

# Nonadenomatous Dysplasia in Barrett Esophagus

## A Clinical, Pathologic, and DNA Content Flow Cytometric Study

Rachel L. Rucker-Schmidt, MD,\* Carissa A. Sanchez,† Patricia L. Blount MD,† ‡  
 Kumran Ayub, MD,§|| ¶ Xiahong Li, PhD,† Peter S. Rabinovitch, MD, PhD,#  
 Brian J. Reid, MD, PhD,† ‡\*\* and Robert D. Odze, MD, FRCPC\*

**Abstract:** Rarely, dysplasia in Barrett's esophagus (BE) is composed of crypts lined by cuboidal-shaped cells that contain a centrally located nucleus, markedly increased nuclear/cytoplasmic ratio, but without nuclear stratification characteristic of conventional "adenomatous" dysplasia. The aim of this study was to evaluate the clinical and pathologic features, natural history, and DNA content flow cytometric abnormalities of BE patients with non-adenomatous dysplasia (NAD) in a cohort of BE patients enrolled in a prospective surveillance program. Eighteen patients with NAD identified over a 6 year period, in a cohort of 270 consecutive patients with BE and without esophageal adenocarcinoma (EA) at baseline, were evaluated for clinical and pathologic features, including association with conventional adenomatous dysplasia and EA, DNA content flow cytometric abnormalities (tetraploidy and aneuploidy) and outcome, over a mean follow-up period of 4.1 years. The findings in the 18 study patients were compared to those in the 252 remaining (control) patients without NAD. Control patients included 228 with metaplasia/indefinite for dysplasia, and 24 with conventional adenomatous dysplasia (13 low-grade, 11 high-grade). The prevalence rate of NAD in our BE cohort was 6.7%. Of the 18 study patients, there were 17 were males and 1 female of mean age 66.7 years. The mean length of BE was 3.9 cm NAD foci were associated with goblet or non-goblet epithelium in 62% and 38% of cases, respectively. Ninety-four percent of patients with NAD (17/18) also had conventional adenomatous dysplasia (four with low-grade, 13 with high-grade) elsewhere in the esophagus at the same endoscopic procedure as the one that detected NAD. Patients with NAD had a significantly shorter length of BE compared to control patients with conventional adenomatous dysplasia (N=24)

( $p=0.03$ ). Patients with NAD also showed a significantly higher rate of DNA content flow cytometric abnormalities compared to the entire cohort of control patients (38% vs. 11%,  $p=0.05$ ). However, no significant differences regarding either flow cytometric abnormalities or progression to EA were found when the NAD patients were compared only to the 24 controls with conventional adenomatous dysplasia. NAD is a high grade histologic variant of intraepithelial neoplasia that is episodic in nature, and shows a high association with conventional adenomatous high-grade dysplasia.

**Key Words:** dysplasia, Barrett esophagus, esophageal adenocarcinoma, aneuploidy, tetraploidy

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Patients with Barrett esophagus (BE) are at increased risk for the development of esophageal adenocarcinoma (EA).<sup>2,3</sup> EA evolves through a metaplasia, dysplasia, carcinoma sequence, although most patients with BE do not progress to EA during their lifetimes.<sup>5</sup> At present, the morphologic identification of dysplasia in endoscopic mucosal biopsies is the standard method of detecting patients at increased risk for EA.<sup>20</sup> Traditionally, dysplasia is categorized as either low-grade or high-grade based on the degree of cytologic and/or architectural atypia of the epithelium.<sup>10,15</sup> Atypical features include nuclear hyperchromasia and pleomorphism, nuclear stratification, increased mitoses, loss of nuclear polarity, and mucin depletion. With higher grades of dysplasia, architectural abnormalities, such as crypt crowding and increased crypt branching, may be present as well.

In general, the features of dysplasia in BE resemble those seen in colonic adenomas because of the presence of elongated pencil-shaped nuclei with clumped chromatin and prominent nuclear stratification. As a result, this type of dysplasia has been referred to as "adenomatous" dysplasia anecdotally. However, rarely, dysplasia does not resemble the type seen in colonic adenomas, but instead is composed of crypts with a more prominent back-to-back gland pattern, containing cells that are more cuboidal in shape with high nuclear/cytoplasmic ratio, round to oval-shaped nuclei with an open chromatin pattern and prominent nucleoli. This type of dysplasia is not characterized by nuclear stratification typical of adenomatous dysplasia, and, as a result, has been termed "nonadenomatous" (NAD) or "foveolar"

From the \*Department of Pathology, Harvard Medical School and Brigham and Women's Hospital, Boston, MA; †Divisions of Human Biology and Public Health Sciences, Fred Hutchinson Cancer Research Center; ‡Departments of Medicine; #Pathology; \*\*Genome Sciences, University of Washington, Seattle, WA; §Provena St Joseph Medical Center, Joliet; ||Adventist Bolingbrook Hospital, Bolingbrook; and ¶Advocate Christ Medical Center, Oak Lawn, IL. Financial Support: NIH P01 CA91955 (C.A.S., P.L.B., K.A., X.L., R.D.O., P.S.R., B.J.R.).

