

Colorectal adenosquamous carcinoma: clinicopathologic analysis of two large cohorts and literature review confirm poor prognosis and reveal prognostic aspects

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Abbreviations: ASC, adenosquamous carcinoma; CI, confidence interval; CRC, colorectal carcinoma; H&E, haematoxylin and eosin; HR, hazard ratio; IHC, Immunohistochemical; ITBCC, International Tumour Budding Consensus Conference; ITWG, International TILs Working Group; LVI, lymphovascular invasion; MMR, mismatch repair; MSI, microsatellite instability; OS, Overall survival; PALGA, Nationwide Dutch Pathology Databank; PCR, polymerase chain reaction; PFS, progression-free survival; TILs, tumour-infiltrating lymphocytes; WHO, World Health Organization.

Aims: We compiled two cohorts of colorectal adenosquamous carcinoma (ASC) to describe its histologic and molecular aspects using modern parameters to compare them with literature reports using meta-analysis of cohorts and individual case series.

Methods and Results: We identified 53 colorectal ASC from 19 North American academic medical centres, in addition to national database reports on 94 Dutch cases. We analysed available clinical, histologic, and immunohistochemical features and patient outcome. ASC comprised 0.02% of colorectal cancers in the Dutch database. The median cohort patient ages at resection were 65 and 69 years (North American and Dutch cohorts, respectively), with a roughly equal male:female ratio. The squamous component represented between 5% and 95% of each tumour, with a median of 50%. Tumour-infiltrating

lymphocytes (TILs) were generally low (66%), and tumour budding was often Bd1 (64%). Most cases were pT3 (55%) or pT4 (40%), with nodal metastases in more than half (58%). Twenty-three cases (43%) metastasized distantly, most commonly to the liver. Mismatch repair (MMR) deficiency was identified in 34% of the cases. Follow-up was available for 48 patients; 13 (27%) had recurrent disease and 29 (60%) died. A total of 31 patients progressed, with median time to progression of 18 months. Available data for the Dutch cohort revealed largely similar findings, as did review of cases in the literature.

Conclusion: Colorectal ASC usually presents at an advanced stage. Despite high rates of MMR deficiency and low tumour budding, TILs were generally low, and there is a high recurrence rate and poor prognosis.

Keywords: adenosquamous, carcinoma, colon, mismatch repair, prognosis

Introduction

Adenosquamous carcinoma (ASC) is a rare subtype of colorectal carcinoma (CRC) of uncertain pathogenesis, with an incidence of <0.1% of all colon cancers.^{1–3} ASC is biphasic histologically, demonstrating both malignant squamous and malignant glandular components. While ASC is formally recognized as a subtype of CRC by the World Health Organization (WHO), the 2019 edition of the WHO Digestive System Tumours classification only contains a single sentence on ASC, emphasizing that little is known about this entity.¹ ASC is more common in the stomach and pancreas; currently, there are around 245 colorectal cases reported in the literature, although relatively few with detailed pathologic analysis.

The first case of colorectal ASC in the literature was reported by Herxheimer in 1907.⁴ In 1971, Comer *et al.* published the first case series, comprising 12 cases of colorectal “adenoacanthoma”.⁵ The literature at this point indicated that ASC presented at an advanced stage, histology showed high-grade features, and the clinical course was aggressive, with an overall 5-year survival of roughly 30%.⁵ In 1995, Frizelle *et al.* published the largest pathology series of colorectal ASC to date, which included 31 cases from a single institution.⁶ With findings comparable to previous studies, they additionally stated that nodal disease imparted a worse prognosis than comparable node-positive conventional colorectal

adenocarcinomas of a similar stage.⁶ A similar pathologic study of 29 cases was published in 2022 by Parente *et al.*,⁷ and a molecular analysis of 22 cases was published in 2023.⁸

Outside of epidemiology-based studies^{2,3,9–11} and two recent additional series with around a dozen cases each,^{12,13} most other colorectal ASC in the literature have been published in small reports of one or two cases. These rarely discuss histologic attributes that in recent decades have been shown to impact survival for CRC patients, including mismatch repair (MMR) status,¹⁴ tumour budding,¹⁵ and tumour-infiltrating lymphocytes (TILs).^{16,17} With relatively few cases reported in the literature, the current study aimed to compile a large cohort of colorectal ASC and describe its clinicopathologic aspects using modern parameters. We were also able to collect similar data for a separate new cohort via a Dutch national registry and report similar aspects.

Materials and Methods

NORTH AMERICAN COHORT

Via departmental archive searches, we retrospectively identified 53 cases of colorectal ASC that had been resected at 19 different academic medical centres. The Institutional Research Board approval was procured at each participating institution. Cases were

included if they demonstrated a clearly malignant squamous component on haematoxylin and eosin (H&E) alongside an adenocarcinoma component on H&E, each of which represented at least 5% of the tumour; conventional colorectal adenocarcinoma with foci of bland (nonmalignant) squamous morules were excluded.¹⁸ Biopsy cases were also excluded.

Existing slides were reviewed locally for the following features: location and size of the mass, gross description, gross perforation, percentage of glandular and squamous components, the presence of precursor adenoma, tumour differentiation, configuration of the tumour border (pushing versus infiltrative), small- and large-vessel lymphovascular invasion (LVI), perineural invasion, tumour budding per the International Tumour Budding Consensus Conference (ITBCC) criteria,¹⁵ TILs in each histologic component per the International TILs Working Group (ITWG) criteria,¹⁷ margin status, tumour deposits, and AJCC 8th Edition pTNM staging. Some data points were missing for a few extramural consultation cases. Immunohistochemical (IHC) slides were also evaluated if available; these had all previously been performed at the originating institutions, under local conditions. Electronic medical records were reviewed for available patient history (including presenting symptoms), data from molecular testing, and follow-up information, including recurrence and outcome at last follow-up.

DUTCH COHORT

Additional data were collected via a national search of cases identified in the Nationwide Dutch Pathology Databank (PALGA) between 1990 and 2023 and recorded under number LZV2023-113. PALGA has nationwide coverage since 1991.¹⁹ Initial selection was based in the following terms: “colon” (SNOMED term T67___) or “rectum” (SNOMED term T68___) or “bowel” (SNOMED term T50100) for all resections of primary carcinomas in combination with primary ASC. Anal carcinomas (SNOMED T69___), metastases, and direct invasion from other organs (Dutch term “doorgroei”) were excluded. Both microscopic descriptions and conclusion texts were evaluated to select cases, as well as the full pathology history per patient. Cases were linked to Statistics Netherlands (Centraal Bureau voor de Statistiek) to retrieve survival data. To calculate the relative incidence, data from the Dutch Cancer Registry were used (<https://nkr-cijfers.iknl.nl>, accessed October 9, 2024). The original search resulted in 84 patients (1990–2023), as well as 15 cases that were coded as squamous cell

carcinomas but described as ASC. Further assessment excluded cases based on incorrect coding of the tumour type ($n = 4$) and direct ingrowth from a primary cervical ASC. Ultimately, we identified 94 verified cases of colorectal ASC for this cohort.

