PhD Thesis

Vascular invasion detected by orcein staining and its significance in different tumours, with emphasis on colorectal cancer

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LIST OF FULL PAPERS THAT SERVED AS THE BASIS OF THE PHD THESIS

I. Sejben I., Bori R., Cserni G.
Venous invasion demonstrated by orcein staining of colorectal carcinoma specimens is associated with the development of distant metastasis.
Journal of Clinical Pathology 2010;**63**.575-578.
IF: 2.475

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Journal of Clinical Pathology 2013;**66**:543-547.
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III. Sejben I., Kocsis L., Török L., Cserni G.
Elastic staining does not assist detection of venous invasion in cutaneous melanoma.
Pathology Research and Practice, 2016;**212**:51-53.
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OTHER PUBLICATIONS

A RELATED TO THE THEME OF THE THESIS

1. Cserni G., Bori R., Sejben I.
   Vascular invasion demonstrated by elastic stain – a common phenomenon in benign granular cell tumors.
   Virchows Archiv, 2009;**454**:211-215.
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2. Bori R., Sejben I., Svébis M., Vajda K., Markó L., Pajkos G., Cserni G.
   Heterogenity of pT3 colorectal carcinomas according to the depth of invasion.
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3. Cserni G., Bori R., Sejben I.
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4. Cserni G., Bori R., Sejben I., Ágoston E.I., Ács B., Szász A.M.
   The Petersen prognostic index revisited in Dukes B colon cancer – interinstitutional differences.
   Pathology, Research and Practice.2016;**212**:73-76.
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1. Cserni G., Bianchi S., Vezzosi V., van Diest P., van Deurzen C., Sejben I., Regitnig P., Asslaber M., Foschini M.P., Sapino A., Castellano I., Callagy G., Arkoumani E., Kulka J., Wells C.A.
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   IF: 1.657

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7. Meretoja T.J., Audisio R.A., Heikkila P.S., Bori R., Sejben I., Regitnig P., Luschin-Ebengreuth G., Zgajnar J., Perhavec V., Gazic B., Lázár G., Takács T., Kővári B., Saidan Z.A., Nadeem R.M., Castellano I., Sapino A., Bianchi S., Vezzossi Vl, Barranger E., Louquy R., Arisio R., Foschini M.P., Imoto S., Kamma H., Tvedskov T.F., Jensen M.B., Cserni G., Leidenius M.H.
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LIST OF ABBREVIATIONS

AJCC	American Joint Committee on Cancer
BLVI	Blood and lymphatic vessel invasion
CI	Confidence interval
CRC	Colorectal carcinoma/cancer
FET	Fisher exact test
H&E	Haematoxylin and eosin
MRI	Magnetic resonance imaging
SD	Standard deviation
TNM	Tumour, lymph node and distant metastasis classification system
VI	Venous invasion

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1. INTRODUCTION

Every year, approximately 1.2 million new cases of colorectal cancer (CRC) are discovered and about 600,000 patients die of the disease all over the world. The incidence of the tumour is high in North America, New Zealand, Australia and Europe [1]. CRC is the second most common cause of cancer-related mortality in both sexes causing about 5,000 deaths per year in Hungary [2]. Its occurrence increases from age 40 and rises sharply above 50. However, cases in younger patients seem to be increasing [1].

Adenocarcinomas comprise more than 90% of CRC. In the WHO classification, the following histopathological variants are recognised: mucinous adenocarcinoma, signet ring cell carcinoma, medullary carcinoma, serrated adenocarcinoma, cribriform comedo-type adenocarcinoma, micropapillary adenocarcinoma, adenosquamous carcinoma, spindle cell carcinoma and undifferentiated carcinoma [3]. Prognosis assessment and adjuvant treatment decisions predominantly rely on Tumour, Node, Metastasis (TNM) staging. The American Joint Committee on Cancer (AJCC), Dukes and modified Astler-Coller staging are based on the same principles and they correspond to each other. The present work used the TNM staging according to the seventh edition of AJCC Staging Manual [4]. The two staging systems of CRC mentioned in this thesis are summarized in Tables 1 and 2 without detailed subdivisions of some categories.

To improve CRC patient management, the prognosticators of these tumours should be more precisely determined. The College of American Pathologists Consensus Statement in 1999 stratified the prognostic parameters into five categories according to the published strength of evidence regarding their prognostic value [5]. Category I includes factors clinically proven, prognostically important and generally used. Factors in category IIA are extensively studied, prognostically important but require further validation. Category IIB contains factors that were promising in multiple studies but lacking sufficient data to be placed into category I or IIA. Category III is for factors not sufficiently studied. Factors well studied but without prognostic value are in category IV.

