DOI: https://doi.org/10.4038/jdp.v17i1.7820 *Journal of Diagnostic Pathology 2022;17(1):1-10.*

Research paper

Comparison of elastic tissue stain with haematoxylin and eosin stain in the detection of venous invasion in colorectal carcinoma in the Sri Lankan setting

P. N. Yogananda[1], S. R. Constantine[1], K. Rajeewan[1], W.R.A.T.J. Ranasinghe[2]

[1] *Department of Pathology, National Hospital of Sri Lanka, Sri Lanka*

[2] *Department of Pathology, Base Hospital Homagama, Sri Lanka*

Submitted on 08.12.2022. Accepted for publication on 13.02.2023.

Abstract

Introduction and objective: Colorectal carcinoma (CRC) is the third leading cause of cancer among both males and females in Sri Lanka. Venous invasion (VI) by the tumour is a well-recognized independent prognostic factor in CRC and a key high-risk factor that determines the need for adjuvant therapy in stage II CRC. It is widely accepted that VI is under-recognised on haematoxylin and eosin (H&E)-stained tissue sections of the CRC resection specimens. The main objective of this study was to evaluate whether the VI detection rate on an elastic tissue stain (ETS) is superior to that of the VI detection rates on a routine H&E stain in CRC resection specimens in a Sri Lankan setting.

Methodology: This was a retrospective, descriptive study with an analytical component carried out at the Department of Histopathology, National Hospital of Sri Lanka. Data were retrieved retrospectively from 386 cases of CRC. The cases with no VI on the H&E stain were subjected to ETS and re-examined for the presence of VI on both H&E and ETS. The results of the two stains were compared statistically using the chi-square test.

Results: VI was present in 152 of the 386 specimens (39.38%) on the initial H&E-stained sections. Of the remaining 234 specimens, ETS enabled the detection of VI in a further 122 cases, increasing the overall VI detection rate to 70.98% of CRC resection specimens (X^2=102.490, P<0.001).

Conclusion: The addition of an ETS enhances the histological detection of VI in CRC resection specimens.

Keywords: colorectal carcinoma, venous invasion, elastic-van-Gieson stain

Introduction

Colorectal cancer (CRC) is the third leading cause of cancer worldwide and has the second-highest mortality rate (1). CRC is also the third leading cause of cancer among both males and females in Sri Lanka (2). The prognosis and treatment options of CRC are determined by the TNM stage. According to the National Comprehensive Cancer Network (NCCN) guidelines - USA (3), surgical resection with curative intent is the mainstay of treatment for stage I and II CRC. Patients with stage III and IV CRC are treated with postoperative adjuvant chemotherapy. However, the NCCN guidelines recommend the use of adjuvant chemotherapy in selected patients with stage II disease having high-risk features that include poor histological differentiation (except those that are MSI-H), lymphovascular invasion, perineural invasion, bowel obstruction, retrieval of fewer than 12 lymph nodes, perforation, and near, intermediate or involved margins (3,4,5). Thus,

Corresponding author: Dr Priyanka Yogananda
Department of Pathology
National Hospital of Sri Lanka, Sri Lanka
priy629@gmail.com

it is the histopathologist's responsibility to provide information on the above high-risk features following a thorough assessment of colorectal resection specimens.

Vessel involvement is one of the key high-risk factors in CRC. This was first described by Brown and Warren in 1938 in their studies on rectal carcinoma resection specimens (6). They were the first to recognize histological evidence of venous invasion (VI) in CRC and demonstrate that VI in the primary tumour is responsible for distal visceral metastases (6). Since then, several studies have assessed the relationship between the clinical behaviour of CRC and the histological finding of VI in these tumours (6). Currently, VI is a well-recognised independent indicator of haematogenic dissemination and cancer-specific survival in CRC (6-11).

Vascular invasion is more specifically defined and categorized as lymphatic invasion, intramural VI (IMVI) and extramural VI (EMVI) in the Royal College of Pathologists (RCPath) dataset for histopathological reporting of CRC (12). It recommends recording the deepest level of VI; extramural or intramural. TNM 8 tumour staging also requires the assessment of VI (13). EMVI is specifically a strong predictor of the unfavourable outcome (11,14-17). There is evidence that IMVI (intramuscular and submucosal) is also a prognosticator of CRC outcome (11,16,18).

