0.1668–0.5588)	0.9981

[a] Values in parentheses are ranges.
[b] P from Wilcoxon rank sum test.

Table 3 Relationship of QFIA variables to subsequent esophageal and cardia cancer risk

QFIA variable	Esophageal cancer			Cardia cancer		
	RR[a]	95% CI	P	RR[a]	95% CI	P
NUC_MAX	1.53	1.27–1.84	0.0001	1.12	0.85–1.48	0.4066
DNA_MAX	1.71	1.46–2.01	0.0001	1.15	0.90–1.47	0.2660
INT_MAX	1.55	1.26–1.89	0.0001	1.14	0.86–1.51	0.3706
NUC_MEAN	1.37	1.15–1.63	0.0005	1.41	1.12–1.76	0.0031
DNA_MEAN	1.44	1.18–1.76	0.0004	1.28	0.99–1.66	0.0613
INT_MEAN	1.19	0.94–1.51	0.1502	1.02	0.76–1.37	0.9151

[a] RR was adjusted for age and sex; RR was for 1 SD change in a QFIA variable.

absolute but arbitrary unit of fluorescence. Non-junk alarms were abnormal cells that were identified during automated analysis, which were confirmed by the cytotechnologist to be intact, nonoverlapping cells.

Statistical Methods. All 1331 subjects (793 women and 538 men) who had interpretable QFIA and cytology readings, follow-up data, and no evidence of cancer at the beginning of the follow-up period were included in this analysis.

The distributions of the QFIA variables were examined and means (SDs) and medians (ranges) were calculated for all subjects and for subjects within categories of age, sex, smoking, alcohol use, education, family history, and nutrition intervention trial treatment group.

The relationship between QFIA values and these descriptive variables and the relationship between QFIA values and the cytological diagnoses were examined using Spearman correlation coefficients. Rank tests were used to compare QFIA values between cases and noncases. Proportional hazard regression models (SAS, PROC PHREG; SAS Institute, Inc., Cary, NC) were used to estimate RRs and 95% CIs, to examine the associations of the QFIA variables and the cytological diagnoses with the risk of subsequent esophageal and cardia cancer. For proportional hazards models, each QFIA variable was divided by its SD to produce a standardized variable that reflected the change in RR for an increase of 1 SD of that variable. The six QFIA variables were modeled individually in three ways (as continuous variables, with indicators for quartiles, and as linear trend variables with quartiles scored 0–3). Multivariate models were used to adjust for potential confounders (age and sex for esophageal cancer; age and smoking for cardia cancer) and to look for effect modification by non-QFIA risk factors. Risks for esophageal and cardia cancers were modeled, first with cytological diagnoses as indicator variables alone and, subsequently, with the addition of each QFIA variable individually to see whether the QFIA variables added predictive value to the traditional cytological evaluation.

Results

One hundred and six new cancers were diagnosed in the study participants during the 3.5-year follow-up period, including 62 squamous cell carcinomas of the esophagus and 44 adenocarcinomas of the gastric cardia. No adenocarcinomas of the esophagus were identified in the study population.

Mean (SD) values for the six QFIA variables, for all subjects and for subjects stratified by the seven descriptive variables, are shown in Table 1. All six QFIA variables increased with age and were consistently higher in females, nonsmokers, nondrinkers, and those with no education. Family history of cancer and nutrition intervention trial treatment were unrelated to the QFIA values. Although a number of correlations between QFIA values and descriptive variables were statistically significant, the magnitudes of these correlations were uniformly low (<0.10; data not shown).

Table 2 compares the median values of the six QFIA variables in subjects who developed esophageal cancer or gastric cardia cancer during the 3.5-year follow-up period (cases) and subjects who did not develop these cancers (noncases). Subjects who developed esophageal cancer had significantly higher values than did noncases for five of the six QFIA variables (INT_MEAN being the exception). Subjects who developed gastric cardia cancer had significantly higher values than noncases for NUC_MEAN, but there were no marked differences between cases and noncases in the other five variables.

