ORIGINAL ARTICLE

Detection of venous invasion in surgical specimens of colorectal carcinoma: the efficacy of various types of tissue blocks

A Sternberg, A Mizrahi, M Amar, G Groisman

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See end of article for authors' affiliations

Correspondence to: Professor A Sternberg, Department of Surgery A, Hillel Yaffe Medical Centre, Hadera 38100, Israel; Sternberg_a@hillel-yaffe.health.gov.il

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Background: Venous invasion (VI) is an important prognosis predictor after colorectal carcinoma (CRC) resection, enabling more accurate staging and influencing postoperative management.
Aims: To assess/compare various tissue block types (perpendicular, tangential, across mesentery (AM), from major vessels or lymph nodes (LNs)) for VI detection in CRC.
Methods: Fifty two CRCs (51 colectomies, one polypectomy) were studied. Tumours were measured, surface area calculated, and colorectum and bowel wall sites recorded. Weigert's staining for elastin facilitated VI detection. VI sites, type, and amount were recorded. Ratios of relative yield of tissue block types to their frequency were calculated.
Results: Average numbers of tissue blocks/colectomy specimen were: perpendicular, 10.2; tangential, 9.1; AM, 3.3; from major vessels, 2.1. Average number of LNs examined was 16.47. VI was detected in 22 tumours. Overall, VI was detected in 16 perpendicular, seven tangential, five AM, and two LN blocks. VI was detected in eight, two, one, and three tumours in perpendicular, tangential, LN, and AM blocks alone, respectively. In seven tumours, VI was identified in multiple tissue block types. The average number of blocks obtained was 39.7, 42.1, and 38 from all tumours, VI positive, and VI negative tumours, respectively ($p=0.0497$). Efficacy to detect VI was 2.151, 2.088, 1.092, 0.172, and 0 for AM, perpendicular, tangential, LN, and mesenteric vessel blocks, respectively.
Conclusions: VI was identified most frequently and in eight cases only in perpendicular blocks. However, extramural VI was detected in six tumours only in blocks cut tangentially, AM, or from harvested LNs. Hence, all these types of blocks should be submitted routinely and scanned for VI.

Distant metastases are the main cause of cancer related death from colorectal carcinoma (CRC). Obviously, access of cancer cells into veins draining the primary tumour is a prerequisite for haematogenous dissemination. Therefore, it is not surprising that the detection of venous invasion (VI) in the surgical specimen has been shown to be associated with an increase in tumour recurrence (especially visceral metastases) and a decrease in patient survival after potentially curative resection of CRC.[1–21] Accordingly, certain investigators believe that in addition to regional lymph node involvement,[22] VI should also be considered an indication for the administration of systemic adjuvant treatment.[16 17 21 23] VI may also be used for the staging of CRC, both as an enhancer of the predictive power of existing classifications,[24–27] or for the creation of a completely new classification.[28] The American joint committee on cancer, the College of American Pathologists, the Association of Coloproctology of Great Britain and Ireland, and the Japanese Society for Cancer of the Colon and Rectum all require routine reporting of the status of VI in CRC specimens.[29–32]

> ''Several investigators advocate cutting the specimen tangentially at the outer perimeter of the tumour and at the corresponding mesenteric border of the bowel wall, thereby transecting the maximum number of veins that drain the tumour, and increasing the chances of identifying venous invasion''

The incidence of VI is directly related to tumour stage and grade, and it varies from 10% to 89.5%.[1–21 33] This variation reflects differences in the characteristics of the tumours that comprise the various reported series, technical differences in specimen processing, and interobserver variation. The histopathological diagnosis of VI is associated with an inherent false negative rate that results from the destruction of some of the invaded veins beyond recognition, sampling errors, and certain aspects of specimen processing (especially the technique of obtaining tissue blocks, the staining method used, and the number of blocks and slides that are examined). This false negative rate has been estimated to be at least 10.5%, and possibly as high as 29.6%.[33] How can we minimise this false negative rate? First, by increasing the number of tissue blocks and the number of slides that are examined. Second, by adding a stain for elastic fibre to the conventional haematoxylin and eosin (H+E) staining, thereby enabling the identification of VI in a considerable proportion of tissue samples that were negative on H+E staining alone.[14 15 18–21 33 34] Third, several investigators advocate cutting the specimen tangentially at the outer perimeter of the tumour and at the corresponding mesenteric border of the bowel wall, thereby transecting the maximum number of veins that drain the tumour, and increasing the chances of identifying VI.[10 12 21]

Our study assesses and compares the contribution of various types of tissue blocks to the diagnosis of VI in surgical specimens of CRC.