The histopathological definition of VI has evolved over time. Talbot et al. defined VI as a tumour present in spaces containing red cells which are lined by endothelial cells and have a surrounding muscle layer (6). Subsequently, this definition was expanded to indicate that venous involvement should be looked for when there is a nodular or elongated tumour island that does not directly continue with the tumour front that is present next to an artery, especially, when there is no obvious adjacent vein (orphan artery sign) or a smooth bordered tumour protrusion into surrounding fat (protruding tongue sign) (12). Currently, this definition has been expanded to include the presence of an elastic layer (demonstrable by an elastic tissue stain/ETS) around these nodular/elongated tumour profiles or tumour protrusions as indicators of VI (6,11,15,19,20).

Some studies have shown that the use of an ETS increases the identification of VI and therefore, this special stain is a better predictor of patient prognosis than VI detected by the H&E stain alone (11,18-19,21). ETS is specifically useful in the detection of VI in cases following neoadjuvant chemotherapy, where the muscle wall of the vein is effaced or disrupted beyond recognition by the H&E stain due to treatment. Most of these studies have shown a two to three-fold increment in VI detection with an ETS (18,20,21, 22-24).

Roxburgh et al. studied 419 cases and showed a three-fold increment in the detection of VI from 18% to 58% following the addition of an ETS (18). One study demonstrated that ETS increases the inter-observer agreement for EMVI compared to H&E stain (21). Several authors have proposed that ETS should be performed routinely in the assessment of CRC resection specimens (19,23-27), and preferably at the first instance, citing low cost, (11,15,23,28,29) and impact on turnaround time and workload (25,27,28). Population-based data have shown that VI detection rates are low especially among pathologists not specializing in gastrointestinal pathology [29] and that the use of ETS enhances VI detection among such pathologists (21).

Both the College of American Pathologists (CAP) and RCPath guideline (UK) have emphasized the usefulness of ETS both in augmenting the detection of VI and enhancing its prognostic value (12,30). The RCPath guideline now recommends close monitoring of VI detection rates and performing a routine ETS in colorectal resection specimens in centres where the rate is consistently below the 30% threshold (12).

Immunohistochemical stains for endothelial markers such as CD31, factor VIII-related antigen and h-caldesmon have been used to detect VI but the value of these markers is questionable in challenging cases where the endothelium is not present (11,31). In addition, studies have not shown an improvement in interobserver agreement with respect to the

detection of VI with immunohistochemical stains (24-26, 32).

Currently, magnetic resonance imaging (MRI) is used as an accepted method of identification of the extent of locoregional spread of CRC for pre-surgical staging as high-resolution scanners can readily detect EMVI (33). The incidence of MRI-detected EMVI in CRC is shown to be comparable to that of pathological assessment done on the excised specimen, with high specificity and moderate sensitivity (12,33).

In Sri Lanka, routine histopathological assessment of CRC specimens is usually done with H&E-stained tissue sections alone. According to the worldwide literature, there is wide variability in the detection of VI with H&E stain in colorectal resection specimens (9%-90%) among different units (11,29). Interobserver variability too is marked, even among specialized gastrointestinal pathologists (20), which is partly attributable to difficulties in identifying VI on the H&E stain due to the destructive effects of tumour emboli within the vein. If a particular vascular space has completely lost its endothelium with the destruction of the muscle wall due to the expansive growth of the tumour within the vessel, it will be difficult to identify it as VI on the H&E stain alone. This problem can be overcome by using an ETS, which stains to highlight the characteristic elastic fibre pattern of the vessel wall around the suspected focus of VI and therefore, facilitates the confirmation or exclusion of VI. Detection of VI using MRI and immunohistochemical methods is not widely acceptable or cost-effective when compared to an ETS in the Sri Lankan setting.

In Sri Lanka, nearly all colorectal specimens are reported by general pathologists and not by specialized gastrointestinal pathologists. Furthermore, CRCs are managed according to the NCCN (USA) guidelines, which highlights the need for histopathological identification of high-risk features in stage II patients to decide on the use of adjuvant chemotherapy (3). Hence, the addition of an ETS to routine practice appears justifiable in the Sri Lankan setting.

The main objective of this study was to evaluate whether using an additional ETS is superior to that of the routine H&E stain alone in the detection of VI in CRC resection specimens in the Sri Lankan setting.

In addition, the data was analysed to determine the associations between VI and other pathological characteristics of CRC, including tumour site, tumour differentiation, the maximum local tumour infiltration (pT stage), presence of metastases in lymph nodes and perineural invasion.

