Brushing cytology with adjunctive FISH and biomarker analyses is highly sensitive and specific in the early detection of low-grade dysplasia in Barrett's Esophagus

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INTRODUCTION

Four-quadrant biopsies are utilized to evaluate the morphology of Barrett's esophagus (BE), and to detect early neoplastic changes in the metaplasia-dysplasia-adenocarcinoma sequence. Studies suggest that brushing cytology analysis seems superior to traditional biopsy interpretation based on sampling error reduction, better patient compliance and less time consumption. In this study, we explore the genomic alterations and protein expression profiles on brushing cytology samples of BE patients. Compared to biopsy, cytologic assessment along with molecular genomic assays, DNA ploidy analysis and protein expression profiles maybe more reliable in the detection of low-grade dysplasia (LGD) in BE, and provide additional objective methods in the surveillance of BE patients.

DESIGN

96 patients visited two independent gastroenterology clinics from 5/01/2015 to 8/31/2016. Results of brushing cytology specimens from these patients were compared with concurrent biopsy samples when available. Immunohistochemical analyses (Ki-67, p53 and AMACR) and FISH assays to detect genomic alterations were applied on the cytology specimens. FISH assay A was composed of a green probe for detecting the copy number of the ERBB2 at 17q12, a golden probe for detecting the copy number of P16 at 9p21, and an aqua control probe specific to the chromosome 7 centromere. FISH assay B is composed of a green probe for detecting the copy number of the MYC gene at 8q24, a red probe for detecting the copy number of the ZNF217 at 20q13, and an aqua control probe specific to the chromosome 7 centromere. Samples were classified as positive based on a 99% confidence threshold (100 cell count) respective for each target: 17q12 (>=2 cells), 8q24 (>= 1 cell), 20q13 (>=1 cell), and 9p21 (>=4 cells). Statistical analysis was performed to determine the concurrence rates between cytology and biopsy results as well as the significance of complementary biomarker and genomic events. Logistic regression model was applied to obtain a scoring system composed of the following events: presence of cytolologic dysplasia, presence of p53 mutation (>5%), increased Ki-67 expression (>5%), loss of p16 gene and presence of aneuploidy (score 0 = negative for all event, score 1= 1/5 event positive, score 2 = 2/5 events positive, score 3 = 3/5 events).

TABLE 1. Patient demographics and clinical characteristics

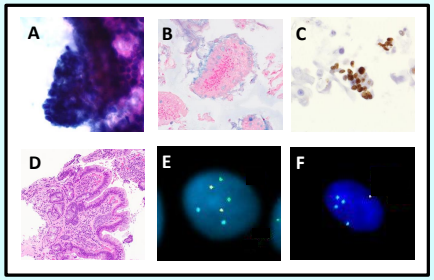
	Female	Male	Overall
Patient number	21	75	96
Medium age (range)	66 (51-81)	61 (20-80)	62.5 (20-81)
BE surveillance	4 (19%)	23 (31%)	27 (28%)

TABLE 2. Cyto-histopathology Correlation

Cyto \ Histo	LGD	ND
LGD	21	9
ND	0	51

ND: negative for dysplasia.

FIGURE 1. Cytology, histopathology and major molecular alterations



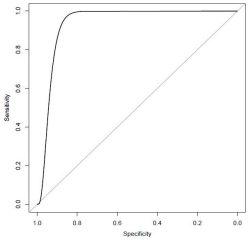
A: Cytologic LGD, H&E 600 x; B: Alcian blue staining shows intestinal metaplasia, 200 x; C: Ki-67 positive in LGD cell block, 600 x; D: Biopsy LGD, H&E 600 x ; E: Cell with normal ploidy; F: Cell with loss of p16.

TABLE 3. Cytologic and molecular events with p values, sensitivity and specificity

	LGD (%)	ND (%)	P value	Sen	Spe
Cytologic LGD	21 (100%)	9 (15%)	<0.05*	0.905	0.867
Ki 67	19 (90%)	6 (10%)	<0.05*	1.000	0.881
P53	18 (86%)	23 (31%)	<0.05*	1.000	0.881
P16	12 (57%)	9 (15%)	<0.05*	0.571	0.850
ERBB1	1 (5%)	2 (3.3%)	0.77	0.048	0.983
Aneuploidy	19 (90%)	6 (10%)	<0.05*	1.000	0.893
H/O BE	9 (43%)	18 (30%)	0.28	N/A	N/A

*: biostatistical significance.
Sen: sensitivity; Spe: specificity; ND: negative for dysplasia.
Ratios, p values, sensitivity and specificity of chromosomal gains of CMYC and ZNF 217, and AMACR protein expression abnormalities were also calculated, however, did not show biostatistical significance.

FIGURE 2. ROC analysis to identify the diagnostic value of new model.



logit[P(bx=1)]=-3.76 + 1.11*score

Area under curve (AUC)= 0.92

P value < 0.005

The ROC curve was adopted using the scoring system (scores 0-5) composed of cytomorphologic abnormalitie, Ki-67, p53, loss of p16 and presence of aneuploidy. P(bx=1) is the probability of biopsy results to be positive.

RESULTS

- All 96 consecutive cases with brush cytology also had biomarker and genomic FISH assay results. 81 of these cases had concurrent histopathology results.
- Among the 81 cases, 30 cases were diagnosed as LGD by cytology, FISH and biomarker assays; however, histopathology showed LGD in only 21 cases (Tables 1 & 2).
- 9 out of 81 cases showed no dysplasia on histopathology despite positive results of LGD by cytology. All of these 9 cytology positive cases except one also had p16 gene loss and significant p53 mutation (> 5%).
- Brushings cytology was highly sensitive (0.905) and specific (0.867) in detecting LGD, on histopathologic correlation.
- Cytologic LGD, aneuploidy, loss of p16 gene, increased Ki-67 expression and p53 mutation, correlate with LGD on biopsy (Figure 1 and Table 3).
- Chromosomal gains of *ERBB2*, *CMYC*, *ZNF217* and AMACR protein expression did not correlate with the presence of LGD (Table 3).
- Our scoring system combining cytologic findings and major molecular alterations display highly positive correlation with the diagnosis of LGD on histopathology (Figure 2).

CONCLUSIONS

- BE brushing cytology is highly sensitive (0.905) in detecting LGD.
- Loss of p16 gene and p53 mutation are common in BE patients with LGD. Chromosomal gains of *ERBB2*, *CMYC*, *ZNF217* and AMACR protein expression did not correlate with the presence of LGD.
- In cases with histopathology and cytology discrepancy, loss of p16 and p53 mutation corroborated with the cytology findings of LGD. This consistency between cytomorphology and p16 gene loss may suggest their early predictive roles in the progression of dysplasia in BE patients.
- We hereby propose a scoring system based on cytologic assessment with adjunctive molecular alterations, along with histopathological correlation.

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