Polish Annals of Medicine

Journal homepage: https://www.paom.pl

Research Paper

# Visualizing the desmoplastic reaction at the front of colorectal cancer – the value of Mallory staining

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Article info

Article history
Received: April 22, 2026
Accepted: June 8, 2026
Available online: July 9, 2026

Keywords
Colorectal cancer
Mallory staining
Desmoplastic reaction

Doi
https://doi.org/10.31648/paom/224271



Abstract

Introduction: Colorectal cancer invasion patterns at the tumor front reflect interactions between cancer cells and the surrounding stromal microenvironment. Two different invasion patterns are recognized: an infiltrative pattern, associated with aggressive tumor behavior, and an expansive pattern characterized by a prominent desmoplastic reaction with collagen fiber accumulation. Because collagen fibers are difficult to distinguish from smooth muscle tissue in routine hematoxylin–eosin staining, additional histochemical methods may improve their visualization.

Aim: This pilot study aimed to evaluate the usefulness of Mallory trichrome staining in the histopathological assessment of collagen fibers at the colorectal cancer invasion front.

Material and methods: Tissue samples from 126 patients who underwent surgical resection for colorectal cancer were analyzed. Based on hematoxylin–eosin staining, representative cases demonstrating a collagen-rich stromal reaction at the tumor margin were identified, and 25 selected samples were additionally examined using Mallory trichrome staining.

Results and discussion: Mallory staining enabled clear visualization and differentiation of collagen fibers from surrounding tissue structures, particularly smooth muscle layers, allowing a more precise identification of the desmoplastic reaction at the tumor invasion front. This improved morphological assessment of the expansive pattern of tumor growth compared with routine hematoxylin–eosin staining alone.

Conclusions: These findings suggest that Mallory trichrome staining may serve as a useful supplementary histochemical technique for evaluating stromal reactions in colorectal cancer. Improved identification of desmoplastic reaction may support more accurate characterization of tumor invasion patterns and contribute to improved pathological assessment.

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## 1. INTRODUCTION

In pathomorphological observations of colorectal cancer (CRC), two different types of cancer invasion into the intestinal wall tissue have been identified. The first is an "infiltrative pattern", which is defined by the invasion of cancer cords or finger-like protrusions into the intestinal wall, with the presence of cellular buds and single cells in the boundary area. The infiltrative tumor border configuration is mostly associated with blood vessel invasion, lymph node metastases, and distant spread to other organs, which corresponds to a more aggressive course of the disease.[1] The most common sites of metastases are the liver, lungs and non-regional lymph nodes.[2]

The second type, an "expansive pattern", is observed in cases with stromal fibrotic reaction and collagen fiber accumulation,[3,4] which forms a physical barrier to cancer cell infiltration.[5] Collagen accumulation in the tissues adjacent to the invasive front of CRC is referred to as a desmoplastic reaction (DR) (4). This response is characterized by upregulated collagen synthesis (particularly types I and III) by cancer-associated fibroblasts (CAFs), contributing to the formation of a fibrotic stroma at the tumor boundary.[6] This connective tissue response is categorized into three types based on the presence of specific CAF products, such as myxoid stroma and immature or mature hyalinized keloid-like collagen.[7] Mature fibrotic stroma and an abundance of collagen fibers have been found to correlate with better survival in CRC patients.[8]

In routine pathomorphological practice, the presence of collagen fibers is assessed using standard hematoxylin and eosin (H&E) staining. However, collagen protein exhibits only weak contrast under a microscope, appearing pale pink and often difficult to distinguish from the similarly colored smooth muscle fibers of the intestinal wall.

## 2. AIM

The aim of this study is to evaluate the usefulness of Mallory staining in the histopathological assessment of CRC tissue specimens. The study investigates whether specific histochemical staining of collagen fibers improves the visualization and delineation of the desmoplastic invasion front in CRC.

## 3. MATERIAL AND METHODS

This single-center pilot study, focusing on CRC margin components, was conducted with the approval of the University Bioethical Commission. CRC and adjacent morphologically unchanged tissue samples from 126 patients who underwent CRC tumor resection were obtained from archival paraffin blocks at the authors' institution. Deparaffinized CRC sections were stained using the H&E method for histopathological evaluation.

Based on the following inclusion criteria: prominent desmoplastic stromal reaction, presence of spindle-shaped fibroblasts, and dense extracellular matrix formation – a subset of 25 samples was selected from the analyzed cohort and stained using the classical Mallory trichrome staining. The Mallory trichrome technique uses aniline blue, acid fuchsin, and orange G to enhance contrast between collagen fibers (stained blue) and other tissue types such as smooth muscle (stained red/carmine).[9]

Histological sections were deparaffinized and stained with 0.1% acid fuchsin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany; cat. no. 8129) for 5 min and rinsed with distilled water. Sections were subsequently immersed in 1% phosphomolybdic acid (Honeywell International Inc., Seelze, Germany, cat. no. 221856) for 2 min, quickly rinsed, and placed in Mallory staining solution, composed of: 2% orange G (cat. no. PA-03-7194-T), 2% oxalic acid (cat. no. 658537), and 0.5% aniline blue diammonium salt (cat. no. 415049; all from Sigma-Aldrich) for 3 min, washed, dehydrated with ethanol, and cleared with xylene. Observations were performed using an Olympus BX41 microscope equipped with an Olympus XC50 camera and Cell software (Olympus, Tokyo, Japan), and representative microphotographs were obtained by a blinded pathologist.

