

Predictors of Progression to Cancer in Barrett's Esophagus: Baseline Histology and Flow Cytometry Identify Low- and High-Risk Patient Subsets

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OBJECTIVE: Barrett's esophagus develops in 5–20% of patients with gastroesophageal reflux disease and predisposes to esophageal adenocarcinoma. The value of endoscopic biopsy surveillance is questioned because most patients do not develop cancer. Furthermore, observer variation in histological diagnosis makes validation of surveillance guidelines difficult because varying histological interpretations may lead to different estimated rates of progression. Thus, objective biomarkers need to be validated for use with histology to stratify patients according to their risk for progression to cancer.

METHODS: We prospectively evaluated patients using a systematic endoscopic biopsy protocol with baseline histological and flow cytometric abnormalities as predictors and cancer as the outcome.

RESULTS: Among patients with negative, indefinite, or low-grade dysplasia, those with neither aneuploidy nor increased 4N fractions had a 0% 5-yr cumulative cancer incidence compared with 28% for those with either aneuploidy or increased 4N. Patients with baseline increased 4N, aneuploidy, and high-grade dysplasia had 5-yr cancer incidences of 56%, 43%, and 59%, respectively. Aneuploidy, increased 4N, or HGD were detected at baseline in all 35 patients who developed cancer within 5 yr.

CONCLUSIONS: A systematic baseline endoscopic biopsy protocol using histology and flow cytometry identifies subsets of patients with Barrett's esophagus at low and high risk for progression to cancer. Patients whose baseline biopsies are negative, indefinite, or low-grade dysplasia without increased 4N or aneuploidy may have surveillance deferred for up to 5 yr. Patients with cytometric abnormalities merit more frequent surveillance, and management of high-grade dysplasia can be individualized. (*Am J Gastroenterol* 2000; 95:1669–1676. © 2000 by Am. Coll. of Gastroenterology)

INTRODUCTION

Gastroesophageal reflux affects 40% of adults in the United States (1). Barrett's esophagus develops in 5–20% of patients with chronic reflux and predisposes to esophageal adenocarcinoma (2–4). Rapid increases in the incidence of esophageal adenocarcinoma have been reported for the United States and regions of Western Europe (5, 6). Unfortunately, most esophageal adenocarcinomas are detected when they are associated with a mortality of >90% (7). Endoscopic surveillance of patients with Barrett's esophagus is recommended because it detects early, curable cancer (8, 9). However, its value and cost-effectiveness are questioned because most patients do not develop cancer (10–22). For example, in 11 studies of 1127 patients, only 39 (3.5%) progressed to cancer, and the incidence of cancer ranged from 1/52 to 1/441 patient-years of follow-up. These observations have led to discrepancies between endoscopic practice, in which most patients are in annual or biennial surveillance (23), and the results of cost-effectiveness analyses, which suggest that 5-yr surveillance intervals are optimal (24). One approach to resolving this dilemma would be to use biomarkers to stratify patient care according to the risk of progressing to cancer.

A five-tier classification of dysplasia and cancer is recommended for endoscopic surveillance of Barrett's esophagus (25). However, prospective studies providing risk estimates for progression to cancer using this classification system are lacking. Furthermore, four studies over more than a decade show observer variation in histological diagnosis, especially for diagnoses less than high-grade (26–29). However, observer agreement increases to 86% when the diagnoses of negative, indefinite, and low-grade are combined (26). Because of these observations, many investigators advocate validation of objective biomarkers to complement histological diagnosis in assessing the risk of progression to cancer (28, 30).

Several previous studies have suggested that flow cytometry may be a useful adjunct to histology in assessing risk in Barrett's esophagus (31–36). Flow cytometry has also been

shown in small studies to predict progression to intermediate endpoints, further suggesting that it may be an objective aid in identifying patients at increased risk (36–38). Some investigators have postulated that combined use of histology and flow cytometry may identify a low-risk population (34), but prospective data are lacking. We investigated the hypothesis that baseline histological and cytometric variables could identify patient subsets at low and high risk for progression to cancer.