Table 3 shows the results of proportional hazard regression models relating standardized continuous QFIA values to later

Table 4 RRs for developing cancer by quartile values of QFIA variables

QFIA Variable	Quartile 1 (reference)	Quartile 2	Quartile 3	Quartile 4	Model χ^2 (P)	P for trend[a]
A. RRs for developing esophageal cancer by quartile values of QFIA variables						
NUC_MAX (μm^2)	1.00	0.82 (0.37–2.12)[b]	2.00 (0.93–4.31)	2.52 (1.21–5.28)	11.454 (0.0095)	0.0021
DNA_MAX (ppu)	1.00	1.87 (0.75–4.69)	2.33 (0.96–5.66)	3.84 (1.67–8.84)	12.868 (0.0049)	0.0006
INT_MAX (ppu/μm^2) × 100	1.00	1.09 (0.47–2.57)	1.92 (0.89–4.14)	2.25 (1.07–4.76)	7.306 (0.0628)	0.0107
NUC_MEAN (μm^2)	1.00	0.79 (0.31–2.01)	1.44 (0.64–3.24)	3.08 (1.50–6.30)	18.217 (0.0004)	0.0002
DNA_MEAN (ppu)	1.00	2.02 (0.91–4.49)	1.33 (0.56–3.15)	2.63 (1.22–5.68)	8.116 (0.0437)	0.0363
INT_MEAN (ppu/μm^2) × 100	1.00	0.63 (0.31–1.30)	0.62 (0.30–1.28)	1.01 (0.53–1.90)	3.378 (0.3369)	0.9957
B. RRs for developing cardia cancer by quartile values of QFIA variables						
NUC_MAX (μm^2)	1.00	1.22 (0.51–2.95)	1.39 (0.59–3.30)	1.37 (0.58–3.26)	0.721 (0.8683)	0.4427
DNA_MAX (ppu)	1.00	1.00 (0.43–2.31)	0.73 (0.29–1.80)	1.28 (0.58–2.82)	1.699 (0.6372)	0.6802
INT_MAX (ppu/μm^2) × 100	1.00	0.56 (0.24–1.34)	0.50 (0.20–1.23)	1.08 (0.52–2.24)	4.898 (0.1794)	0.8717
NUC_MEAN (μm^2)	1.00	2.21 (0.77–6.36)	1.85 (0.62–5.52)	3.86 (1.44–10.34)	9.418 (0.0242)	0.0072
DNA_MEAN (ppu)	1.00	1.21 (0.52–2.79)	0.50 (0.17–1.45)	1.73 (0.80–3.77)	7.388 (0.0605)	0.3345
INT_MEAN (ppu/μm^2) × 100	1.00	1.09 (0.50–2.38)	0.57 (0.22–1.45)	1.01 (0.45–2.24)	2.419 (0.4901)	0.6781

[a] P from a linear trend variable, with quartiles scored 0–3.
[b] Values in parentheses are 95% CIs.

Table 5 RR for developing esophageal and cardia cancer by initial cytological diagnosis

Cytological diagnosis (n = 1315)	Esophageal cancer		Cardia cancer	
	No. of cancer cases	RR[a] (95% CI)	No. of cancer cases	RR (95% CI)
Normal (n = 74)	1	1.00	2	1.00
Hyperplasia (n = 330)	9	2.05 (0.26–16.21)	8	0.91 (0.19–4.26)
Dysplasia 1 (n = 505)	18	2.69 (0.36–20.13)	12	0.89 (0.20–3.97)
Dysplasia 2 (n = 241)	15	4.72 (0.62–35.70)	8	1.24 (0.26–5.83)
Near cancer (n = 165)	18	8.67 (1.16–64.92)	14	3.28 (0.74–14.41)
P[b]	0.0001		0.0030	

[a] RR was adjusted for age and sex.
[b] P from a linear trend variable, with diagnoses scored 0–4.

esophageal and cardia cancer risk. Five of the six QFIA variables (INT_MEAN being the exception) were significant predictors of esophageal cancer. DNA_MAX (RR = 1.71 for an increase of 1 SD) was the most predictive of these QFIA variables. NUC_MEAN was the only QFIA variable that was a significant predictor of cardia cancer.

Univariate models relating the descriptive variables shown in Table 1 to subsequent cancer risk showed significant associations for age and sex with esophageal cancer risk and for age and smoking with cardia cancer risk (data not shown), and so these variables were included in multivariate analyses; no confounding effects or modifications due to these factors were observed.

Table 4, A and B, give RRs for development of esophageal and cardia cancer by quartile values of the standardized QFIA variables. For esophageal cancer (Table 4A), there was a significant trend of increasing risk with increasing values for five of the six variables (INT_MEAN being the exception), and the risks for persons in the fourth quartiles were approximately 2–4 times higher than were the risks for persons in the first quartile. For cardia cancer (Table 4B), there was a significant trend only for NUC_MEAN.

