

## Detection of vascular invasion in colorectal cancer by using Weigert's stain for elastic fibers

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### ABSTRACT

**Objective:** Venous invasion is stage independent prognostic risk factor for distant metastasis in colorectal cancer. Elastic stain is among the various ancillary techniques that were described to increase the sensitivity of detection of vascular invasion. This combined prospective and retrospective study is aimed to assess the sensitivity of elastic tissue stain to detect venous invasion in comparison to the routine H & E stained sections, and to correlate intramural and extramural venous invasion with the Dukes staging system.

**Method:** Serial sections from 42 cases of colorectal cancer diagnosed between the years 2007 and 2009 were examined by using H & E and Weigert's stain. A comparison was drawn between the two stains in regard to venous invasion; the results were correlated with Dukes stage for colorectal cancer.

**Results:** The mean age of sampled patients was 50 years. Venous invasion was detected in 16 (38%) cases by the use of H & E stain, which was increased to 35 (83%) cases by the use of Weigert's stain for elastic tissue with a clear statistical significance ( $P < 0.001$ ). Venous invasion was correlated with Dukes B & C cases with a P value of more than 0.05 & less than 0.01 respectively.

**Conclusion:** The use of elastic tissue stain on one tissue block increases significantly the frequency of detection of vascular invasion of colorectal cancer.

**Keywords:** Venous invasion, colorectal cancer, Weigert's stain, elastic fibers

### الخلاصة

**الأهداف:** تعد اختراقات الأوعية الدموية لسرطان القولون والمستقيم من عوامل الخطورة الانذارية لانتشار السرطان عن طريق الدم والذي لا يعتمد على مرحلة المرض. تعتبر صبغة الألياف المطاطية واحدة من التقنيات المساعدة المتعددة لزيادة حساسية الكشف عن اختراقات الأوعية الدموية. تهدف الدراسة الاستقبلية والتراجعية الى تقييم حساسية صبغة الألياف المطاطية في الكشف عن اختراقات الأوعية الدموية ومقارنتها مع مقاطع الأنسجة المصبوغة بصبغة هيماتوكزيلين-أيوسين الروتينية مع ربط اختراقات الاوردة ضمن الجدارية وخارج الجدارية مع مرحلة نظام ديوك.

**طريقة العمل:** تم أخذ مقاطع متسلسلة لـ (٤٢) حالة لسرطان القولون والمستقيم المشخصة بين ٢٠٠٧-٢٠٠٩ وتم فحصها بعد صبغ المقاطع النسيجية بصبغتي هيماتوكزيلين-أيوسين وفيكريت للألياف المطاطية وتمت مقارنة نسبة وجود اختراقات الأوعية الوريدية باستخدام الصبغتين وتم ربط النتائج بمرحلة ديوك لسرطان القولون والمستقيم.

**النتائج:** كان معدل أعمار المرضى ٥٠ سنة. وجدت اختراقات الأوعية الوريدية في ١٦ حالة (بنسبة ٣٨ %) باستخدام هيماتوكزيلين-أيوسين بينما ارتفعت القيمة الى ٣٥ حالة (بنسبة ٨٣ %) بعد استخدام صبغة الألياف المطاطية لنفس المقاطع النسيجية مع قيمة احصائية واضحة (قيمة P اقل من ٠,٠٠١) كما ربطت الاختراقات الوريدية بمرحتي ديوك B و C مع قيمة P أكثر من ٠,٠٥ واقل من ٠,٠١ بالتتابع.

**الاستنتاج:** استخدام صبغة الألياف المطاطية لمقطع نسيجي واحد يزيد من أهمية تكرار الكشف عن الاختراقات الوريدية لسرطان القولون والمستقيم.

Univariate and multivariate analyses have widely described venous invasion to be an adverse, significant, stage-independent prognostic risk factor for colorectal cancer<sup>(1,2,3)</sup>. It has crucial role in the development of micro-metastases and ultimately macroscopic tumour growth at secondary sites<sup>(4,5)</sup>. Furthermore; it determines the need for further surgical treatment or adjuvant therapy<sup>(1,2,6)</sup>.