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Correspondence: Robert D. Odze, MD, FRCPC, Department of Pathology, Harvard Medical School and Brigham and Women's Hospital, 75 Francis St. Boston, MA 02115 (e-mail: mailto:rodze@partners.org).

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dysplasia anecdotally.<sup>12</sup> The histologic features have been noted, briefly, in previous reports.<sup>10,15</sup> However, no specific clinical, pathologic, or outcome study has been reported on this entity. Therefore, the aim of this study was to systematically evaluate the prevalence, clinical and pathologic features, and natural history, of a prospectively acquired cohort of high-risk patients with BE and NAD who have been clinically followed with a standardized and rigorous endoscopic surveillance protocol.

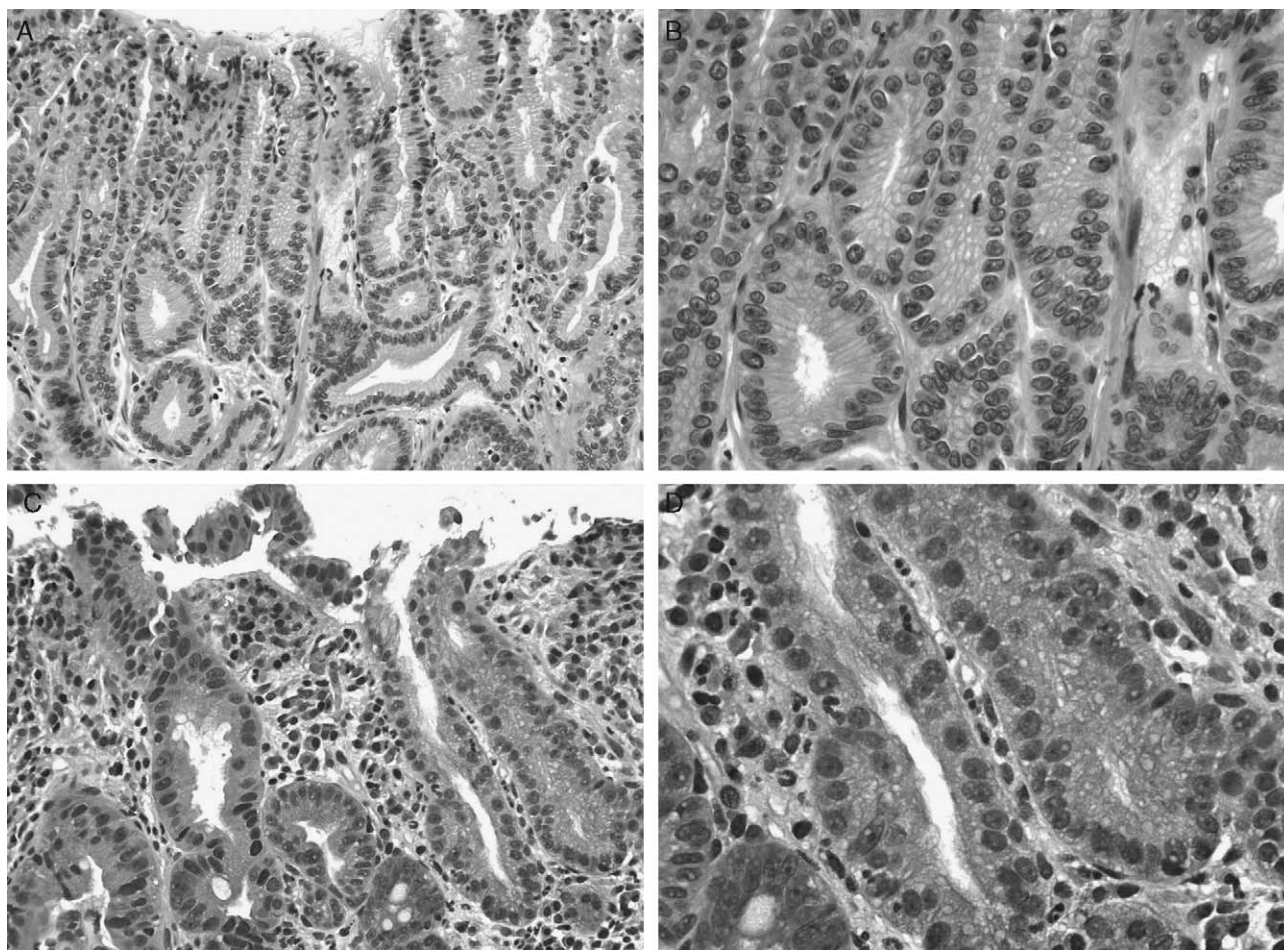
## MATERIALS AND METHODS

### Patient Cohort and Study Design

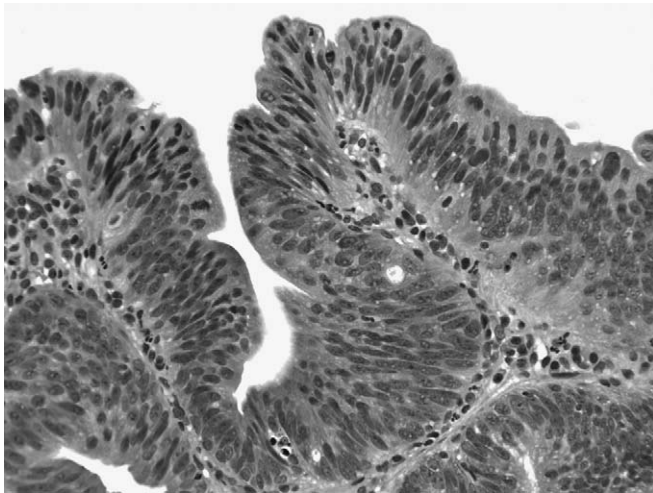
The Seattle Barrett's Esophagus Study is an ongoing cohort study in which clinical, endoscopic, pathologic, and DNA content flow cytometric data are evaluated prospectively. The Seattle Barrett's Esophagus

Study was approved by the Human Subjects Division of the University of Washington in 1983 and renewed annually thereafter with reciprocity from the Fred Hutchinson Cancer Research Center (FHCRC) Institutional Review Board (IRB) from 1993 to 2001. Since 2001, the study has been approved by the FHCRC IRB with reciprocity from the University of Washington Human Subjects Division.

