

# Adenosquamous carcinoma of the colon with an aberrant MLH1 immunostaining pattern: a diagnostic pitfall in mismatch repair assessment

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## Case Report

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# Abstract

## Background

Adenosquamous carcinoma (ASC) is a rare histological subtype of colorectal cancer (CRC) that carries a poor prognosis and has been associated with a distinct molecular profile - higher frequency of mismatch repair deficiency (dMMR), PD-L1 overexpression, and KRAS mutations. With recent advances in CRC therapeutics, molecular characterization of rare histological subtypes has become increasingly important. Here, we report a case of colorectal ASC with a distinctive dMMR immunohistochemical profile and provide a review of the literature on this rare colorectal neoplasm.

## Case Presentation:

We report the case of a 64-year-old woman who presented with a three-month history of worsening abdominal pain and recent-onset hematochezia. Abdominal CT revealed a large mass involving the right colon. She underwent a total pelvic exenteration. Histopathology showed a high-grade, poorly differentiated tumor with mixed glandular and squamous components, staged as pT4b pN1a. Immunohistochemistry confirmed both glandular and squamous differentiation. Mismatch repair protein analysis showed loss of PMS2 expression and a granular, dot-like MLH1 staining pattern—interpreted as loss of expression and consistent with a known clone-specific artifact—while MSH2 and MSH6 were retained, supporting a dMMR phenotype. The patient received mFLOX chemotherapy but died four months postoperatively due to disease-related complications.

## Discussion

This case illustrates the classic clinicopathological features of ASC, alongside a dMMR immunoprofile that may carry therapeutic relevance given the efficacy of immune checkpoint inhibitors in MSI-high colorectal tumors. The findings also underscore the importance of correctly recognizing clone-specific MLH1 staining artifacts, as misinterpretation could falsely suggest isolated PMS2 loss and trigger unnecessary germline testing for Lynch syndrome. Given the rarity of ASC and its limited molecular characterization, accurate MMR status assessment remains critical for guiding treatment decisions and avoiding diagnostic pitfalls.

## Background

Colorectal cancer (CRC) is a significant medical and economic burden worldwide and is among the most common malignancies in Brazil. Despite advances in screening programs, early detection, and awareness of modifiable risk factors, CRC remains one of the leading causes of cancer-related mortality, particularly among younger patients (1, 2). Although most CRCs are adenocarcinomas, other histological subtypes exist, including adenosquamous carcinoma (ASC), a rare subtype comprising less than 1% of

gastrointestinal cancers and less than 0.1% of all diagnosed CRCs. This histological subtype usually occurs in older individuals, is frequently diagnosed at an advanced stage, is associated with aggressive biological behavior and poor prognosis (3). In addition, a distinct molecular profile has been reported, including a higher frequency of mismatch repair deficiency (dMMR), PD-L1 overexpression, and KRAS mutations compared with conventional colorectal adenocarcinoma (3, 4). With recent advances in CRC therapeutics, including new chemotherapy regimens and immune checkpoint inhibitors, molecular characterization of rare histological subtypes has become increasingly important. Here, we report a case of colorectal ASC with a distinctive dMMR immunohistochemical profile and provide a review of the literature on this rare colorectal neoplasm.

## Case presentation

A 64-year-old woman presented to the emergency department with a three-month history of progressively worsening right lower quadrant pain. One week prior to admission, she developed hematochezia. She was an active smoker, and her past medical history was significant for previous cholecystectomy, appendectomy, and cesarean section.

On physical examination, the patient reported severe abdominal pain, but there were no signs of peritonitis. Abdominal palpation revealed a palpable mass in the right iliac fossa. An abdominal computed tomography (CT) scan (Fig. 1) demonstrated irregular parietal thickening of the right colon, extending from the ileocecal valve to the proximal hepatic flexure and measuring 13.0 × 11.0 × 8.2 cm. The lesion was in contact with the right anterior abdominal wall in the iliac fossa region. Multiple regional lymphadenopathies were also identified, measuring up to 1.6 cm.