In many occasions, no distinction between venous and lymphatic invasion is made<sup>2,7</sup> and the detection of venous invasion in colorectal cancer specimens may be exceedingly difficult using the conventional haematoxylin and eosin (H & E) staining alone, which typically requires the identification of a single or groups of tumour cells within an endothelial-lined space and/or containing red blood cells<sup>(7,8)</sup>. The difficulty in the diagnosis might arise from histological artifacts that mimic vessel wall invasion or vascular destruction by tumour beyond recognition in addition to the interobserver variation<sup>(7)</sup>. This may be responsible in part to the disparities in the reported prevalence of this feature which is ranging from 10% to 90%<sup>(8,9)</sup>.

Few studies have considered the prognostic impact of intramural and extramural venous invasion<sup>(7)</sup>, although the updated protocol for the examination of colorectal cancer specimen has recommended the reporting of the anatomic location of venous invasion<sup>(2,10)</sup>.

In this regard, several authors have advocated the use of various ancillary techniques to improve the chance of detection of venous invasion such as increasing the number of tumour blocks and the number of slides examined and cutting tangential sections to transect the maximum number of veins that drain the tumour<sup>(6,8)</sup>. However; to date, no widely accepted standard or guidelines for the pathological evaluation of vessel invasion are in place<sup>(2,4)</sup>.

The use of elastic tissue stains in microscopical assessment has been proposed to be a sensitive technique for increasing the

rate of detection of venous invasion within the tumour in a considerable proportion of tissue samples that are negative or suspicious on H & E staining alone<sup>(1,6,8-10)</sup>.

This combined prospective and retrospective study is aimed to assess the sensitivity of elastic tissue stain to detect venous invasion in comparison to the routine H & E stained sections, and to correlate intramural and extramural venous invasion with the Duke's staging system.

### Material and Methods

Forty two of surgically resected colorectal cancer cases between the years 2007 and 2009 were collected from histopathological laboratories of AL-Jamhuri Teaching Hospital and private laboratories in Nineveh province. The demographic data related to age and sex of patients, site of primary tumour and Dukes stage were obtained from the request forms and reports of histopathology. The available blocks of tissue that had been embedded in paraffin were retrieved from the archive.

The work was conducted at the Department of Pathology, College of Medicine, University of Mosul. Two serial sections of 4-5 $\mu$  thickness were cut from each block, one section is stained with routine H & E stain the other with Weigert's stain for elastic fibers and counterstained with H & E.

All sections were scrutinized for the presence of venous invasion, which was defined as the presence of tumour cells within an endothelial lined space surrounded by a rim of smooth muscle and/or containing red blood cells on the basis of H & E stained sections.

By Weigert's stain for elastic tissue, venous invasion is defined as the presence of tumour deposits within a space with elastic fibers in its wall (which appears deep purple or black), Assessment of the location of venous invasion, whether intramural or extramural, was also done. Intramural venous invasion is defined as venous invasion within tumour, submucosa and muscularis propria, while extramural

venous invasion is located within the serosa or pericolic fat. When venous invasion was in doubt, the case was considered as negative.

The statistical analysis to compare the two stains was done by the use of  $\chi^2$  test. A P-value < 0.05 was considered to be statistically significant.

## Results

The mean age of sampled patients was 50 years (range is 18- 70 years). Of the 42 cases of colorectal cancer, 16 were positive for venous invasion by the use of H & E stain. Of the remaining negative group, twenty were positive after the incorporation of Weigert's stain for elastic tissue.

Overall, the total frequency of venous invasion increased from 16/42 (38%) as detected by H & E stain only to 35/42 (83%) after the use of Weigert's stain for elastic fibers with a (P<0.001) (table 1; fig 1).

Regarding intramural and extramural venous invasion, the frequency of detection after using Weigert's stain increased from 9/42 to 31/42 and 9/42 to 16/42 respectively (P<0.001 and P<0.05 ) (table 1; fig 2,3,4).

Of the 16 cases with Dukes B disease, review of H & E and elastic tissue stained slides revealed venous invasion in 7 & 13 cases respectively; however the results did not reach statistical significance ( P>0.05, table 2). While in Dukes C cases, eight of 23 cases were deemed positive on H & E stained slides whereas 20 cases of this group were confirmed by Weigert's stain for elastic fibers (P<0.01, table 2).

The study confirmed that the use of elastic stain on at least one tissue block increases the overall yield of venous invasion by 40% (table 3).