Methodology

This was a retrospective, descriptive study with an analytical component carried out on 386 consecutive colon resection specimens (right hemicolectomies, transverse colectomies, left hemicolectomies, sigmoid colectomies, abdominoperineal resections and total colectomies) performed for CRC, reported at the Histopathology Department at National Hospital of Sri Lanka from January 2017 to February 2021. This included specimens from patients who had received neoadjuvant chemotherapy. Cases which showed pathological complete tumour response to chemotherapy on histological examination were excluded from the study.

Histopathology request forms and relevant histopathology reports were traced and clinicopathological data were extracted and recorded in the data extraction sheet. The information extracted included age, gender, tumour site, tumour differentiation, tumour stage (pT stage), perineural invasion, lymphatic invasion, VI, the total count of lymph nodes extracted, number of lymph nodes showing tumour metastases and TNM stage (according to AJCC 8th edition). Ethical clearance was obtained from the Ethics Review Committee, National Hospital of Sri Lanka.

Of the 386 cases included in the study, 152 had VI in the original histopathology report which was based on the review of H&E-stained tissue sections of the tumour. All the H&E-stained tumour-containing sections of the cases which were signed out as negative for the VI were

reviewed. Sections which showed features suspicious for VI and sections of the tumour front in which there were no suspicious foci on morphology were selected and subjected to Elastic Van-Gieson stain (Verhoeff elastic tissue stain-EVG) followed by counterstaining with H&E stain. Cases showing nodular or elongated tumour cell islands which were not directly continuous with the tumour front, observed near an artery without an adjacent vein (orphan artery sign) or tumour nodule with a smooth border protruding into the surrounding pericolic fat (protruding tongue sign) were considered suspicious for VI (12). Based on the above selection criteria, ETS was performed on one to four sections from each of the cases that were originally reported as negative for VI.

Following ETS, sections were examined for evidence of VI. Cases showing a convincing elastic layer, stained in black, around the rounded or elongated tumour profile or tumour protrusions were considered positive for VI (11, 15, 18, 19). They were categorized as EMVI and IMVI.

Statistical comparisons between the categories were carried out using chi-square (X^2 test). The level of statistical significance was regarded as < 0.05 for this study.

Results

Three hundred and eighty-six (386) colon resection specimens were studied, and 41/386 patients had received neoadjuvant therapy. The pathological parameters of the study population are summarized in Table 1.

The presence of VI had been identified and recorded in 152/386 (39.38%) colorectal specimens on initial H&E-stained sections, and 16/152 (10.53%) were reported to show IMVI alone.

Out of 234 cases, which were negative for VI on initial H&E-stained section examination, 122 showed VI following the EVG stain (52.14%) (Figure 1). This increased the overall VI rate from 39.38% to 70.98% (274/386). There was a significant increment in the rate of detection of VI after EVG stain on CRC specimens (X^2 = 102.490, p<0.001). The H&E stain alone showed VI in 13/42 (30.95%) cases following post-neo-adjuvant therapy and EVG stain enabled the identification of VI in another 15 cases resulting in a statistically significant increase in the VI detection rate bringing it to 66.67% (28/42) in this subcategory (X^2 = 25.066, p<0.001).

Of the 122 newly identified cases with VI, an additional 53 cases showed evidence of IMVI alone, increasing the number of cases with IMVI alone from 10.53% (16/152) to 25.18% (69/274), and this was a statistically significant increase (X^2 = 250.421, p<0.001) (Figure 2).

There was a statistically significant association between VI and other pathological characteristics that indicate aggressive behaviour in CRC, namely, tumour site, maximum local tumour infiltration (pT stage), presence of metastases in lymph nodes and