Endoscopic biopsy protocols used in the Seattle Barrett's Esophagus Study have been published previously.<sup>6</sup> Four quadrant biopsies for histology were taken every 1 cm (for high-risk patients with high-grade dysplasia or DNA content abnormalities) or every 2 cm (for low-risk patients without high-grade dysplasia or DNA content abnormalities) at intervals ranging from every 6 months (for high-risk patients) to 3 years, as described previously.<sup>16</sup> Endoscopic biopsies from every



**FIGURE 1.** A, Medium power view of a focus of NAD in a patient with BE. The epithelium shows a back-to-back arrangement of crypts containing basally located, round, oval-shaped nuclei, open chromatin pattern, and prominent nucleoli. Loss of cell polarity is minimal, except for focally at the surface epithelium. There is no significant nuclear stratification. B, High power view showing the nuclear features, which consist of oval-shaped irregular nuclei, peripheral condensation of chromatin, open chromatin pattern and prominent nucleoli. C, Another BE patient with NAD showing less of a back-to-back crypt pattern and slightly more chronic inflammation in the lamina propria. In this particular biopsy, the bases of the crypts show a slight degree of crypt branching. D, High power view of the same area showing the characteristic nuclear features of NAD. BE indicates Barrett esophagus; NAD, nonadenomatous.



**FIGURE 2.** Focus of conventional adenomatous dysplasia (high-grade) in a patient with NAD. In contrast to NAD, conventional adenomatous dysplasia shows enlarged hyperchromatic pencil-shaped nuclei with clumped chromatin and prominent stratification. In this case, full thickness nuclear stratification and significant loss of cell polarity warrant a diagnosis of high-grade conventional adenomatous dysplasia. NAD indicates nonadenomatous.

2-cm, for flow cytometry, were placed into media with 10% DMSO (dimethyl sulfoxide) within 15 seconds and held on ice until frozen and stored at  $-70^{\circ}\text{C}$ .