The patient subsequently underwent en bloc resection of the distal ileum, right colon, urinary bladder, and internal reproductive organs, with creation of a diverting ileostomy.

Gross examination revealed a large tumor mass measuring 13.1 × 8.5 × 5.2 cm, primarily centered in the cecum. The lesion exhibited an ulceroinfiltrative growth, extending distally along the colon and resulting in luminal obstruction. On sectioning, the tumor was seen to invade through the colonic serosa, with direct extension into the anterior abdominal wall musculature, as well as involvement of the right ovary and fallopian tube.

Histologic examination revealed a high-grade, poorly differentiated epithelial neoplasm composed of admixed glandular and squamous components, with the squamous component accounting for approximately 45% of the tumor (Fig. 2).

The neoplasm displayed a combination of tubular, trabecular, cribriform, and solid growth patterns, along with a focal discohesive single-cell component. Tumor cells showed marked cytologic atypia. A desmoplastic stromal reaction and tumor necrosis were also present, together with a moderate intratumoral lymphocytic infiltrate. Perineural and intramural and extramural lymphovascular invasion were identified. The final pathological stage was pT4b pN1a, with negative surgical margins.

Immunohistochemistry showed diffuse CAM5.2 positivity in the neoplastic cells, while CK5/6 and p63 labeled the squamous component, confirming squamous differentiation. Among the mismatch repair (MMR) proteins, PMS2 showed loss of nuclear expression, whereas MSH2 and MSH6 were retained. MLH1 displayed a granular, dot-like nuclear pattern, interpreted as loss of expression (Fig. 3).

Following diagnosis, chemotherapy with mFLOX (Folinic acid, fluorouracil and oxiplatin) was initiated. The patient experienced multiple complications requiring repeated hospitalizations and died four months after surgery.

## Discussion and conclusions

ASC is a rare subtype of CRC, comprising only 0.06–0.18% of all colorectal cancer diagnoses (5). The first case was reported by Herxheimer in 1907 and was described as a biphasic tumor containing both adenocarcinoma and squamous cell carcinoma components. Several hypotheses regarding tumor histogenesis have been proposed, including origin of the squamous component from undifferentiated colonic cells, malignant transformation of ectopic squamous cells within the colonic mucosa, and squamous differentiation arising from normal glandular epithelium or from a pre-existing colonic adenocarcinoma (5, 6).

This histological subtype is associated with aggressive biological behavior, and most cases are diagnosed at advanced stages. Reported series have shown that 36.6–46.2% of cases present with stage IV disease. Five-year survival rates vary considerably across studies, with a reported mean overall survival of 39.4 months, decreasing to as low as 8.5 months in patients with stage IV disease (4).

Histologically, two architectural patterns have been described: the composite type, in which the glandular and squamous components are haphazardly admixed, and the collision type, in which two adjacent but histologically distinct tumors coexist in a “side-by-side” pattern. No specific percentage cutoff is required for either histological component (5, 6).

ASC harbors molecular alterations that overlap with those observed in conventional colorectal adenocarcinoma, including mutations in KRAS, APC, TP53, and BRAF. However, a higher prevalence of microsatellite instability (MSI) and MMR deficiency has also been reported in this tumor subtype (3). Recognition of dMMR status in ASC may have direct therapeutic implications, as immune checkpoint inhibitors have demonstrated substantial efficacy in MSI-high colorectal carcinomas.

The case described in this report harbors typical clinicopathological features of ASC, including right-sided colonic origin, advanced disease stage at diagnosis, and a prominent poorly differentiated component. The immunohistochemical profile demonstrated loss of MLH1 and PMS2 expression, with retained expression of MSH2 and MSH6, consistent with a dMMR tumor.