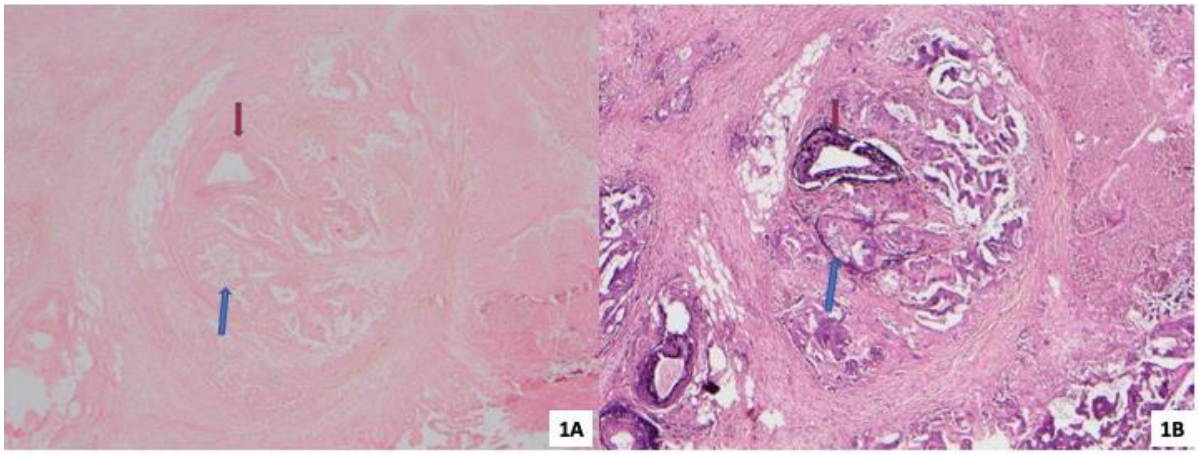

Figure 1. Extramural VI
1A: H&E-stained section (x100) 1B: EVG-stained section (x100) (blue arrow - vein, red arrow - artery)

Clinicopathological characteristics of the tumour		All cases (n=386) (%)	Cases with VI (%)	Chi-square (p-value)
Tumor site	Caecum	41	26 (63.41)	**X^2 = 15.699 p=0.047**
	Ascending colon	28	17 (60.71)	
	Hepatic flexure	9	16 (69.57)	
	Transverse colon	23	6 (66.67)	
	Splenic flexure	8	8 (100.00)	
	Descending colon	1	9 (56.25)	
	Sigmoid colon	111	85 (76.58)	
	Rectosigmoid junction	44	25 (56.82)	
	Rectum	106	82 (77.36)	
Tumour type + Differentiation	Adenocarcinoma, well-differentiated	6	3 (50.00)	X^2 = 7.300 p=0.199
	Adenocarcinoma, moderately differentiated	344	245 (71.22)	
	Adenocarcinoma, poorly differentiated	8	7 (87.50)	
	Mucinous carcinoma, moderately differentiated	26	19 (73.08)	
	Mucinous carcinoma, poorly differentiated	1	0 (0%)	
	Signet-ring cell carcinoma	1	0 (0%)	
Tumour stage (T)	T1	16	7 (43.75)	**X^2= 20.398 p<0.0001**
	T2	49	25 (51.02)	
	T3	242	184 (76.03)	
	T4a	63	44 (69.84)	
	T4b	16	14 (87.50)	
Lymph node stage (N)	N0	210	119 (56.67)	**X^2 = 55.672 p<0.001**
	N1c	11	11 (100.00)	
	N1a	51	42 (82.35)	
	N1b	35	25 (71.43)	
	N2a	32	32 (100.00)	
	N2b	47	45 (95.74)	
Perineural invasion	Absent (Pn0)	320	211 (65.94)	**X^2 = 23.145 p<0.001**
	Present (Pn1)	66	63 (95.45)	

Table 1. The association between the pathological characteristics of the study population and the presence of VI

perineural invasion. There was no association with tumour differentiation.

The frequency of VI differed remarkably according to the primary location of the tumour. It was 100% in splenic flexure CRCs and 56.25% in descending colon CRCs (X^2= 15.699, p=0.047).

VI was present in 87.50% (14/16) of pT4b tumours and 43.75% (7/16) of pT1 tumours. There was a significant difference in the VI rate according to the local extent of the tumour (X^2= 20.398, p<0.0001).

The occurrence of VI was significantly associated with the nodal stage based on tumour metastases into lymph nodes. Tumours with no detectable tumour metastases showed a VI rate of 56.67% (119/210) while tumours with lymph node metastases showed a VI rate of >70% at least (X^2 = 55.672, p<0.001).

VI was detected in 95.45% (63/66) of CRC which showed perineural invasion and was present in only 65.94% (211/320) of tumours without perineural invasion. There was a significant difference in VI in tumours with and without perineural invasion (X^2 = 23.145, p<0.001).

Of all the CRCs, the majority were moderately differentiated adenocarcinoma, NOS and VI was present in 71.22% (245/344). This value is close to the final overall VI rate of 70.98% (274/386) in this study. The number of cases with other categories of tumour differentiation was equal to or less than 26 in number. There was no statistically significant difference in VI rate between different tumour differentiation categories (X^2 = 7.300, p=0.199). This could be due to the limited number of cases in most of these tumour subcategories.

