JCP Online First, published on May 3, 2012 as 10.1136/jclinpath-2011-200552

Absence of extramural venous invasion is an excellent predictor of metastasis-free survival in colorectal carcinoma stage II—a study using tangential tissue sectioning

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Accepted 20 March 2012

ABSTRACT

Aims Extramural venous invasion (EVI) is an important predictor of haematogenous metastasis in colorectal cancer (CRC). However, remarkable discrepancies in incidence rates indicate major problems regarding EVI assessment. The present prospective study applies tangential vessel preparation to CRC resection specimens and correlates results of EVI with metachronous haematogenous metastatic (MHM) spread.

Methods Stage II CRC diagnosed at the Institute of Pathology, University Teaching Hospital Feldkirch, Austria over a period of 30 months were analysed and tangential sectioning of the pericolonic tissue was performed. Confirmation, or exclusion of MHM, as assessed by computerised tomography, sonography or biopsy, was recorded.

Results In 50/79 (63%) cases EVI was detected. In 13/50 (26%), MHM developed. Of the 29/79 (37%) patients without EVI, only one (3.5%) developed MHM. Statistically, the rate of MHM for patients with EVI was independent of adjuvant chemotherapy.

Conclusions Tangential sectioning of the tumour periphery in CRC stage II yields a high rate of histologically evaluable extramural veins and permits proper assessment of EVI. Absence of EVI is significantly associated with metastasis-free survival, a finding of potential therapeutic value. On the other hand, one-third of the patients with EVI and circumferential tumour growth develop MHM, a setting in which the option for adjuvant chemotherapy should be considered. This study emphasises the importance of tangential sectioning of the invasive tumour front in CRC compared with the recommended perpendicular technique. The sensitivity and specificity of this method regarding MHM are characterised.

INTRODUCTION

Colorectal carcinoma (CRC) is the third most commonly diagnosed cancer worldwide, representing nearly 10% of all malignancies, and is a major cause of cancer-related mortality.[1] Treatment decisions and estimation of patient prognosis are largely based on the assessment of tumour stage according to the tumour node metastasis (TNM) system. However, patients with CRC stage II pose a significant therapeutic management problem. Considerable controversy exists regarding the role of adjuvant chemotherapy in this setting. Stage II patients with a high-risk profile may benefit from adjuvant chemotherapy (AdC) and show prolonged disease-free survival.[2] Various efforts using molecular markers and/or gene expression profiling have recently attempted to better define patients with an increased risk of dissemination.[3] Until now, none of these methods has been clinically established. Recognised clinicopathological factors associated with high risk include T4 tumours, poorly differentiated tumours, perforation, obstruction, small numbers of lymph nodes examined and extramural venous invasion (EVI).[4] Interestingly, the reported prevalence of EVI in CRC resection specimens vary remarkably, ranging from 8.7% to 52%.[5–10] This indicates major technical problems in properly assessing this important parameter. We have shown that tangential sectioning of the extramural adipose tissue surrounding the tumour permits detection of EVI at much higher rates than the usual tissue sectioning methods.[10] In this prospective study, we have analysed the specificity and sensitivity of EVI with this technical approach as a predictor of metachronous haematogenous metastasis (MHM) in CRC stage II. We show that this method of vessel preparation confers substantially greater accuracy in prediction of metastatic spread in CRC.

PATIENTS AND METHODS

CRC cases submitted to the Institute of Pathology, University Teaching Hospital, Feldkirch, over a period of 30 months (n=317) were used. After fixation in 10% neutral buffered formalin, tumour specimens were bread-sliced at 7 mm intervals. Subsequently, the tumour periphery was cut tangentially with 3 mm thickness under close inspection ('shell-like' sectioning) as described previously (figure 1).[10]

Tissue was embedded in paraffin and sections cut at 4 μm were stained with H&E, from the region surrounding the tumour periphery additionally with Elastica–van Gieson technique. All available slides were reviewed by a gastrointestinal pathologist (KD) blinded for patient status. Tumours were classified according to the current WHO criteria for tumours of the digestive tract.[11] Serial sections were evaluated for cases with suspected invasion of the serosa or adjacent organs, or for tumours with a small distance to the radial specimen margin.