For this study, we analyzed 270 of the 309 patients in the cohort, prospectively, from July 2001 to February 2008. Thirty-nine patients without endoscopically visible BE or EA at their baseline endoscopy were excluded from the analysis. The 270 remaining BE patients were categorized as either “study” (ie, those with NAD) or “control” patients (ie, those without NAD). The 2 groups of patients were compared for their clinical, endoscopic, pathologic, natural history, and DNA content flow cytometric data. All patients had metaplastic columnar epithelium, with goblet cells, in esophageal biopsies confirmed by evaluation of one of the authors (R.D.O.) at the Brigham and Women’s Hospital, Boston, MA. Clinical features evaluated include sex, age at the time of diagnosis, and the length of BE as defined by the total centimeter of Barrett mucosa spanning the ora serrata

and the distal end of the esophagus or lower esophageal sphincter. The follow-up interval was calculated as the length of time (in mo) from the patient’s initial diagnosis of NAD for the study patients, and from the baseline biopsy in the control patients, to the time of the most recent follow-up endoscopy, or endoscopic mucosal resection.

**Pathology Methods**

NAD was defined by the presence of crypts with a back-to-back gland pattern, cuboidal-shaped cells with a high nuclear/cytoplasmic ratio, slight pleomorphism, round-shaped to oval-shaped nuclei, and, in many instances, prominent nucleoli (Fig. 1). In contrast to adenomatous dysplasia, NAD does not show elongated pencil-shaped nuclei with clumped chromatin, nor stratification of the nuclei, features more typical of colonic adenomas (Fig. 2). In addition, none of the NAD foci were associated with erosions, ulcers or active (neutrophilic) inflammation in the lamina propria or epithelium. Adenomatous dysplasia was categorized as either low-grade or high-grade according to previously published criteria.<sup>18</sup> EA was defined by the presence of neoplastic cells infiltrating the lamina propria and/or submucosa.

A total of 44 BE biopsies (range, 1 to 8) from 28 surveillance endoscopies (range, 1 to 4 endoscopies/patient) in the 18 study patients with NAD were evaluated for a wide variety of pathologic features including the distribution of dysplastic foci (focal = present in 1 biopsy, diffuse = present in  $\geq 1$  biopsy), location of crypt and/or surface epithelial involvement, cell type of the adjacent nondysplastic epithelium (nongoblet versus goblet cell), relationship to the neo-squamocolumnar junction, and relationship to areas of conventional (adenomatous) dysplasia when present. Adenomatous dysplasia was assessed in NAD patient biopsies obtained from either the same, or subsequent, endoscopic procedures during the study period, and graded as either low-grade or high-grade.

**Flow Cytometry Methods**

Fresh frozen biopsies obtained from the endoscopic procedure from each of the study and control patients were evaluated prospectively for DNA content flow cytometry.<sup>14,16</sup> Cell cycle analysis was performed on 71 BE biopsies from 13 of 18 study patients with NAD and

**TABLE 1.** Clinical Features of Study Patients and Controls

Patient Group	N	Male/Female Ratio	Mean Age (Range)	Feature	
				Mean Length of Barrett’s in cm (Range)	Mean Follow-up (mo)
Nonadenomatous dysplasia	18	17/1	66.7 (44-80)	3.9 (0.1-7.0)	52
Controls	252	206/46	64 (33-94)	5.0 (0-17)	46
Metaplasia/indefinite	228	186/42	64 (41-94)	4.9 (0-17)	46
Adenomatous dysplasia	24	20/4	63 (35-88)	6.0 (0-15)*	44
Low-grade	13/24	11/2	65 (41-80)	5.8 (0-11)	48
High-grade	11/24	9/2	60 (35-88)	7.0 (1-15)	36

\*P = 0.03 Nonadenomatous dysplasia (n = 18) versus adenomatous dysplasia (n = 24).

**TABLE 2.** Histologic Features of 18 Study Patients With Nonadenomatous Dysplasia

Feature	Prevalence (%)
Nonadenomatous dysplasia involvement of crypts and surface	43/47 (91.5)
Nonadenomatous dysplasia involvement of crypts only	4/47 (8.5)
Associated with nongoblet epithelium	18/47 (38)
Associated with goblet cell epithelium	29/47 (62)
Adjacent to squamous mucosa	24/47 (51)
Beneath squamous mucosa	2/47 (4)
No association with squamous mucosa	21/47 (45)
Significant architectural distortion	8/47 (17)*
Adenomatous dysplasia on current biopsy	17/18 (94)
Low-grade	4/17 (25)
High-grade	13/17 (76)

\*8 foci from 2 patients.