Discussion

It has long been recognised that VI is an independent prognostic factor in CRC, which predicts patient survival (11, 26, 34). Hence, it is of paramount importance to detect VI precisely during the histological assessment of CRC resection specimens. Individual histopathology reporting centres should take measures to maintain the VI detection rate at an acceptable level. This fact is emphasized by both the RCPath (UK) and the CAP (USA) guidelines for CRC resection specimen examination. Further, the RCPath guidelines indicate that an individual centre should maintain its VI detection rate at a minimum of 30% and if it is less than this, an ancillary study should be incorporated into the routine histopathological examination of CRC resection specimens (12).

Several ancillary techniques have been studied for their efficacy in the detection of VI. These include ETS to highlight the muscle wall of the veins, an immunohistochemical marker to highlight the endothelium of the vessel wall

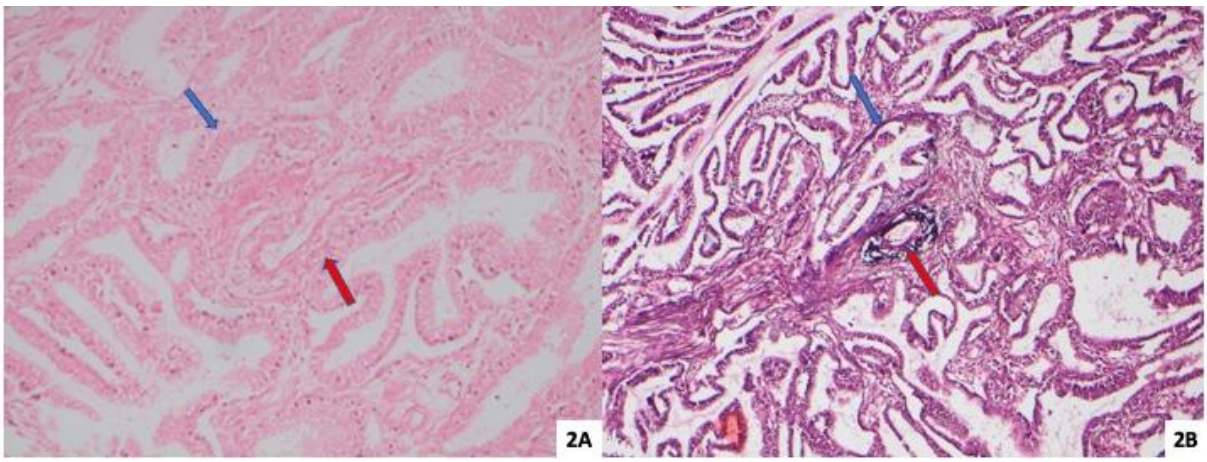

Figure 2. Intramural VI
2A: H&E-stained section (x100) 2B: EVG-stained section (x100) (blue arrow - vein, red arrow - artery)

and MRI to detect veins invaded by the tumour tissue (11, 12, 25, 31, 33, 35, 36).

Several studies have evaluated the usefulness of the ETS in improving the VI detection rate during routine practice and its prognostic importance, and most of these studies have shown a clear increment in the VI detection rate (11,18,19,21,27). A study of 89 CRC specimens conducted by Sejben et al. reported an incidence of 70.4% for VI following the addition of orcein ETS (24). More recently, Duduyemi et al. reported a VI detection rate of 78% with the Verhoeff–Van Gieson elastic stain in their study cohort of 41 cases of pT1-pT3 stage CRC (37).

The present study too demonstrates that the VI detection rate could be increased by adding an ETS. The VI rate based on H&E-stained tissue sections alone was increased to 70.98% following the addition of the ETS.

ETS facilitates the detection of both IMVI and EMVI and this study demonstrated a significant improvement in IMVI when compared to the H&E stain alone. On H&E stain, EMVI (this included the cases which showed EMVI alone or both EMVI and IMVI) was present in 136/152 (89.47%) VI positive cases and IMVI alone was reported in 16/152 (10.53%) cases. Following the EVG stain, the total number of cases showing IMVI alone increased up to 69 out of the total VI-positive cases (69/274, 25.18%). One study showed an appreciable correlation between IMVI and decreased cancer-specific survival in CRC (38). Although it is unclear whether there is a relationship between IMVI and the prognosis of CRC, this study highlights that adding EVG stain to the routine H&E stain can improve the detection of some cases of CRC having IMVI which would otherwise have not been detected by H&E-staining alone (7,16,39).

