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Original contribution

Adenoma-like adenocarcinoma: clinicopathologic characterization of a newly recognized subtype of colorectal carcinoma☆,☆☆

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Summary The 5th edition of the World Health Organization (WHO) Classification of Tumours (Digestive System) recognizes a new subtype of colorectal adenocarcinoma, called adenoma-like adenocarcinoma. In this study, we sought to determine its clinicopathologic associations and how it is comparable with adenocarcinoma, of no special type (NOS). We retrospectively reviewed all available archival slides of stage I–III colonic adenocarcinoma resection specimens at our institution from 2013 to 2016.Ninety-one cases were classified as adenoma-like adenocarcinoma, and 251 cases were classified as adenocarcinoma, NOS. Of the adenoma-like adenocarcinoma cases, a majority (65 cases, 71%) were composed exclusively of adenoma-like features, designated as pure adenoma-like adenocarcinoma, whereas in the rest, the component of adenoma-like morphology was more than 50% but less than 100%, designated as mixed adenoma-like adenocarcinoma. Compared with adenocarcinoma, NOS, adenoma-like adenocarcinoma cases were significantly associated with the absence of tumor budding ($P < 0.001$), the absence of an immature/myxoid desmoplastic reaction ($P < 0.001$), the presence of intraepithelial tumor-infiltrating lymphocytes ($P = 0.006$), involvement of fewer lymph nodes ($P < 0.001$), fewer tumor deposits ($P = 0.042$), lower pT stage ($P = 0.047$), lower pN stage ($P < 0.001$), and consequently the pTNM prognostic group ($P < 0.001$), as well as better recurrence-free survival (RFS), as per univariate analysis than adenocarcinoma, NOS cases ($P = 0.026$) but not as per multivariate analysis. However, mixed adenoma-like adenocarcinoma had a worse RFS than pure adenoma-like adenocarcinoma (hazard ratio = 1.639, 95% confidence interval = 0.494–5.437). Our findings not only support the importance of distinguishing this new

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subtype of colorectal adenocarcinoma but also raise the question whether mixed adenoma-like adenocarcinoma cases should be included in this category, and if so, whether 50% is an appropriate cutoff, as currently defined by the WHO.

1. Introduction

The 5th edition of the World Health Organization (WHO) Classification of Tumours (Digestive System) has recognized a new subtype of colorectal adenocarcinoma, called adenoma-like adenocarcinoma [1]. Over the last few decades, several publications attempted subclassifying colorectal adenocarcinoma based on a distinctive histologic appearance, such as villous tumor [2,3], invasive papillary adenocarcinoma [4], and villous adenocarcinoma [5]. Based on the morphologic descriptions, it appears that some of them could be referring to this subtype that is now termed adenoma-like adenocarcinoma. Yao et al. [3] noted a well-differentiated morphology with less p53 protein expression in 17 villous carcinoma cases, but unfortunately no follow-up data were available. A concomitant study with 20 cases noted that these colorectal adenocarcinoma cases with a villous morphology were associated with involvement of fewer lymph nodes [2]. Another report of this new subtype of colorectal carcinoma was made by Palazzo et al. [4] in their description of two cases designated as invasive papillary adenocarcinoma. In their limited number of cases, an aggressive behavior was noted, with one patient dying of disease [4]. A more comprehensive review of 36 cases demonstrated an association with a favorable prognosis and raised the importance of awareness of this entity, given its diagnostic challenge due to its well-differentiated morphology [5]. In 2016, the term adenoma-like adenocarcinoma was introduced by Gonzalez et al. [6]. In their study, they noted frequent *KRAS* mutations and a lower rate of metastasis in this group of tumors.

Currently, adenoma-like adenocarcinoma cases are defined as per the WHO as an *invasive adenocarcinoma in which ≥50% of the invasive areas have an adenoma-like aspect with villous structures, with a low-grade aspect*. [1] The tumor is also associated with a pushing border and with minimal desmoplastic reaction [1]. In this study, we present the largest cohort of this entity and sought to validate the clinicopathologic implications. For this purpose, we used a large comparison group of colonic adenocarcinomas of no special type (adenocarcinoma, NOS).

2. Materials and methods

2.1. Patient population

Approval from the Institutional Review Board of Washington University School of Medicine was obtained before initiating the study. A retrospective search was performed for all colonic adenocarcinoma resection specimens in our database from 2013 to 2016. Cases with clinical or pathologic stage IV disease as per the American Joint Committee on Cancer 8th Edition [7] were excluded, to appropriately assess for recurrence-free survival (RFS). Rectal carcinomas were also excluded because majority of these cases receive neoadjuvant therapy and could alter morphologic assessments. A total of 372 cases of colonic adenocarcinoma stage I–III were identified, whose archival slides were available for review. Of those 372 cases, 30 cases were excluded from the study because they represented a special subtype of colorectal carcinoma (22 cases of mucinous adenocarcinoma, 7 cases of medullary carcinoma, and one case of serrated adenocarcinoma). The tumor location was recorded as right sided when the tumor involved anywhere from the cecum to the proximal two-thirds of the transverse colon and as left sided when the tumor involved from the distal third of the transverse colon up to the rectum. The relevant clinical information was obtained from the electronic medical chart.

2.2. Histopathologic characteristics

All the tumor slides were reviewed by one pathologist in training (I.A.G.) and one gastrointestinal (GI) pathologist (D.C.) simultaneously using a multiheader microscope, blinded to the clinical information. The findings recorded were based on consensus. The specimens were grossed as per our institutional protocol that consists of one tumor section submitted per centimeter of the greatest tumor dimension, and if the tumor is 3 cm or less, the entire tumor is submitted for histopathologic evaluation.