1,397 BE biopsies from 235 of 252 control patients. Tetraploidy and aneuploidy were defined as described previously.<sup>16</sup> Flow cytometric data was not restricted to 1 biopsy per 2-cm level of esophagus.

### Statistical Analysis

Statistical comparisons of clinical, histologic, and flow cytometric data for discrete counts were performed using Fischer exact test. Nonpaired *t* test was used for comparison of means. A *P* value of < 0.05 was considered the threshold for statistical significance. All of the analyses were performed with Statistical Analysis System (SAS) software (version 9.0; SAS Institute, Carrey, North Carolina, NC).

## RESULTS

### Clinical Data

The clinical and endoscopic features of the study patients with NAD and controls are summarized in Table 1. NAD was observed in 18 (6.7%) of 270 BE patients evaluated prospectively. The 252 controls were further subdivided into patients with or without conventional adenomatous dysplasia for further comparison

with the NAD study group. At their baseline endoscopy, the control group included 228 of 252 (91%) with metaplasia or indefinite for dysplasia and 24 (10%) with adenomatous dysplasia (13 low-grade and 11 high-grade). Seventeen of the 18 (94%) NAD patients also had associated adenomatous dysplasia (four low-grade and 13 high-grade). The study group with NAD consisted of 17 males and 1 female of mean age 66.7 years (range, 44 to 80 y). The control patients without NAD consisted of 206 males and 46 females with a mean age of 64 (range, 33 to 94 y). The mean length of BE in the NAD patients was 3.9 cm (range, 0.1 to 7.0 cm) compared with a mean of 6.0 cm (range, 0.1 to 15 cm) in the subgroup of control patients with adenomatous dysplasia (*P* = 0.03). Otherwise, there were no significant differences in the clinical or endoscopic features between the study patients with NAD compared with the control patients in total, or with the subgroups of controls. Study patients were followed for a mean of 52 months (range, 10 to 78), which was similar to the mean of 46 months (range, 1 to 80) for the control group.

### Pathology Results

Of the 18 study patients, NAD was documented in 44 separate mucosal biopsies (range, 1 to 8) from 28 surveillance endoscopies (range, 1 to 4 endoscopies/patient) (Fig. 1). A summary of the pathologic characteristics of NAD in the 18 study cases is provided in Table 2. In 13 patients, NAD was detected in 1 biopsy fragment and in 5, it was detected in multiple (> 1) biopsies from different levels of the esophagus. NAD showed goblet cell differentiation in 4 cases, and mucinous differentiation in 3. Foci of NAD most commonly involved both the crypts and surface epithelium (full crypt involvement), but involved only the bases of the crypts in 4 biopsies (8.5%). NAD was more frequently associated with metaplastic epithelium with goblet cells (62%) compared with nongoblet epithelium (38%). It was most commonly found adjacent to the neo-squamocolumnar junction (51%). None of the NAD foci were associated with active inflammation or ulceration.

Most (17/18; 94%) patients with NAD also had foci of conventional adenomatous dysplasia in the same endoscopic procedure in which the NAD was initially

**TABLE 3.** DNA Content Flow Cytometric Results of Study and Control Patients

Patient Group	N	Flow Abnormality		
		Tetraploidy or Aneuploidy (1 Abnormality)	Tetraploidy and Aneuploidy (2 Abnormalities)	Tetraploidy and/or Aneuploidy (1, 2, or Both Abnormalities)
Nonadenomatous dysplasia	13	3 (23%)	2 (15%)	5 (38%)
Controls	235	21 (8%)*	4 (2%)†	25 (11%)†
Metaplasia/indefinite	218	17 (7%)*	2 (0.9%)†	19 (8%)†
Adenomatous dysplasia	17	4 (24%)	2 (12%)	6 (35%)
Low-grade	9	3 (33%)	1 (11%)	4 (44%)
High-grade	8	1 (13%)	1 (13%)	2 (25%)

\**P* < 0.09 versus nonadenomatous dysplasia.

†*P* < 0.05 versus nonadenomatous dysplasia.