There is no agreement regarding how many blocks from each CRC should be stained with ETS as the chosen number of blocks varies according to the tumour size and the presence of suspicious foci in the H&E stain (6,8,11,15,17,18). One study selected only one tissue block from each case which was most likely to have VI (34). In the present study, one to four blocks were subjected to ETS depending on the number of blocks containing the tumour and H&E sections showing suspicious areas of VI in each case. In most cases, three blocks were stained with ETS.

Whether to take a section for ETS at the initial tumour section cutting for H&E stain or following examination of the tumour with H&E stain, as a second step is yet to be agreed upon. In this study, there were several cases in which the focus suspicious of VI was lost in the comparative elastic-stained section where sections for ETS were taken on a second occasion. This is most probably due to the trimming of the wax block. On the other hand, if sections are taken from all the tumour blocks present in an individual case and stained with ETS at first instance, it would be a waste of resources if VI is detected on the H&E stain or there are no suspicious foci in the tissue blocks. Therefore, cutting spare sections from the tumour blocks at initial sectioning and performing ETS on selected sections from cases which are negative for VI on H&E stain could be considered.

There was an association between VI and other pathological characteristics that indicate an aggressive behaviour in CRC, namely maximum local infiltration of tumour (pT stage), presence of lymph node metastasis and perineural invasion. A significant association was also identified between VI and the tumour site. There was no significant association between VI and tumour differentiation. This is most likely due to the fewer number of cases (n=16) in the tumour differentiation categories other than moderately differentiated adenocarcinoma.

Messenger et al. in their review article titled “Developments in the assessment of venous invasion in colorectal cancer: implications for future practice and patient outcome” addressed the importance of performing an

ETS in the detection of VI in CRC following neoadjuvant treatment (11). The current study supports this view by showing a significant increment of VI detection from 30.95% to 66.67% following the addition of ETS compared to H&E-stain alone in tumours that had been treated with neoadjuvant therapy.

Conclusion

The addition of an ETS significantly improved the histological identification of VI in the evaluation of CRC resection specimens. It was found to increase the visualization of IMVI.

Considering the prognostic importance of the histological detection of VI in CRC, the use of the ETS, using at least one spare tumour section is recommended in all cases that are equivocal for VI on the H&E-stained sections.

References

1 World Health Organization. Global Cancer Observatory, Cancer Today, Cancer fact sheets [Internet]. 150 Cours Albert Thomas, 69372 Lyon CEDEX 08, France: IARC; 2019 Feb [cited 2019 Sep 12]. https://gco.iarc.fr

2. National Cancer Control Program: Cancer incidence data Sri Lanka 2019 [Internet]. Colombo, SL: Ministry of Health, Nutrition and Indigenous Medicine; 21st publication, Nov 2021 [cited 2022 Dec 19]. https://www.nccp.health.gov.lk/storage/post/pdfs/Cancer%20Incidence%20Data%20Book-2019_compressed.pdf

3. Benson AB, Venook AP, Al-Hawary MM, et al. NCCN Guidelines Insights: Colon Cancer, Version 2.2018. J Natl Compr Canc Netw. 2018;16(4):359-369. https://doi.org/10.6004/jnccn.2018.0021.

4. Puppa G, Sonzogni A, Colombari R, Pelosi G. TNM staging system of colorectal carcinoma: a critical appraisal of challenging issues. Arch Pathol Lab Med 2010 Jun:134(6):837-52. https://doi.org/10.5858/134.6.837

5. Dotan E, Cohen SJ. Challenges in the management of stage II colon cancer, Semin Oncol. 2011 August;38(4):511-20. https://doi.org/10.1053/j.seminoncol.2011.05.005

6. Talbot IC, Ritchie S, Leighton M, Hughes AO, Bussey HJ, Morson BC. Invasion of veins by carcinoma of rectum: method of detection, histological features and significance. Histopathology 1981 Mar; 5(2): 141-63. https://doi.org/10.1111/j.1365-2559.1981.tb01774.x

7. Petersen VC, Baxter KJ, Love SB, Shepherd NA. Identification of objective pathological prognostic determinants and models of prognosis in Dukes' B colon cancer. Gut. 2002;51(1):65-69. https://doi.org/10.1136/gut.51.1.65

8. Seefeld PH, Bargen JA. The spread of carcinoma of the rectum: invasion of lymphatics, veins and nerves. Ann Surg. 1943;118(1):76-90. https://doi.org/10.1097/00000658-194311810-00005

9. Chapuis PH, Dent OF, Fisher R, Newland RC, Pheils MT, Smyth E, et al. A multivariant analysis of clinical and pathological variables in prognosis after resection of large bowel cancer. Br J Surg. 1985;72(9):698-702. https://doi.org/10.1002/bjs.1800720909