The tumor morphology was reviewed and categorized as per the 5th edition of the WHO Classification of Tumours (Digestive System) [1]. Recognition of adenoma-like adenocarcinoma was made based on the tumor description provided by the WHO, wherein the invasive carcinoma consisted of an adenoma-like morphology, with low-grade cytologic features, and no to little surrounding desmoplastic reaction [1] (Fig. 1). We subclassified these cases as pure, when the tumor was entirely composed of adenoma-like morphology, and mixed, when another component of adenocarcinoma was admixed, but at least 50% of the tumors showed adenoma-like features, in accordance with the WHO definition (Fig. 2). The percentage of this tumor component was based on eyeballing and considering all tumor slides of

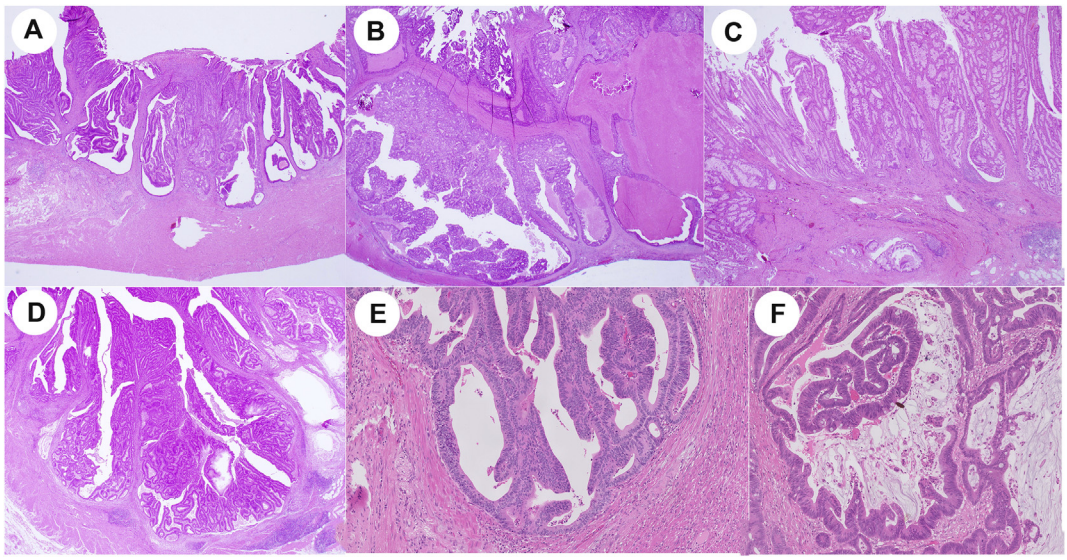

Fig. 1 Representative photomicrographs of separate cases of pure adenoma-like adenocarcinoma cases (A–F, including a pT3 tumor in panel B).

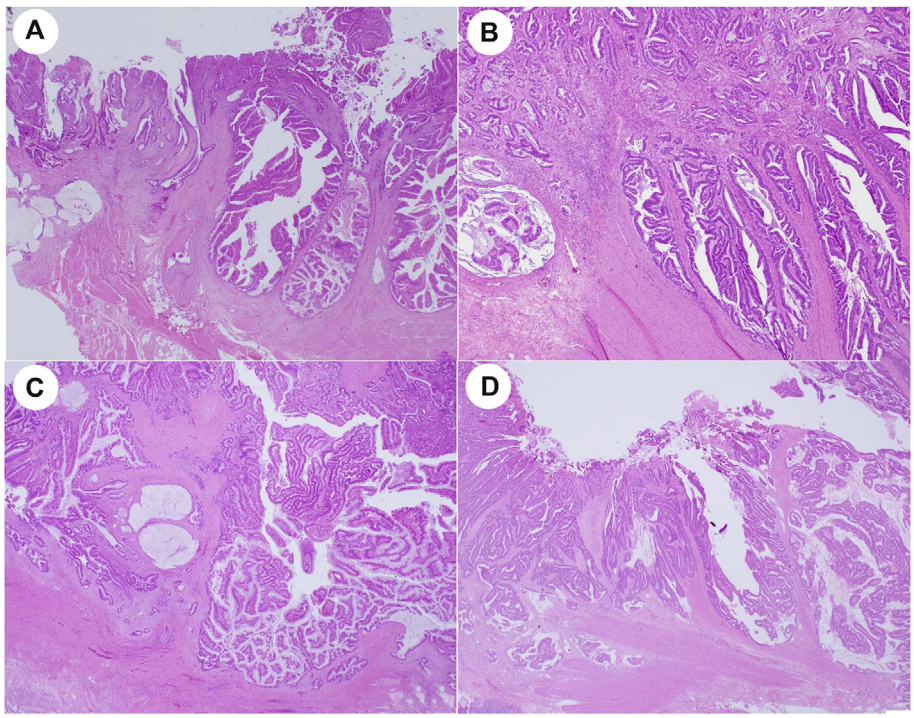

Fig. 2 Representative photomicrographs of separate cases of mixed adenoma-like adenocarcinoma (with adenoma-like areas and conventional invasive areas [A–D]).

each case. In the mixed category, a percentage of the adenoma-like morphology was assigned to each case. Other special types of colorectal adenocarcinoma as defined by the WHO were excluded from the study.