Table 1 TNM staging of CRC

Primary Tumour (T)	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ
T1	Tumour invades submucosa
T2	Tumour invades muscularis propria
T3	Tumour invades through the muscularis propria into pericolorectal tissues
T4a	Tumour penetrates to the surface of the visceral peritoneum
T4b	Tumour directly invades or is adherent to other organs or structures
Regional Lymph Nodes (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in 1–3 regional lymph nodes
N2	Metastasis in four or more regional lymph nodes
Distant Metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis
M1a	Metastasis confined to one organ or site
M1b	Metastases in more than one organ/site or the peritoneum

Table 2 AJCC and Dukes stages of CRC

AJCC stage	intramural invasion	extramural invasion	regional lymph node metastasis	distant metastasis	**Dukes stage**
I	yes	no	no	no	A
II	yes	yes	no	no	B
III	yes	yes/no	yes	no	C
IV	yes	yes/no	yes/no	yes	

Blood and lymphatic vessel invasion (BLVI) has been established to be a category I prognostic factor in CRC along with local extent of tumour invasion, regional lymph node metastases, residual tumour, preoperative serum carcinoembryonal antigen level and radial margin involvement [5]. BLVI has not only prognostic importance but may also influence

therapy. Dukes B colon cancer patients are often recommended adjuvant chemotherapy on the basis of clinico-pathological factors, such as bowel obstruction, less than 12 lymph nodes examined, high grade, BLVI, perineural invasion, localized perforation and positive, close or undetermined resection margins [6].

The terminology of BLVI is somewhat variable from author to author. Although the meaning of blood vessel invasion, lymphatic invasion or venous invasion (VI) is clear, other terms are used in different ways. With common terms, invasion of lymphatic and/or blood vessels are referred to as lymphovascular/lymph-vascular/angiolymphatic invasion. However, some authors use lympovascular invasion to describe small vessel invasion which encompasses lymphatic, blood capillary and small venule involvement. In contrast, involvement of veins and arteries is referred to as large vessel invasion. The term vascular invasion can be interpreted as involvement of blood vessels only or the invasion of blood and lymphatic vessels together.

Elastica stainings are reported to be useful in the evaluation of VI in colorectal [7-12], gastric [13, 14] and oesophageal cancers [15]. The use of elastica stains can provide a more precise identification of VI in CRC compared to routine haematoxylin and eosin (H&E) stain. And the demonstration of venous involvement by an elastic stain in colorectal carcinoma specimens is associated with a higher risk of distant metastasis [16-18]. We undertook a two-phase study to investigate the prognostic significance of VI demonstrated by orcein stain in CRC. Along the same lines, we tried to visualize VI in sections from cutaneous melanoma specimens using orcein staining.

Although BLVI is an important sign of aggressive behaviour in tumours, sometimes it may be found in some benign conditions too. Using orcein stain, Cserni et al. discovered VI in four out of five cases of benign granular cell tumour [19]. Other benign lesions such as congenital melanocytic naevus, Spitz naevus, sclerosing adenosis of the breast, vasitis nodosa or neurofibromatosis could show this feature without adverse prognostic effect. In these instances, the subendothelial layers of the vessels are involved [20].

2. AIMS

2.1 To investigate the frequency of VI in CRC specimens detected on H&E and orcein-stained slides separately and to compare the results.

2.2 To assess VI found on H&E and orcein stain and its relation to distant metastasis in CRC.

2.3 To confirm the association between VI identified by orcein staining in CRC and the development of distant metastasis after an extended follow-up period.

2.4 To look for differences between cancers with VI that develop distant metastases and those without metastases despite venous involvement.

2.5 To determine the benefit of orcein elastic staining of primary cutaneous melanoma specimens in detecting VI.

3. MATERIALS AND METHODS

3.1 THE STUDY OF ORCEIN DETECTED VI AND ITS ASSOCIATION WITH DISTANT METASTASES IN CRC

In the first phase of our CRC study, CRC resection specimens received at the Department of Pathology of the Bács-Kiskun County Teaching Hosptital in 2007 were retrospectively collected from an institutional database. The tumours were staged according to the seventh edition (2010) of TNM classification. Besides the classification into pT categories reflecting the depth of invasion or invasion of the peritoneum or nearby organs, and pN categories reflecting the nodal status, the initial M categories for the presence or absence of distant metastases were derived from data available at the multidisciplinary meetings. For the initial M categories, intraoperative biopsy and histology proven metastases (pM) and clinically detected metastases (M) were considered. For the initial staging, patients were evaluated routinely by chest radiograms and abdominal ultrasound, and further imaging studies were performed when required. During follow-up, the M category evaluation was also complemented by autopsy-derived data and patients' charts were used as source.