Patients treated with AdC received 5-fluorouracil-based combination therapies. Treatment was

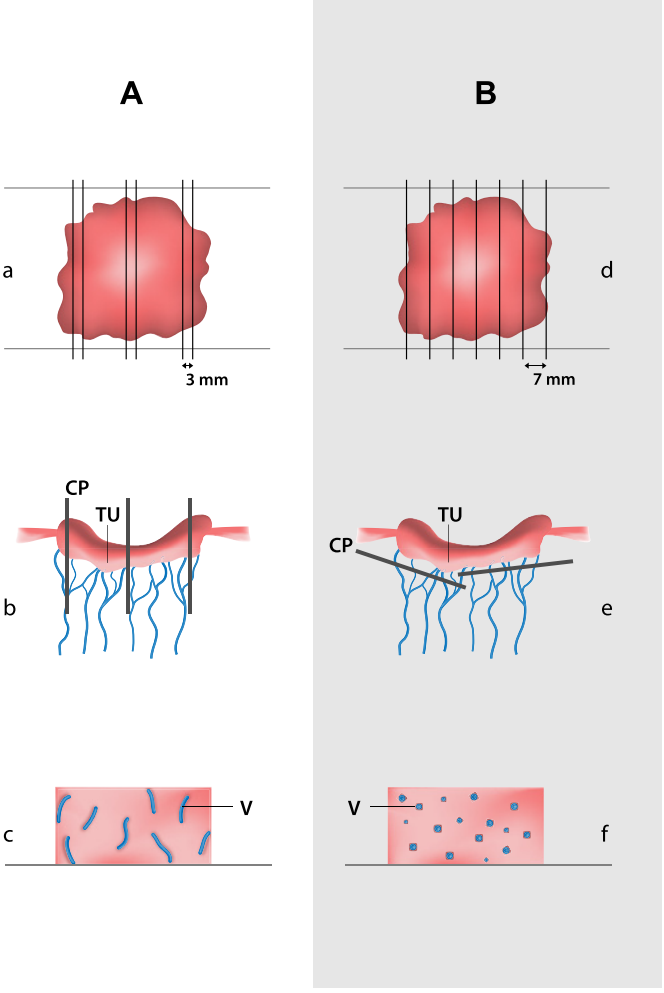

Figure 1 Comparison of the conventional (A) and our tangential (B) method of dissection. In (A) several narrow vertical planes of section through the tumour (a) and the adjacent mesocolon/mesorectum (b) with single longitudinally cut veins (c). In (B) vertical sectioning of the entire tumour (7-mm-thick sections) (d), followed by sectioning perpendicular to the primary plane of the mesocolon/mesorectum (e) with a higher number of veins detectable on cross-section (f). TU: tumour; CP: cutting plane; V: veins.

assessed retrospectively. The follow-up time indicates the period between initial diagnosis and confirmation or exclusion of MHM, as assessed by computerised tomography, sonography and biopsy.

Sensitivity, specificity and positive and negative predictive values of EVI status were estimated, with 95% CI. Statistical calculations were performed using SPSS V.19.0 software (SPSS Inc,).

RESULTS

Patient characteristics

Among 91 stage II CRCs, 79 qualified for further evaluation. Twelve patients were excluded due to missing follow-up, multiple CRC or death within 3 months after surgery. The median age was 68 years (range: 23–88). The median follow-up period was 49 months for those without MHM (range: 8–92), and 15 months for patients with MHM (range: 5–81). All clinicopathological characteristics in relation to EVI status are shown in table 1.

Extramural venous invasion and metachronous haematogenous metastasis

Compared with the conventional perpendicular method of assessment in which tissue at the tumour margin is sampled in a plane that passes through the centre of the tumour, tangential sampling of the adipose tissue near the extramural invasive tumour front reveals far greater numbers of vessels (figure 2). Thereby, veins are easily identified due to their proximity to arteries. Figure 2 shows a representative specimen from a patient who developed MHM. Using this technique, 50/79 (63%) patients were found to have EVI. We included a control cohort consisting of 50 specimens, which were macroscopically dissected by the conventional method; EVI was detected in only 16/50 (32%) of these cases. The number of veins with EVI varied. For cases with EVI, a mean of 2.5 extramural veins with tumour invasion was detected (in 9/13 of cases with MHM, the range of veins with tumour infiltration was 1–2, and in the remaining 4/13 cases the range was 3–5 veins). In one case, fully 10 tumour-invaded veins were detected, but without MHM. Elastica–van Gieson stains confirmed the results observed on H&E stains. One-third of the patients with EVI and circumferential tumour growth developed MHM. The ratio of pT1-2 to pT3-4, and the ratio of well/moderate to poor tumour differentiation, was independent of the EVI status.