Pathologic tumor stage, nodal involvement, tumor deposits, lymphovascular invasion (LVI), and perineural invasion (PNI) were verified and recorded based on the guidelines published in the current College of American Pathologists (CAP) protocol (version 4.1.0.0). Tumor grading was recorded as low-grade and high-grade based on the least differentiated component and not based on the overall percentage of gland formation (as per the latest WHO classification system). Tumor budding (TB) was recorded as present when single tumor cells or clusters of 4 or fewer tumor cells were identified

Table 1 Patient and tumor characteristics.

Clinical and pathologic characteristics	All, N = 342	ACA, NOS, N = 251	AA, N = 91	*P*
Age, mean ± SD	65.2 ± 12.6	64.2 ± 12.3	67.9 ± 13.1	**0.009**
Gender, n, %				
Male	179, 52%	133, 53%	46, 51%	0.690
Female	163, 48%	118, 47%	45, 49%	
Race, n, %				
Caucasian	288, 84%	208, 83%	80, 88%	0.417
African American	45, 13%	35, 14%	10, 11%	
Other[a]	9, 3%	8, 3%	1, 1%	
History of other cancer, n, %				
No	293, 86%	219, 87%	74, 81%	0.166
Yes	49, 14%	32, 13%	17, 19%	
Lynch syndrome, n, %				
No	336, 98%	249, 99%	87, 96%	**0.045**
Yes	6, 2%	2, 1%	4, 4%	
Location, n, %				
Right colon	204, 60%	147, 59%	57, 63%	0.498
Left colon	138, 40%	104, 41%	34, 37%	
Adjuvant chemotherapy, n, %				
No	244, 72%	167, 67%	77, 86%	**< 0.001**
Yes	97, 28%	84, 33%	13, 14%	
Tumor grade, n, %				
Low	294, 86%	206, 82%	88, 97%	**< 0.001**
High	48, 14%	45, 18%	3, 3%	
Lymphovascular invasion, n, %				
No	217, 63%	143, 57%	74, 81%	**< 0.001**
Yes	125, 37%	108, 43%	17, 19%	
Perineural invasion, n, %				
No	289, 85%	204, 81%	85, 93%	**0.006**
Yes	53, 15%	47, 19%	6, 7%	
Tumor budding, n, %				
Low (0−4)	272, 80%	184, 73%	88, 97%	**< 0.001**
Intermediate (5−9)	31, 9%	30, 12%	1, 1%	
High (≥10)	39, 11%	37, 15%	2, 2%	
Desmoplastic reaction, n, %				
Immature/myxoid	66, 19%	58, 23%	8, 9%	**< 0.001**
Intermediate	133, 39%	109, 44%	24, 26%	
Mature	143, 42%	84, 33%	59, 65%	
Intraepithelial tumor-infiltrating lymphocytes, n, %				
No	228, 67%	178, 71%	50, 55%	**0.006**
Yes	114, 33%	73, 29%	41, 45%	
Tumor deposits, n, %				
No	303, 89%	217, 86%	86, 95%	**0.038**
Yes	39, 11%	34, 14%	5, 5%	
Number of tumor deposits, mean ± SD	0.4 ± 2.1	0.5 ± 2.4	0.2 ± 0.9	**0.042**
MMR status, n, % (n = 298)				
Retained	245, 82%	187, 84%	58, 77%	0.201
Lost	53, 18%	36, 16%	17, 23%	
***BRAF* gene, n, % (n = 38)**				
Wild-type	23, 61%	18, 64%	5, 50%	0.473
V600E	15, 39%	10, 36%	5, 50%	
***KRAS* gene, n, % (n = 28)**				
Wild-type	16, 57%	16, 64%	0	0.067
Codon 12 mutation	12, 43%	9, 36%	3, 100%	
pT stage, n, %				
pT1	25, 7%	18, 7%	7, 8%	**0.047**
pT2	71, 21%	50, 20%	21, 23%	
pT3	202, 59%	143, 57%	59, 65%	

Table 1 (*continued*)

Clinical and pathologic characteristics	All, N = 342	ACA, NOS, N = 251	AA, N = 91	*P*
pT4	44,13%	40, 16%	4, 4%	
pN stage, n, %				
pN0	218, 64%	145, 58%	73, 80%	**< 0.001**
pN1	81, 24%	70, 28%	11, 12%	
pN2	43, 13%	36, 14%	7, 8%	
Number of LNs involved, mean ± SD	1.2 ± 2.6	1.4 ± 2.8	0.5 ± 1.3	**< 0.001**
Total number of LNs examined, mean ± SD	20.1 ± 7.9	19.9 ± 7.9	20.6 ± 8.6	0.423
pTNM prognostic stage group, n, %				
I	79, 23%	55, 22%	24, 26%	**< 0.001**
II	139, 41%	90, 36%	49, 54%	
III	124,36%	106, 42%	18, 20%	

NOTE. Percentages might not total to 100% owing to rounding. Bold value indicates significant p-value.
Abbreviations: ACA, adenocarcinoma; NOS, no special type; AA, adenoma-like adenocarcinoma; MMR, mismatch repair; LN, lymph node; SD, standard deviation.
[a] Includes Asian and Hispanic.

at the tumor edge [8]. TB was also classified based on the number of buds present into three categories: 0−4 (low), 5−9 (intermediate), and ≥10 buds (high) per 0.785 mm^2. Cases containing 4 or more intraepithelial lymphocytes infiltrating tumor, per high-power field, were designated as being positive for intraepithelial tumor-infiltrating lymphocytes (iTILs) [9−12]. The presence and type of desmoplastic reaction at the invasive tumor edge was noted and classified as immature/myxoid, intermediate, and mature desmoplastic reaction as per the proposed assessment by Ueno et al. [13−15] and recently validated by our group [11]. The expression pattern of DNA mismatch repair (MMR) proteins were obtained from the pathology reports. Retained MMR was defined as any nuclear expression of MSH2, MSH6, PMS2, and MLH1 in the tumor cells, and MMR loss (dMMR) was defined as the complete absence of nuclear expression of any protein in the tumor cells.

