Original Article

The Use of Special Stains in the Detection of Vascular Invasion in Cases of Colon Cancer in Resource-Limited Settings in Africa

Babatunde M. Duduyemi, Derrick Andoh[1], Ernest Adankwah[1], Hannah Nyarko[2], Divine Agyemang[1]

Departments of Pathology, [1]Medical Diagnostics and [2]Biochemistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Abstract

Background: Vascular invasion (VI) is a well-established independent prognostic factor in colorectal cancer (CRC) associated with the hematogenous spread and high risk of mortality. Its accurate detection is essential in identifying a high-risk group of patients who may benefit from adjuvant therapy and also to detect the risk of disease recurrence. The aim of the study was to determine the value of an elastic tissue stain in assessing vascular invasion in CRC. **Materials and Methods:** Formalin-fixed paraffin-embedded histologically confirmed CRC blocks were retrieved from the archives of the Histopathology Department of the Komfo Anokye Teaching Hospital. Duplicate sections were made from each block and divided into two, with one part of the sections stained with hematoxylin and eosin (H and E) and the other with Verhoeff–Van Gieson (VVG) stain. Both H and E and VVG-stained sections were assessed for vascular invasion. Findings were put in tables and charts. **Results:** Forty-one cases were used for our study, comprising 40 adenocarcinomas (six well differentiated, 26 moderately differentiated, and eight poorly differentiated) and one high-grade non-Hodgkin large cell lymphoma. The mean age of our patient was 55 years, with a male-to-female ratio of 1:1.4. Of the 41 sections stained with H and E, venous invasion (VI) was detected in 14 cases (34%), whereas VVG was detected vascular invasion in 18 cases of the 27 undetected by H and E in addition to all the 14 cases detected by H and E (78%). **Conclusion:** The application of the VVG elastic tissue stain enhanced the sensitivity of detecting VI as compared to the use of H and E alone, and thus, recommendations should be made to include elastic tissue stains in CRC pathology reporting to bring therapeutic benefits to patients in our environment.

Keywords: Colon cancer, hematoxylin and eosin, venous invasion, Verhoeff–Van Gieson

Received on: 24-01-20 **Review completed on:** 23-03-20 **Accepted on:** 25-04-20 **Published on:** 08-08-20

Introduction

It is an established fact that the balance between the rate of apoptosis and the rate of proliferation in colon cancer cells plays an important role in the formation of neoplasm and its progression.[1] The formation of new vessels from preexisting vessels (angiogenesis) is essential for the growth and development of new cancer cells. However, since cells within proliferative lesions initially lack angiogenic ability and restricting their capability for expansion, they tend to upregulate the rate of angiogenesis for tumor growth and metastasis.

Vascular invasion is the spread of cancer cells into blood vessels and/or lymphatic vessels. It is an established independent prognostic factor in colorectal cancer (CRC) associated with poor outcome.[2] There are two distinct elements of vascular invasion, namely venous invasion and lymphatic vessel invasion. Differentiating between the two types of vascular invasion is important, as the current literature stresses the different clinical implications associated with them.

Venous invasion is associated with the presence of visceral metastasis[3] and was described by Talbot *et al.* as a rounded mass of tumor in an endothelium-lined space either surrounded by a rim of smooth muscle or containing red blood cells.[2] The venous invasion may also be suspected when a rounded

Access this article online

Quick Response Code:

Website: www.atpjournal.org

DOI: 10.4103/atp.atp_1_20

Address for correspondence: Dr. Babatunde M. Duduyemi, Department of Pathology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
E-mail: tundeduduyemi@gmail.com

How to cite this article: 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.

or elongated tumor profile is identified adjacent to an artery, especially when no separate accompanying vein can be identified (the "orphan" artery sign), or where smooth tongues of tumor extend into pericolic/perirectal fat ("protruding tongue" sign). The presence of lymphatic invasion, on the other hand, is associated with lymph node metastasis.[4]

The accurate detection of vascular invasion is of particular importance in identifying a high-risk group who might benefit from adjuvant treatment.[5,6] Methods aimed at accurately detecting vascular invasion have been the subject of many research studies. Immunohistochemical markers of endothelial cells such as CD31 and CD34 have proven to be essential in the identification of lymphatic and small blood vessel invasion but are limited in its application due to its high cost.[7] However, many researchers recommend the use of the more cheaply histochemical elastic tissue stains, which can highlight elastic fibers in vessel walls and can allow accurate detection of venous invasion.