Of the 50 patients with EVI, 13 developed MHM (26%). Importantly, only one patient out of 29 without EVI developed MHM (3.5%). Notably, patients with EVI showed no significant difference in the rate of MHM whether they were treated with AdC or not. The data regarding EVI, AdC and MHM are shown in table 2.

The sensitivity of our dissection method for detection of EVI among all patients that developed MHM was 0.93 (95% CI 0.69 to 0.99). The specificity was 0.43 (95% CI 0.32 to 0.55). The positive predictive value of EVI status was 0.26 (95% CI 0.16 to 0.40) and the negative predictive value was 0.97 (95% CI 0.83 to 0.99); and the OR was 9.8.

In a statistical analysis of the associations of localisation, AdC, tumour manifestation and EVI with metastasis, only EVI appeared to have a significant effect (p=0.01).

DISCUSSION

Stage II CRC poses considerable difficulties in therapeutic strategy. Various approaches, including gene expression profiling and/or multi-parameter immunohistochemical analyses, have been proposed to facilitate decision-making, but to date, consensus on the value of these assays is lacking.[3 12 13] By contrast, EVI is a prerequisite for the development of MHM, is clearly associated with decreased overall survival and is an essential prognostic factor for patient survival according to the Union Internationale contre le cancer.[7 14 15] However, the reported prevalence of EVI in CRC resection specimens in stage II vary remarkably, ranging from 8.7% to 52% (table 3), This indicates major problems in properly assessing this important parameter.[16] A recent review analysing practice patterns in CRC reports that 70% of pathologists detect venous invasion in <10% of resection specimens.[17] This is well below the guidelines proposed by the Royal College of Pathologists, stating that EVI should be detected in at least 25% of all CRC specimens.[18] Messenger *et al* recognise that an assessment of tissue sectioning methods merits further investigation since apparent confusion between the distinction of tangential and perpendicular sectioning limits reliable conclusions.[17] In the present study, we show that 63% of CRC stage II have EVI

Table 1 Extramural venous invasion (EVI) and metachronous metastasis status (M), according to the clinicopathological characteristics for patients with stage II colorectal carcinoma (%)

Characteristic	EVI−M0 (n=28)	EVI+M0 (n=37)	EVI−M1 (n=1)	EVI+M1 (n=13)
Age (years)				
<68	14 (50)	20 (54)	1 (100)	6 (46)
≥68	14 (50)	17 (46)	0 (0)	7 (54)
Gender				
Male	13 (47)	18 (49)	1 (100)	9 (70)
Female	15 (53)	19 (51)	0 (0)	4 (30)
Tumour differentiation				
Well/moderate	23 (82)	33 (89)	1 (100)	12 (92)
Poor	5 (18)	4 (11)	0 (0)	1 (8)
T-stage				
pT3	26 (93)	33 (89)	0 (0)	12 (92)
pT4	2 (7)	4 (11)	1 (100)	1 (8)
Localisation				
Colon	22 (79)	25 (68)	1 (100)	7 (54)
Rectum	6 (21)	12 (32)	0 (0)	6 (46)
Adjuvant chemotherapy				
No	19 (68)	18 (49)	0 (0)	6 (46)
Yes	7 (25)	18 (49)	0 (0)	5 (38)
Unknown	2 (7)	1 (2)	1 (100)	2 (16)
Lymph nodes				
<12	9 (32)	14 (38)	0 (0)	5 (39)
≥12	19 (68)	23 (62)	1 (100)	8 (61)
Tumour manifestation				
Circumferential	6 (21)	14 (38)	0 (0)	7 (54)
Focal	22 (79)	23 (62)	1 (100)	6 (46)
Metastasis				
No	28 (100)	37 (100)	0 (0)	0 (0)
Yes	0 (0)	0 (0)	1 (100)	13 (100)

when tangential dissection of invasive tumour front in the mesocolon/mesorectum is applied.