MATERIALS AND METHODS

Patients

Patients eligible for this prospective study had metaplastic columnar epithelium in esophageal biopsies, no history of esophageal malignancy, a baseline endoscopy, and at least one follow-up evaluation. A total of 327 patients, including 265 men and 62 women of median age 62 yr (range, 22–83 yr), who had baseline histology and at least one follow-up evaluation (1208 endoscopies) in the Seattle Barrett's Esophagus Project, were evaluated prospectively over a 15-yr period between July 1, 1983, and June 30, 1998. The baseline endoscopy for histology was defined as the first in these 327 patients. A 322 patient subset (260 men, 62 women) had baseline histology and flow cytometry with at least one prospective follow-up evaluation (1193 endoscopies). The baseline endoscopy for cytometric variables and direct comparison of histological and cytometric variables was defined as the first with flow cytometry in the 322 patients. The baseline histological diagnoses differ slightly for the 327 patients evaluated for histology alone compared with the 322 evaluated for direct comparison with cytometry because the latter set contained five fewer patients and six patients whose diagnoses changed between the two baselines. Median intervals between endoscopies for patients with baseline negative, indefinite, low-grade dysplasia (LGD), and high-grade dysplasia (HGD) were 24.4, 18.2, 15.7, and 4.6 months, respectively. Median and mean follow-up times were 2.4 and 3.9 yr, respectively (range, 17 days to 13 yr) among those without cancer at last contact. There were 1200 patient-years of follow-up in these patients. Patients were counseled concerning risks and benefits of surveillance and were informed of alternatives, including surgery for HGD. The study was approved by the University of Washington Human Subjects Division in 1982 and renewed annually thereafter, with reciprocity from the Fred Hutchinson Cancer Research Center since 1993.

Endoscopy and Biopsy

From 1985 to 1998, four-quadrant biopsies were taken with "jumbo" biopsy forceps at ≥ 2 -cm intervals in the Barrett's segment (39). One biopsy at each level was divided for histology and flow cytometry. After 1992, four-quadrant biopsies were typically taken at 1-cm intervals in patients with a previous diagnosis of HGD. Multiple biopsies were taken of endoscopic abnormalities.

Histology

Biopsies were fixed, processed, and interpreted prospectively by a single observer who was blinded to the flow cytometric results, as described previously (9, 31, 38). Patients were classified according to the maximum abnormality (negative, indefinite, LGD, HGD, cancer) in any biopsy at a given endoscopy (40). We also analyzed a modified protocol of histological biopsies based on only the half biopsy split for flow cytometry at each level.

DNA Content Flow Cytometry

Biopsies were processed for flow cytometry and analyzed by the computer program Multicycle (Phoenix Flow Systems, San Diego, CA), as described previously (31, 38, 41). Flow cytometric histograms were interpreted by a single observer who was blinded to the histologic results, as described previously (31, 38). To prevent autolysis, which can cause false-positive near-diploid aneuploidy (42), biopsies were placed on ice before freezing in 10% DMSO at -70°C . Aggregation of nuclei results in false-positive 4N elevations, and therefore we used a single-step detergent technique with trituration and monitoring of clumping at the 6N position on a 10X scale (38). An aneuploid population was defined as described previously (38). The 4N fractions $>6\%$ (within the range 3.85N–4.1N) were classified as abnormal, as described previously (31, 33, 38). Flow cytometric results are based on one-half biopsy from each level of the Barrett's segment.

Statistical Analysis

The Kaplan-Meier estimator (43) was used for cancer cumulative incidence curves and estimates with censoring at time of last surveillance endoscopy. Corresponding 95% confidence intervals were based on Greenwood standard error estimates. Exact binomial confidence limits were used in place of Greenwood confidence limits for groups without observed cancer incidence. Relative risk (RR) estimates were obtained from univariable Cox proportional hazards models (43) of cancer outcome. Cancer outcome and censoring times were defined relative to baseline endoscopy. Group comparison p values were based on the Wald test of the null hypothesis, and the corresponding $\text{RR} = 1.0$. Pearson χ^2 test statistics were used for comparison of proportions involving other than disease outcome. The p values were not corrected for multiple comparisons.