Table (1): Detection of venous invasion by H & E stain vs. Weigert's stain for elastic fibers.

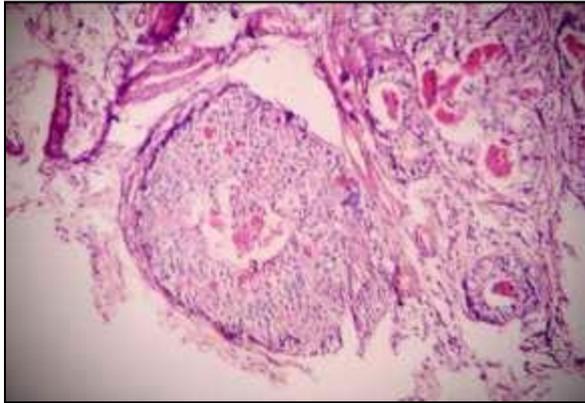
No.	Intramural VI		Extramural VI		Overall	
	H & E(%)	Elastic stain (%)	H & E (%)	Elastic stain (%)	H & E (%)	Elastic Stain (%)
42	9 (21)	31 (74)	9 (21)	16 (38)	16 (38)	35 (83)

Table (2): Detection of vascular invasion with H & E stain vs. Weigert's stain in relation to Dukes staging system.

Stage	No	Intramural		Extramural		Overall	
		H & E (%)	Elastic Stain (%)	H & E (%)	Elastic Stain (%)	H & E (%)	Elastic Stain (%)
Dukes A	3	1	2	-----	-----	1	2
Dukes B	16	5 (31)	13 (81)	3 (19)	6 (38)	7 (44)	13 (81)
Dukes C	23	3 (13)	16 (70)	6 (26)	10 (43)	8 (35)	20 (87)

Table (3): Relation between the detection of venous invasion with the use of elastic tissue stain and the number of the available tissue blocks.

No. of blocks	No. of cases	Venous invasion by elastic stain
1	20	8 (40%)
2-4	22	12 (54%)
Total	42	20 (48%)



Figure(1) A: Adenocarcinoma of the colon with no evidence of venous invasion (H & E x 200).

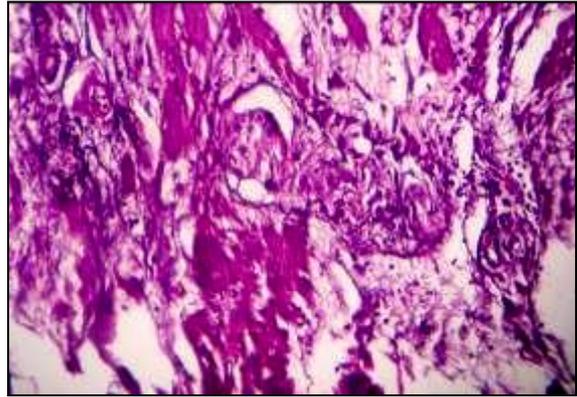


Figure (2) B: Intramural venous invasion is identified when elastic tissue stain is added (Weigert's stain x 200).

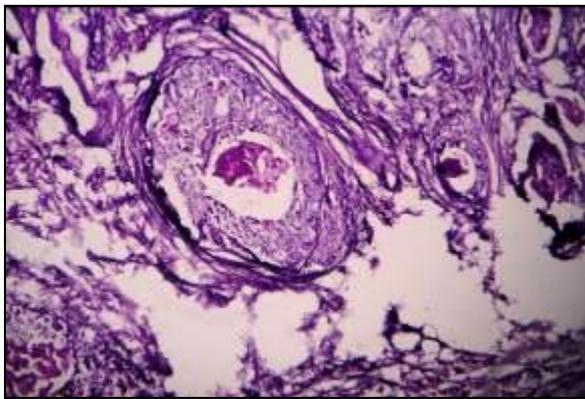


Figure (1) B: Adenocarcinoma of the colon with malignant glands surrounded by concentric layers of elastic fibers (Weigert's stain x 200).