STATISTICAL ANALYSIS

Statistical analysis was performed on the North American cohort by one of the authors (S.M.J.). Patient and clinicopathological characteristics were summarized with frequencies and percentages or means, medians, and ranges, as appropriate. For analysis of outcomes during follow-up, progression was defined as a recurrence or death from any cause. Risk of progression was compared with key characteristics using Cox proportional-hazards regression models, and hazard ratios (HRs) were reported along with P -values from likelihood ratio tests. The median progression-free survival (PFS), along with PFS at 1 and 5 years after tumour resection, was summarized using the Kaplan–Meier method. Overall survival (OS) was analysed and summarized similarly. Estimates were summarized with 95% confidence intervals (CIs). P -values less than 0.05 were considered statistically significant. These analyses were performed using SAS v. 9.4 (SAS Institute, Cary, NC, USA) or R (Vienna, Austria).

Similar statistical analysis was performed on the Dutch cohort by one of the authors (I.D.N.). OS was defined as the interval between the date of diagnosis until the date of death or until last follow-up. Patients who were alive at the end of follow-up were censored in survival analyses. Factors influencing PFS and OS were investigated with univariate Cox regression analysis. P -values less than 0.05 were considered statistically significant. These analyses were performed using SPSS (v. 29; IBM, Armonk, NY, USA).

LITERATURE REVIEW

We conducted a literature review of case reports and small case series of colorectal ASC (individual case series). Some publications were excluded after we could not access them via several locations (generally older and/or non-English publications). We additionally excluded cases where the diagnosis of colorectal ASC seemed potentially in doubt. Larger, well-described case series^{5–7,12,13} were separately analysed (meta-analysis). Two smaller series where individual case data and photomicrographs were not provided were excluded.^{20,21} The meta-analysis was performed with all available studies on OS in terms of

HR with 95% CI. A random-effects model with inverse variance weighting of studies was used.

Results

NORTH AMERICAN COHORT: CLINICAL FINDINGS

Clinical findings of the 53 North American cases are summarized in Table 1. Median patient age at tumour resection was 65 years (range: 29–94 years), and the male-to-female ratio was 1:1.1. The majority of patients were reported as white (42 of 47 with demographic data available, 89%), and the most common presenting symptoms included abdominal pain (20 of 46 patients, 43%), gastrointestinal bleeding/anaemia (17 of 46, 37%), and weight loss (11 of 46, 24%). Four patients (9%) were asymptomatic at presentation, with the ASC being an incidental finding at time of colonoscopy. Known risk factors for CRC included smoking history (8 of 46, 17%), obesity (4 of 46, 9%), and inflammatory bowel disease (3 of 46, 7%). Calcium levels were available for 34 (64%) patients at the time of diagnosis, with one patient (3%) demonstrating mild hypercalcemia.

Seven patients received neoadjuvant therapy (13%), and 25 of 40 patients (63%) received adjuvant therapy. Follow-up data were available for 48 patients, among whom 29 died (14 from disease, 15 of other or unknown cause), and 19 were alive (15 with no evidence of disease, four with disease). Available follow-up ranged from <1 month to 232 months (median: 17 months). OS was estimated to be 65% (95% CI: 53%–81%) at 1 year and 38% (95% CI: 24%–58%) at 5 years after tumour resection (median OS: 24 months, 95% CI: 13–96 months). A total of 13 patients experienced disease recurrence, and 31 patients progressed (recurrence and/or death). Median time to progression was 18 months (95% CI: 11–84 months), with 1-year PFS of 59% (95% CI: 46%–75%).

DUTCH COHORT: CLINICAL FINDINGS

The 94 cases of colorectal ASC from the Dutch cohort represented 0.02% of all CRC cases in the Netherlands during the period of study (1990–2023). Clinical findings are summarized in Table 1. In line with the first cohort, the male-to-female ratio was 1:1. The average was slightly older (median age: 69 years, range: 30–89 years). Risk factor data were limited, although three patients had inflammatory bowel

Table 1. Clinical findings in cases of colorectal adenosquamous carcinoma

	North American cohort (<i>n</i> = 53)	Dutch cohort (<i>n</i> = 94)
Age (years)		
Mean	64	67
Median	65	69
Range	29–94	30–89
Sex (%)		
Male	25 (47)	47 (50)
Female	28 (53)	47 (50)
Race/Ethnicity (%)		
White	42/47 (89)	n/a
Black	3/47 (6)	
Asian	1/47 (2)	
Hispanic	1/47 (2)	
Presenting symptoms (%)		
Abdominal pain	20/46 (43)	n/a
Gastrointestinal bleeding/anaemia	17/46 (37)	
Weight loss	11/46 (24)	
Fatigue	7/46 (15)	
Change in bowel habits	6/46 (13)	
Nausea/vomiting	5/46 (11)	
Asymptomatic	4/46 (9)	
Known risk factors (%)		
Smoking	8/46 (17)	n/a
Obesity	4/46 (9)	n/a
Inflammatory bowel disease	3/46 (7)	3 (3)
Family history of colon cancer	2/46 (4)	4 (4)
None	30/46 (65)	n/a
Neoadjuvant treatment (%)		
Yes	7 (13)	8/93 (9)
No	46 (87)	85/93 (91)

Continued

Table 1. (Continued)

	North American cohort (<i>n</i> = 53)	Dutch cohort (<i>n</i> = 94)
Adjuvant treatment (%)		
Yes	25/40 (63)	n/a
No	15/40 (38)	
Follow-up length (<i>n</i> = 48) (months)		
Median	17	16
Range	<1–232	0–248
Recurrence (%)		
Yes	13/48 (27)	20 (24)
No	35/48 (73)	62 (76)
Status at last follow-up (%)		
Alive		
No evidence of disease	15/48 (31)	39/93 (42)
With disease	4/48 (8)	
Dead		
Died of disease	14/48 (29)	54/93 (58)
Died of other/ unknown cause	15/48 (31)	
Death (any cause) (<i>n</i> = 29)		
1-year OS (95% CI)*	65% (53%, 81%)	69% (59%, 79%)
2-year OS (95% CI)	49% (36%, 66%)	59% (48%, 70%)
Median OS (95% CI) (months)	24 (13–96)	56 (21–90)
Progression (recurrence and/or death) (<i>n</i> = 31)		
1-year PFS (95% CI)	59% (46%, 75%)	67% (57%, 77%)
2-year PFS (95% CI)	42% (30%, 60%)	59% (48%, 70%)
Median PFS (95% CI) (months)	18 (11–84)	56 (23–89)

*OS (overall survival) and PFS (progression-free survival) percentages and medians, presented with Kaplan–Meier estimate and 95% CI (confidence interval).

disease. Nine patients (including two patients with Lynch syndrome and one with inflammatory bowel disease) presented with multiple synchronous CRC.