MATERIALS AND METHODS

Fifty two CRCs (51 colectomy, one polypectomy) resected for cure or palliation from 50 consecutive patients (31 women,

Abbreviations: CRC, colorectal carcinoma; H+E, haematoxylin and eosin; LN, lymph node; VI, venous invasion

19 men; age, 35–90 years; average, 69.7) were examined according to the following protocol. The tumours were measured, drawn, and their surface area was calculated. Tumour sites along the colorectum and in the bowel wall were recorded. Perpendicular tissue blocks, cut either parallel or at right angles to the longitudinal axis of the bowel, and including the full thickness of the bowel wall, were submitted from the zone of transition from normal mucosa to tumour, and from the point of maximum tumour penetration. If the invading border of the tumour is thought of as a target for shooting practice, with the area of maximum tumour penetration at its centre (the bull's eye), tangential tissue blocks were obtained from the area of maximum tumour penetration and, where possible, also from the second and third rings surrounding it. Tissue blocks were taken from sections across the mesentery (first near the mesenteric border of the bowel corresponding to the tumour and then, where possible, also at a distance from the bowel wall) from large mesenteric vessels and from all lymph nodes (LNs), which were harvested by manual dissection. The microscopic diagnosis of VI in H+E stained tissue was based on the detection of tumour cells within an endothelium lined space, surrounded by a rim of smooth muscle and/or containing red blood cells. When present, an adjacent artery of similar size verified that the involved vascular structure was indeed a vein. When VI was not identified on H+E stained slides, Weigert's stain for elastic fibres was added and a second search for VI was carried out. The following characteristics of VI were recorded: site—intramural, extramural, or both; type—filling (when tumour cells fill the vascular lumen), floating (when tumour cells are located in the centre of the vascular lumen and have no contact with the vessel wall), infiltrating (when tumour cells are seen infiltrating the vessel wall), or combinations of these types; and quantity—minimal (one to two involved veins), intermediate (three to four involved veins), or massive (five or more involved veins). The efficacy of the various types of tissue blocks to detect VI was calculated as the ratio of the relative yield of each block type (percentage) divided by its frequency (percentage of the total number of tissue blocks), and the results were compared.

RESULTS

Tumour sites along the colorectum were as follows: caecum, eight cases; ascending colon, seven cases; hepatic flexure, one case; transverse colon, splenic flexure, and descending colon, four each; sigmoid colon, 16 cases; rectosigmoid, four cases; midrectum, one case; lower rectum, three cases. Tumour sites in the bowel wall were as follows: circular, 26 cases; mesenteric, three cases; mesenteric and one side wall, nine cases; mesenteric and both side walls, four cases; antimesenteric and both side walls, eight cases; one side wall, one case. The tumour TNM stages (sixth edition)[29] were: I, five cases; IIA, 18 cases; IIB, two cases; IIIB, 11 cases; IIIC, six cases; and IV, 10 cases. The average number of examined LNs was 16.47. The average number of blocks for each colectomy specimen was: perpendicular, 10.2; tangential, 9.1; across the mesentery, 3.3; and from large mesenteric vessels, 2.1.

Table 1 The efficacy of the various types of tissue blocks to detect VI

	Frequency (%)	Yield (%)	Efficacy
Perpendicular	24.8	51.7	2.088
Tangential	22.1	24.1	1.092
Across the mesentery	8	17.2	2.151
From mesenteric vessels	5.1	0	0
From mesenteric LNs	40	6.9	0.172

Frequency refers to the percentage of the total number of tissue blocks. Yield refers to the percentage of the total number of blocks in which VI was detected. Efficacy refers to the ratio of yield to frequency.
LN, lymph node; VI, venous invasion.