All tumour blocks were prospectively and routinely assessed for VI in slides stained with orcein. Briefly, after deparaffination, the slides were kept overnight in a solution of 0.1 g orcein (Reanal, Budapest, Hungary) in 100 ml 70% ethanol and 2 ml concentrated hydrochloric acid. This was followed by differentiation in 70 % ethanol, dehydration, clearing in xylene and mounting. First the H&E-stained slides were assessed for VI, and this was followed by the examination of the synchronously stained orcein slides (Figure 1). The results on VI were reported as detected on H&E or orcein. The tumours were therefore also categorised according to the V classification of the TNM: V1 for the presence of microscopically detected VI, and V0 for its absence. Follow-up data were available up to April 2009. The following cases were excluded from the analysis: intramucosal carcinomas with no invasion beyond the muscularis mucosae (pTis according to the TNM classification), recurrent cancers, cases treated with neoadjuvant therapies before histopathological evaluation, cases with synchronous or metachronous cancers elsewhere in the body, and MX cases due to either lack of follow-up data or perioperative death without autopsy.

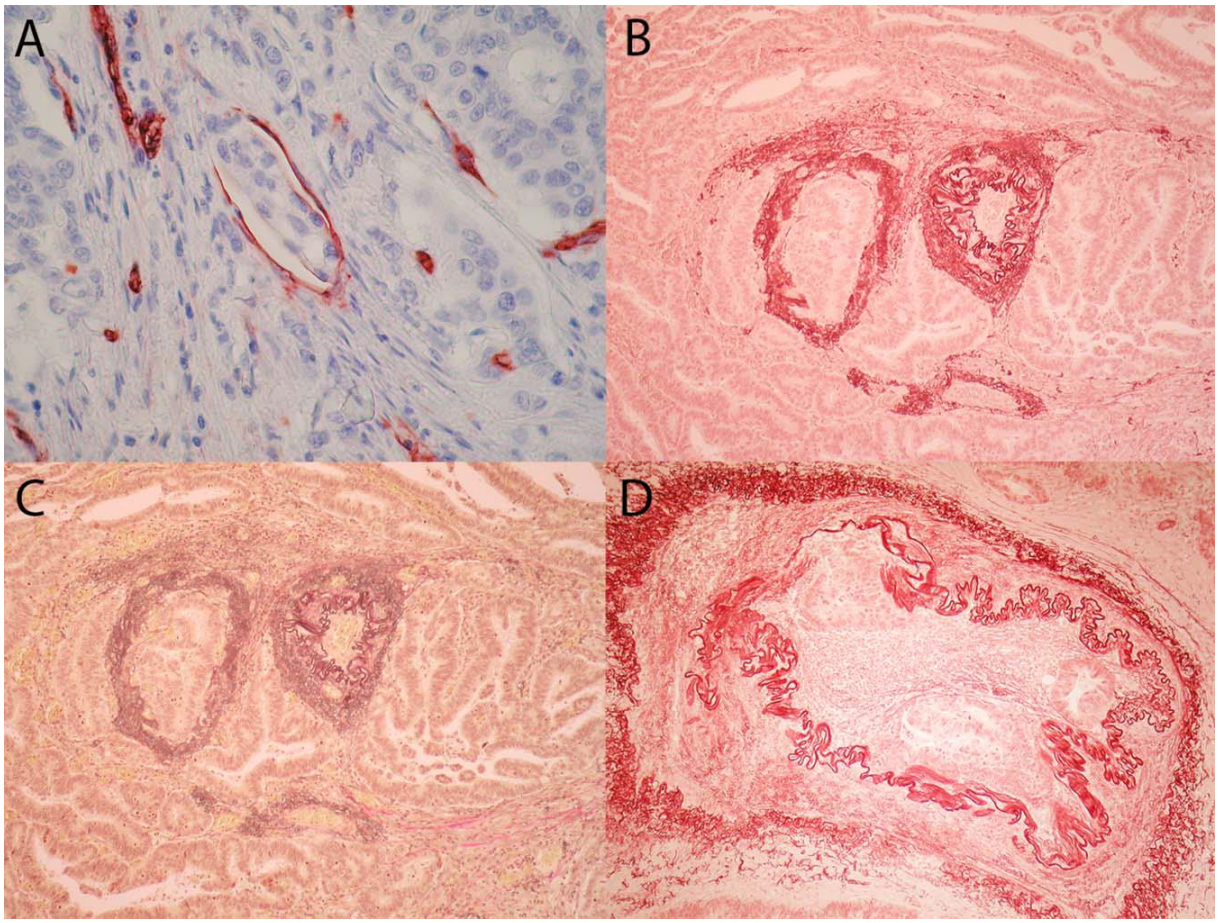


Figure 1 Types of vessel invasion in CRC. A: Small vessel (i.e. capillary) invasion. B and C: VI stained by orcein and elastica van Gieson, respectively. Right to the vein invaded by carcinoma is an accompanying artery, which helps the recognition of VI. D: Invasion of an artery. (A: CD34 ×400, B: Orcein ×100, C: Elastica van Gieson ×100, D: Orcein ×100).