RESULTS

Baseline Histology

In the full 327-patient population, 42 cancers were detected, including 35 diagnosed within 5 yr of baseline.

HISTOLOGICAL ABNORMALITIES LESS THAN HIGH-GRADE DYSPLASIA. Five of 129 patients with baseline negative for dysplasia progressed to cancer (Fig. 1A). The cumulative incidence of cancer 5 and 8 yr after a baseline endoscopy in which all biopsies were negative was

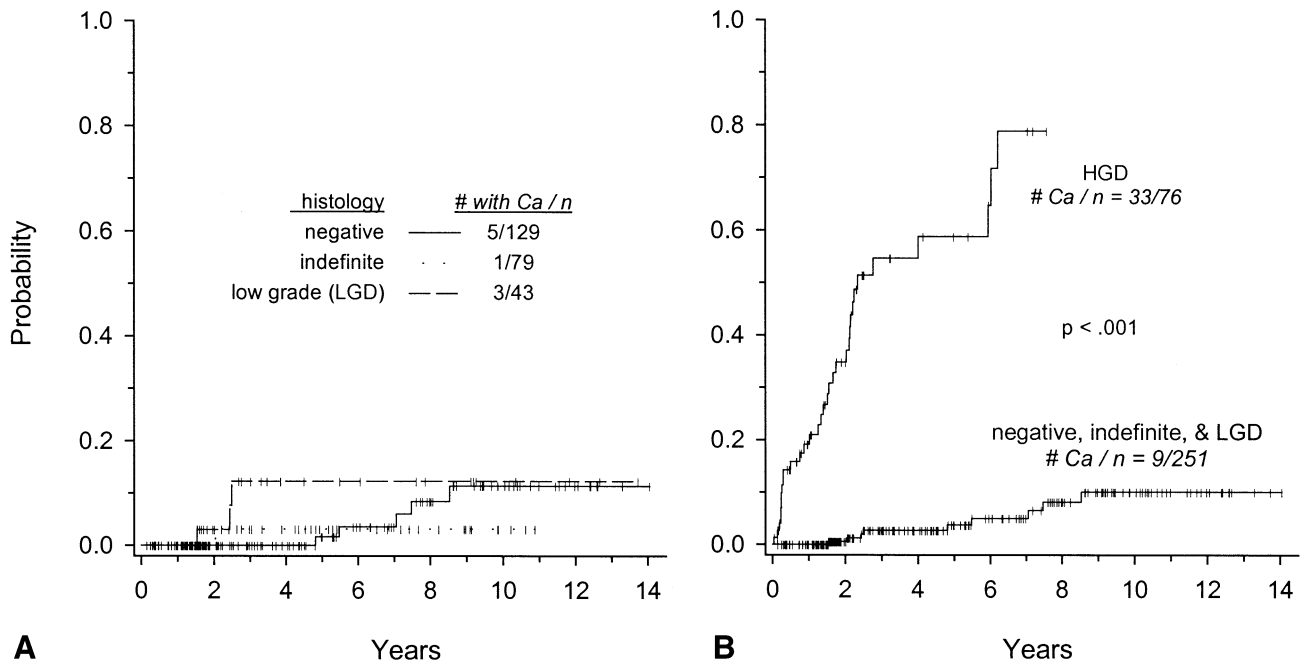


Figure 1. (A) Cumulative incidence of cancer after baseline histological diagnoses of negative, indefinite, and low-grade dysplasia. (B) Cumulative incidence of cancer after baseline histological diagnoses of negative, indefinite, low-grade dysplasia combined and high-grade dysplasia. Tick marks indicate time of last follow-up endoscopy for patients without cancer at last endoscopy.