Our data show an even higher percentage of EVI in stage II CRC compared with previous observations in stage II CRC, and support the view that the conventional perpendicular preparation technique greatly underestimates the true prevalence of EVI in CRC (table 3). We thus propose that if the EVI status is not assessed properly, a correct prediction of the haematogenous metastatic potential of a given CRC is currently not possible.

A significant advantage of our method is that it permits quicker and more reliable assessment of the mesocolic/mesorectal veins. Veins and arteries run parallel to each other in the

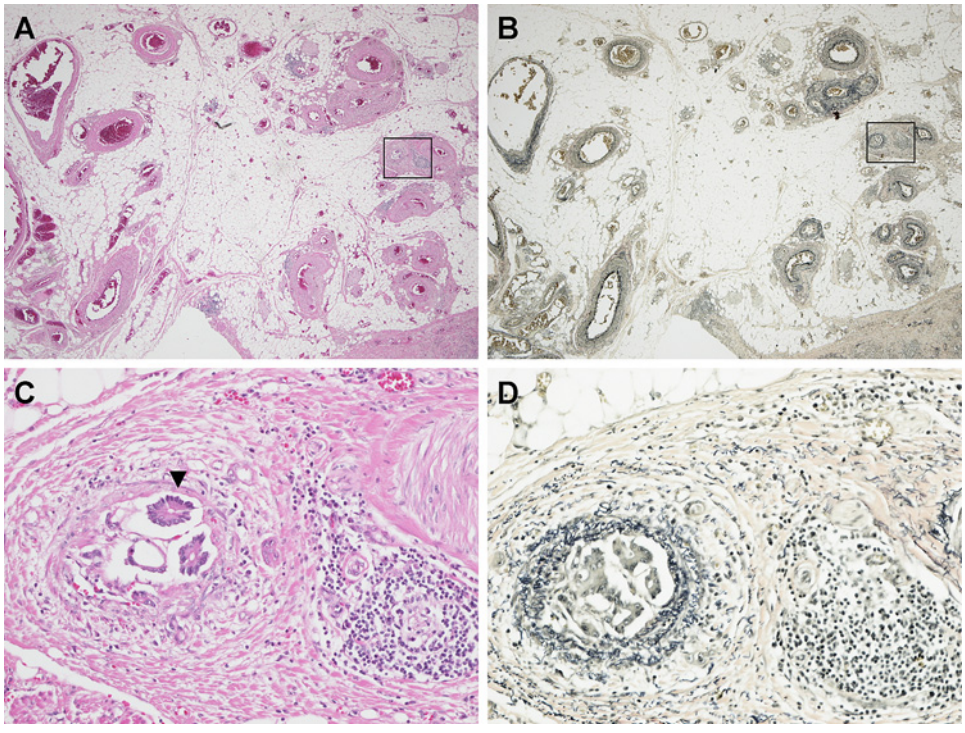

Figure 2 Tangential section of the peritumoural mesocolic tissue showing numerous vessels on cross-section stained with H&E (A) and Elastica−van Gieson (B). Original magnification: 20×. The rectangles in A and B indicate the higher magnified areas in C and D. Extramural venous invasion of a colorectal adenocarcinoma (arrowhead) stained with H&E (C) and Elastica−van Gieson (D). Original magnification: 200×.