The association of VI or nodal involvement and distant metastases was evaluated with the Fisher exact test (FET) (Vassar-Stats, Vassar College, Poughkeepsie, New York, USA). Significance level was set at $p<0.05$ (two tailed).

In the second phase of our study, the same series of tumours was reanalysed after a follow-up period extending to February 2014. Patients lost to follow-up were censored, and their metastasis status was recorded according to the last follow-up month. Parameters of cases demonstrating VI with (M1) and without (M0) distant non-peritoneal metastasis at the

time of diagnosis or later during follow-up were compared with each other. The orcein stained slides of the CRCs in the compared subsets were reviewed with the number and localisation (intramural or extramural) of VI being recorded. VI grade/density was determined by the overall number of involved veins divided by the number of orcein stained slides (number of tumour blocks). For the nodal status, the current TNM recommendations were used, and isolated tumour cells were considered a subset of the pN0 (node-negative) category. Contingency tables were used for assessing the associations of nodal or VI status and distant metastasis; the Fisher exact test and the t-test for independent samples were used for the comparison of categorical and continuous variables, respectively. Significance level was set at $p<0.05$, and all tests were two tailed. All statistical analyses were performed on VassarStats (VassarStats 1998-2005, Vassar College Poughkeepsie, New York, USA).

3.2 THE STUDY OF UTILITY OF ORCEIN STAINING TO DETECT VI IN CUTANEOUS MELANOMA

We conducted a second study to evaluate the usefulness of orcein stain to detect VI in cutaneous melanomas. Forty cases of primary cutaneous melanoma in vertical growth phase, surgically resected between January 2006 and September 2013 were retrieved from the institutional archives. Only cases with minimum tumour thickness of 3 mm were included. All tumours were at least Clark level III. All tumour blocks were stained originally with H&E and subsequently with orcein as described above. Immunohistochemistry was not used to identify the endothelium. Two pathologists simultaneously assessed the slides for vascular invasion at a multiheaded microscope. First the H&E-stained slides then those stained with orcein were examined. The results with the two staining methods were recorded separately and compared with the original reports.

These two studies were retrospective with no influence on the treatment or outcome of patients who were disindentified. Both studies were approved by both the institutional data safety manager and the local institutional ethical committee.

4.RESULTS

4.1 THE STUDY OF ORCEIN DETECTED VI AND ITS ASSOCIATION WITH DISTANT METASTASES IN CRC

After the exclusion of the cases mentioned in Materials and Methods, 89 patients (47 men and 42 women) remained for analysis. These included 16 patients with rectal tumours and 73 with colon tumours. The median number of orcein-stained slides was six. VI was detected in 16/89 (18%) cases on H&E-stained slides, whereas it was found to be present in 63/89 (71%) cases after the evaluation of orcein-stained slides (Figure 1). VI was more common with greater pT categories and was also more common with nodal involvement; all tumours classified as pN2 had VI. The initial staging results are shown in Table 3. Eleven cases had distant metastases at the initial staging. The median number of lymph nodes examined was 18 (range 4-55). There were 46 (52%) node-positive cases, nine of the M1 tumours were among these, whereas two metastatic cases belonged to the node-negative patients.

The lymph node status (pN0 versus node positive) nearly showed an association with an initial M1 status (p=0.05). VI detected by H&E was not associated with an initial M1 status (p=1), but VI detected by orcein stain showed a significant association with synchronously detected distant metastasis (p=0.029).

Ten M0 cases with less than 6 months of follow-up after surgery were excluded from subsequent analyses. Of the remaining 79 tumours 14 (18%) demonstrated VI on H&E slides and this number rose to 56 (71%) after the analysis of orcein-stained slides. Chemotherapy was administered to 45 patients (30 with VI and nodal involvement, 11 with VI alone, three with nodal involvement alone, and one with no VI or nodal metastasis) and no systemic therapy was given to 34 patients (six with VI and nodal involvement, eight with VI alone, one with nodal involvement alone, and the remaining 19 with no VI or nodal metastasis). For the 68 cases that were initially M0, the median follow-up time was 17 months (range 6-28 months); during this period nine patients with colon cancer developed distant metastases after 4-22 months (median 9 months) following surgery (Table 4).