1.7% (95% CI = 0.2, 11) and 8.4% (95% CI = 3.2, 21), respectively (Table 1). One of 79 patients with baseline indefinite for dysplasia progressed to cancer (Fig. 1A), a cumulative incidence of 3% (95% CI = 0.5, 20) at both 5 and 8 yr, which was not significantly different from negative for dysplasia (Table 1). Three of 43 patients with baseline LGD developed cancer, for a cumulative incidence of 12% (95% CI = 4, 34) at 5 and 8 yr (Fig. 1A, Table 1). The incidence rate was not significantly different among baseline LGD patients than among baseline negative and indefinite patients ($p = 0.14$).

Because there were no significant differences in cancer incidence among negative, indefinite, and LGD groups, and because an observer variation study showed highest reproducibility when these diagnoses were consolidated (26), we combined these patient groups for subsequent analyses (Fig. 1B, Table 1). The 5-yr cumulative cancer incidence for the combined diagnoses of negative, indefinite, and LGD was 4% (95% CI = 1.6, 9).

HIGH-GRADE DYSPLASIA. The 5-yr cumulative cancer incidence among 76 patients with baseline HGD was 59% (95% CI = 44, 74) (Fig. 1B, Table 1). The RR of cancer for baseline HGD compared with negative, indefinite, and LGD was 28 (95% CI = 13, 63) ($p < 0.001$).

Baseline Flow Cytometry

In the 322 patients with baseline flow cytometry, 41 cancers were diagnosed, including 34 within 5 yr of the baseline endoscopy.

DIPLOID CELL POPULATIONS ONLY. Of 322 patients, 241 (75%) had neither baseline aneuploidy nor increased 4N fractions; 12 of the 241 progressed to cancer (Fig. 2, Table 1). The RR of cancer in patients with aneuploidy, increased 4N, or both, compared to those with neither was 11 (95% CI = 5.5, 21) ($p < 0.001$).

CYTOMETRIC ABNORMALITIES. Of 322 patients, 48 (15%) had baseline increased 4N; 18 of the 48 progressed to cancer (Fig. 2, Table 1). The RR of cancer for patients with increased 4N compared to those without was 7.5 (95% CI = 4.0, 14) ($p < 0.001$). A total of 53 (16%) had baseline aneuploidy and 17 developed cancer (Fig. 2, Table 1). The RR of cancer in patients with aneuploidy compared to those without was 5.0 (95% CI = 2.7, 9.4) ($p < 0.001$).

Baseline Histology and Flow Cytometry Combined

Histological and cytometric results were compared directly for the first endoscopy with flow cytometry in the 322-patient subset. The only significant difference between the 322-patient subset and the 327-patient population was that in the former, four of 44 patients with LGD progressed to cancer (5-yr cumulative cancer incidence of 20% [95% CI = 7.5, 48], $p = 0.02$ for LGD vs negative and indefinite).

NEGATIVE, INDEFINITE, AND LGD WITH AND WITHOUT ANEUPLOIDY OR INCREASED 4N. Of 120 patients who were negative for dysplasia, 110 (92%) had neither aneuploidy nor increased 4N at baseline. Only two progressed to cancer, compared with one of 10 patients with

Table 1. Cumulative Incidence of Cancer After Baseline Diagnoses (95% Confidence Intervals [CI]*)