Table 2 Metachronous haematogenous metastasis (MHM) as related to extramural venous invasion (EVI) and adjuvant chemotherapy (AdC) for stage II colorectal carcinoma

	EVI negative (n=29)				EVI positive (n=50)			
	AdC (−)	AdC (+)	AdC unknown	Total	AdC (−)	AdC (+)	AdC unknown	Total
MHM (−)	19	7	2	28	18	18	1	37
MHM (+)	0	0	1	1	6	5	2	13

mesocolon, and are perpendicular to the bowel wall. Therefore, they are much easier to identify on tangential sections. Additional special stains for assisting the identification of venous invasion, as advised by other groups, improve the detection of veins, especially for cases with extensive formation of tumour stroma.[19 20]

Using this method of dissection to assess EVI, absence of EVI proves to be a highly specific (97%) predictor of metastasis-free survival in stage II CRC; the number of invaded veins itself was not significant for MHM. The risk of developing MHM in our study was well below 5% when EVI was not present. For example, in one study, MHM has even been reported in up to 84% of patients without EVI, once more indicating major flaws in the assessment of EVI.[8] The probability of false negative cases in these studies is much higher, since conventional perpendicular sections of the tumour and surrounding tissue run parallel to the mesocolic/mesorectal vessels, and thus, detect these only by chance and not in a reproducible and systematic fashion as demonstrated by our technique.[21 22]

The positive predictive value of MHM in stage II CRC for patients with venous invasion amounted to 26% in our cohort, which is well in line with other reports.[8] Overall, such prognostic power is currently only achieved by the combination of various factors (eg, TNM stage, tumour localisation, immunohistochemical results, extent of necrosis, tumour budding).[8] Merkel *et al* found that in emergency presentations of stage II CRC there is a special risk, resulting in a 38.1% rate of metastasis.[8] Circumferential tumour manifestation with stenosis is a characteristic likely to cause an emergency presentation. For our group of patients with both circular tumour manifestation and EVI, the incidence rate of MHM increased to 33%. However, due to the small patient number, this finding must be interpreted with caution. A possible explanation is that hyperactivity of the bowel caused by stenosis may lead to a mechanical break-off of intravenous tumour plugs, with dissemination. Among our study subjects, circumferential tumour growth was associated with metastasis only when EVI was present.

Our study suggests that the EVI status may warrant consideration when making clinical decisions regarding the necessity of AdC. The high negative predictive value for MHM suggests that AdC is not indicated for patients without EVI. However, this conclusion may only be applicable when EVI status is properly and systematically assessed. For approximately one-third of the patients with stage II CRC who had both EVI and circumferential tumour growth, especially in the setting of an emergency operation, AdC seems to be justified due to the higher incidence of MHM. Implications for the remaining patients with EVI are presently not clear. In our study, only a small number of patients received AdC, thereby limiting detailed deductions. However, patients with EVI demonstrated no significant difference of MHM with or without AdC. The number of tumour-infiltrated veins was not an indicator of MHM. It remains to be determined in a larger cohort if closely meshed checkups with imaging techniques may be an alternative to AdC for patients with EVI as the only risk factor, or if these patients should be managed as stage III CRC patients.

Currently, EVI is reported as suggested by the TNM classification, but the localisation (intra/extramural) and method for dissection of CRC specimens is not specified.[23 24] Our data show that the EVI status is a very good predictor of MHM in CRC, provided it is technically assessed correctly. Critical rethinking of the traditional macroscopic dissection of CRC specimens is warranted. We propose tangential sectioning as part of a new standard.

Table 3 Studies reporting the prevalence of venous tumour invasion for stage II colorectal carcinoma

Group	Year of publication	Case number	Prevalence (%)
Dukes *et al**	1941	574	8.7
Krasna *et al**	1988	30	20
Talbot *et al*†	1981	264	36
Merkel *et al*†	2001	305	10.2
Peterson *et al*	2002	268	34
Dirschmid *et al*†	1996	31	52

*No separation between intra-and extramural venous invasion.
†Only extramural venous invasion.

Take-home messages

- Extramural venous invasion in colorectal cancer is a recognised clinicopathological factor associated with high risk of hematogenous dissemination.
- The incidence of extramural venous invasion in colorectal cancer varies remarkably between studies.
- Tangential preparation of the invasive tumour front is far more accurate than the generally recommended perpendicular preparation for assessment of extramural venous invasion.
- Critical rethinking of the traditional macroscopic dissection of colorectal specimens is warranted and we propose tangential sectioning as part of a new standard.

Contributors All authors have contributed to the work, read and revised the text and approved the contents of the manuscript.

Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

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J Clin Pathol published online May 3, 2012
doi: 10.1136/jclinpath-2011-200552