Recurrence occurred in 20 patients (24%), with median time to recurrence of 6 months. At last follow-up, 39 of 93 patients (42%) were alive and the other 54 (58%) were dead.

HISTOLOGIC FINDINGS

All North American cases underwent rigorous review of clinicopathologic features. In the Dutch cohort, a limited amount of key histologic data were available for analysis based on sign-out reports. This information is summarized in Table 2. For the North American cohort, most tumours (34, 64%) were located in the right colon, and median tumour size was 6.0 cm (mean: 6.8 cm; range: 2.5–15.0 cm), with eight cases (15%) larger than 10.0 cm. Grossly, tumours were most commonly described as ulcerated (29, 55%), and 20 (38%) were nearly circumferential. In the Dutch cohort, most tumours were again located in the right colon (54 of 91, 59%), with a smaller median size of 4.5 cm (mean: 5.1 cm; range: 0.6–14 cm).

Histologically, the North American cases either showed an admixture of glandular and squamous elements (39 cases, 74%; Figure 1A) or less intermixed, discrete regions of the two components (14 cases, 26%; Figure 1B). Squamous and glandular features were roughly equal overall, as both components comprised a median of 50% and a range of 5%–95% of each tumour. Twenty-five of the cases (47%) underwent IHC staining with a squamous marker (CK5/6, p63, p40) at diagnosis, all of which were positive in the squamous component.

All cases were either moderately (36% for the North American cases, 40% for the Dutch cases) or poorly differentiated (64% and 60%, respectively), with differentiation reported as the less differentiated of the two components. Small-vessel LVI was present in 68% and 26% (Figure 1C), large-vessel LVI was present in 34% and 16% (Figure 1D), and perineural invasion was present in 38% and 6%, respectively (Figure 1E). Four (8%) and eight (10%) cases had a positive surgical resection margin. Tumour budding was evaluated in the adenocarcinoma component of the North American cases, and 34 (64%) were Bd1 (0–4 buds), while 11 (21%) were Bd2 (5–9 buds), and 8 (15%) were Bd3 (>9 buds). Among the 34 Bd1 cases, 12 (35%) were reported as not showing any tumour buds. TILs were evaluated in both the adenocarcinoma and squamous components of the North American cases; most (35, 66%) demonstrated low TILs overall (Figure 1F), and 42 (79%) showed the same TIL categorization in both components. The

Table 2. Pathologic findings in cases of colorectal adenosquamous carcinoma

	North American cohort (<i>n</i> = 53)	Dutch cohort (<i>n</i> = 94)
Tumour location (%)		
Right colon	34 (64)	54/91 (59)
Left colon	11 (21)	22/91 (24)
Rectum	8 (15)	15/91 (16)
Tumour size (cm)		
Mean	6.8	5.1
Median	6.0	4.5
Range	2.5–15.0	0.6–14.0
Gross description (%)		
Ulcerated/fungating	29 (55)	n/a
Circumferential	20 (38)	
Perforated	9 (17)	
Percent squamous by histology (%)		
Mean	52	n/a
Median	50	
Range	5–95	
Configuration of components (%)		
Discrete squamous and glandular	14 (26)	n/a
Intermixed squamous and glandular	39 (74)	
Precursor lesions (%)		
Tubular adenoma	5 (9)	n/a
Tubulovillous adenoma	2 (4)	
Villous adenoma	3 (8)	
Traditional serrated adenoma	1 (2)	
Flat intestinal-type dysplasia	1 (2)	
None observed	41 (77)	
Differentiation (%)		
Well-differentiated	0 (0)	0 (0)
Moderately differentiated	19 (36)	24 (40)

Continued

Table 2. (Continued)

	North American cohort (<i>n</i> = 53)	Dutch cohort (<i>n</i> = 94)
Poorly differentiated	34 (64)	36 (60)
Border configuration		
Infiltrative	36/51 (71)	n/a
Pushing	15/51 (29)	
Small vessel lymphovascular invasion (%)		
Present	36 (68)	21/82 (26)
Absent	17 (32)	61/82 (74)
Large vessel lymphovascular invasion (%)		
Present	18 (34)	13/82 (16)
Absent	35 (66)	69/82 (84)
Perineural invasion (%)		
Present	20 (38)	5/82 (6)
Absent	33 (62)	77/82 (94)
Tumour budding (%)		
Bd1	34 (64)	n/a
Bd2	11 (21)	
Bd3	8 (15)	
Margin status (%)		
Negative	49/53 (92)	69/77 (90)
Positive	4/53 (8)	8/77 (10)
TILs overall (%)		
Low	35 (66)	n/a
Intermediate	9 (17)	
High	9 (17)	
TILs in glandular component (%)		
Low	33 (62)	
Intermediate	10 (19)	
High	10 (19)	
TILs in squamous component (%)		
Low	35 (66)	
Intermediate	8 (15)	
High	10 (19)	

Continued

Table 2. (Continued)

	North American cohort (<i>n</i> = 53)	Dutch cohort (<i>n</i> = 94)
Total lymph nodes		
Mean	25	17
Median	19	14
Range	2–140	0–79
Positive lymph nodes		
Mean	4	2
Median	1	1
Range	0–21	0–20
Tumour deposits (%)		
Yes	9 (17)	10/79 (13)
No	44 (83)	69/79 (87)
T-category stage (%)		
pT1	0 (0)	4/81 (5)
pT2	3 (6)	8/81 (10)
pT3	29 (55)	44/81 (54)
pT4	21 (40)	25/81 (31)
N-category stage (%)		
pN0	22 (42)	34/79 (43)
pN1	14 (26)	28/79 (35)
pN2	17 (32)	17/79 (22)
M-category stage (%)		
pM0 or pMX	30 (57)	71 (76)
pM1	23 (43)	23 (24)
Distant metastasis sites (%)		
Liver	18/23 (78)	7/23 (30)
Peritoneum	4/23 (17)	5/23 (22)
Omentum	2/23 (9)	2/23 (8)
Non-regional lymph nodes	2/23 (9)	4/23 (16)
Adrenal gland	1/23 (4)	—
Abdominal muscles	1/23 (4)	—
Lung	1/23 (4)	2/23 (8)
Mesentery	1/23 (4)	—

Continued

Table 2. (Continued)

	North American cohort (<i>n</i> = 53)	Dutch cohort (<i>n</i> = 94)
Uterus and ovaries	1/23 (4)	1/23 (4)
Bladder	—	1/23 (4)
MMR protein expression (%)		
Retained	27/41 (66)	18/27 (67)
Deficient*	14/41 (34)	9/27 (33)
KRAS mutation status (%)		
Positive	7/20 (35)	4/9 (44)
Negative	13/20 (65)	5/9 (56)
BRAF mutation status (%)		
Positive	8/23 (35)	0/7 (0)
Negative	15/23 (65)	7/7 (100)

MMR, mismatch repair; TILs, tumour-infiltrating lymphocytes.