**TABLE 4.** Progression Status of Study and Control Patients Upon Follow-up

Patient Group	N	Maximum Diagnosis in Follow-up			
		Metaplasia/Indefinite (%)	Dysplasia Grade		EA (%)
			Low (%)	High (%)	
Nonadenomatous dysplasia	18	1 (6)	0 (0)	14 (78)	3 (17)
Concurrent low-grade†	4			3 (75)	1 (25)
Concurrent high-grade†	13			11 (84)	2 (15)
Controls	252	181 (72)	41 (16)	18 (7)	12 (5)
Metaplasia/indefinite	228	181 (79)	35 (15)	5 (2)	7 (3)*
Adenomatous dysplasia	24	—	6 (25)	13 (54)	5 (21)
Low-grade	13	—	6 (46)	4 (31)	3 (23)
High-grade	11	—	—	9 (82)	2 (18)

\* $P < 0.05$  versus nonadenomatous dysplasia.

†Refers to concurrent adenomatous dysplasia.

EA indicates esophageal adenocarcinoma.

discovered (Fig. 2). Of the 17 NAD patients with conventional adenomatous dysplasia, 4 had associated low-grade and 13 had associated high-grade as the highest degree of dysplasia other than NAD.

### DNA Content Flow Cytometric Results

DNA content flow cytometric data was available for 13 (72%) of the 18 NAD patients within 6 months of the endoscopy and for 235 (93%) of the 252 controls at their baseline endoscopy. The flow cytometric data is summarized in Table 3. In five of 13 (38%) NAD patients we detected either tetraploidy or aneuploidy, or both, in biopsies obtained at endoscopy. In 3 patients with NAD (23%), we detected tetraploidy in 2 and aneuploidy in 1. However, 2 (15%) patients with NAD had both tetraploidy and aneuploidy detected at endoscopy. In the control group, we detected a significantly lower proportion of DNA content flow cytometric abnormalities of tetraploidy and/or aneuploidy, in 25 (11%) of patients ( $P < 0.05$ ). Twenty-one (8%) patients had either tetraploidy or aneuploidy detected at endoscopy, and 4 (2%) had both tetraploidy and aneuploidy detected. When analyzed and compared with the control subgroups, a significant increase in flow cytometric abnormalities were observed in the NAD group compared with the controls with metaplasia/indefinite for dysplasia group ( $P < 0.05$ ). However, no significant differences were observed by flow cytometry between the study patients with NAD and the control patients with conventional adenomatous dysplasia, or between the study patients and each individual control group with either low-grade or high-grade adenomatous dysplasia.

### Follow-up Data

Table 4 summarizes the follow-up data for the BE patients evaluated in this study. Eighteen study patients with NAD underwent 56 endoscopies during the study period (range, 1 to 5 per patient). The 252 control BE

patients without NAD underwent 846 endoscopies during the same time period (range, 1 to 13).

Of the 18 patients with NAD, 1 (6%) showed no evidence of dysplasia upon follow-up, 14 (78%) showed conventional adenomatous dysplasia and 3 (17%) developed EA. Seventeen of 18 (94%) had concurrent conventional adenomatous dysplasia. Three of 4 (75%) NAD patients with concurrent low-grade adenomatous dysplasia progressed from the initial NAD observation to high-grade adenomatous dysplasia over a mean of 23.7 months and 1 progressed to EA at 11.6 months. Two (15%) of the 13 NAD patients with concurrent conventional adenomatous high-grade dysplasia progressed to EA over 37.1 months. The follow-up of the 4 NAD patients with concurrent low-grade adenomatous dysplasia was similar to the 13 NAD patients with associated conventional high-grade dysplasia in which 11 (85%) showed further foci of high-grade adenomatous dysplasia and 2 (15%) developed EA upon follow-up.

Separate follow-up comparisons were performed on the study patients with NAD and the control patients with either conventional low-grade or high-grade adenomatous dysplasia. As noted in Table 4, the development of conventional adenomatous dysplasia and EA was significantly higher in patients with NAD (17%) compared with the control group (5%) in total, and when compared with the subgroup of control patients with metaplasia or indefinite for dysplasia in their baseline biopsies ( $P < 0.05$ ). However, no differences were noted in the frequency of development of high-grade adenomatous dysplasia or EA between the patients with NAD and the control patients with adenomatous dysplasia in their baseline biopsies. Of the control patients with adenomatous dysplasia, 13 (54%) and 5 (21%) ultimately developed these changes within a mean of 19.8 and 18.0 months, respectively. Further, the prevalence rate and follow-up time to EA detection was similar between the study patients with NAD compared with the control patients without NAD, but with either conventional low-grade or high-grade adenomatous dysplasia.