Table 3 The distribution of pT, pN, M and V categories at initial staging according to the TNM classification

		M0		M1		All
		V0	V1	V0	V1	
pT1	pN0	1	1	0	0	2
	pN1, pN2	0	0	0	0	0
pT2	pN0	5	1	0	0	6
	pN1, pN2	0	0	0	0	0
pT3	pN0	13	16	0	1	30
	pN1	4	14	0	2	20
	pN2	0	9	0	3	12
pT4	pN0	2	2	0	1	5
	pN1	1	7	0	1	9
	pN2	0	2	0	3	5
All		26	52	0	11	89

pT, pathological T category in the TNM (tumour, node, metastases) system.
V classification: V0, absence of VI; V1, presence of microscopically detected VI.

Table 4 Data for cases with distant metastases occurring during follow-up

Case	pT	Quantitative nodal status*	V(H&E)	V(orcein)**	Systemic treatment
1	pT3	0/31	V1	V1(9)	No
2	pT3	12/32	V0	V1(2)	Yes
3	pT4	1/23	V0	V1(3)	No
4	pT4	3/21	V1	V1(10)	No
5	pT3	3/9	V1	V1(4)	Yes
6	pT3	0/11	V0	V1(4)	Yes
7	pT3	5/28	V0	V1(4)	Yes
8	pT3	5/15	V0	V1(3)	Yes
9	pT3	0/14	V0	V1(8)	No

*The ratios reflect the number of involved lymph nodes divided by the number of lymph nodes examined.
**The values in parentheses reflect the number of invaded veins counted on orcein-stained slides.
V (H&E), venous invasion based on H&E-stained slides; V (orcein), VI based on orcein-stained slides.
V classification: V0, absence of VI; V1, presence of microscopically detected VI.

Five of the 20 finally metastatic cases were detected in the node-negative group, all the 20 metastatic cases had VI detected by the orcein stain. Synchronous or metachronous distant metastases were associated with lymph node involvement and VI detected by orcein ($p=0.02$ and $p=0.001$, respectively), whereas H&E detected VI showed no associations ($p=0.31$).

Considering the presence of VI or lymph node metastasis as predictors of distant metastasis (either detected at the time of initial staging or during follow-up), the predictive values of VI detected by H&E or orcein and nodal involvement are shown in Table 5.

Table 5 Overall characteristics of the nodal status or VI to predict distant metastases

M1 at initial staging (n=89)				
	pN1/pN2	V1(H&E)	V1(orcein)	pN1/pN2/V1
PPV	0.2	0.13	0.17	0.16
NPV	0.95	0.88	1	1
Sensitivity	0.82	0.18	1	1
Specificity	0.53	0.82	0.33	0.27
Accuracy	0.56	0.74	0.42	0.36
M1 at initial staging or later (n=79)				
	pN1/pN2	V1(H&E)	V1(orcein)	pN1/pN2/V1
PPV	0.37	0.36	0.36	0.33
NPV	0.87	0.78	1	1
Sensitivity	0.75	0.26	1	1
Specificity	0.56	0.85	0.39	0.32
Accuracy	0.61	0.71	0.54	0.49

M1, presence of distant metastasis; NPV, negative predictive value; PPV, positive predictive value; V1, presence of microscopically detected VI.

In the second phase of the CRC study, the median extended follow-up time for the repeated analysis was 48 months. On review of the original data and slides, two patients in the group positive for VI had to have their status changed. One was recognized to have a single instance of pseudo-VI with partial circumferential elastic fibres inconsistent with VI. The other had only highly elevated carcinoembryonal antigen levels without imaging evidence of distant metastasis but with locoregional recurrence 14 months following surgery, when she was lost to follow-up. The first patient was considered further as having no VI, the second as having no evidence of distant metastasis (M0). Of the original series, three metastatic patients were excluded due to the diagnosis of pancreatic, prostatic and both of these carcinomas, respectively, and one case originally considered for synchronous metastases only was omitted as no follow-up was available. Two patients (of the original seven from the 2010 series) with no follow-up data for the initial analysis were found to have such data on the longer term, and were therefore added to the cases, leaving 87 CRC patients (45 males and 42 females; median

age 71 years, range 29-88 years; 16 with rectal and 71 with colon cancer) for further analysis of the association between metastatic disease and VI.