2.3. Statistical analysis

The clinical characteristics were summarized using descriptive statistics. Overall survival (OS) was defined as the years from the date of surgery to death. Alive patients were censored at the last follow-up. RFS was defined as the years from the date of surgery to recurrence. Alive patients without recurrence were considered as a competing event at the last follow-up. The macro %findcut was used to find a best cut point of the percentage of adenoma-like adenocarcinomas for the 26 mixed cases. This approach gave the largest difference of log-rank test statistics between subjects in the two groups. Recurrence-free probabilities were calculated using cumulative incidence curves using the Fine and Gray approach. Differences between the different categories were determined using the Fine and Gray approach. Cause-Specific Analysis of competing risks was used to evaluate the relationship of the variables of interest for RFS analysis. The variables with $P < 0.25$ from univariate models were considered in the multivariable model. The stages included pathological N stage, T stage, and pTNM American Joint Committee on Cancer prognostic stage grouping. Given the possible correlation among these, the prognostic stage grouping had the highest priority. The final multivariable model was built using the backward stepwise selection approach to identify all significant risk factors. Factors significant at a 10% level were kept in the final model. All statistical tests were two sided using an α of 0.05 level of significance. SAS Version 9.4 (Cary, NC) was used to perform all statistical analyses.

3. Results

3.1. Patient and tumor characteristics: adenoma-like adenocarcinoma

The mean age of diagnosis for adenoma-like adenocarcinoma was 67.9 (±13.1) years and presented evenly in men (46 patients, 51%) and women (45 patients, 49%), and 88% of the patients were Caucasian (Table 1). In 17 patients (19%), a prior history of another neoplasm was noted, with breast cancer being the most common (4 patients). Four patients (4%) had a prior diagnosis of Lynch syndrome. Most of the cases (57 patients, 63%) were located in the right colon, with 47% of the cases located in the cecum and ascending colon. In the left-sided tumors, the most common location was the sigmoid colon (20 cases, 22%). Most of the cases were resected via right hemicolectomy (45 patients, 49%), followed by sigmoidectomy (11 patients, 12%) and extended right hemicolectomy (8 patients, 9%). Other procedures included extended left hemicolectomy, subtotal colectomy, and total abdominal colectomy. All cases had negative proximal, distal, and radial surgical resection margins. None of the cases received neoadjuvant therapy, but 13 patients (15%) received adjuvant chemotherapy.

LVI and PNI were present in 17 cases (19%) and 6 cases (7%), respectively. Three cases were classified as high-grade based on extensive cribriforming in the least differentiated area of the tumor. Intermediate and high TB was identified in 1 (1%) and 2 (2%) cases, respectively, all of which were identified in mixed adenoma-like adenocarcinoma. Only 8 cases (9%) showed an immature/myxoid desmoplastic reaction, and most of the cases (59 cases, 65%) had a mature stromal reaction. As expected, none of the pure adenoma-like adenocarcinomas show either TB or a desmoplastic reaction. iTILs were identified in 41 cases (45%). In only 5 cases, tumor deposits were identified, with a mean number of tumor deposits of 0.2 (±0.9, range: 0−6). The majority of the cases were of pT3 stage (65%), followed by pT2 (23%), pT1 (8%), and pT4 (4%), and most of the cases were of pN0 stage (80%), followed by pN1 (12%) and pN2 (8%). The mean number of lymph nodes involved was 0.5 (±1.3, range: 0−6), with a mean number of lymph nodes examined being 20.6 (±8.6). Forty-nine cases (54%) were of pTNM prognostic stage groups II, followed by group I (26%) and group III (20%). MMR proteins were retained in 58 cases (77%). Among dMMR cases, the more common proteins lost were PMS2 and MLH1 (14 cases, 83%). Molecular studies were available only in a limited number of patients, with *BRAF* gene mutation (V600E) reported in 5 tumors and *KRAS* gene mutation (codon 12) reported in three tumors.

3.2. Patient and tumor characteristics: adenocarcinoma, NOS

A total of 251 adenocarcinoma, NOS cases were included. The mean age of diagnosis was 64.2 years (±12.3), with 53% of the cases presenting in men (Table 1). In 32 cases (13%), a prior history of another neoplasm was noted, with breast cancer being the most common (9 patients). Eighty-three percent of the patients were Caucasian. Two patients (1%) had a prior diagnosis of Lynch syndrome. The cases were fairly evenly distributed to the right side (59%) and left side (41%), with the most common specific location being the sigmoid colon (73 cases, 29%). The other two most common locations were the ascending colon (51 cases, 20%) and the cecum (48 cases, 19%). The two most common surgical procedures were right hemicolectomy (111 cases, 44%) and left hemicolectomy (60 cases, 24%). All the cases had negative proximal, distal, and radial surgical resection margins. None of the cases received neoadjuvant therapy, but 84 cases (33%) received adjuvant chemotherapy, with Folinic acid, Fluorouracil, and Oxaliplatin (FOLFOX) being the most common regimen (45 cases, 54%).

LVI was identified in 43% of the cases, and PNI was identified in 19% of the cases. Majority of the cases (86%) were low-grade (exclusion of the tumor invasive front with TB and poorly differentiated clusters were performed during review, based on the new WHO recommendation). Low

Table 2 Clinicopathologic characteristics of pure and mixed adenoma-like adenocarcinoma.