## DISCUSSION

Most cases of dysplasia arising in BE are characterized by a proliferation of cells with elongated pencil-shaped hyperchromatic nuclei with clumped chromatin, and stratification, features that resemble the appearance of dysplastic cells in colonic adenomas.<sup>10,15</sup> As a result, this has been referred to, rather anecdotally, as “adenomatous” dysplasia. However, rarely, BE-related dysplasia may be composed of cells with cuboidal, instead of elongated, shape, round to oval nuclei with an open chromatin pattern and prominent nucleoli, a high nuclear/cytoplasmic ratio, but without nuclear stratification characteristic of adenomatous dysplasia. Unfortunately, neither the association of the 2 types of dysplasia nor the clinical significance of NAD has been investigated previously. Thus, the purpose of this study was to evaluate the clinical, morphologic, DNA content flow cytometric abnormalities, and, particularly, the natural history of patients with NAD.

In this study, the clinical, pathologic, DNA content flow cytometric and follow-up findings of 18 BE patients with at least 1 focus of NAD documented in endoscopic surveillance mucosal biopsies of the esophagus were evaluated and compared with a control group of 252 BE patients without NAD. In addition, to compare and contrast the features of patients with NAD to the controls with adenomatous dysplasia, separate analyses were performed between the study patients with NAD and the subgroup of control patients with adenomatous dysplasia, either low-grade or high-grade. The prevalence rate of NAD in our high-risk BE cohort was 6.7%. NAD occurred in goblet rich epithelium in 62% of foci, and nongoblet epithelium in 38% of foci. One of the striking findings of our study was the close association between NAD and conventional adenomatous dysplasia; 94% of the patients (17/18) with NAD also had conventional adenomatous dysplasia. Of the foci of associated adenomatous dysplasia, 77% were high-grade and 23% were low-grade. Thirty-eight percent of NAD patients showed at least 1 DNA content flow abnormality (tetraploidy and/or aneuploidy) in biopsies obtained at the same endoscopic procedure and although this value was significantly higher than control patients without dysplasia or with indefinite for dysplasia, no significant differences were observed compared with control patients with adenomatous dysplasia, either low-grade or high-grade. Upon follow-up, all study patients, except 1 (95%), developed further foci of adenomatous high-grade dysplasia (78%) or invasive carcinoma (17%). Only 1 (7%) of 14 patients developed further foci of NAD. Progression to EA in patients with NAD and associated adenomatous dysplasia was similar to control patients with adenomatous dysplasia. The only statistically significant results occurred when a comparison was made between NAD patients as a group with the subset of controls without dysplasia. On the basis of these data, we conclude that NAD is a high-grade histologic variant of intraepithelial neoplasia and should be managed similarly

to patients with conventional adenomatous high-grade dysplasia.

This is the first study to objectively evaluate the biologic characteristics of NAD. In the original BE-associated dysplasia interobserver study by Reid et al<sup>15</sup> in 1988, which was based on a newly conceived classification of dysplasia into negative, indefinite, and positive (either low-grade or high-grade), NAD was not specifically evaluated. In fact, the number of cases of dysplasia that had adenomatous-like nuclear stratification versus non-stratified nuclei was not documented. The authors of that study commented that “although nuclear stratification was found frequently in association with dysplasia, stratification alone was insufficient to make the diagnosis (of dysplasia).” Interestingly, the case illustrated in Figure 13 of that study shows cytologic features similar to the NAD cases evaluated in our current study. In the Reid et al study, that case was felt to represent low-grade dysplasia by half of the observers and high-grade dysplasia by the other half. The lack of nuclear stratification led the observers to conclude that the cytologic features were low-grade, but the observers who considered the histologic features as indicative of high-grade, did so on the basis of architectural distortion, such as the presence of a compact back-to-back gland arrangement. In fact, the authors noted that some morphologists were reluctant to assign a diagnosis of high-grade dysplasia in what they perceived to be an absence of both architectural and cytologic abnormalities. However, others felt that either one or the other of these features alone was sufficient to render a diagnosis of high-grade dysplasia.

In the only other study that evaluated specific histologic features of dysplasia in BE, Montgomery et al<sup>10</sup> formulated more stringent criteria for dysplasia in BE and tested the reproducibility of 125 cases with 12 gastrointestinal pathologists. In contrast to the results by Reid et al, in the study by Montgomery et al, high-grade dysplasia was determined to be present if either high-grade cytologic changes or significant architectural distortion (characterized by little or no lamina propria between the crypts) were identified in biopsy specimens. However, in that study, distinction between adenomatous and NAD features was not specifically addressed. In fact, the criteria for dysplasia in that study included “stratification often extending from the epithelial basement membrane to the luminal surface.” However, the authors did note that “prominent irregular nuclei with irregularly clumped chromatin and irregular nucleoli are a feature of high-grade dysplasia.” These are features reminiscent of NAD in our study. Interestingly, Figure 10 in the Montgomery et al paper, representing a case of dysplasia fixed in Hollande’s fixative shows focal changes of NAD characterized by cuboidal, instead of elongated, cells, with round-oval shaped nuclei and lack of nuclear stratification. In that study, it was felt that this focus of dysplasia could not be graded accurately. Of the 24 readings, 11 observers diagnosed low-grade dysplasia, 10 high-grade dysplasia, and 3 intramucosal