10. Morris M, Platell C, de Boer B, Mccaul K, Iacopetta B. Population-based study of prognostic factors in stage II colonic cancer. Br J Surg. 2006;93(7):866-71. https://doi.org/10.1002/bjs.5345

11. Messenger DE, Driman DK, Krisch R. Developments in the assessment of venous invasion in colorectal cancer: implications for future practice and patient outcome. Hum Pathol. 2012;43(7):965-73. https://doi.org/10.1016/j.humpath.2011.11.015

12. Loughrey MB QP, Quirke P, Shepherd NA. The Royal College of Pathologists: standards and datasets for reporting cancers: datasets for colorectal cancer histopathology reports.

4th ed. London: The Royal College of Pathologists, 2018.

13. Brierley JD, Gospodarowicz MK, Wittekind C, eds. International Union Against Cancer (UICC). TNM classification of malignant tumours, 8th ed, Oxford: John Wiley & Sons, 2017.

14. Maughan NJ, Morris E, Forman D, Quirke P. The validity of the Royal College of Pathologists' colorectal cancer minimum datasets within a population. Br J Cancer. 2007;97(10):1393-98.
https://doi.org/10.1038/sj.bjc.6604036

15. Roxburgh CS, McMillan DC, Richards CH, et al. The clinical utility of the combination of T stage and venous invasion to predict survival in patients undergoing surgery for colorectal cancer. Ann Surg. 2014;259(6):1156-65.
https://doi.org/10.1097/SLA.0000000000000229

16.Betge J, Pollheimer MJ, Lindtner RA, Kornprat P, Schlemmer A, Rehak P, et al. Intramural and extramural vascular invasion in colorectal cancer: prognostic significance and quality of pathology reporting. Cancer 2012;118(3):628-638.
https://doi.org/10.1002/cncr.26310

17. Van Wyk HC, Roxburgh CS, Horgan PG, Foulis AF, McMillan DC. The detection and role of lymphatic and blood vessel invasion in predicting survival in patients with node negative operable primary colorectal cancer. Crit Rev Oncol Hematol. 2014;90(1):77-90.
http://dx.doi.org/10.1016/j.critrevonc.2013.11.004

18.Roxburgh CS, Mcmillan DC, Anderson JH, McKee RF, Horgan PG, Foulis AK. Elastica staining for venous invasion results in superior prediction of cancer-specific survival in colorectal cancer. Ann Surg 2010;252:989-97.
https://doi.org/10.1097/SLA.0b013e3181f1c60d

19.Roxburgh CS, Foulis AK. The prognostic benefits of routine staining with elastica to increase detection of venous invasion in colorectal cancer specimens. J Clin Pathol. 2011;64(12):1142.
https://doi.org/10.1136/jclinpath-2011-200284

20.Hwang C, Lee S, Kim A, Kim YG, Ahn SJ, Park DY. Venous invasion in colorectal cancer: Impact of morphological findings on detection rate. Cancer Res Treat 2016 Oct;48(4):1222-28. doi:10.4143/crt.2015.429.
https://doi.org/10.4143/crt.2015.429

21.Kirsch R, Messenger DE, Riddle RH, Pollett A, Cook M, Al-Haddad S, et al. Venous invasion in colorectal cancer: impact of an elastin stain on detection and interobserver agreement among gastrointestinal and nongastrointestinal pathologists. Am J Surg Pathol 2013;37:200-210.
https://doi.org/10.1097/PAS.0b013e31826a92cd

22.Inoue T, Mori M, Shimono R, Kuwano H, Sugimachi K. Vascular invasion of colorectal carcinoma readily visible with certain stains. Dis Colon Rectum. 1992;35(1):34-39.
https://doi.org/10.1007/BF02053336

23.Vass DG, Anisworth R, Andrson JH, Murry D, Foulis AK. The value of an elastin tissue stain in detecting venous invasion in colorectal cancer. J Clin Pathol. 2004;57(7):769-72.
https://doi.org/10.1136/jcp.2003.015826

24.Sejben I, Bori R, Cserni G. Venous invasion demonstrated by orcein staining of colorectal carcinoma specimens is associated with the development of distant metastasis. JClin Pathol.2010;63(7):575-78.
https://doi.org/10.1136/jcp.2010.075846