Clinical and pathologic characteristics	Pure AA, N = 65	Mixed AA, N = 26	*P*
Age, mean ± SD	67 ± 13.4	68 ± 12.6	0.661
Gender, n, %			
Male	30, 46%	16, 62%	0.185
Female	35, 54%	10, 38%	
Race, n, %			
Caucasian	60, 92%	20, 77%	**0.038**
African American	4, 6%	6, 23%	
Other[a]	1, 2%	0	
Location, n, %			
Right colon	40, 62%	17, 65%	0.732
Left colon	25, 38%	9, 35%	
Tumor grade, n, %			
Low	64, 98%	24, 92%	0.195
High	1, 2%	2, 8%	
Lymphovascular invasion, n, %			
No	56, 86%	18, 69%	0.077
Yes	9, 14%	8, 31%	
Perineural invasion, n, %			
No	61, 94%	24, 92%	1.000
Yes	4, 6%	2, 8%	
Tumor budding, n, %			
Low (0−4)	66, 100%	23, 88%	**0.021**
Intermediate (5−9)	0	1, 4%	
High (≥10)	0	2, 8%	
Desmoplastic reaction, n, %			
Immature/myxoid	0	8, 31%	**<0.001**
Intermediate	15, 23%	9, 35%	
Mature	50, 77%	9, 35%	
Intraepithelial tumor-infiltrating lymphocytes, n, %			
No	31, 48%	19, 73%	**0.028**
Yes	34, 52%	7, 27%	
Tumor deposits, n, %			
No	62, 95%	24, 92%	0.622
Yes	3, 5%	2, 8%	
Number of tumor deposits, mean ± SD	0.2 ± 0.9	0.2 ± 0.6	0.586
MMR status, n, %			
Retained	42, 76%	16, 80%	1.0
Lost	13, 24%	4, 20%	
pT stage, n, %			
pT1	7, 11%	0	0.192
pT2	15, 23%	6, 23%	
pT3	39, 60%	20, 77%	
pT4	4, 6%	0	
pN stage, n, %			
pN0	54, 83%	19, 73%	0.264
pN1	8, 12%	3, 12%	
pN2	3, 5%	4, 15%	
Number of LNs involved, mean ± SD	0.4 ± 1.1	0.8 ± 1.8	0.518
Total number of LNs examined, mean ± SD	20.8 ± 9.1	19.9 ± 7.1	0.755
pTNM prognostic stage group, n, %			
I	19, 29%	5, 19%	0.439
II	35, 54%	14, 54%	

Table 2 (*continued*)

Clinical and pathologic characteristics	Pure AA, N = 65	Mixed AA, N = 26	*P*
III	11, 17%	7, 27%	

NOTE. Percentages might not total to 100% owing to rounding.
Bold value indicates significant p-value.
Abbreviations: AA, adenoma-like adenocarcinoma; MMR, mismatch repair; LN, lymph node; SD, standard deviation.
[a] Includes Asian and Hispanic.

TB was present in 184 cases (73%), intermediate TB was present in 30 cases (12%), and high TB was present in 37 cases (15%). A myxoid/immature desmoplastic reaction was noted in 58 cases (23%). iTILs were identified in 73 cases (29%). Tumor deposits were seen in 34 cases (14%), with a mean number of tumor deposits of 0.5 (±2.4, range: 0−34). The majority of the cases were of pT3 stage (57%), followed by pT2 (20%), pT4 (16%), and pT1 (7%). One hundred forty-five cases (48%) had no nodal disease (pN0), 70 cases (28%) were considered pN1, and 36 cases (14%) were considered pN2. The mean numbers of lymph nodes involved were 1.4 (±2.8, range: 0−23), with a mean number of lymph nodes examined being 19.9 (±7.9). One hundred six cases (42%) were of pTNM prognostic stage group III, followed by group II (36%) and group I (22%). MMR proteins were retained in 187 cases (84%). Among dMMR cases, the more common proteins lost were PMS2 and MLH1 (28 cases, 73%). Limited molecular information was available. *BRAF* (V600E) gene mutation was reported in 10 of 28 tumors (36%), and *KRAS* (codon 12) gene mutation was reported in 9 of 25 tumors (36%).

3.3. Clinicopathologic comparisons between adenoma-like adenocarcinoma and adenocarcinoma, NOS

Adenoma-like adenocarcinoma and adenocarcinoma, NOS cases were similar in gender, race, and a prior history of cancers. However, adenoma-like adenocarcinoma cases presented at an older age compared with adenocarcinoma, NOS cases (67.9 vs. 64.2 years, $P = 0.009$). Adenoma-like adenocarcinoma cases were more likely to have a history of Lynch syndrome and the presence of iTILs than adenocarcinoma, NOS ($P = 0.006$), although there was no significant difference between the groups in terms of overall MMR expression. They were significantly less likely to have poor differentiation, LVI, PNI, TB, myxoid/immature desmoplastic reaction, and tumor deposits. Adenoma-like adenocarcinoma cases were also significantly associated with lower pT stage, pN stage, the number of lymph nodes involved, and the pTNM prognostic stage group. Consequently, they were significantly less likely to receive adjuvant chemotherapy ($P < 0.001$). There was no significant difference in tumor location or *BRAF* or *KRAS* status between adenoma-like adenocarcinoma and adenocarcinoma, NOS cases. The details are recorded in Table 1.