MSI can be assessed using PCR-based amplification of microsatellite markers or by next-generation sequencing-based approaches. However, molecular testing may not be widely available in many settings,

particularly in low- and middle-income countries. In this context, immunohistochemistry (IHC) for MMR proteins plays a fundamental role in the evaluation of MMR deficiency and the identification of tumors potentially harboring MSI (9) .

In this case, there was loss of MLH1 and PMS2 expression, with the granular, dot-like MLH1 staining pattern representing an immunohistochemical artifact associated with the Ventana-Roche M1 clone used in the present case. This staining pattern has previously been reported at a low frequency, occurring in approximately 6.3% of colorectal carcinomas evaluated in the study by Dasgupta et al. This phenomenon has been proposed to result from a non-functional MLH1 protein variant with preserved antigenicity. Failure to recognize this MLH1 staining pattern may lead to an erroneous interpretation of the MMR protein profile and consequently prompt unnecessary germline mutational analysis. In particular, misinterpreting the MLH1 staining pattern as retained expression could result in an apparent isolated PMS2 loss, a finding associated with an increased risk of Lynch syndrome and that may therefore trigger further germline evaluation (10, 11).

A limitation of this study is that we were unable to repeat the MLH1 immunohistochemical analysis using a different antibody clone or confirm microsatellite instability through molecular testing.

Our case report highlights the main clinical and histopathological characteristics of this rare tumor subtype, which is associated with aggressive clinical behavior. Due to the limited number of cases described in the literature, its molecular profile remains poorly characterized. The reported higher prevalence of ASC with a dMMR phenotype highlights the importance of assessing MMR status in this tumor subtype, particularly given the potential therapeutic implications of MSI-high/dMMR status. This case also underscores the importance of accurate interpretation of MMR immunohistochemical staining patterns. Recognition of clone-specific staining artifacts is essential to avoid misinterpretation of the MMR profile and inappropriate downstream testing (7–9).

## Abbreviations

ASC  
adenosquamous carcinoma  
CT  
computed tomography  
CRC  
colorectal cancer  
dMMR  
mismatch repair deficiency  
MMR  
mismatch repair  
MSI  
microsatellite instability

mFLOX  
folinic acid, fluorouracil and oxiplatin  
PCR  
polymerase chain reaction

## Declarations

**Ethics approval and consent to participate:** Given that this report includes only non-identifiable histopathological, immunohistochemical and radiologic findings, the requirement for patient consent for publication was waived by the Ethics Committee of the Department of Pathology, Faculdade de Medicina da Universidade de São Paulo (FMUSP), in accordance with institutional policy for the use of de-identified pathological material in scientific publications.

**Consent to Publish declaration:** not applicable.

**Availability of data and materials:** All data analyzed during this study are included in this published article.

**Competing interests:** The authors declare that they have no competing interests

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**Author contributions:** Beatriz Gioppo Betini and Wilker Dias Martins were responsible for the conception and design of the case report, conducted macroscopic evaluation, dissection, and sampling of the resected specimen for histopathological analysis. Wilker Dias Martins performed the histopathological and immunohistochemical analysis and interpretation. Beatriz Gioppo Betini drafted the initial manuscript. Wilker Dias Martins critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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## Figures



**Figure 1**

CT scan demonstrating an infiltrative tumor mass centered in the right colon, measuring 13 cm in its greatest dimension.