adenocarcinoma. Similarly, in a follow-up study from the same group of investigators based on the cases from the initial diagnostic variability study, distinction between adenomatous dysplasia and NAD was not made with regard to outcome.<sup>11</sup>

Most forms of dysplasia in the gastrointestinal tract, such as that which occurs in the stomach or colon, are composed of columnar cells with elongated pencil-shaped nuclei with stratification.<sup>13,18</sup> However, NAD histologic variants of dysplasia have been described in other organs, such as the stomach.<sup>13</sup> For instance, 1 type of gastric dysplasia is composed of glands of variable size and shape, and cells that are cuboidal or low-columnar with clear eosinophilic cytoplasm and round-oval shaped vesicular nuclei with nucleoli. This type of dysplasia has been referred to as “Type 2,” “hyperplastic” or “foveolar” dysplasia.<sup>13</sup> The nuclear features of gastric foveolar dysplasia are reminiscent of NAD in BE. Although no outcome studies have been performed on foveolar dysplasia of the stomach, some authors have noted an association with poorly differentiated adenocarcinoma of the intestinal type, and nonmetaplastic foveolar, or pyloric gland type, gastric epithelium. Interestingly, 38% of our patients developed NAD within nongoblet epithelium. Although, historically, dysplasia in BE has been noted to develop predominantly in intestinalized epithelium (ie, epithelium with goblet cells), a recent study by Takubo et al<sup>19</sup> showed that 70% of 141 cases of primary small (<2 cm) adenocarcinomas of the esophagus were situated adjacent to cardia or fundic-type mucosa rather than intestinal-type mucosa with goblet cells.

Previous studies by our group, as well as others, have shown that neoplastic progression in BE arises as a result of acquired genetic instability, and subsequent evolution of clonal populations with accumulated genetic lesions.<sup>1,4,7-9,14,16,17</sup> DNA content flow cytometry to identify patients with tetraploidy or aneuploidy has been shown to be an effective adjunct to histology in helping to stratify patients with BE into low-risk and high-risk subsets. For instance, a DNA content flow cytometric study by Reid and colleagues<sup>16</sup> in 2000 showed that patients without detectable DNA content abnormalities in their baseline biopsies had a 0% incidence of adenocarcinoma compared with a 28% incidence rate in BE patients with either aneuploidy or increased 4N fraction (tetraploidy). In our current study, 38% of the patients with NAD had increased tetraploidy or aneuploidy, which was statistically similar to the control patients with adenomatous dysplasia. Thus, based on this data, we do not feel that the presence of DNA content flow abnormalities, and specifically tetraploidy or aneuploidy, are associated with the development of NAD and probably reflect a general underlying instability of the epithelium in BE.

One of the limitations of this study is based on the fact that the Seattle Barrett’s esophagus cohort is a high-risk population of patients with BE, and, thus, may not necessarily be representative of the general population.

Thus, it is probable that the prevalence rate of NAD in our study, and of dysplasia in general, is higher in this cohort compared to the general population. Another difficulty of interpretation of our data is that the majority of patients with NAD (94%) in our study also had conventional adenomatous dysplasia in other biopsies from the same endoscopic procedure. Therefore, it is difficult to distinguish the contribution of adenomatous versus NAD when evaluating flow cytometric abnormalities or clinical follow up. Flow cytometric data and outcome data were statistically similar in the study and control groups, and a much larger study would be required to determine the independent significance of NAD and adenomatous dysplasia with regard to patient management.

In summary, we described the clinical, pathologic, DNA content flow cytometric, and outcome data of a cohort of high-risk BE patients who had NAD in their esophageal mucosal biopsies. NAD is a high-grade histologic variant of intraepithelial neoplasia, is often episodic in nature, and shows a high association with conventional adenomatous high-grade dysplasia. Further, molecular and prospective studies should be done to characterize the molecular phenotype that leads to NAD and the potential long-term sequela of patients with this type of dysplasia.

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