3.4. Clinicopathologic comparisons between pure and mixed adenoma-like adenocarcinoma

A total of 91 cases of adenoma-like adenocarcinoma was reported; of which, 26 (29%) were considered as mixed adenoma-like adenocarcinoma (see Table 2). There was no difference in age or gender between pure or mixed adenoma-like adenocarcinoma, but patients of African-American descent were more commonly diagnosed with mixed adenoma-like adenocarcinoma (23% vs 6%, $P = 0.038$) (Table 2). There was no preference for right or left colon between pure and mixed adenoma-like adenocarcinomas ($P = 0.732$). No significant difference was identified in LVI and PNI. All pure adenoma-like adenocarcinomas showed low TB compared with 12% of the mixed cases, which had intermediate or high TB ($P = 0.021$). None of the pure cases showed a myxoid/immature desmoplastic reaction in contrast to mixed cases, among which 31% had a myxoid/immature desmoplastic reaction ($P < 0.001$). iTILs were significantly associated with pure cases ($P = 0.028$). There was no difference in pathologic T or N stage or in pTNM prognostic groups between pure and mixed cases.

3.5. Histopathologic features of adenoma-like adenocarcinoma in biopsies

The prior biopsies were available for review in 23 cases (35%) of pure adenoma-like adenocarcinoma. Eleven cases (46%) were interpreted as an adenoma only, although 3 of these cases had multiple (1−3) biopsies, all of which were also considered to represent adenomas. In these biopsies, no high-grade dysplasia was identified. The remaining 12 cases were diagnosed as well-differentiated adenocarcinoma arising in an adenoma. Two of them were repeat biopsies after prior diagnoses of adenoma. Histologic analyses of these positive biopsies on review yielded four clues to diagnosis of adenocarcinoma in this group of cases. Five of them featured evidence of deeper invasion in an oriented specimen (Fig. 3A), 5 of them featured glands surrounded by altered stroma (dense eosinophilic) instead of the lamina propria (Fig. 3B), 3 of them had adjacent large vessels, one with thrombus (Fig. 3C) and others with associated ulceration, and only one showed focal desmoplastic reaction, while most of the other fragments resembled a tubulovillous adenoma (Fig. 3D).

3.6. Adenoma-like adenocarcinoma and RFS

Adenocarcinoma, NOS cases had a worse RFS than adenoma-like adenocarcinoma, when using the current WHO definition (hazard ratio [HR] = 2.07, 95%

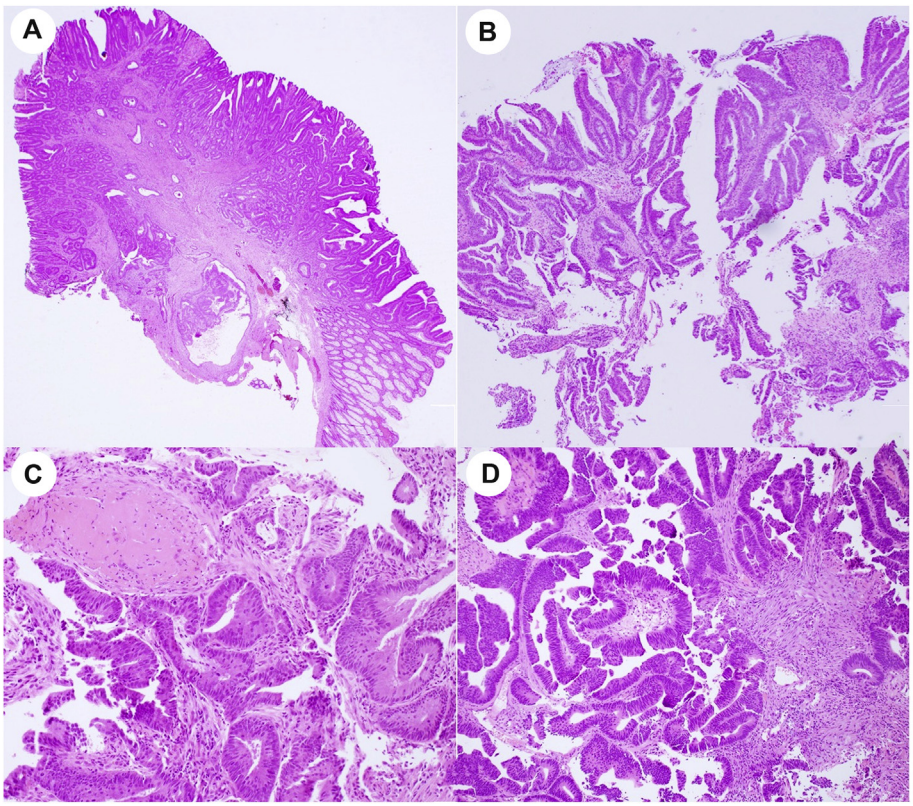

Fig. 3 Four separate biopsy patterns diagnostic of adenocarcinoma, in the setting of adenoma-like adenocarcinoma. Deeper infiltration of well-differentiated invasive glands into collagenous but not desmoplastic stroma, in a large oriented biopsy (A). Villiform low-grade neoplastic epithelium with underlying collagenous stroma, not the lamina propria (B). Low-grade neoplastic epithelium associated with vascular thrombosis (C). Area of focal desmoplastic reaction, within other fragments that resembled tubulovillous adenoma (D).