Venous invasion (VI), despite its prognostic importance, has since its discovery remained one of the most poorly performed aspects of colorectal pathology reporting. Recent studies have shown that there is marked variability in the incidence of VI based on the assessment of venous invasion in CRC specimens on hematoxylin and eosin (H and E) alone as compared to the use of the more cheaper histochemical elastin stain.[8] It is also reported that accurate detection of VI can be challenging on routine H and E slides, especially when the muscular wall of the vein is destroyed beyond morphologic recognition, and unless key morphologic features are sought, VI can easily be overlooked. It is worth noting that other techniques involving immunohistochemical markers of endothelial cells such as CD31 and CD34 have proven to be essential in the identification of lymphatic and small blood vessel invasion.[7] However, their application is limited since the endothelium of many veins are destroyed and are also expensive compared to elastic tissue stains.

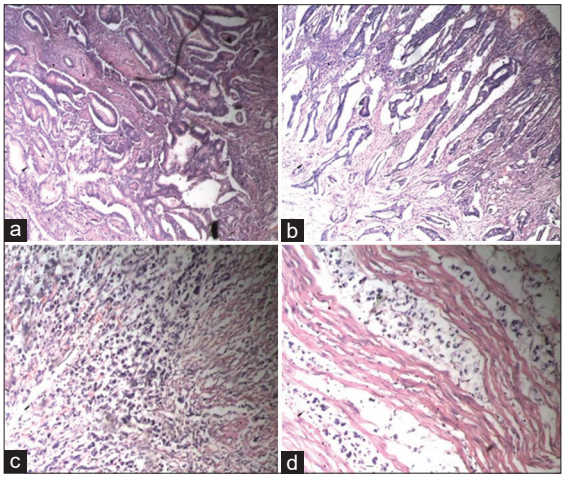

Figure 1: Photomicrographs showing hematoxylin and eosin-stained sections of different grades of colorectal cancer. (a) Well-differentiated adenocarcinoma of the colon (×40). (b) Moderately differentiated adenocarcinoma of the colon (×40). (c) Poorly differentiated adenocarcinoma of the colon (×100). (d) Poorly differentiated signet ring cell tumor (×100)

Strategies to improve the detection of vascular invasion in CRC have been the subject of many recent studies with a lot of researchers advocating for the extensive use of cost-effective histochemical elastic stains, which highlight elastic fibers in the walls of veins (but not lymphatic vessels) and allow a more accurate identification of venous invasion decreasing interobserver variability. Based on evidence obtained from a study conducted by Roxburgh *et al.,* elastic tissue stains have been found to be superior to H and E in the assessment of venous invasion with reports indicating a two- to three-fold increase in VI detection with elastin stain as compared to H and E alone.[9]

Materials and Methods

Study design

This study was a cross-sectional study, which involved sampling of different stages of colon cancer cases reported between the periods of 2012–2015 and assessing their incidence of vascular invasion.

Study site

Samples were collected from the Pathology Department of the Komfo Anokye Teaching Hospital (KATH), Kumasi, Ashanti Region. Histological processing and assessment was also conducted at the histopathology laboratory of the pathology

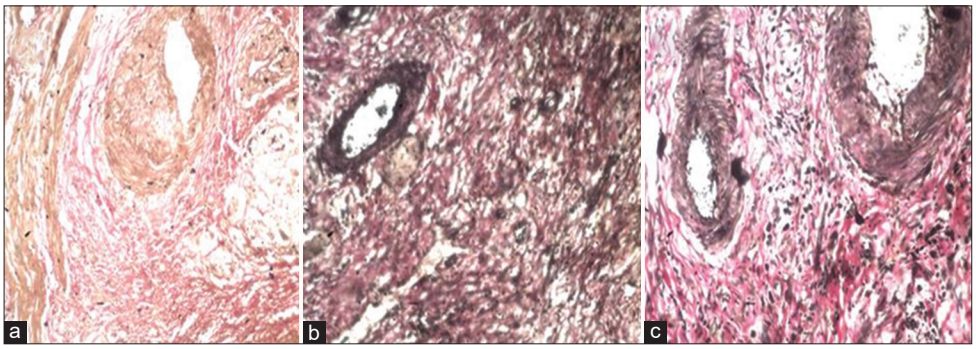

Figure 2: Photomicrographs showing H & E (a) and Verhoeff–Van Gieson (b, c) stained sections with evidence of vascular invasion of colorectal cancer (×100). Blue arrow shows the well-stained vascular wall, and black arrow illustrating a tumor deposit within an endothelial space

department in the same hospital and also at the Cancer and Infectious Diseases Laboratory, Kwame Nkrumah University of Science and Technology (KNUST).

Ethical issues

Ethical clearance for approval to begin the study was sought from the Research and Development Unit, KATH, and the Committee on Human Research, Publication, and Ethics, KNUST, School of Medical Sciences.