*For simplicity, this includes one case where MMR immunohistochemistry was not performed, but PCR testing revealed microsatellite instability.

majority of the North American cases (36 of 51, 71%) showed an infiltrative rather than pushing border.

Tumour pT-category staging was most commonly pT3 (55% and 54%) or pT4 (40% and 31%, respectively). Most cases (58% and 57%) had nodal metastasis, although tumour deposits were less common (17% and 13%, respectively). Distant metastases were identified in 23 (43%) of the North American cases and 23 (24%) of the Dutch cases, with the liver the most common site (18 of 23 [78%] and 7 of 23 [30%]). Histology of distant metastatic lesions was available for 20 Dutch cases; conventional adenocarcinoma was the most frequently reported (*n* = 9, 45%), followed by conventional squamous cell carcinoma (*n* = 7, 35%) and ASC (*n* = 4, 20%).

IHC for MMR proteins was performed in 40 North American cases and 27 Dutch cases, and polymerase chain reaction (PCR) for microsatellite instability (MSI) was performed in one additional North American case. MMR deficiency or MSI was identified in 14 (34%) North American cases and nine (33%) Dutch cases. Loss of MMR protein expression was always seen in both the adenocarcinoma and squamous components (Figure 2A–D). Among the North American cases, abnormal patterns included MLH1/PMS2 loss in eight, MSH2/MSH6 loss in three, isolated

PMS2 loss in one, and loss of MSH6 in both components with additional MLH1/PMS2 loss in the adenocarcinoma component in one. The case tested by PCR showed MSI. Among the 14 abnormal North American cases, overall TILs were low in seven (50%), compared to 28 of 39 (72%) MMR-proficient cases ($P = 0.19$). In the North American cohort, *KRAS* mutations were identified in 7 of 20 cases tested (35%), and *BRAF* mutations were identified in 8 of 23 cases tested (35%), of which five (63%) showed MMR deficiency. In the Dutch cohort, *KRAS* mutations were identified in four of nine cases tested (44%), and *BRAF* mutations were identified in none of the seven cases tested (0%).

PROGNOSTIC FEATURES

Univariate analysis of prognostic factors relating to PFS and OS in the North American cohort are shown in Table 3. PFS (Figure 3A) was significantly impacted by large-vessel LVI (HR: 2.80, 95% CI: 1.33–5.97, $P = 0.007$; Figure 3B), pN2 vs. pN0 disease (HR: 2.67, 95% CI: 1.16–6.49, $P = 0.022$), pM1 disease (HR: 2.36, 95% CI: 1.05–5.64, $P = 0.038$), and *KRAS* mutation (HR: 4.40, 95% CI: 1.22–17.70,

$P = 0.024$). Similarly, OS (Figure 3C) was significantly impacted by large-vessel LVI (HR: 3.60, 95% CI: 1.63–8.18, $P = 0.002$), pN2 versus pN0 disease (HR: 2.56, 95% CI: 1.09–6.47, $P = 0.031$), pM1 disease (HR: 3.20, 95% CI: 1.37–8.11, $P = 0.007$; Figure 3D), and *KRAS* mutation (HR: 3.87, 95% CI: 1.08–15.51, $P = 0.038$).

A similar analysis was performed on the Dutch cases (see Table S1), albeit limited by the available features. For PFS, pT stage, tumour deposits, and margin status were prognostic, as well as neoadjuvant therapy. For OS, pN2 disease and pM1 disease were similarly prognostic in this analysis, although other factors were not. Increasing size and a positive margin were negatively prognostic in this analysis, unlike in the North American cohort.

LITERATURE REVIEW OF CASE REPORTS

We identified 101 cases of colorectal ASC reported in small cases or case series.^{22–93} Selected data on these cases are compiled in Table S2. Cases where the article could not be retrieved^{4,94–120} were not included, nor were complex cases with additional components or considerations.^{49,121,122} Some earlier cases were

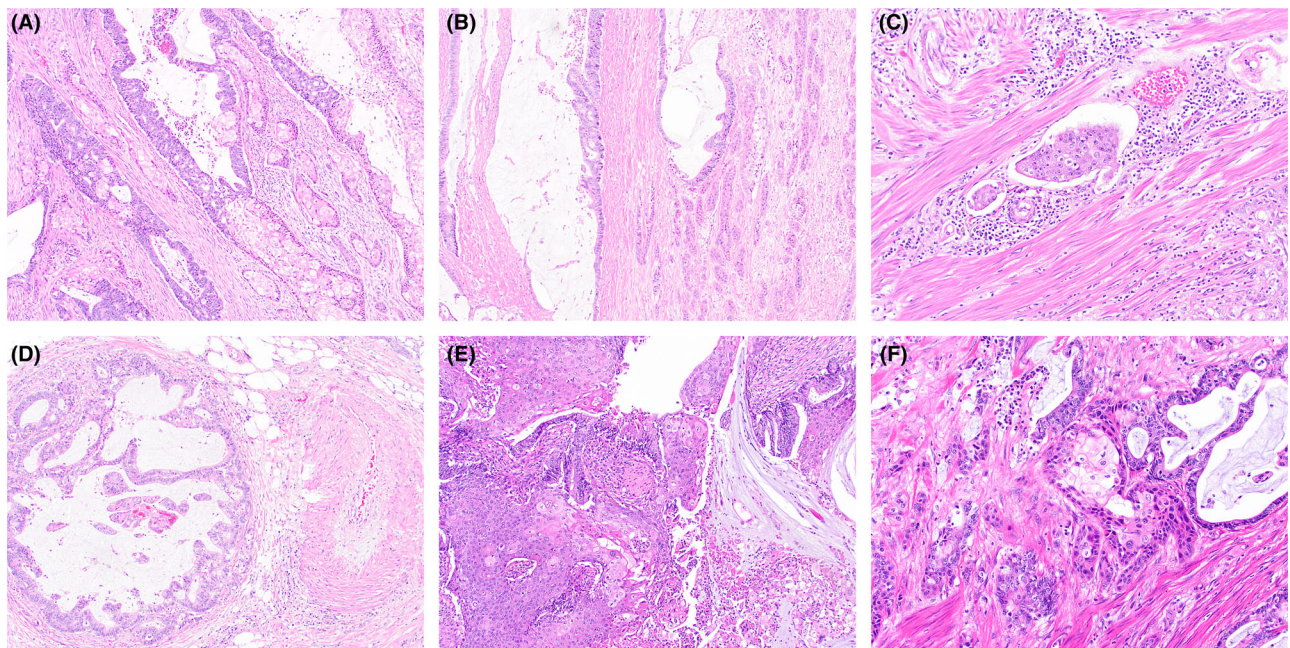


Figure 1. Histologic features of colorectal adenosquamous carcinoma. (A) The majority of cases in our North American cohort demonstrated admixing of the malignant glandular and malignant squamous components. (B) In some cases, the two components were more discrete and distinct. (C) Small-vessel lymphovascular invasion (LVI) was seen in most cases. (D) Large-vessel LVI was less common, but still seen in around one-third of cases. It was a negative prognostic factor for both progression-free and overall survival. (E) Perineural invasion was present in 38% of cases. (F) Around two-thirds of cases showed low tumour-infiltrating lymphocytes in both the glandular and the squamous components.