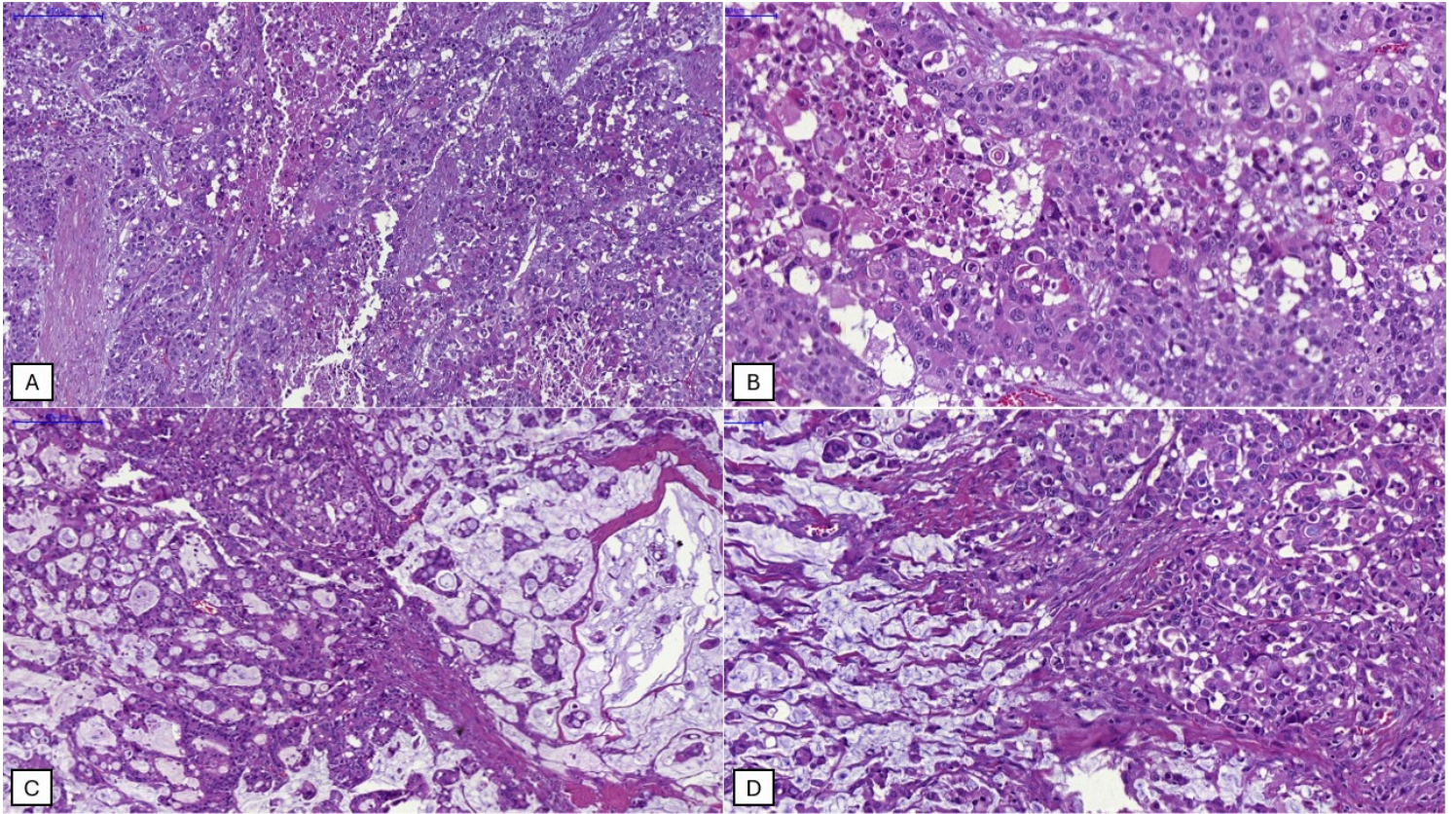


**Figure 2**

Poorly differentiated carcinoma exhibiting squamous differentiation (figure A; original magnification 100x) with keratinization and intercellular bridges (figure B; original magnification 200x). Glandular component displaying tubular and cribriform growth patterns, extracellular mucin (figure C; original magnification 100x), and poorly cohesive cells, including signet ring cells (figure C; original magnification 200x).