Variables	3 Yr	5 Yr	8 Yr
Histological variables			
Negative	0 (0.4, 7)	1.7 (0.2, 11)	8.4 (3.2, 21)
Indefinite	3.1 (0.5, 20)	3.1 (0.5, 20)	3.1 (0.5, 20)
LGD	12 (4, 34)	12 (4, 34)	12 (4, 34)
Baseline HGD	55 (41, 69)	59 (44, 74)	
Negative/Indefinite/LGD	2.7 (1, 7.1)	3.8 (1.6, 9)	8.1 (3.9, 16)
Cytometric variables			
Aneuploid			
No	9 (5.4, 14)	11 (6.7, 16)	17 (11, 26)
Yes	43 (28, 62)	43 (28, 62)	58
4N abnormality			
No	9 (6, 15)	9 (6, 15)	16 (10, 25)
Yes	44 (28, 64)	56 (37, 77)	74
Aneuploid/4N abnormality			
-/-	5 (2.4, 9.5)	5 (2.4, 9.5)	11 (5.8, 19)
+/-	41 (24, 64)	41 (24, 64)	53
-/+	43 (24, 68)	62	81
+/+	46	46	
Either positive	43 (31, 58)	49 (36, 65)	65
Combined variables (histology, aneuploid, 4N)			
Negative histology			
Cytometry negative	0 (0, 5.5)	0 (0, 7.1)	5 (1.3, 19)
Either aneuploid or 4N positive	0 (0, 46)	0	50
Indefinite			
Cytometry negative	0 (0, 13)	0 (0, 18)	6.7 (1.0, 39)
Either aneuploid or 4N positive	17	17	17
LGD			
Cytometry negative	0 (0, 25)	0 (0, 37)	0
Either aneuploid or 4N positive	47	73	
Baseline HGD			
Cytometry negative	42	42	
Either aneuploid or 4N positive	61 (44, 78)	66 (48, 83)	
Negative/Indefinite/LGD			
Cytometry negative	0 (0, 3.4)	0 (0, 4.7)	5 (1.6, 14)
Either aneuploid or 4N positive	20 (8.1, 46)	28 (12, 55)	46

* 95% CI provided when more than five patients at risk for esophageal adenocarcinoma are still followed.

HGD = high-grade dysplasia; LGD = low-grade dysplasia.

aneuploidy or increased 4N ($p = 0.05$). Of 83 patients whose baseline biopsies were indefinite, 72 had neither aneuploidy nor increased 4N (87%). Only one progressed to cancer compared with one of 11 with aneuploidy or increased 4N ($p = 0.26$). Of 44 patients with baseline LGD, 33 (75%) had neither aneuploidy nor increased 4N. None of these 33 progressed to cancer, compared with four of 11 who had aneuploidy or increased 4N ($p < 0.001$) (Table 1).

Of 247 patients whose baseline diagnoses were negative, indefinite, or LGD, 215 (87%) had neither aneuploidy nor increased 4N. The 5-yr cumulative cancer incidence was 0 (95% CI = 0, 4.7) for those without aneuploidy or increased 4N compared with 28% (95% CI = 12, 55) for those with either cytometric abnormality (Fig. 3A, Table 1). Among patients with negative, indefinite, or LGD, the RR of cancer for aneuploidy and/or increased 4N *versus* neither was 19 (95% CI = 4.7, 78) ($p < 0.001$).

HIGH-GRADE DYSPLASIA WITH AND WITHOUT ANEUPLOIDY OR INCREASED 4N. Of 75 patients with baseline HGD, 49 (65%) had baseline aneuploidy, in-

creased 4N, or both. The relative risk of cancer after baseline aneuploidy and/or increased 4N *versus* neither was 1.7 (95% CI = 0.76, 3.7) ($p = 0.19$). (Fig. 3B, Table 1).

Modified Biopsy Protocol

Because many endoscopists take fewer biopsies than in our protocol, frequently using small forceps, we reanalyzed results using only histological and cytometric data from halves of "jumbo" biopsies divided at each level in the 322 patients with both baseline histology and flow cytometry. Our four-quadrant protocol detected baseline aneuploidy, increased 4N, or HGD in all 34 patients (100%; 95% CI = 90, 100) who developed cancer within 5 yr. However, a protocol using only a half biopsy each for histology and flow cytometry detected 20 (59%; 95% CI = 41, 75) with baseline HGD, 26 (76%; 95% CI = 59, 89) with aneuploidy and/or increased 4N, and 28 (82%; 95% CI = 65, 93) with baseline aneuploidy, increased 4N, or HGD.