VI was detected in 22 of 52 tumours. In 13 tumours, VI was detected in tissue stained with H+E alone, whereas in nine tumours it was identified only after adding Weigert's elastic stain. In seven of the 13 cases of VI diagnosed by H+E alone, the addition of the elastic stain enabled the identification of additional involved veins.

The TNM stages of the tumours in which VI was detected were: I, one case; IIA, two cases; IIIB, six cases; IIIC, four cases; and IV, nine cases. The incidence of VI in each TNM stage was: I, 20%; IIA, 11.1%; IIIB, 54.5%; IIIC, 66.7%; and IV, 90%. The sites of VI were as follows: extramural, 16 cases; intramural, four cases; and intramural and extramural, two cases. The types of VI (alone or in combinations) were as follows: filling, 12 cases; floating and infiltrating, 10 cases each. The extent of VI was as follows: minimal (one to two involved veins), 13 cases; moderate (three to four involved veins), five cases; massive (more than four involved veins), four cases.

Overall, VI was identified in 30 tissue blocks: 16 perpendicular blocks, seven tangential blocks, five across mesentery blocks, and two blocks from LNs. Perpendicular blocks alone demonstrated VI in eight of 21 tumours, tangential blocks alone in two of 21 tumours, across the mesentery blocks alone in three of 21 tumours, and blocks from LNs alone in one of 21 tumours. In the remaining seven tumours VI was detected in multiple types of tissue blocks.

The average overall number of tissue blocks that were submitted was 39.7 from the 51 colectomy specimens, 42.1 from the 21 VI positive tumours, and 38 from the 30 VI negative tumours. This difference was significant ($p = 0.0497$).

Table 1 presents the efficacy of the various types of tissue blocks to detect VI.

DISCUSSION

The number of invaded veins that are identified in CRC specimens is typically small.[18 20 33] Consequently, the effect of sampling errors on the detection of VI is significant. The hypothesis that cutting the specimen tangentially at the periphery of the tumour and across the mesentery increases the chances of diagnosing VI is based upon knowledge of the anatomical course of the veins that drain the bowel wall, and upon the logical assumption that by examining more veins the chances of detecting invaded veins are improved.[10 12 21]

We found that most instances of VI were identified in perpendicular tissue blocks (in 15 of 21 tumours). Furthermore, in eight of the 21 tumours VI was detected only in the perpendicular blocks.

However, in six of the 21 tumours, VI was diagnosed only in tissue blocks that were cut tangentially, across the mesentery, or from harvested mesenteric LNs. These findings support both the hypothesis that tangentially cut tissue blocks increase the rate of diagnosis of VI in CRC, and the recommendation that they should be submitted routinely.

As would be expected, our study also shows that the submission of a greater number of tissue blocks for histopathological evaluation increases the chances of diagnosing VI significantly. However, our data indicate that the efficacy of the various types of tissue blocks to detect VI is affected by the anatomical orientation of each block type, and it is not just the result of examining more tissue samples. The data presented in table 1 indicate that across the mesentery blocks are the most efficacious in detecting VI; although they comprised only 8% of the total number of submitted tissue blocks, they contributed 17.2% of detected VI (efficacy, 2.151). This finding supports the concept that submitting

tissue blocks that are anatomically oriented to transect numerous veins increases the likelihood of detecting VI. Surprisingly, and contrary to this concept, perpendicular blocks were almost as efficacious (2.088), whereas blocks cut tangentially from the outer perimeter of the tumour had an efficacy of 1.092 only. These findings suggest that the route of the venous drainage from the primary tumour to the veins at the mesenteric border of the bowel wall runs predominantly through intramural veins, and only a considerably smaller fraction goes through veins situated around the outer perimeter of the tumour.