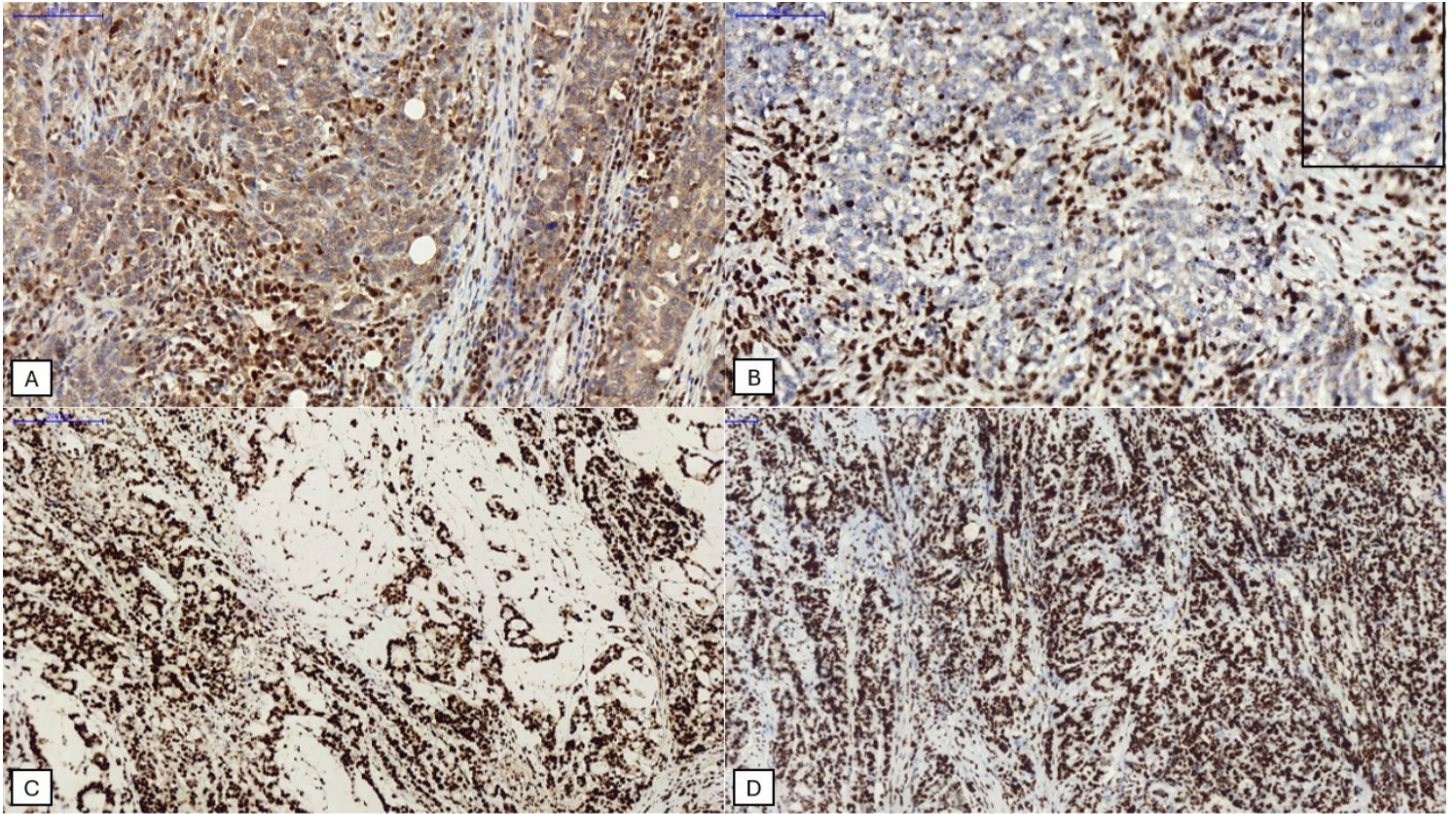


**Figure 3**

Absence of nuclear PMS2 staining, with nonspecific cytoplasmic expression (figure A; original magnification 200x). Granular, dot-like nuclear MLH1 staining, using the M1-clone, better appreciated in the higher magnification (upper right panel) (figure B; original magnification 200x). Positive MSH2 (figure C; original magnification 100x) and MSH6 staining (figure D; original magnification 200x).