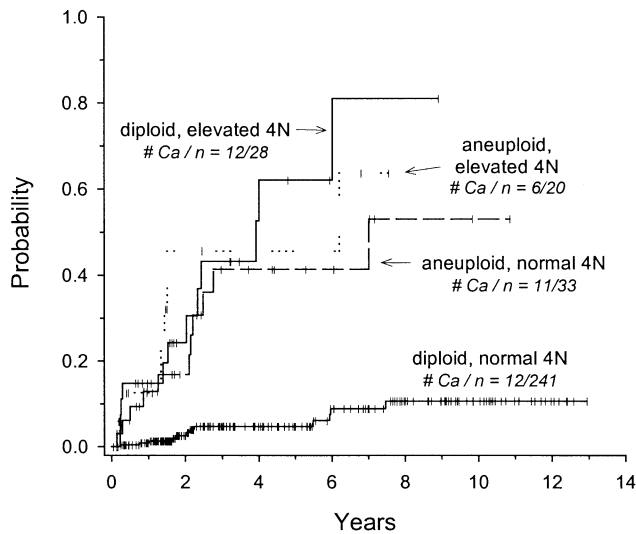


Figure 2. Cumulative incidence of cancer after baseline flow cytometric diagnoses of diploid, normal 4N, elevated 4N without aneuploidy, aneuploidy without elevated 4N, elevated 4N, and aneuploidy.

DISCUSSION

Most patients with Barrett's esophagus derive no increase in life expectancy from expensive and invasive programs for early detection or prevention of malignancy because they will not develop cancer (10–22). By identifying patient subsets with different incidences of cancer, our study should improve management of the malignant risk in Barrett's esophagus. A baseline endoscopic biopsy protocol using histology and flow cytometry identified a large, benign subset, comprising 66% of our patients, with a low risk for cancer. No patient with negative, indefinite, or LGD whose biopsies had neither aneuploidy nor increased 4N developed cancer within 5 yr. This benign subset may constitute greater percentages of Barrett's patients in practices with fewer high-risk referrals. Counseling, reassurance, and lengthened surveillance intervals for these low-risk patients could reduce patient anxiety and decrease cost of medical care. There is no evidence that such low-risk patients will benefit from endoscopic ablative therapies or antireflux surgeries intended, in theory, to diminish the risk of esophageal adenocarcinoma (44–46), and such interventions do not seem to be indicated in the routine clinical setting at the present time. Conversely, patients without HGD but with aneuploidy or increased 4N had a substantial risk for developing cancer (RR = 19). Baseline increased 4N, aneuploidy, and HGD had 5-yr cumulative cancer incidences of 56%, 43%, and 59%, respectively. Adjusting for age and sex did not influence the estimated relationship of histological and cytometric measures to cancer outcome.

The risk of cancer in HGD seems to be variable, and comparison of our results with those of Sontag *et al.* and Weston *et al.* indicates that the risk of baseline HGD may vary among centers (47, 48). Some HGD patients remain

free of cancer indefinitely, whereas others progress rapidly. Sontag *et al.* found a lower risk of cancer in baseline HGD (prevalent HGD) and in follow-up (incident HGD). Our study design evaluated only baseline predictors of progression to cancer. However, in 27 incident HGD patients, we found a 5-yr cumulative incidence of cancer of 31% (95% CI = 14, 60), which was lower than our baseline HGD ($p = 0.01$) and seemed closer to the results of Sontag *et al.* and Weston *et al.*, although theirs were not presented with time-dependent analyses (data not shown). Abnormal cytometric results at the time of HGD diagnosis were more frequent in baseline (65%) than incident HGD (27%) ($p < 0.001$) but were not statistically significant predictors of cancer in either category of HGD. Patients who had aneuploidy or increased 4N in baseline (prevalent) and incident HGD had 3-yr cancer incidences of 61% (95% CI = 44, 78) and 58% (95% CI = 16, 99), respectively, compared to 42% (95% CI = 23, 68) and 7.7% (95% CI = 1.1, 43) for baseline and incident HGD without cytometric abnormalities, respectively. It is clear that there is a great incentive to find biomarkers that distinguish the subset of HGD patients who will progress from those who will not, and there is considerable evidence that not all HGD patients should be subjected to invasive intervention (47). The higher cancer incidence in our baseline HGD patients might be due to lead-time bias or referral of high-risk cases because our center has been one of the few willing to survey HGD.