The initial stages, metachronous metastasis rates and their association with VI are represented in Table 6. Sixty-one patients (70 %; 95% confidence interval (CI): 59-79%) had VI identified with the orcein stain, 18(21%; CI: 13-31%) also had VI identified on H&E-stained slides. Fourty-six patients received chemotherapy (29 with both VI and nodal involvement, 12 with VI alone, 4 with nodal metastasis alone and 1 without VI or nodal disease) and 41 (19 with no VI or nodal involvement, 10 with only VI, 2 with only nodal disease and 10 with both nodal involvement and VI) did not. Thirty-one patients have died, 18 of or with disease and 13 of unrelated causes.

In addition to the 10 patients initially staged as M1 on the basis of distant non-peritoneal metastasis, further 16 patients developed distant metastasis after a median of 24 months [mean±standard deviation(SD):34±26, range: 13-83 months] following surgery. Two metachronous metastatic cases (both node-positive and one positive for VI) had only peritoneal involvement. As these are unlikely to be explained by VI or nodal involvement, they were not considered as distant (haematogenous) metastasis for the purpose of the analyses, where only non-peritoneal distant metastases were taken into account.

Table 6 The distribution of pT, pN, M and V categories at initial staging according to the TNM classification and the cases in which distant metastases developed during follow up (metachronous distant metastatic cases)

		Initial stage					Metachronous distant metastatic cases	
		M0		M1		All		
		V0	V1	V0	V1		V0	V1
pT1	pN0	1	1	0	0	2	0	0
	pN1,pN2	0	0	0	0	0	0	0
pT2	pN0	5	1	0	0	6	0	0
	pN1,pN2	0	0	0	0	0	0	0
pT3	pN0	12	16	0	1	29	1	3
	pN1	5	12	0	2	19	0	3
	pN2	0	8	0	3	11	0	2
pT4	pN0	2	2	0	1	5	1	0
	pN1	1	7	0	1	9	0	4
	pN2	0	4	0	2	6	0	0
All		26	51	0	10	87	2	12

pT, pN and M: staging categories in the pTNM system
V classification: V0, absence of VI; V1, presence of microscopically detected VI.

If nodal status and the presence of any VI detected by orcein were tests to predict the development of distant spread (excluding peritoneal spread), on the basis of our data, the accuracy, sensitivity and specificity of these would be 59.8%, 70.8%, 55.6% and 52.9%, 91.7%, 38.1% respectively. The associations with distant metastasis of a node-positive status (FET, p=0.03) and of VI detected by orcein staining (FET, p=0.008) were significant. H&E detected VI showed no significant association with metastases (FET, p=0.24).

Of the 61 cases with identified VI, 39 did not develop distant non-peritoneal metastasis (V1M0 group) and 22 had either synchronous (n=10) or metachronous (n=12) distant non-peritoneal metastasis (V1M1 group). These groups are compared in Table 7. Of the parameters analysed, the age, gender ratio, pT or pN categories, the number of lymph nodes examined or involved, the number of cases with H&E detected VI and the number of orcein stained slides showed no significant differences. The average follow-up period of

patients in the V1M0 group was greater, whereas their mean VI density and the proportion of cases having extramural rather than solely intramural VI was lower than in the V1M1 group.

Using a non-inclusive cut-off of 1 for VI density (i.e. at least one instance of orcein-detected VI per slide on average considered significant; n=14 with this feature), the association with distant non-peritoneal metastasis was found to be significant (FET, p=0.025) and the accuracy, sensitivity and specificity were 70.5%, 40.9% and 87.2%, respectively. Considering only orcein-detected extramural VI, the association with distant non-peritoneal metastasis was significant (Table 7), and the accuracy, sensitivity and specificity were 60.0%, 90.9% and 42.1%, respectively.

Table 7 Comparison of patients with VI (V1) without (M0) and with (M1) distant non-peritoneal metastasis

	V1M0 (n=39)	V1M1 (n=22)	p
Age (mean ± SD)	66.7 ± 13.9	68.9 ± 11.7	0.54
Gender (M/F)	19/20	13/9	0.59
Follow-up (mean ± SD)	49.6 ± 30.9	27.1 ± 22.8	0.004
pT categories (pT1/pT2/pT3/pT4)	1/1/28/9	0/0/14/8	0.75
pN categories (pN0/pN1/pN2)	17/12/10	5/10/7	0.33
H&E-detected VI	11	7	0.78
Orcein stained slides per case (mean ± SD)	6.2 ± 1.7	6.2 ± 1.1	0.89
Number of LNs examined (mean ± SD)	21.2 ± 11.1	20.0 ± 7.5	0.61
Number of positive LNs (mean ± SD)	2.6 ± 3.9	3.2 ± 3.9	0.52
VI density (mean ± SD)	0.59 ± 0.53	1.23 ± 1.21	0.025
IM only / EM with or without IM*	16/22	2/20	0.009

M: male, F: female, LN: lymph node, IM: intramural, EM: extramural
*One case could not be classified adequately as intramural VI was identified, but VI of undeterminable localization was found in addition

Regarding the relation between the localisation of VI and its density, the mean (±SD) VI density was higher in cases with extramural VI (n=42) than in cases having only intramural VI (n=18) (1.05±0.98 versus 0.31±0.16; $p<0.0001$), and there were also more cases with VI detected on H&E slides in CRCs with extramural VI (17 versus 1, p=0.01).