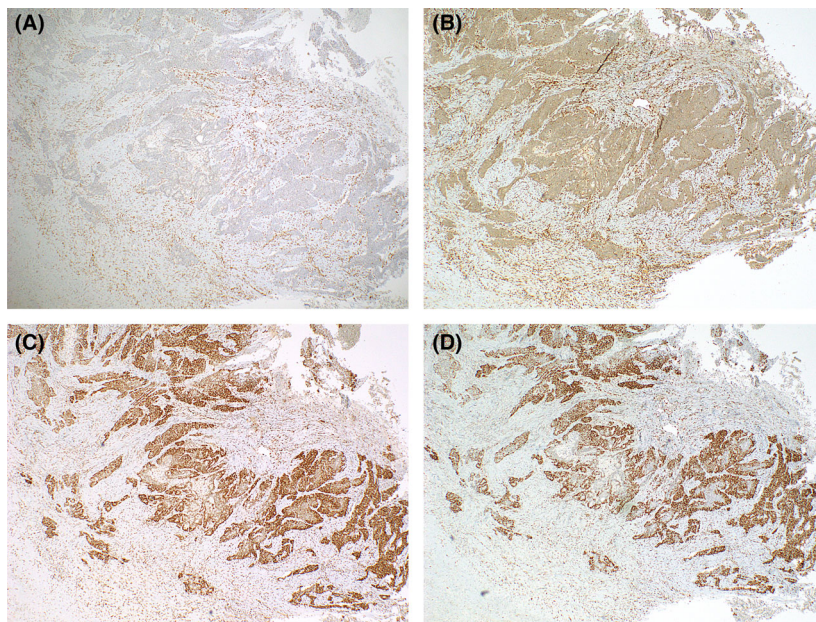


Figure 2. A high percentage of adenosquamous carcinomas demonstrates mismatch repair or microsatellite instability: 34% of the North American cohort and 33% of the Dutch cohort. This exemplary case demonstrates loss of expression of MLH1 (A) and PMS2 (B) proteins by immunohistochemistry, with retention of MSH2 (C) and MSH6 (D). Tumour-infiltrating lymphocytes serve as a positive internal control.

described as “adenocanthomas”. While this historical term generally refers to an adenocarcinoma with squamous metaplasia, these cases were included when the term was clearly meant to indicate an adenocarcinoma containing a malignant squamous component.^{5,22–29,31}

Among the compiled literature cases, the median patient age was 59 years (range: 12–94 years) and the male-to-female ratio was 1.2:1. Patient symptoms included abdominal pain (48, 48%), evidence of gastrointestinal bleeding (33, 33%), and weight loss (23, 23%); no patient was noted as being asymptomatic. Eight patients (8%) were specifically reported as having clinically notable hypercalcemia. Risk factors were generally not reported, although 10 patients (10%) had ulcerative colitis. The most common tumour sites included sigmoid (19, 19%) and rectum (18, 18%), and median tumour size was 6.0 cm (range: 1.5–13.0 cm). LVI was noted as present in 14 cases and perineural invasion in three cases, although many reports did not mention this parameter. Sixteen cases provided a histologic estimate of component percentages, with the squamous component on average representing 27% of each case. The pT-category stage was pT3 or pT4 in 48 of 59 (81%) cases with that information. Fifty-seven of 71 (80%) cases had nodal metastases at presentation, and 33 (33%) cases had distant metastases at presentation

(with liver by far the most common site). Six of 63 (10%) patients received neoadjuvant therapy, and 33 of 57 (58%) received adjuvant therapy. MMR IHC was only reported in 10 cases, with loss of staining in four (40%); one additional case was MSI-high by PCR. *KRAS* and *BRAF* mutations were described in a few cases, along with scattered other mutations (including one *GNAS*-mutant case and one *NTRK*-mutant case). Recurrence occurred for 25 of 57 (44%) cases. Follow-up data were provided in 78 cases, with 41 (53%) dead of disease. The median PFS was 7 months (95% CI: 1.1–12.8 months), and the median OS was 14 months (95% CI: 7.3–20.7 months). Eight cases were autopsy reports.

LITERATURE REVIEW OF LARGER STUDIES

We identified eight population- or registry-based studies^{2,3,6,9–11,13,30} that reported on 1269 patients with colorectal ASC. These cannot all be considered unique patients, due to overlap in registries (reported within individual registries and SEER) and some potential overlap with the North American cohort (Figure 4A). All except one study are based on data from the USA. The incidence varied between 0.06% and 0.09% of reported CRC, which is higher than in our Dutch cohort (Figure 4B). We visualized the clinicopathologic characteristics for the different studies in

Table 3. Univariate analysis of factors influencing unadjusted progression-free and overall survival: North American cohort