Endoscopic surveillance based on a five-tiered classification system for dysplasia and cancer has been recommended for clinical management of Barrett's esophagus, but this classification has not been shown to be reproducible in formal, blinded studies (26–29). For example, reanalysis of data from eight readers in a previous study (26) showed an average, pair-wise agreement of 48% in a five-tiered classification. Pending validation of the reproducibility of our classification criteria in formal, blinded multicenter studies, we recommend caution in therapeutic interventions for diagnoses other than cancer, based on our observations of HGD outcome, because there is evidence that other centers may have different rates of progression depending on diagnostic criteria or other factors (47, 48). In the absence of consensus, we recommend that HGD be confirmed by at least two experienced pathologists on at least two endoscopic evaluations before considering therapeutic intervention.

Future studies may refine histological criteria for dysplasia by using progression to cancer as a "gold standard," but flow cytometry presently complements histology in risk assessment. For example, observer agreement seems to be highest (86%) when the diagnoses of negative, indefinite, and LGD are combined (26), and flow cytometry separates these patients into low- and high-risk subsets with 5-yr cancer incidences of 0% and 28%, respectively. It might be suggested only patients with LGD need flow-cytometric analysis, but this ignores the potential for low reproducibility of this histological diagnosis, and many of our LGD

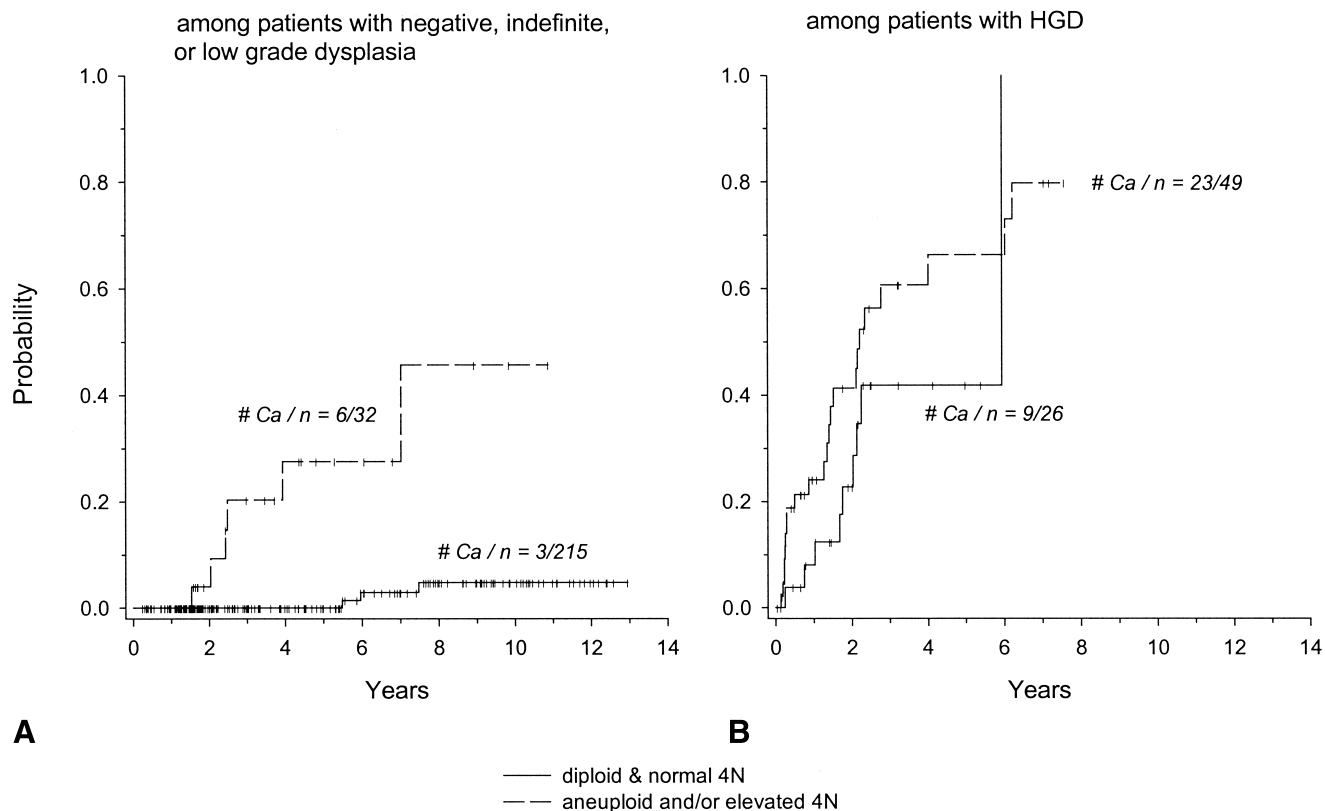


Figure 3. Cumulative incidence of cancer with histology and flow cytometry combined. (A) Negative, indefinite, or low-grade dysplasia with neither aneuploidy nor elevated 4N fractions or with either aneuploidy or elevated 4N. (B) Baseline high-grade dysplasia with neither aneuploidy nor elevated 4N or with either aneuploidy or elevated 4N.

patients might be diagnosed differently by other observers (26–29). Our data, as well as previously published data (12, 15, 25), are equivocal as to whether or not LGD is a significant predictor of cancer. However, Sontag *et al.* recently reported a 19-yr prospective study in which only 18 of 848 patients (2.1%) with LGD progressed to cancer (49). Thus, the data from both this and our own prospective studies suggest that only a small subset of patients with LGD will progress to cancer in cases of Barrett's esophagus.