The cytological diagnoses were significantly positively correlated with all QFIA variables except INT_MEAN, but the magnitudes of all of these correlations were low (<0.15; data not shown). Table 5 gives RRs for development of esophageal and cardia cancer by cytological diagnosis. There was a significant trend for increasing cancer risk with increasing severity of cytological diagnosis for both cancers, but the trend was more pronounced for cancer of the esophagus.

The RRs for cytological categories plus each of the standardized QFIA variables are shown in Table 6, A (esophageal cancer) and B (cardia cancer). Although baseline cytology was a strong predictor of subsequent esophageal cancer risk, five of the six QFIA variables were significant predictors of risk, even after adjustment for cytological diagnosis.

Table 7A shows that persons with both cytological dys-

Table 6 RR for developing cancer by cytological diagnosis and QFIA variables

QFIA variables	RR[a] (95% CI)					
	Normal	Hyperplasia	Dysplasia 1	Dysplasia 2	Near cancer	QFIA variables[b]
A. RR for developing esophageal cancer by cytological diagnosis and QFIA variables						
NUC_MAX	1.00	2.29 (0.29–18.06)	2.67 (0.36–20.02)	4.53 (0.60–34.31)	7.93 (1.06–59.50)	1.48 (1.23–1.79)
DNA_MAX	1.00	2.31 (0.29–18.26)	2.62 (0.35–19.67)	4.28 (0.56–32.46)	7.51 (1.00–56.44)	1.65 (1.40–1.94)
INT_MAX	1.00	2.10 (0.27–16.55)	2.46 (0.33–18.44)	4.24 (0.56–32.15)	8.36 (1.12–62.63)	1.55 (1.26–1.91)
NUC_MEAN	1.00	2.21 (0.28–17.48)	2.77 (0.37–20.77)	4.72 (0.62–35.76)	7.96 (1.061–59.74)	1.30 (1.09–1.55)
DNA_MEAN	1.00	2.13 (0.27–16.81)	2.60 (0.35–19.46)	4.53 (0.60–34.31)	7.96 (1.06–59.75)	1.40 (1.14–1.71)
INT_MEAN	1.00	2.08 (0.26–16.38)	2.69 (0.36–20.16)	4.79 (0.63–36.28)	8.79 (1.17–65.88)	1.19 (0.92–1.53)
B. RR for developing cardia cancer by cytological diagnosis and QFIA variables						
NUC_MAX	1.00	0.94 (0.20–4.43)	0.91 (0.20–4.09)	1.27 (0.27–5.99)	3.45 (0.78–15.26)	1.07 (0.81–1.41)
DNA_MAX	1.00	0.94 (0.20–4.41)	0.90 (0.20–4.04)	1.23 (0.26–5.84)	3.39 (0.77–15.00)	1.11 (0.86–1.43)
INT_MAX	1.00	0.93 (0.20–4.37)	0.89 (0.20–3.99)	1.23 (0.26–5.82)	3.44 (0.78–15.17)	1.15 (0.86–1.54)
NUC_MEAN	1.00	0.97 (0.21–4.60)	0.91 (0.20–4.07)	1.22 (0.26–5.76)	3.14 (0.71–13.84)	1.32 (1.05–1.66)
DNA_MEAN	1.00	0.92 (0.20–4.35)	0.87 (0.19–3.88)	1.19 (0.25–5.62)	3.23 (0.73–14.27)	1.27 (0.97–1.66)
INT_MEAN	1.00	0.94 (0.20–4.41)	0.92 (0.21–4.12)	1.29 (0.27–6.08)	3.52 (0.80–15.53)	1.02 (0.75–1.41)

[a] RR was adjusted for age and sex.
[b] RR for 1 SD change in a QFIA variable.