## 4. RESULTS

The examination of CRC tissue specimens from a cohort of 126 patients enabled the preparation of reference figures illustrating two invasion patterns at the CRC invasion front: the infiltrative pattern of cancer invasion (Figure 1a) and the expansive pattern (Figure 1b). Mallory staining of a selected subset of 25 patients from the analysed cohort demonstrated a clearly visible accumulation of collagen fibers at the CRC invasion margin (Figure 1c, 1d). These findings suggest that Mallory trichrome staining may serve as a useful supplementary histochemical technique for evaluating stromal reactions in colorectal cancer.

## 5. DISCUSSION

This study highlights the diagnostic value of Mallory staining in the histopathological evaluation of CRC. This technique clearly differentiates collagen fibers from surrounding tissue structures, making it a practical tool for assessing the desmoplastic reaction at the tumor invasion front. In contrast to H&E staining, which provides poor contrast between collagen and smooth muscle fibers, the Mallory staining offers enhanced visualization and facilitates more accurate tissue interpretation. The presence of a desmoplastic reaction at the invasive front has emerged as a prognostic marker in CRC,[7,8] as it reflects the biological response of the tumor microenvironment, particularly the activation of cancer-associated fibroblasts and remodeling of the extracellular matrix.

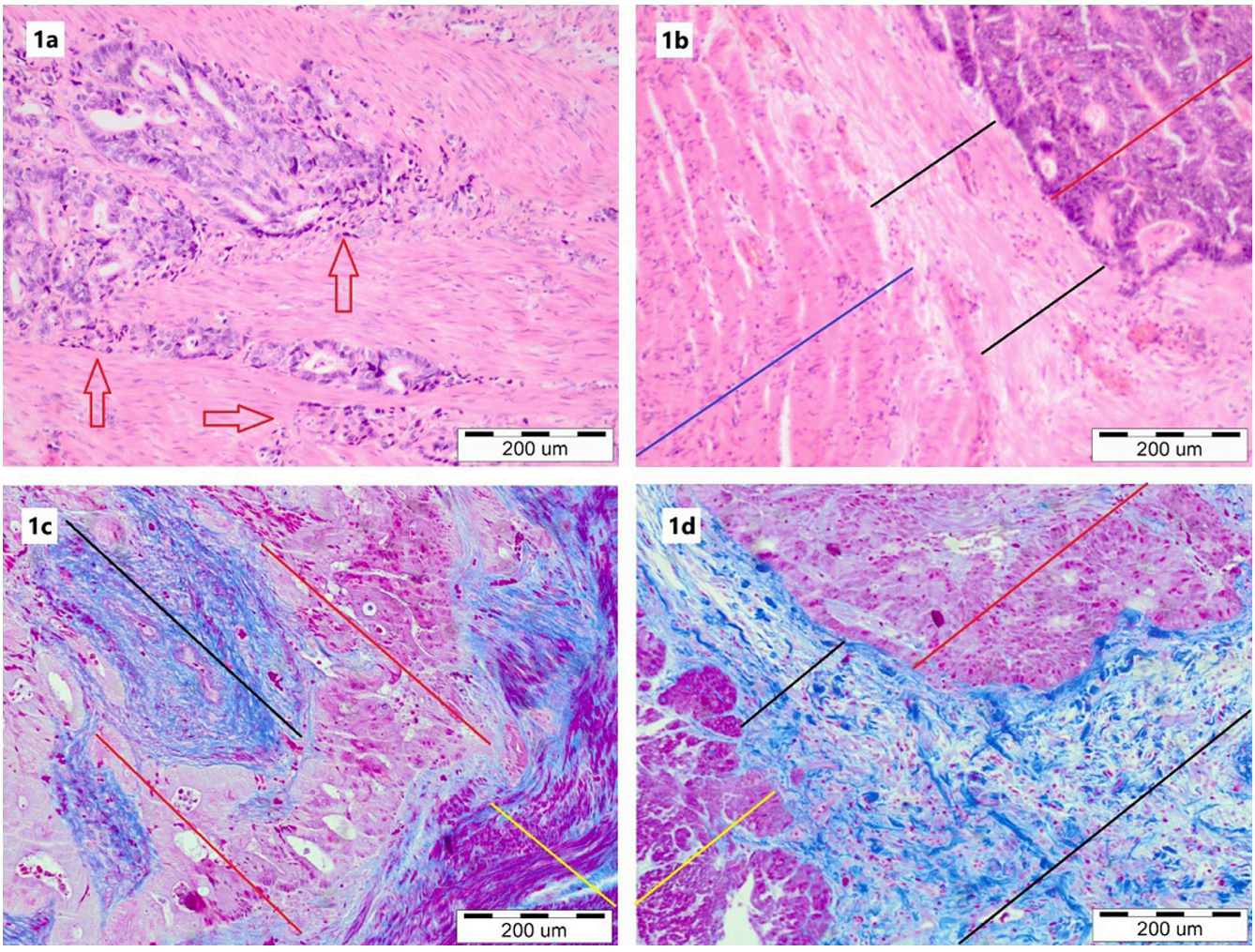


**Figure 1. The haematoxylin–eosin (a, b) and Mallory trichrome (c, d) staining reveals the types of colon cancer infiltration in the large intestine wall. (a) Infiltrative cancer growth pattern, cancer cell cords and clusters (red arrows) invade the muscularis externa directly with the absence of any boundary between cancer and muscle tissue. (b) Expansive cancer growth pattern, the tumor mass is demarcated by a layer of fibrous connective tissue – DR (c) Mallory staining clearly shows DR at the CRC tumor boundaries; collagen fibers (blue) are mixed with smooth muscle cells (magenta), muscularis propria is displaced and damaged. (d) Prominent desmoplastic reaction and preserved layer of muscularis propria. Legend: tumor tissue – red arrows or lines; desmoplastic reaction – black lines; muscularis externa – blue or yellow lines. Magnification: 100×. Patients: Figure 1a, 56-year-old male, rectum cancer (TNM stage IIa, T3N0M0, G2); Figure 1b and 1d: 71-year-old male, rectum cancer (TNM stage IIIb, T3N1M0, G2); Figure 1c – 74-year-old male, rectum cancer (TNM stage IIc, T4bN0M0, G2).**

The most widely used pathological assessment of stage of CRC progression is based on the TNM staging system, provided by the WHO Classification of Tumors – Digestive System Tumors, 5th Edition, 2019 and the American Joint Committee on Cancer (AJCC Staging Manual, 8th Edition), Union for International Cancer Control (UICC).[10,11] The pathomorphological report must include T-staging that reflects the depth of tumor invasion into the layers of the colonic wall (T1–T3), and potential infiltration to the adjacent organs or structures (T4), an evaluation of features of cellular atypia and grading.