	Progression-free survival			Overall survival		
	HR	95% CI	P-value	HR	95% CI	P-value
Male sex	1.28	0.63–2.63	0.50	1.48	0.71–3.19	0.30
Age (per 1-year increase)	0.99	0.97–1.02	0.49	0.99	0.96–1.02	0.37
Left-sided	0.73	0.33–1.51	0.40	0.55	0.23–1.19	0.13
Size (per 1-cm increase)	1.04	0.93–1.16	0.45	1.08	0.96–1.20	0.20
Grossly ulcerated	1.51	0.73–3.21	0.27	1.54	0.72–3.39	0.26
Grossly circumferential	1.12	0.52–2.31	0.77	1.33	0.61–2.82	0.47
Grossly perforated	1.88	0.69–4.37	0.20	2.26	0.82–5.40	0.11
Percentage squamous	1.00	0.99–1.01	0.98	1.00	0.99–1.02	0.73
Poorly differentiated	1.75	0.83–4.01	0.15	1.77	0.81–4.26	0.16
Pushing border	0.78	0.31–1.73	0.56	0.80	0.32–1.80	0.61
Small vessel LVI	2.08	0.91–5.62	0.084	1.67	0.72–4.53	0.24
Large vessel LVI	2.80	1.33–5.97	0.007*	3.60	1.63–8.18	0.002*
Perineural invasion	1.86	0.91–3.78	0.09	1.63	0.77–3.39	0.20
Positive margin	1.01	0.16–3.44	0.99	0.92	0.15–3.15	0.91
Budding		0.87–4.73	0.094	Reference	0.69–4.01	0.23
Bd1	Reference	0.61–3.80	0.32	1.73	0.49–3.43	0.51
Bd2	2.09			1.39		
Bd3	1.60					
Low overall TILs	2.09	0.97–5.02	0.061	1.84	0.84–4.46	0.13
Neoadjuvant therapy	0.44	0.10–1.27	0.14	0.39	0.09–1.13	0.087
Adjuvant therapy	0.66	0.31–1.47	0.30	0.54	0.25–1.22	0.14
pT4	1.82	0.89–3.76	0.10	1.87	0.89–3.96	0.098
pN category	Reference	0.77–5.46	0.15	Reference	1.09–6.47	0.021*
pN0	2.07	1.16–6.49	0.022*	2.21		
pN1	2.67			2.56		
pN2						
Tumour deposits	2.06	0.80–4.67	0.13	2.23	0.86–5.12	0.094
pM1	2.36	1.05–5.64	0.038*	3.20	1.37–8.11	0.007*
MMR deficiency	1.18	0.47–2.73	0.71	1.48	0.58–3.54	0.39
KRAS mutation	4.40	1.22–17.70	0.024*	3.87	1.08–15.51	0.038*
BRAF mutation	1.36	0.36–4.35	0.62	0.84	0.18–2.95	0.80

CI, confidence interval; HR, hazard ratio; LVI, lymphovascular invasion; MMR, mismatch repair; TILs, tumour-infiltrating lymphocytes.

*Statistical significance.

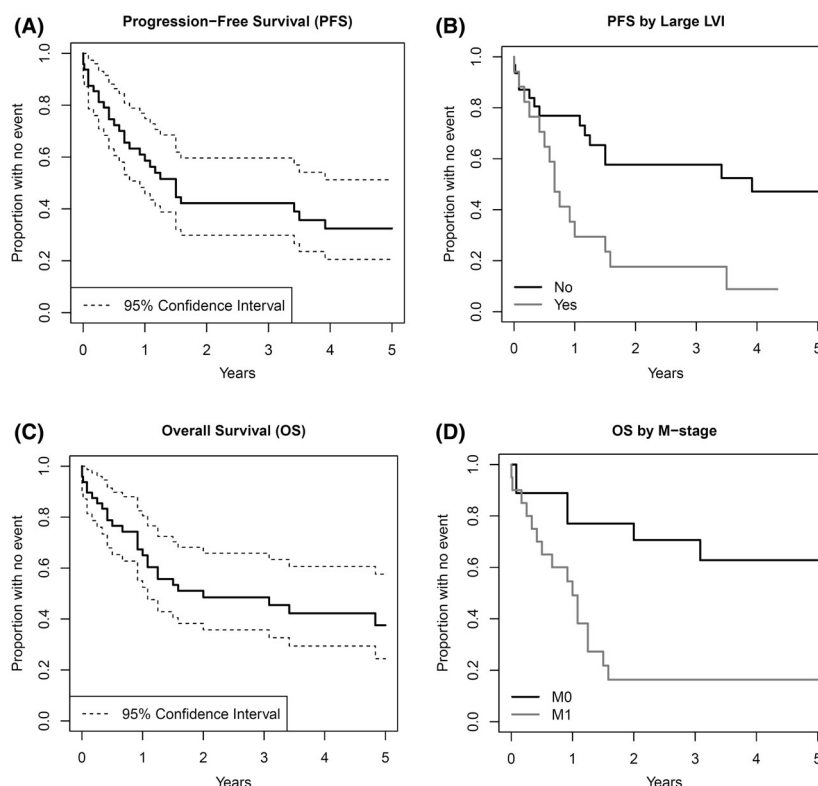


Figure 3. Outcome in North American patients with adenosquamous carcinoma. (A) Progression-free survival was 59% at 1 year and 33% at 5 years. (B) Large-vessel lymphovascular invasion (LVI) was significantly associated with decreased progression-free survival. (C) Overall survival was 65% at 1 year and 38% at 5 years. (D) Distant metastatic disease was significantly associated with decreased overall survival.

comparison with our cohorts (Figure 4C). We did not perform statistical analysis due to overlap in patients in the cohorts. Survival in patients with ASC was consistently worse than in patients with conventional CRC (HR: 1.7, 95% CI: 1.5–1.8, $P = 0.002$; Figure 4D) for all stages, although the effect in stage IV CRC was less pronounced. Of the four studies included in this forest plot, two were stage-matched,^{2,3} one provided data allowing our forest plot to provide stage-matched information,¹⁰ and one provided specific comparison data for stage III tumours.⁹ Limited data were available on factors that determined outcome within the group of ASC,