Table 7 RR for developing cancer by cytological diagnosis and QFIA variable values

Cytological Diagnosis	DNA_MAX		NUC_MEAN	
	Low (≤0.584)	High (>0.584)	Low (≤148.2)	High (>148.2)
A. RR for developing esophageal cancer by cytological diagnosis and DNA_MAX value				
<Dysplasia	1.00 (reference) [2/224][a]	5.07 (1.08–23.87) [8/180]		
≥Dysplasia	4.42 (1.02–19.15) [17/436]	8.28 (1.99–34.47) [34/475]		
B. RR for developing cardia cancer by cytological diagnosis and NUC_MEAN Value				
<Dysplasia			1.00 [3/237]	3.42 (0.89–13.24) [7/167]
≥Dysplasia			2.64 (0.76–9.18) [14/427]	3.35 (1.00–11.27) [20/4840]

[a] Values in parentheses are 95% CIs; values in brackets are no. of cancer cases/total no. of subjects.

plasia and a high QFIA value for DNA_MAX had a greater risk of esophageal cancer than did persons with just one of these risk factors. Compared to persons with neither risk factor, those with both risk factors had 8-fold greater risk of esophageal cancer. For cardia cancer (Table 7B), a higher QFIA value for NUC_MEAN added little predictive value beyond that for cytological dysplasia alone.

Discussion

Esophageal cancer and gastric cardia cancer are common fatal malignancies in China (1, 2). The prognosis of these tumors is poor because symptomatic cases are usually too advanced to be cured (22). Esophageal balloon cytology was developed in China to screen high-risk individuals to look for early, curable esophageal and gastric cardia cancers and to identify people who are at increased risk for developing these cancers in the future (4–10).

Recently, the sensitivity of traditional esophageal cytology has been questioned, and ways to improve its accuracy are being sought. One possible way is to have the slides examined by QFIA, a method that can quantitate morphometric and biochemical parameters and molecular markers at the single-cell level (12). The presence of excess DNA and enlarged nuclei are known characteristics of many kinds of malignant and premalignant cells, and QFIA can measure these variables objectively and reproducibly. Several studies have shown that QFIA is a useful methodology for the early detection of cells

exfoliated from colorectal and bladder cancers (13–18). Our study is the first to evaluate whether QFIA measurements in esophageal balloon samples can predict subsequent risk of esophageal or gastric cardia cancer.

Here, we examined the relationship of six calculated QFIA variables to several patient characteristics and to the development of squamous cell carcinoma of the esophagus and adenocarcinoma of the gastric cardia during the 3.5 years after a cytological examination of a high risk population in China. Most importantly, we evaluated what predictive value QFIA added beyond that of traditional cytology.

We found that all six QFIA variables increased significantly with age and were correlated at low levels with several other potential cancer risk factors but that adjustment for these risk factors did not appreciably change the estimates of the QFIA variable with cancer.

Subjects who developed esophageal cancer during the 3.5-year follow-up period had significantly higher values than did noncases for nearly all of the QFIA variables and DNA_MAX, the highest measured DNA content for a single cell in the specimen, appeared to be the most predictive of the QFIA variables for esophageal cancer. The combination of both cytological dysplasia and high DNA_MAX permitted the best prediction of all, with a RR of >8, compared to persons who were negative on both tests. As a screening measure, cytological dysplasia with a high DNA_MAX had a sensitivity of 56% (34 of 61 cases) and a specificity of 65% for diagnosing individuals who would develop esophageal cancer within the next 3.5 years. Just over 7% of persons in this group developed esophageal cancer during the follow-up period.

The only QFIA variable that was significantly associated with subsequent risk of cardia cancer was NUC_MEAN. The values for this variable were significantly higher in cases than in noncases, and there was a significant trend for increasing cancer risk with increasing values. Screening test sensitivity for predicting cardia cancer was lower, however, than for esophageal cancer. The sensitivity of cytological dysplasia with a high NUC_MEAN was 45%, with a specificity of 64% for identifying individuals who would progress to cardia cancer during the follow-up period. A possible reason why most of the QFIA variables were less predictive for cardia cancer than they were for esophageal cancer is that the esophageal balloon was designed primarily to sample the esophagus, and it may have sampled the cardia less completely (8). Consistent with this possibility was the finding that the traditional cytological diagnoses were also less predictive for cardia cancer than they were for esophageal cancer.

In conclusion, our study suggests that analysis of esophageal balloon cytology smears by QFIA can improve the ability of traditional cytology to identify individuals who are at increased risk for developing squamous esophageal cancer. This implies that QFIA may be a clinically useful tool for an early detection program in a high-risk population. Additional studies are needed to confirm and refine these observations.

Acknowledgments

We gratefully acknowledge Mary Ann Whitlock (Westat, Inc.) for computational support in the data management and analysis.

References

1. Li, M. X., and Li, B. Recent progress in research on esophageal cancer in China. Adv. Cancer Res., *33:* 173–249, 1980.