25.Kojima M, Shimazaki H, Iwaya K, Kage M, Akiba J, Ohkura Y, et al. Pathological diagnostic criterion of blood and lymphatic vessel invasion in colorectal cancer: a framework for developing an objective pathological diagnostic system using the delphi method, from the Pathology Working Group of the Japanese Society for Cancer of the Colon and Rectum. J Clin Pathol. 2013;66(7):551-58.
https://doi.org/10.1136/jclinpath-2012-201076

26.Minisky B, Miles C. The clinical significance of vascular invasion in colorectal cancer. Dis

Colon Rectum. 1989;32(9):794-803. https://doi.org/10.1007/BF02562132

27.Abdulkader M, Abdulla K, Rakha E, Kaye P. Routine elastic staining assists detection of vascular invasion in colorectal cancer. Histopathology. 2006;49(50 487-492. https://doi.org/10.1111/j.1365-2559.2006.02533.x

28.Dawson H, Kirsch R, Driman DK, Messenger De, Assarzadegan N, Riddlell Rh. Optimizing the detection of venous invasion in colorectal cancer: the Ontario, Canada, experience and beyond. Front Oncol. 2014;4:354. https://doi.org/10.3389/fonc.2014.00354

29.Messenger DE, Driman DK, McLeod RS, Riddell RH, Kirsch R. Current practice patterns among pathologists in the assessment of venous invasion in colorectal cancer. J Clin Pathol 2011:64:983-89. https://doi.org/10.1136/jclinpath-2011-200156

30.Kakar S, Shi C, Berho ME, Driman DK, Fitzgibbons P, Frankel WL, et al. College of American Pathologists protocol for the examination if specimens from patients with primary carcinoma of the colon and rectum. Version 4.0.0.1. College of American Pathologists. Published June 2017. Accessed September 13, 2019.

31.Kingston EF, Goulding H, Bateman AC. Vascular invasion is underrecognized in colorectal cancer using conventional hematoxylin and eosin staining. Dis Colon Rectum.2007;50(11):1867-72. https://doi.org/10.1007/s10350-007-9021-6

32.Harris EI, Lewin DN, Wang HL, Lauwers GY, Srivastava A, Shyr Y, et al. Lymphovascular invasion in colorectal cancer: an interobserver variability study. Am J Surg Pathol. 2008;32(12):1816-21. https://doi.org/10.1097/PAS.0b013e3181816083

33. Jhaveri, K. S., Hosseini-Nik, H, Thipphavong S, Assarzadegan N, Meneszes RJ, Kennedy ED, et al. MRI detection of extramural venous invasion in rectal cancer: correlation with histopathology using Elastin stain. Am.J.Roenogenol 2016;206:747-55. https://doi.org/10.2214/AJR.15.15568

34.Suzuki A, Togashi K, Nokubi M, et al. Evaluation of venous invasion by Elastica van Gieson stain and tumor budding predicts local and distant metastases in patients with T1 stage colorectal cancer. Am J Surg Pathol. 2009;33(11):1601-1607. https://doi.org/10.1097/PAS.0b013e3181ae29d6

35.Ervine AJ, McBride HA, Kelly PJ, Loughrey MB. Double immunohistochemistry enhances detection of lymphatic and venous invasion in early-stage colorectal cancer. Virchows Arch. 2015;467(3):265-71. https://doi.org/10.1007/s00428-015-1792-x

36.Ekinci Ö, Öğüt B, Çelik B, Dursun A. Compared With Elastin Stains, h-Caldesmon and Desmin Offer Superior Detection of Vessel Invasion in Gastric, Pancreatic, and Colorectal Adenocarcinomas. International Journal of Surgical Pathology. 2018;26(4):318-326. https://doi.org/10.1177/1066896917752442

37.Duduyemi BM, Andoh D, Adankwah E, Nyarko H, Agyemang D. The use of special stains in the detection of vascular invasion in cases of colon cancer in resource-limited settings in Africa. Ann Trop Pathol 2020;11:21-4 https://www.atpjournal.org/text.asp?2020/11/1/21/291702

38.Knijn N, van Exsel UEM, de Noo ME, Nagtegaal ID. The value of intramural vascular invasion in colorectal cancer - a systematic review and meta-analysis. Histopathology. 2018;72(5):721-728. https://doi.org/10.1111/his.13404

39. Leijssen LGJ, Dinaux AM, Amri R, Taylor MS, Deshpande V, Bordeianou LG, Kunitake H, Berger DL. Impact of intramural and extramural vascular invasion on stage II-III colon cancer outcomes. J Surg Oncol. 2019;119(6):749-757. https://doi.org/10.1002/jso.25367