> ''Our data indicate that the efficacy of the various types of tissue blocks to detect venous invasion is affected by the anatomical orientation of each block type, and it is not just the result of examining more tissue samples''

Blocks from mesenteric LNs comprised 40% of the total number of submitted blocks, yet they detected only 6.9% of VI (efficacy, 0.172). Nevertheless, in our study two blocks from LNs showed VI and, more importantly, in one of these VI was identified only in the block from the LN. Therefore, mesenteric LNs should be routinely scanned not only for metastases, but also for VI.

The fact that VI was not seen in blocks from large mesenteric vessels is probably the result of the a priori small chance that a tissue slide will catch a small embolus of cancer cells (floating type of VI) at any random point along a larger collecting vein. However, because the route of haematogenous dissemination must traverse these larger mesenteric veins, we believe that blocks should be submitted from them and scanned for VI.

Our study taught us that the number of tangential blocks that may be cut from the outer perimeter of a primary CRC depends on several variables; namely, the surface area of the tumour, its depth of penetration, the configuration of its penetrating edge, and whether it is situated in a peritonealised segment of the colorectum or in one of its retroperitoneal parts (for example, lower two thirds of the rectum; posterior or posterolateral aspects of the ascending or descending colon). As a result, it is not possible to define a recommended number of tissue blocks that should be submitted for each tumour surface area.

It has been shown that the addition of a stain for elastic fibres facilitates the detection of VI considerably.[14 15 18–21 33 34] In a previous study, the addition of Weigert's stain for elastic fibres enabled us to detect VI in 15 of 39 CRC specimens in which VI had not been identifiable with H+E alone.[33] Similarly, in our present study, VI was identified in nine of the 22 VI positive tumours only after the elastic stain was added. Furthermore, the elastic stain increased the number of involved veins in seven of the 13 cases in which VI had been diagnosed by H+E alone.

The characteristics of VI in our study (overall incidence, incidence for each TNM stage, site, type, and extent) are similar to most previously reported series. Nevertheless, it is noteworthy that the submission of tissue blocks that are cut tangentially, across the mesentery, or from mesenteric vessels and LNs increases the relative detection of extramural VI.

With regard to postoperative systemic treatment for the 22 patients with VI positive tumours, systemic chemotherapy was indicated for nine of these patients who underwent palliative resection of stage IV tumours. Of the 13 patients who underwent potentially curative surgery, according to present standards of treatment,[22] adjuvant systemic chemotherapy was indicated for the 10 patients with node positive disease, whereas the remaining three patients (who had stage I and IIA tumours) would not be candidates for adjuvant systemic treatment. However, if we regard VI as an indication for the administration of adjuvant systemic chemotherapy, the detection of VI would influence the postoperative management of 23.1% of the patients who underwent potentially curative surgery in our study.

In conclusion, to minimise the false negative rate associated with the histopathological diagnosis of VI in CRC, thereby improving staging and therapeutic decisions for the individual patient, we recommend that tangentially cut tissue blocks from the outer perimeter of the tumour, across the mesentery, and from mesenteric vessels and LNs should be routinely submitted as an integral part of the specimen evaluation. In addition when VI is not detected in tissue stained with H+E alone, a stain for elastic fibres should be added, and a second search for VI carried out.

Take home messages

- Vascular invasion (VI) is of prognostic relevance in colorectal carcinoma (CRC) and can be used to guide postoperative treatment after potential curative resection
- To minimise the false negative rate associated with the histopathological diagnosis of VI in CRC, tangentially cut tissue blocks from the outer perimeter of the tumour, across the mesentery, and from mesenteric vessels and LNs should be routinely submitted as an integral part of specimen evaluation
- When VI is not detected in tissue stained with haematoxylin and eosin alone, a stain for elastic fibres should be added, and a second search for VI carried out

Authors' affiliations

A Sternberg, Department of Surgery A, Hillel Yaffe Medical Centre, Hadera 38100, and the Rappaport Faculty of Medicine, the Technion, Haifa, Israel
A Mizrahi, M Amar, G Groisman, Department of Pathology, Hillel Yaffe Medical Centre, Hadera, and the Rappaport Faculty of Medicine

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