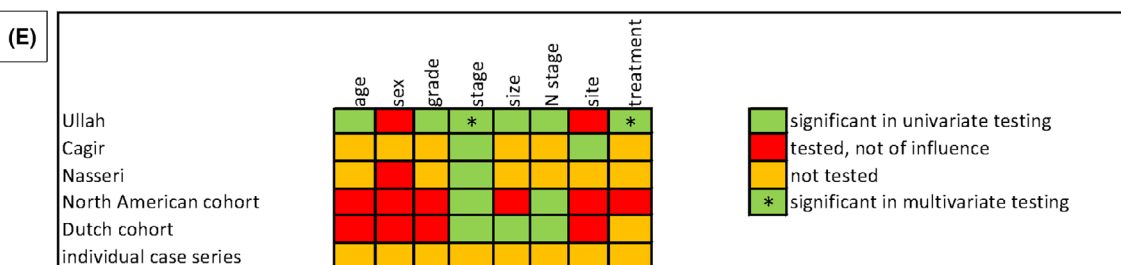
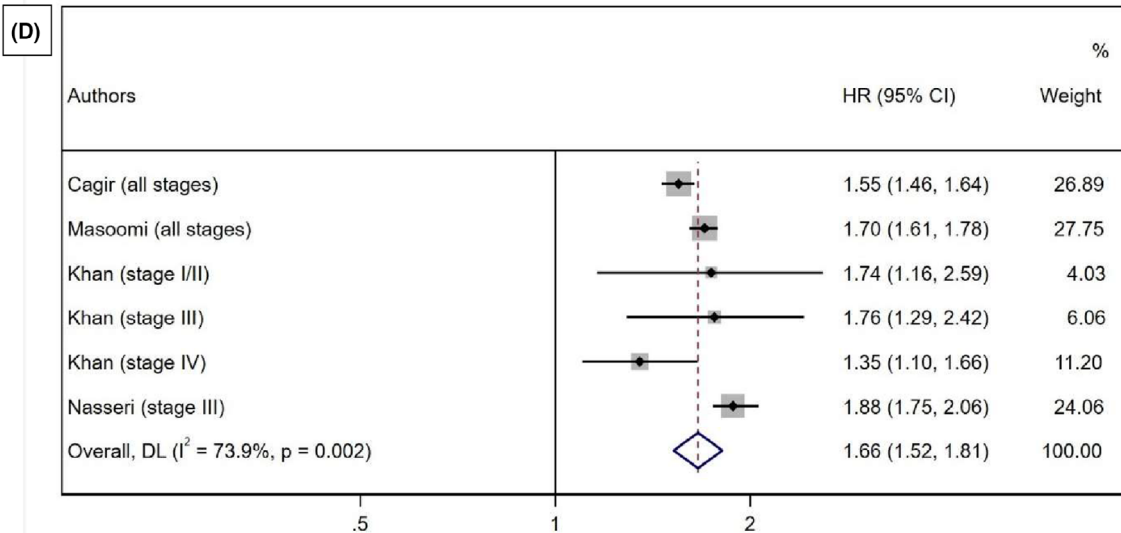
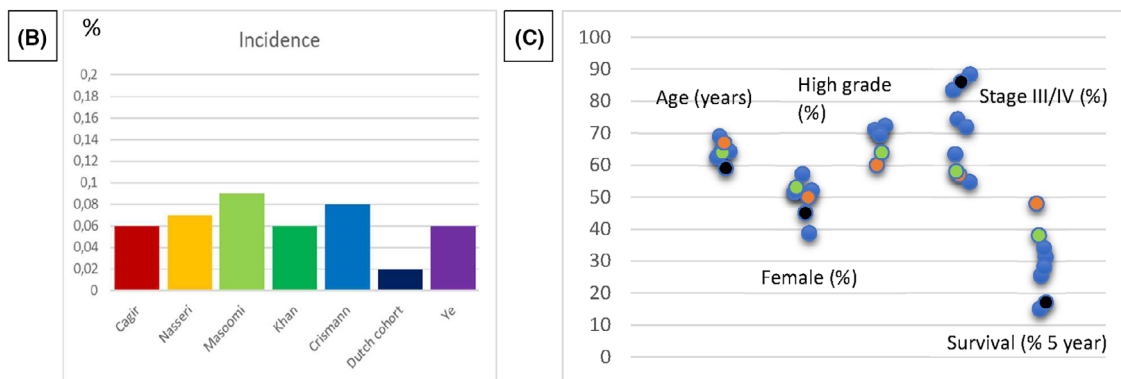
although stage was consistently deemed relevant (Figure 4E).

Discussion

Relatively few cases of colorectal ASC have been reported in the pathology literature with detailed pathologic analysis, many of those being decades old. Our study of 53 North American cases and 94 Dutch cases, all newly reported, reflects some of the previously reported findings (as shown in our literature review) but contains novel insights as well. Conventional CRC has a median age at diagnosis of around

Figure 4. Overview of results from the cohort studies. (A) Basic data of the included studies. *Individual case series; publication years instead of incident years are given. (B) Incidence data of adenosquamous carcinoma (ASC) in the total cohorts of colorectal cancer patients. (C) Summary of the main features of the different cohorts (in blue) compared to the cohorts in this study (North American cohort, green; Dutch cohort, orange; individual case series, black; registry series, blue). (D) Forest plot for survival of patients with ASC compared to colorectal adenocarcinoma not otherwise specified (CI, confidence interval; HR, hazard ratio). (E) Factors that were reported as significantly influencing survival in patients with colorectal ASC in cohorts from the literature and the current study. [Colour figure can be viewed at wileyonlinelibrary.com]

(A)	First author	Year	database/cohort	inclusion years	country	n
	Crismann ³⁰	1978	Cincinnati Registry	unknown	USA	5
	Frizelle ^b	2001	Mayo clinic tissue registry	1907-1992	USA	31
	Ye ¹³	2024	Sixth Affiliated Hospital	2012-2020	China	94
	Masoomi ³	2012	California Cancer Registry	1994-2004	USA	99
	Cagir ²	1999	SEER	1973-1992	USA	145
	Khan ¹⁰	2022	SEER	2000-2017	USA	279
	Ullah ¹¹	2024	SEER	2000-2020	USA	284
	Nasseri ⁹	2021	National Cancer Database	2004-2016	USA	332
	Current study		North American cohort	2000-2023	North America	53
			Dutch cohort	1990-2023	Netherlands	94
			Literature cohort	1953-2023*	Global	101



66–69 years,¹²³ and we found ASC to occur in a similar to slightly younger age range (median 65 years in our North American cohort, 69 years in our Dutch cohort, and 59 years in our literature review), a relationship that has been reported in some database reviews.^{13–15} Some small case series have also reported median ages in the fifth or sixth decade.^{12,13,21} Most of our data show a roughly equal male to female distribution; interestingly, most case series skew male^{6,7,12,13,20} but most large database reviews skew female.^{2,9,10} Our data also indicate a predilection for the right colon, which is similarly supported by some,^{2,7,13} but not all other series. Interestingly, 9% of our North American patients were asymptomatic (similar to 7% in a recent smaller case series⁷), and two of the four asymptomatic patients presented with pT3 disease. The majority of our ASC cases (60%–64%) were poorly differentiated, in keeping with prior reports, which is notably higher than CRC overall, for which the rate of poorly differentiated cases is roughly 20%.³ The macroscopic appearance of ASC is similar to that of conventional CRC. Our North American cohort and the reviewed literature cases found a median size of 6.0 cm, similar to that in case series where size data were reported.^{6,7,13} Occasional cases in the literature noted a link between colorectal ASC and ulcerative colitis^{24,28,30,38,44,54,77,84} or hypercalcemia,^{32,35,36,40,43,46,50} although this was uncommonly encountered in our cohorts.

Most important, the evidence in essentially all reported literature indicates that colorectal ASC generally presents at an advanced stage and has a poor stage-matched prognosis.^{2,3,6,7,9–13,20,21} Our study also found poor rates of PFS and OS in ASC. Our North American cohort demonstrated an all-stage estimated OS of 65% at 1 year and 38% at 5 years after resection, which is lower than reported survival rates for all-stage colorectal cancer in the literature (78.6% 1-year OS per National Health Service England data,¹²⁴ and 63%–67% 5-year OS per Surveillance, Epidemiology, and End Results data¹²⁵).