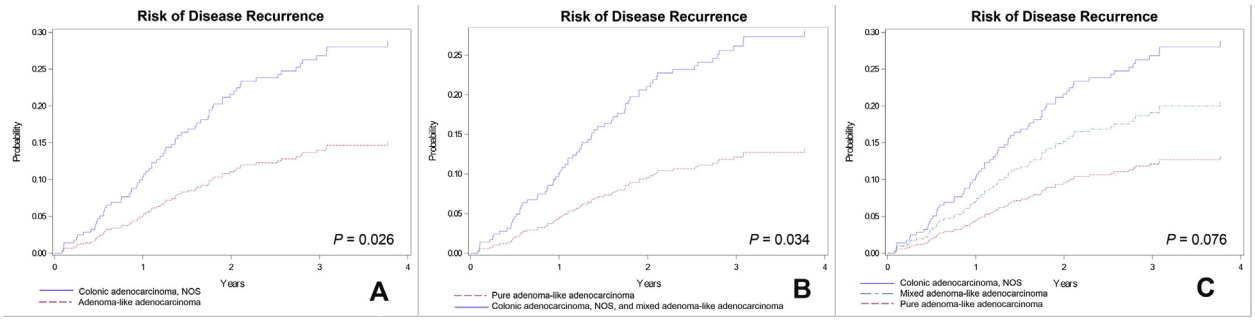

Fig. 4 Risk of disease recurrence plots. Adenoma-like adenocarcinoma compared with colonic adenocarcinoma, NOS cases (A). Pure adenoma-like adenocarcinoma compared with all other cases (B). Comparison of pure adenoma-like adenocarcinoma, mixed adenoma-like adenocarcinoma, and colonic adenocarcinoma, NOS cases (C). NOS, no special type.

confidence interval [CI] = 1.09−3.94) (Fig. 4A). In addition, pure adenoma-like adenocarcinoma cases had a longer RFS than the rest of the cases (HR = 0.43, 95% CI = 0.19−0.94) (Fig. 4B). Importantly, mixed adenoma-like adenocarcinoma cases had a worse RFS than pure adenoma-like adenocarcinoma cases (HR = 1.64, 95% CI = 0.49−5.44) and a better RFS than adenocarcinoma, NOS cases (HR = 0.68, 95% CI = 0.26−1.79) (Fig. 4C). For the 26 mixed cases, a macro %findcut method was used to find the largest difference of log-rank test statistics from OS between the two groups. Using the method described by Contal and O'Quigley [16], the best cut point was found to be 70%. Using the cut point of 70%, cases with more than 70% of pure morphology were associated with a better RFS than those with lower than 70% of pure morphology (P = 0.002).

On univariate Cause-Specific Analysis of competing risks, it was found that age ($P = 0.081$), adenoma-like adenocarcinoma ($P = 0.035$), TB ($P = 0.004$), iTILs ($P = 0.002$), LVI ($P < 0.001$), PNI ($P = 0.006$), tumor deposits ($P < 0.001$), myxoid/immature desmoplastic reaction ($P = 0.002$), pT stage ($P < 0.001$), pN stage ($P < 0.001$), and pTNM prognostic groups ($P < 0.001$) were associated with RFS (Table 3). On multivariable Cause-Specific Analysis of competing risks, the absence of iTILs (HR = 2.558, 95% CI: 1.38−4.83) was associated with a worse RFS, and lower pTNM prognostic groups (HR = 0.075; 95% CI: 0.023−0.241) were associated with a better RFS.

When only the TNM prognostic stage group II cases were compared between these two groups, there was no difference in survival in terms of RFS ($P = 0.57$), supporting the fact that the diagnosis of adenoma-like adenocarcinoma is not an independent prognostic factor, but the difference in RFS is primarily due to differences in T stage, N stage, TB, and other prognostic parameters rather than due to intrinsic tumor type.

Table 3 Cause-Specific Analysis of competing risks models of recurrence-free survival.

Clinical and pathologic characteristics	Univariate analysis	Multivariate analysis	
	P	HR (95% CI)	*P*
Age	**0.081**	1.032 (1.011−1.053)	**0.003**
Gender	0.190		
Tumor location	0.984		
Adenoma-like adenocarcinoma	**0.035**		
Tumor budding	**<0.001**		
Intraepithelial tumor-infiltrating lymphocytes	**0.002**		**0.010**
Yes		1	
No		2.332 (1.221−4.453)	
Lymphovascular invasion	**<0.001**		
Perineural invasion	**0.006**		
Tumor deposits	**<0.001**		
Stromal reaction	**<0.001**		**<0.001**
Immature/myxoid		3.085 (1.629−5.843)	
Intermediate		0.932 (0.466−1.864)	
Mature		1	
MMR expression	0.744		
pT stage	**<0.001**		
pN stage	**<0.001**		
pTNM prognostic stage group	**<0.001**		**<0.001**
I		0.118 (0.035−0.394)	
II		0.349 (0.196−0.622)	
III		1	

Abbreviations: HR, hazard ratio; CI, confidence interval; MMR, mismatch repair.
Bold value indicates significant p-value.

4. Discussion

The 2019 WHO classification recognized adenoma-like adenocarcinoma as a new subtype of colorectal carcinoma, which is characterized by a distinct low-grade morphology mimicking adenoma, but with a pushing invasion into deeper colonic tissue [1]. To our knowledge, our study reports the largest cohort of adenoma-like adenocarcinoma cases in the colon and also presents a comprehensive comparison with adenocarcinoma, NOS cases. Carcinomas of other special types were not included in this study. Adenoma-like adenocarcinoma cases in our cohort were identified in an older population than adenocarcinoma, NOS ($P = 0.009$) cases, were significantly associated with a better RFS than adenocarcinoma, NOS cases, and were inversely associated with known pathologic poor prognosticators in colorectal carcinoma such as LVI, PNI, tumor deposits, lymph node metastasis, higher pathologic tumor stage, TB [8,17−20], and immature/myxoid desmoplastic reaction [13,14,21]. Adenoma-like adenocarcinomas also showed higher incidence of iTILs than adenocarcinomas, NOS ($P = 0.006$). However, on multivariate analysis, it was found that the presence of adenoma-like adenocarcinoma morphology was not significantly associated with RFS. Nonetheless, these findings validate a separate category of colorectal adenocarcinoma for these cases, as has been incorporated by the 5th edition of the WHO Classification of Tumours (Digestive System) [1].