Four studies over more than a decade have shown little progress in resolving sources of histological irreproducibility, especially in biopsies that are less than high grade (26–29). We are more hopeful that flow cytometry can be performed as an objective measure. There is an extensive body of literature on reducing sources of interlaboratory variation in flow cytometry, including sample handling, preparation, instrumentation, computer analysis, and histogram interpretation (50–52). Recent studies have shown that flow cytometry can be reproducible; for example, one large study found 94% interlaboratory agreement in a five-tiered classification system of ploidy (53). We are optimistic that interlaboratory differences in instrumentation will not be insurmountable, as we have found excellent agreement between ICP-22 (Partec GmbH, Münster, Germany) and Coulter Elite instruments (Beckman Coulter, Fullerton, CA) (data not shown). Furthermore, our finding of a 6% thresh-

old for elevated 4N fractions associated with histological abnormalities has been reproduced by another laboratory (33), and another laboratory also observed, during a prospective study of 30 patients, that individuals without flow cytometric aneuploidy did not progress to cancer during 5 yr (36). Although observer variation in flow-cytometric histogram interpretation seems to be less than for the diagnosis of dysplasia (54), we recommend confirmation of aneuploidy or increased 4N by a laboratory experienced in flow-cytometric analyses of Barrett's samples. We do not recommend surgical or endoscopic ablative therapy based solely on flow-cytometric findings. However, patients with aneuploidy or increased 4N may benefit from more frequent surveillance.

Our results demonstrate that aneuploidy, increased 4N fractions, and HGD are predictors of progression to cancer in Barrett's esophagus and that a four-quadrant biopsy protocol is superior to a more limited protocol. We and others have shown that endoscopic biopsy surveillance detects early, curable Barrett's adenocarcinomas (8, 9), and 95% of the cancers detected in the present study were T1 lesions. However, there is debate concerning optimal patient management because mortality from esophagectomy varies among institutions (55, 56), alternative therapies have not been shown to be superior in preventing or curing cancer, and prospective studies provide apparently different esti-

mates of rates of progression (47, 48). Our data support use of flow cytometry with histology in the evaluation of cancer risk in Barrett's esophagus. Patients who undergo our biopsy protocol and have negative, indefinite, or LGD biopsies without increased 4N or aneuploidy may have subsequent surveillance deferred for up to 5 yr. More frequent surveillance can be reserved for patients with cytometric abnormalities, and management of HGD can be individualized based on careful risk-benefit calculations (9).

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