While larger database reviews exist, our study reports the largest cohort of colorectal ASC with detailed clinicopathologic review, including some specific findings that were significantly related to patient outcome. LVI is a known poor prognostic factor in CRC, particularly in early-stage disease.¹²⁶ We found a high rate (68%) of small-vessel LVI in our North American cohort, which was carefully re-reviewed, although the rate was lower in the Dutch cohort (26%), which relied on existing sign-out reports. Similarly, large-vessel LVI was found in 34% of North

American cases and reported in 16% of Dutch cases. While small-vessel LVI was not prognostic in our ASC series, perhaps due to underreporting that could not be assessed on independent review, large-vessel LVI was found to be significantly associated with both poor PFS and poor OS in the North American cohort. This study is the first to report LVI as a statistically significant prognostic factor in colorectal ASC, and is consistent with recent reports that large-vessel LVI in particular indicates poor prognosis in CRC.^{127,128} Distant metastatic disease was unsurprisingly also associated with poor OS in both of our ASC cohorts, and indeed stage appears to be the most consistently reported indicator of poor outcome in the literature.^{2,9,11} Tumour size was also significantly prognostic on univariate analysis in our Dutch cohort and in the second-largest database series.¹¹

Review of our North American cases showed that the mean percentage of squamous and glandular components was each 50%; only one other study has evaluated this, reporting that the mean squamous percentage was 38%.⁷ While tumour biology might be expected to differ between majority-squamous ASC and majority-glandular ASC, the percentage of the squamous component was not prognostic on survival analysis. Tumour budding, the presence of single tumour cells or clusters of up to four cells at the advancing edge of the tumour, is a well-established prognostic factor in CRC, particularly in early-stage disease.¹⁵ We evaluated the glandular components of the cases in our North American cohort using the 2016 ITBCC criteria and found that 64% had the lowest possible tumour budding score (Bd1). This usually implies a good prognosis in certain staging scenarios for conventional CRC, but this finding appears to be at odds with the poor prognosis noted in ASC patients, perhaps because ASC tends to present at an advanced stage or because prognosis is in part influenced by the squamous component. Metastatic foci in our cohort were sometimes glandular, sometimes squamous, and sometimes comprised of both components, a finding also reported by Parente *et al.*⁷

MMR status is considered an important prognostic factor for CRC, in addition to having relevance to treatment options and potential genetic syndromes for CRC patients.^{129,130} Patients with MMR-deficient (and as a result, MSI) CRC generally have an improved prognosis.¹³¹ The typical rate of MMR deficiency in sporadic CRC is ~15%.¹³¹ In contrast, our study of colorectal ASC found an MMR/MSI deficiency rate more than double this; one-third of our cases in both cohorts showed MSI or loss of MMR

protein expression, most commonly of MLH1 and PMS2, and always in both the glandular and squamous components. This rate is also higher than reported MMR deficiency rates in squamous cell carcinoma in other organs.¹³² Our literature review also identified occasional cases with MMR deficiency,^{70,71,76,77,79,86} although MMR testing was not reported in the vast majority of reviewed cases. In their recent series of 11 cases, Ye *et al.* reported MMR deficiency in 3 of 7 (42%) tested tumours.¹³ In contrast, a recent molecular analysis of 22 ASC cases by Angerilli *et al.* included MMR IHC on all cases, finding only one (5%) to be MMR-deficient⁸; the reason for this wide discrepancy between our two cohorts and their cohort is unclear. To our knowledge, the MMR status has not been thoroughly studied in ASC of other sites where the entity is more frequent (e.g. pancreas). Of our 23 cases with MMR loss or MSI, 13 patients were dead on last follow-up, suggesting that this is not a favourable prognostic feature in this subtype of CRC. Beyond MMR status, most molecular data for colorectal ASC come from the study by Angerilli *et al.*,⁸ which mainly reported mutations in *TP53*, *APC*, *KRAS*, *BRAF*, and *GNAS*, all of which (except *GNAS*) are commonly mutated in conventional CRC¹³³; a similar number of cases in our series had been tested for molecular abnormalities during routine evaluation, with *KRAS* and *BRAF* mutations in respectively 38% and 27% in our cases, which are respectively similar to and higher than reported figures for conventional CRC.¹³³

Finally, an additional key prognostic indicator for CRC is the presence of TILs at the advancing edge of the tumour. A higher percentage of TILs present indicates a higher level of tumour-induced immune response and generally a better prognosis.^{134–136} Using the ITWG criteria for TIL assessment, 66% of the cases in our North American cohort showed a low stromal TIL score (0%–10%). This was true of both the overall tumour assessment as well as when assessing the squamous and glandular components separately. The low TIL score for ASC is consistent with the poor prognosis expected for these patients and may also help explain why patient outcome was poor despite high rates of MMR deficiency. Studies have shown that MMR-deficient CRC also tend to show a higher amount of TILs.¹³⁷ However, MMR-deficient CRC with low TILs (such as in our cases) demonstrates a worse prognosis than MMR-deficient CRC with high TILs.¹³⁷ Accordingly, a recent study of ITWG-scored TILs in CRC found the TIL score to be prognostic, regardless of MMR status.¹⁷ These findings appear relevant to our ASC cohort, with high

rates of MMR deficiency but low TILs and poor outcome. Whether our finding of low tumour budding factors into this complex interaction currently remains unclear. We do note that MMR status and budding status were not found to be prognostic factors in this study, although low TILs trended toward poor PFS ($P = 0.061$).

Our study has some limitations, most of which resulted from the rarity of colorectal ASC. The relatively small size of our cohorts should be kept in mind when judging our conclusions regarding the behaviour and the prognostic significance of certain clinicopathologic findings in ASC. We note that, although the number of cases included may seem low, we included all available cases from 19 different academic institutions in North America and from a national database in the Netherlands, and we contacted multiple additional North American institutions that did not have cases to contribute. Additionally, many of the included cases were evaluated on a consultation basis, resulting in further limitations, such as metastatic lesions not available for histology review, limited follow-up data, and the lack of molecular data. Finally, some clinicopathologic differences were seen in the North American and Dutch cohorts. The reason for this is unclear, although it may simply relate to different patient populations.

In conclusion, colorectal ASC often presents at an advanced stage and has a poor prognosis, as previously reported, although some patients were asymptomatic. The squamous and glandular components are often approximately equal, which should facilitate diagnosis on resection, if not on biopsy. Stage is a consistent prognostic indicator, and other potential indicators include size and large-vessel LVI. Low tumour budding and MMR deficiency often indicate improved prognosis in CRC overall, but this does not appear to be the case for ASC, which has high rates of Bd1 budding and MMR deficiency but also shows low TILs.

Author contributions

R.S.G. and I.D.N. provided study concept and design; R.K.H. and R.S.G. drafted the article; all authors provided acquisition of data, analysis, and interpretation of data; were involved in revision of the article; and approved the final article.

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Conflict of interest

The authors report no relevant conflicts of interest.

Data availability statement

The datasets used and analysed during the current study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. A univariate analysis of factors influencing unadjusted progression-free and overall survival in the Dutch cohort of colorectal adenosquamous carcinoma.

Table S2. Clinicopathologic details of 101 additional cases of colorectal adenosquamous carcinoma previously reported in the literature.