In our study, adenoma-like adenocarcinoma was not only significantly associated with the absence of established poor pathologic prognosticators compared with adenocarcinoma, NOS but also associated with novel and newly recognized prognostic parameters such as TB, immature/myxoid desmoplastic reaction, and iTILs. TB is a relatively recently established poor pathologic prognosticator in colorectal carcinoma [8,11,17,20,22]. Similarly, immature/myxoid desmoplastic reaction has been associated with a poor prognosis in colorectal cancer [11,13−15,21]. Adenoma-like adenocarcinoma cases were associated with the absence of TB and immature/myxoid desmoplastic reaction, with none of the pure adenoma-like adenocarcinomas showing any evidence of these features. Contrary to TB and immature/myxoid desmoplastic reaction, TILs have been reported to be associated with a better

RFS [11,23−25]. Adenoma-like adenocarcinoma cases in our cohort were significantly associated with iTILs compared with the adenocarcinoma, NOS cases ($P = 0.006$). In addition, on univariate and multivariate analysis, it was found that iTILs were significantly associated with RFS.

The WHO has suggested a cutoff of 50% of adenoma-like morphology to be present in the tumor to be classified as adenoma-like adenocarcinoma [1]. In the initial description of these cases by Yao et al. [3], cases were defined macroscopically as having 80% or more of the tumor surface composed by a "*shaggy*" or "*velvety structure*," and no microscopic percentage cutoff was used. Later on, Loy and Kaplan [5] in their study assigned a score to the cases depending on the percentage of villous architecture: 1−25% (score 1), 26−50% (score 2), 51−75% (score 3), and 76−100% (score 4); cases with score 3 and 4 were designated as villous adenocarcinomas. In their cohort, 1 patient of 35 died owing to disease; however, this patient presented with multiple synchronous colorectal adenocarcinomas. Unfortunately, RFS information was not provided.

In our study, the adenoma-like adenocarcinoma cases were divided into pure (100% adenoma-like morphology) and mixed (≥50% but less than 100% adenoma-like morphology). Pure adenoma-like adenocarcinoma had a significantly better survival than mixed adenoma-like adenocarcinoma and adenocarcinoma, NOS combined ($P = 0.03$). Using a three-tier system, a trend in differences of survival is seen between the three groups ($P = 0.076$), with the mixed group showing intermediate prognosis. The statistical significance is probably not reached owing to the low number of mixed cases. Nonetheless, this raises the question whether mixed cases should be classified into the adenoma-like adenocarcinoma subgroup or should be left in the adenocarcinoma, NOS category. If mixed cases are to be considered in adenoma-like adenocarcinoma, this also raises the question whether 50% is an appropriate cutoff. Our result suggests a 70% cutoff using the macro %findcut method; however, some limitations of this method include a low number of mixed cases, and a range of 1−99% in all cases should be used for obtaining an unbiased percentage cutoff. Our study did not incorporate interobserver reproducibility because the cases were reviewed by two pathologists simultaneously using a multiheader microscope, and the percentages assigned for the purposes of this study were determined by consensus. Although it was not difficult to identify the adenoma-like adenocarcinoma components in the resection specimens, we can foresee that in clinical practice, the percentage determination and appropriate classification might pose significant challenges for mixed adenoma-like adenocarcinoma cases.

In our series, we noted a much higher prevalence of this entity than what was reported by Gonzalez et al. [6] (3.5%). This could be due to differences in racial prevalence (84% of our patient population is Caucasian, with most of the remainder being African Americans and with only 3% belonging to other races). The racial profile may be significantly different in other hospitals, but an objective comparison cannot be made because the racial distribution of the previous studies was not reported. The higher prevalence can also be related to exclusion of stage IV and rectal adenocarcinoma cases from our cohort. Because adenoma-like adenocarcinoma cases in our cohort had a lower pT and pN stage and significantly lower LVI, PNI, tumor deposits, and TB than adenocarcinoma, NOS cases, including stage IV cases would most likely have lowered the percentage of these cases. The incidence of this group of tumors involving the rectum is unfortunately not known. Adenoma-like adenocarcinoma in our cohort was more commonly located in the right colon (63%). Similar findings were seen in the studies by Yao et al. [3] and Gonzalez et al. [6], as 58% and 63% of their cases were located in the right colon, respectively. In contrast, 61% of the cases in the study by Loy and Kaplan [5] were located in the sigmoid colon or rectum. Combining our current cohort with the prior reported cases, a total of 181 cases have been reported; of which, 101 cases (55.8%) were located in the right colon [3−6]. Therefore, excluding rectal adenocarcinomas from our cohort could also explain a higher percentage of this special group of tumors.

In summary, we present the largest cohort of adenoma-like adenocarcinoma cases and provide a comprehensive comparison with a large cohort of adenocarcinoma, NOS cases. Adenoma-like adenocarcinoma cases were significantly associated with a better RFS than adenocarcinoma, NOS cases and showed significantly less association with poor pathologic prognosticators. These tumors, on the other hand, were associated with iTILs, which is an independent good prognosticator as per multivariate analysis in this cohort. Our study also highlights a better survival for pure adenoma-like adenocarcinoma than for mixed adenoma-like adenocarcinoma.

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