However, most cases with VI (n=37) had both intramural and extramural VI detected by the orcein stain, with two additional cases having extramural VI and VI of undeterminable localisation and one further case with intramural VI and VI of undeterminable localisation. The VI density of cases with intramural VI (n=56) and those only extramural VI (n=3) was not significantly different.

4.2 THE STUDY OF UTILITY OF ORCEIN STAINING TO DETECT VI IN CUTANEOUS MELANOMA

The depth of invasion of the melanomas studied ranged between 3.1 and 18 mm. On average, 4 tumour-containing blocks were examined from each case (range 1-7). Vascular invasion was detected in 10 of 40 cases on H&E-stained slides. Blood vessel and lymphatic involvement could not be differentiated on H&E-stained slides. Orcein stain highlights venous involvement (Figure 2). Definite VI was identified with orcein in 5 cases. All but one definite VIs detected with orcein were recognised on H&E-stained slides, too. Six cases of vascular invasion detected with H&E were not seen with orcein. There was only one case where orcein contributed to the identification of more veins involved, but part of these was revealed by H&E, too (Table 8).

To sum up, in contrast to our experience in colorectal, appendiceal and gastric cancers, orcein stain did not improve the detection rate of VI. Furthermore, the detection of VI was made difficult by remaining elastic fibres of actinic dermal elastosis, or the invasion of adnexal structures mimicking vascular invasion on orcein stain.