2. Li, J. Y. Epidemiology of esophageal cancer in China. Natl. Cancer Inst. Monogr., *62:* 113–120, 1982.

3. Rosenberg, J. C., Roth J. A., Lichter, A. S., and Kelsen, D. P. Cancer of the esophagus. *In:* V. T. Devita Jr., S. Hellman, and S. A. Rosenberg (eds.). Cancer Principles and Practice of Oncology, pp. 621–657. Philadelphia: J. B. Lippincott Co., 1985.

4. 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.

5. Shu, Y. J. Cytopathology of the esophagus: an overview of esophageal cytopathology in China. Acta Cytol., *27:* 7–16, 1983.

6. Shen, Q. Diagnostic cytology and early detection. *In:* G. J. Huang and Y. K. Wu (eds.). Carcinoma of the esophagus and gastric cardia, pp. 155–190. Berlin: Springer-Verlag, 1984.

7. Shu, Y. J. The cytopathology of esophageal carcinoma. New York: Masson, 1980.

8. 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., Gou, 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.

9. 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.

10. 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. Acta Cytol., *38:* 183–192, 1994.

11. Dawsey, S. M., Shen, Q., Nieberg, R. K., Liu, S. F., English, S. A., Cao, J., Zhou, B., Wang, G. Q., Lewin, K. J., Liu, F. S., Roth, M. J., and Taylor, P. R. Studies of esophageal balloon cytology in Linxian, China. Cancer Epidemiol. Biomark. Prev., *6:* 121–130, 1997.

12. Hemstreet, G. P., Bonner, R. B., Hurst, R. E., and O'Dowd, G. A. Cytology of bladder cancer. *In:* N. J. Vogelzang, P. T. Scardino, W. U. Shipley, and D. S. Coffey (eds.), Comprehensive Textbook of Genitourinary Oncology, pp. 338–350. Baltimore, MD: Williams & Wilkins, 1996.

13. Bass, R. A., Hemstreet, G. P., Honker, N. A., Hurst, R. E., and Doggett, R. S. DNA cytometry and cytology by quantitative fluorescence image analysis in symptomatic bladder cancer patients. Int. J. Cancer, *40:* 698–705, 1987.

14. West, S. S., Hemstreet, G. P., Hurst, R. E., Bass, R. A., and Doggett, R. S. Detection of DNA aneuploidy by quantitative fluorescence image analysis: potential in screening for occupational bladder cancer. *In:* H. K. Dillon, and M. Ho (eds.). Biological Monitoring of Exposure to Chemicals: Organic Chemicals, Vol. I, pp. 327–341. New York: John Wiley & Sons, Inc., 1987.

15. Parry, W. L., and Hemstreet, G. P. Cancer detection by quantitative fluorescence image analysis. J. Urol., *139:* 270–274, 1988.

16. Hemstreet, G. P., Schulte, P. A., Ringen, K., Stringer, W., and Altekruse, E. B. DNA hyperploidy as a marker for biological response to bladder carcinogen exposure. Int. J. Cancer, *42:* 817–820, 1988.

17. Hemstreet, G. P., Hurst, R. E., Bass, R. A., and Rao, J. Y. Quantitative fluorescence image analysis in bladder cancer screening. J. Occup. Med., *9:* 822–828, 1990.

18. McGowan, P. F., Hurst, R. E., and Bass, R. A. QFIA of exfoliated colorectal cells: potential for early detection of colorectal cancer. Am. J. Surg., *159:* 172–177, 1990.

19. 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., Li, G. Y., 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.

20. Hemstreet, G. P., West, S. S., Weems, W. L., Echols, C. K., McFarland, S., Lewn, J., and Lindseth, G. Quantitative fluorescence measurements of AO-stained normal and malignant bladder cells. Int. J. Cancer, *31:* 577–585, 1983.

21. Hemstreet, G. P., Rao, J. Y., Hurst, R. E., Bonner, R. B., Jones, P. L., Vaidya, A. M., Fradet, Y., Moon, R. C., and Kelloff, G. J. Intermediate endpoint biomarkers for chemoprevention. J. Cell. Biochem. Suppl., *161:* 93–110, 1992.

22. Wu, Y. K., Huang, G. J., Shao, L. F., Zhang, Y. D., and Lin, X. S. Progress in the study an surgical treatment of cancer of the esophagus in China. J. Thorac. Cardiovasc. Surg., *84:* 325–333, 1982.