Sample collection

Inventory on CRC cases was made using the biopsy logbook of the pathology department of KATH. The data regarding laboratory number, age, gender, histological diagnosis, and grade obtained from the logbook were used to retrieve the colon cancer sample blocks from the archives of the department.

Sample selection

Of 110 cases of CRC abstracted for the study, only 41 cases were found to be suitable. The tissue blocks from 41 cases were then selected, cut and stained with H and E and Verhoeff–Van Gieson (VVG); and then assessed for vascular invasion.

Results

The mean age of our study was 55 years, with an age range of 0–99 years peak age incidence of 50–59 years. The male-to-female ratio was found to be 1:1.4.

Forty cases (97.6%) were adenocarcinomas (six well differentiated, 26 moderately differentiated, and eight poorly differentiated) and one (2.4%) high-grade non-Hodgkin large cell lymphoma.

Staging of the cases using TNM pathologic staging showed that 12 (29%) were pT1, 16 (39%) were pT2, and 13 (32%) were pT3 tumors. Information on the node and distant metastasis was not available.

Forty-one sections, each of H and E and VVG, were assessed for the detection of venous invasion. Fourteen were positive for venous invasion on H and E [Table 1]. The distribution of venous invasion, according to the grade of tumor is shown in Table 2. For the other 41 cases stained with the VVG elastic tissue stain, the venous invasion was detected in 32 cases. The distribution of detection in relation to the grade of tumor is also shown in Table 2. The highest detection rate in both stains was seen in Grade 1 (well-differentiated) tumor. The frequency of venous invasion as detected on H and E alone was 34% (14/41), and on the VVG-stained sections was 78% (32/41). The results indicated an increase of 44% in the detection rate of the venous invasion when the two stains were combined [Figure 1a-d] shows the photomicrographs of different grades of CRC while [Figure 2a-c] shows vascular invasion using H&E and VVG.

Table 1: Detection of vascular invasion on hematoxylin and eosin and Verhoeff-Van Gieson

Vascular invasion	H and E (41 cases) (%)	VVG (27 cases undetected by H and E) (%)
Present	14 (34.1)	18 (66.7)
Absent	27 (65.9)	9 (33.3)
Total	41 (100)	27 (100)

H and E: Hematoxylin and eosin, VVG: Verhoeff-Van Gieson

Table 2: Detection of vascular invasion on hematoxylin and eosin and Verhoeff-Van Gieson in relation to the grade of colorectal cancer

Grade	*n*	Vascular invasion			
		H and E		VVG	
		Present	Absent	Present	Absent
Grade I	6	5	1	6	0
Grade II	26	5	21	18	8
Grade III	9	4	5	8	1
Total	41	14	27	32	9

H and E: Hematoxylin and eosin, VVG: Verhoeff-Van Gieson

Discussion

The vascular invasion has been noted as a negative prognostic factor, and hence, its accurate detection is of particular importance in identifying a high-risk group who might benefit from adjuvant treatment.[5,6] Methods aimed at accurately detecting vascular invasion have been the subject of many research studies with many research works proposing more conventional and convenient methods of accurately detecting vascular invasion.

This study supports the addition of elastic tissue stain to enhance the rate of detection of vascular invasion in CRC specimens. The average age of our patients was in the 6th decade of life, which agreed with most reported worldwide, but a decade more than reports from studies in Sub-Saharan Africa.[10]

Most of our cases (85%) are high-grade cancers (Grades II and III). This finding is similar to most reported, where CRC in our environment has been found to be of higher grade, more aggressive than those from the Western world.[11]

Using a TNM staging system by the WHO, pT2 and pT3 are more than pT1 in our cases. This shows that the cancers are relatively more advanced, and therefore, portends poor prognosis than pT1, which is early cancer.

The combined detection rate of 78% for the two stains (routine H and E and VVG) have proven increased sensitivity and accuracy in the detection and assessment of vascular invasion. The results from the current study are similar to the results of a study conducted by Sternberg *et al*. where 81 CRC specimens were used. It was reported that the addition of an elastic fiber stain enabled the identification of venous invasion in 15 (38.5%) of 39 specimens, which were previously negative for venous invasion on H and E stain only, bringing the total incidence of venous invasion in their study to 70.4%.[12]

A lot of studies demonstrating that the influence of adding elastic tissue stains to improve the detection of vascular invasion has been carried out by many investigators. Results from a study done by Howlett *et al*., who used Movat pentachrome on 92 cases of CRC showed that venous invasion was identified in 22 out of 50 cases (44%), which were initially grouped as negative on the basis of H and E stain only. On the combination H and E and elastic tissue stains, the overall detection rate increased from 18% to 62%.[13] In a similar study, Vass *et al*. compared Miller's elastic-stained sections to H and E-stained sections in a retrospective analysis of 75 colorectal carcinomas. They reported an increased rate of detection 30% vascular invasion overall when intramural and extramural results were combined after an elastic tissue stain was utilized.[14]