Assessment of the invasive cancer potential at the molecular level has led to the expansion of recommendations regarding the characterization of tumor infiltration. The presence or absence of certain molecular features directly complements the pathological TNM staging system. A clearly adverse prognostic factor is the presence of vascular invasion (EMVI), defined as the identification of cancer cells within the lumen of small capillaries, venules, or lymphatic vessels – whether located in the bowel wall or the surrounding mesentery.[11] Another important indicator of aggressiveness is perineural invasion (PNI), characterized by the presence of cancer tissue near nerves, involving at least 33% of the nerve circumference, or the presence of tumor cells within the epineurium, perineurium and endoneurium of the nerve sheath. The occurrence of PNI suggests a higher invasive

potential of the cancer cells and distant hematogenous and/or lymphatic metastases.[12,13]

Desmoplastic reaction at the invasive front of CRC is histologically classified into three patterns – mature, intermediate, and immature, based on the presence of keloid-like collagen bundles and/or myxoid stroma.[14] This grading has been shown to have prognostic value, as immature desmoplastic reaction is associated with significantly worse outcomes (recurrence-free and overall survival) compared with intermediate and mature DRs, and remains an independent predictor across multicenter and prospective validations.[15] International references support the relevance of desmoplastic reaction in CRC pathology; however, major international guidelines do not currently include desmoplastic reaction assessment as mandatory, unlike tumor budding. Assessment of desmoplastic reaction is an optional element requiring wider standardization.[16,17]

## 6. CONCLUSIONS

This pilot study emphasises that Mallory staining may be a valuable supplementary technique for identifying collagen fibers and improving the visualization of the DR at the CRC invasion front. The presence of DR has prognostic significance, and its identification in postoperative pathology reports may support the distinction between infiltrative and expansive growth patterns.

### Acknowledgements
The Authors gratefully thank Anna Koprowicz, M.Sc. for her excellent technical assistance.

### Ethics approval
All procedures performed in studies involving human participants were conducted in accordance with institutional ethics committee standards and the Declaration of Helsinki. Ethical approval was granted by the Bioethical Commission of the University (no. 40/2010). This analysis, focusing on CRC margin components, was conducted under approval no. 22/2024 of the same Commission.

### Informed consent
Informed consent was obtained from all participants included in the study.

### Conflict of interest
None declared.

### Funding
None declared.

### Author contributions
Study design: JU-S, ZK, JG
Data collection: ZK, JG
Data interpretation: ZK, JG
Manuscript preparation: JU-S, JG
Literature search: JU-S

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