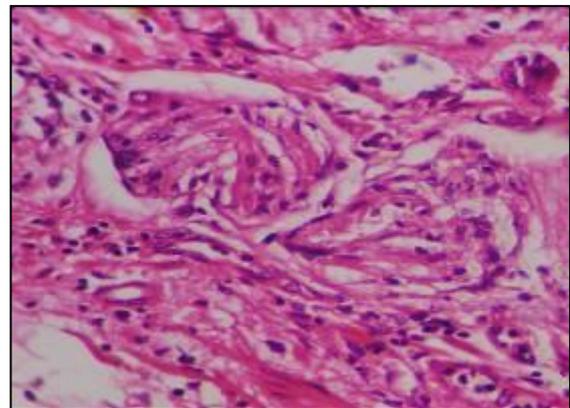


Figure (3) A: Intramural venous invasion which is difficult to be detected using H & E stain alone (H & E x800).

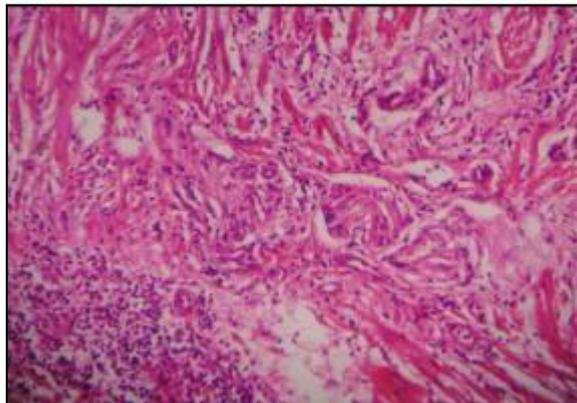


Figure (2) A: Intramural venous invasion which is difficult to be detected using H & E stain alone (H & E x200).

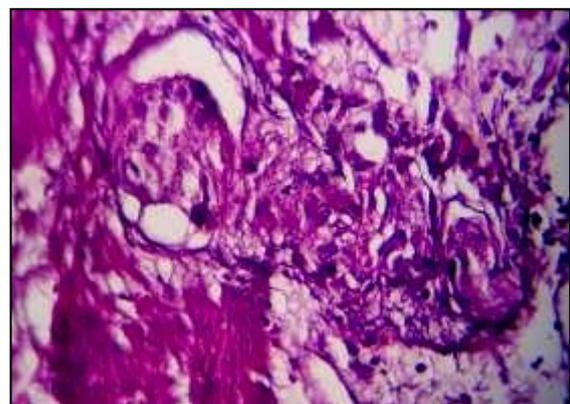


Figure (3) B: Intramural venous invasion is identified when elastic tissue stain is added (Weigert's stain x 800).

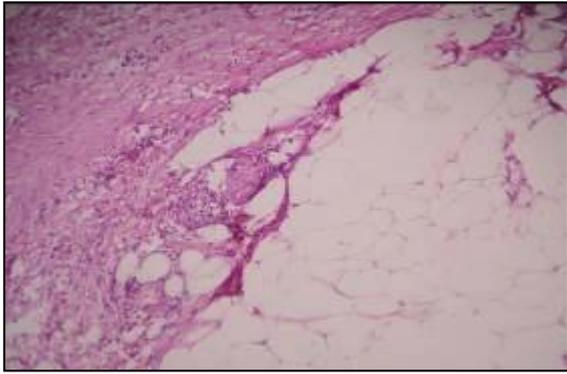


Figure (4) A: Extramural venous invasion, the tumour cells are invading the serosa and venous invasion is difficult to be detected on H & E stain alone (H & E x 200).

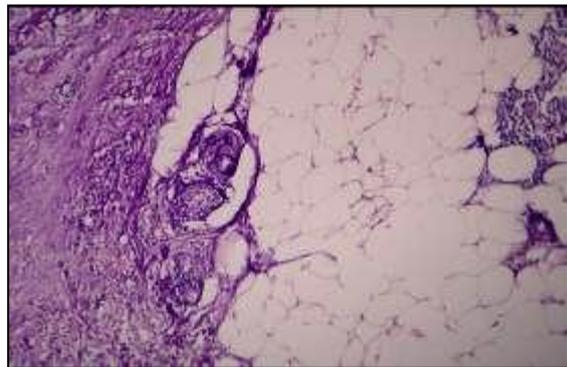


Figure (4) B: With the addition of elastic tissue stain it is possible to detect elastic fibers around the extramurally invading cancer cells (Weigert's stain x 200).