The VVG-stained sections are showing evidence of vascular invasion, a condition addressed by evidence from numerous multivariate analyses to be an independent negative prognostic factor in CRC.[15,16] In a series of studies identifying the exact location of the involved vessels (that is, whether extramural or intramural), it was found that the extramural type was most predictive of survival, although the prognostic importance of the intramural type remains unclear.[17,18] However, Petersen *et al*. showed that both intramural and extramural venous invasion influence the prognostic index in Dukes' B colon carcinomas, which correlates with survival. Our cases showed both intramural and extramural vascular involvement, although this was not quantified independently. This finding would offer patients a high prognostic index, thus necessitating the need to be considered for adjuvant chemotherapy.[6]

Conclusion

The findings of this study suggest that serial sections of tumor blocks in CRC or other cancers should be stained with an elastic stain in addition to routine H and E for the detection of vascular invasion. The application of the VVG elastic tissue stain enhanced the sensitivity of detecting venous invasion as compared to the use of H and E alone, and thus, recommendations should be made to include elastic tissue stains in cancer pathology reporting to bring therapeutic benefits to patients in our environment.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Chen JM, Li WH, Wang JD, Feng YD, Wu JH, Gong JP. Cell balance between apoptosis and proliferation in colon cancer and its correlation with prognosis. Ai Zheng 2005;24:554-8.
2. Talbot IC, Ritchie S, Leighton MH, Hughes AO, Bussey HJ, Morson BC. The clinical significance of invasion of veins by rectal cancer. Br J Surg 1980;67:439-42.
3. Suzuki A, Togashi K, Nokubi M, Koinuma K, Miyakura Y, Horie H, *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;33:1601-7.
4. Kim JC, Roh SA, Lee KH, Namgung H, Kim JR, Kim JS. Genetic and pathologic changes associated with lymphovascular invasion of colorectal adenocarcinoma. Clin Exp Metastasis 2005;22:421-8.
5. Desolneux G, Burtin P, Lermite E, Bergamaschi R, Hamy A, Arnaud JP. Prognostic factors in node-negative colorectal cancer: A retrospective study from a prospective database. Int J Colorectal Dis 2010;25:829-34.
6. 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;51:65-9.
7. Liang P, Nakada I, Hong JW, Tabuchi T, Motohashi G, Takemura A, *et al*. Prognostic significance of immunohistochemically detected blood and lymphatic vessel invasion in colorectal carcinoma: Its impact on prognosis. Ann Surg Oncol 2007;14:470-7.
8. Kirsch R, Messenger DE, Riddell 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;37:200-10.
9. 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.
10. Duduyemi BM, Oluwasola AO, Akang EE, Thomas-Ogunniyi JO. A 16-year review of clinico-pathological pattern of colorectal carcinoma at University College Hospital, Ibadan. Niger J Gastroenterol Hepatol 2011;3:7-14.
11. Dakubo JC, Naaeder SB, Tettey Y, Gyasi RK. Colorectal carcinoma: An update of current trends in Accra. West Afr J Med 2010;29:178-83.
12. Sternberg A, Mizrahi A, Amar M, Groisman G. Detection of venous invasion in surgical specimens of colorectal carcinoma: The efficacy of various types of tissue blocks. J Clin Pathol 2006;59:207-10.
13. Howlett CJ, Tweedie EJ, Driman DK. Use of an elastic stain to show venous invasion in colorectal carcinoma: A simple technique for detection of an important prognostic factor. J Clin Pathol 2009;62:1021-5.
14. Vass DG, Ainsworth R, Anderson JH, Murray D, Foulis AK. The value of an elastic tissue stain in detecting venous invasion in colorectal cancer. J Clin Pathol 2004;57:769-72.
15. Chapuis PH, Dent OF, Fisher R, Newland RC, Pheils MT, Smyth E, *et al*. A multivariate analysis of clinical and pathological variables in prognosis after resection of large bowel cancer. Br J Surg 1985;72:698-702.
16. Michelassi F, Block GE, Vannucci L, Montag A, Chappell R. A 5- to 21-year follow-up and analysis of 250 patients with rectal adenocarcinoma. Ann Surg 1988;208:379-89.
17. Sternberg A, Amar M, Alfici R, Groisman G. Conclusions from a study of venous invasion in stage IV colorectal adenocarcinoma. J Clin Pathol 2002;55:17-21.
18. 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;5:141-63.