## Discussion

The influence of adding different elastic tissue stains to increase the detection rate of vascular invasion in colorectal cancer had been demonstrated by a number of investigators<sup>(8-11)</sup>. In a most recent study done by Howlett et al who used Movat pentachrome on 92 cases of colorectal cancer, venous invasion was identified in 22 out of 50 cases (44%) which were initially grouped as negative on the basis of H & E stain only with an overall increase in the detection rate from 18% to 62% when comparing H & E and elastic tissue stains respectively<sup>(10)</sup>. In another study done by Vass et al. the addition of H & E/elastic stain on 75 cases of colorectal cancer specimens increased the overall detection rate from 21 (27%) to 43 (57%)<sup>(9)</sup>.

Current study supports the use of elastic tissue stain on at least one tissue section to improve the detection of venous invasion in colorectal cancer. Venous invasion was identified in 35 cases, twenty of which were initially labeled as negative on the basis of H & E stain only. This led to an increase of the overall frequency of venous invasion from 38% to 83%. These results are comparable to these found in a previous similar study done by Sternberg et al. in which the addition of Weigert's stain had enabled the diagnosis of 15 (39%) of 39 specimens which were initially negative on H&E sections, raising the overall incidence of venous invasion to 70.4%<sup>(6,11)</sup>. Furthermore; in another comparable study done by Inoue et al. the frequency of venous invasion was 31% as detected by H & E stain and increased to 81% after the incorporation of a Verhoeff van Gieson which was correlated with subsequent development of haematogenous metastasis<sup>(9)</sup>.

However; it has been proposed that the difference in the definition of vascular invasion, the type of stain used and perhaps the metastatic potential of the cells once they have gained access to lymphovascular spaces may be responsible for the variation in the frequency of vascular invasion<sup>(12)</sup>.

It is firmly established that extramural venous invasion is an independent indicator of poor prognosis<sup>(2,11,12)</sup>. In this study, the detection rate increased from 21% to 38% after the addition of elastic tissue stain; the results were mostly comparable with those observed by Vass et al. who demonstrated an increase of frequency of extramural vascular invasion from 24% to 43% after adding elastic fiber stain on tissue sections<sup>(9)</sup>.

On the other hand; the prognostic significance of intramural vascular invasion is more controversial<sup>(9,10)</sup>. A number of studies had demonstrated this type of vascular invasion at a lower frequency than the extramural type. This controversy and variability in the frequency of identification of intramural vascular invasion is attributed in part to the destruction of some veins beyond recognition by the invading tumour cells<sup>(2,11)</sup> and the location of these vessels at the midst

of the tumour and away from its leading edge<sup>(9)</sup>. Some studies regards the presence of vascular invasion as an indicator for the risk of haematogenous metastasis irrespective of its site<sup>(2,11)</sup>. In this study, intramural vascular invasion was found in nine cases (21%) of the H & E stained slides, the figure has raised to 31 cases (74%) when sections were stained with Weigert's stain for elastic tissue. A comparable result was observed by Vass et al, who reported increases in detection of intramural vascular invasion of 29% when elastic stain was utilized<sup>(9,10)</sup>.

Previous studies recognized vascular invasion by tumour to be as good as a prognostic factor as Dukes stage and its presence may influence the therapeutic decision particularly in Dukes stage B disease<sup>(1,9)</sup>. Accordingly; many physicians now accept the recommendation of adjuvant chemotherapy for patients with Dukes B tumour with synchronous vascular invasion<sup>(1,9,13)</sup>.

In this present study, there were 16 cases with Dukes B disease, vascular invasion was detected in seven cases on the basis of H & E stain which increased to 13 cases when elastic stain was added. These data are similar to those found by Vass et al who demonstrated vascular invasion in four & 14 cases out of 27 cases with Dukes B disease on review of H & E and elastic stain respectively<sup>(9)</sup>. Despite this similarity; this factor did not reach a statistical significance which may be the result of the small number of cases harboring Dukes B tumour in the current study. It is also obvious that this study elicited vascular invasion at comparable values for Dukes B & C with the use of elastic stain, the results were also comparable for intramural and extramural types of vascular invasion for the two stages as this pathological marker is a stage-independent marker.

In this setting the use of elastic stain on at least one tissue block enables the identification of vascular invasion in many cases that were initially negative on the H & E stained sections thereby improving the therapeutic decision and patient outcome.

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