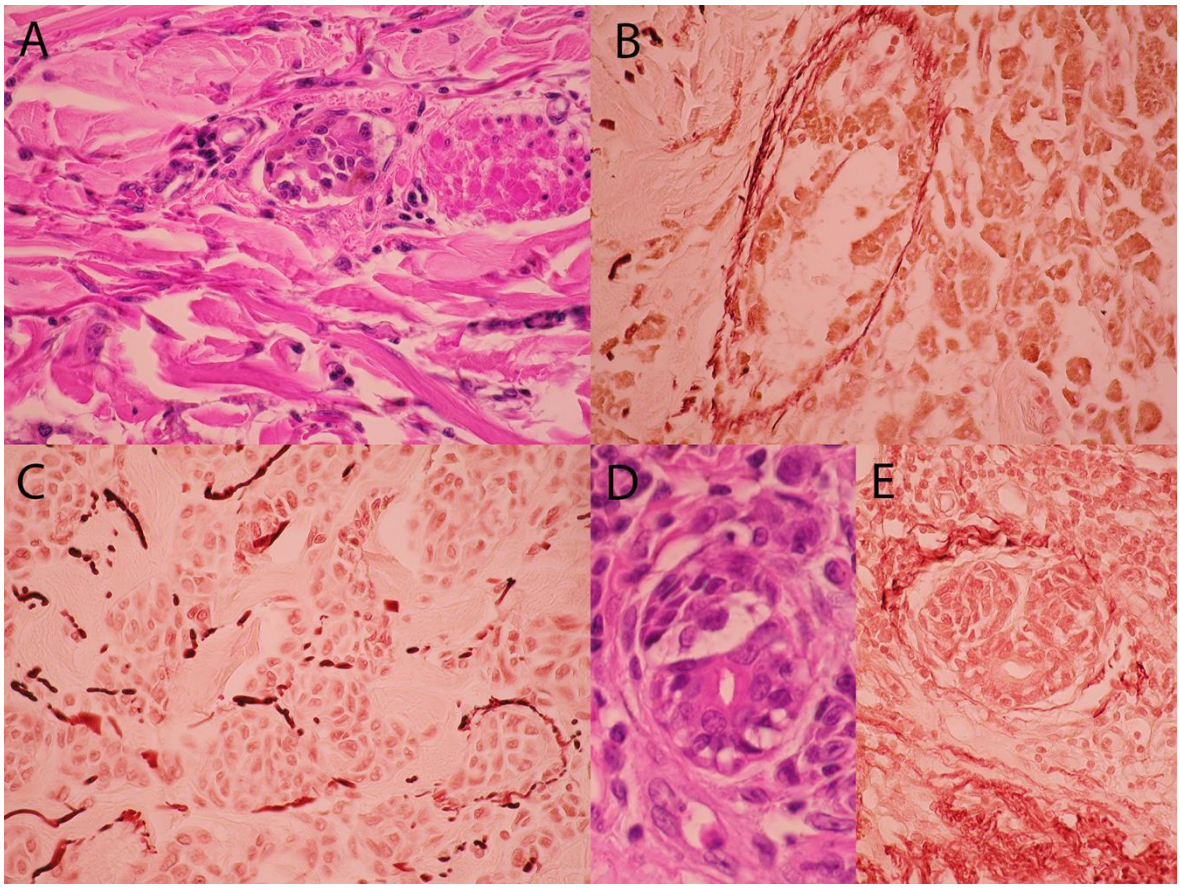


Figure 2 Examples of detected vascular invasion and its mimics in melanoma. A: Capillary type peritumoural vascular invasion detected on H&E stain. B: Venous type intratumoural vascular invasion detected by orcein in the same case as A. C: Elastotic dermal debris preventing the adequate evaluation of vascular invasion on orcein stain. The semicircular arrangement of the elastic fibres in the lower right area could be (mis)interpreted as vascular invasion. D and E: Sweat glands invaded by melanoma cells as seen in H&E (D) and orcein (E) stains, respectively. The periglandular elastic layer may simulate vascular invasion, too.

Table 8 Melanoma cases showing vascular invasion

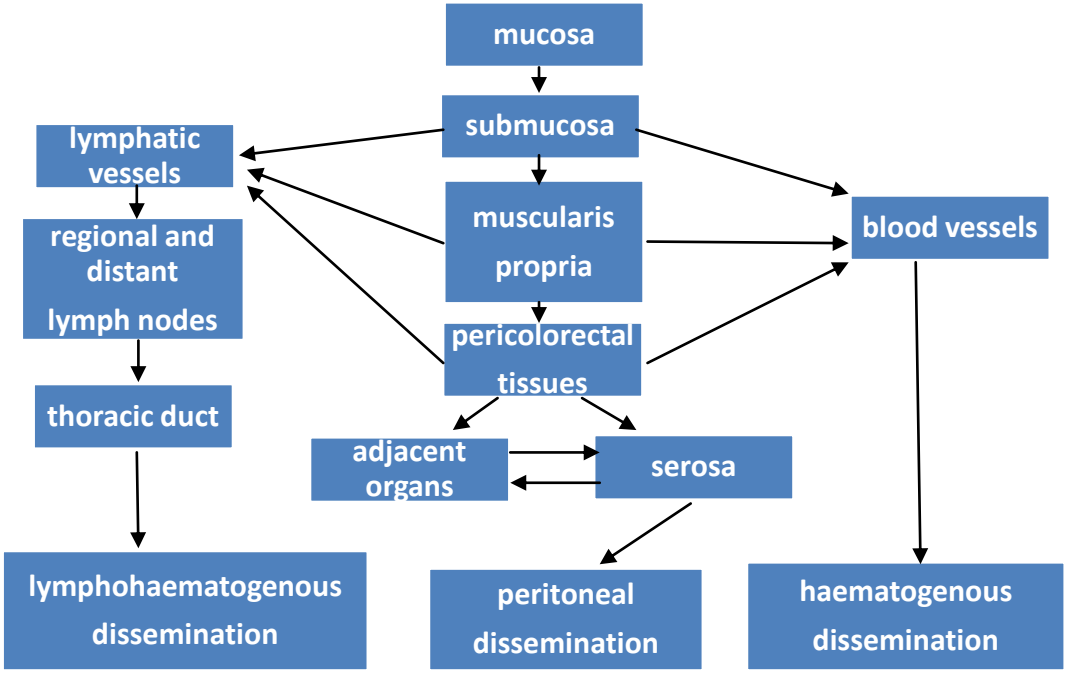
case number	tumour thickness in mm	Clark level	number of vascular invasions with H&E	number of vascular invasions with orcein
1.	5.10	4	3	0
2.	5.80	5	5	4
3.	3.20	4	0	1
4.	3.10	4	1	1
5.	10.00	5	1	0
6.	4.75	5	1	0
7.	5.80	5	2	0
8.	6.90	5	2	16
9.	8.00	4	6	0
10.	18.00	4	2	3
11.	3.60	4	2	0

5. DISCUSSION

Mortality caused by CRC after potentially curative resection is most often related to local or distant recurrence. Spreading of cancer cells follows a variety of routes: direct spread, transperitoneal spread, implantation, lymphatic spread, haematogenous spread and venous extension [21]. Lymphatic routes may combine with haematogenous ones leading to lymphohaematogenous spreading. Haematogenous, lymphohaematogenous and peritoneal spreading are the three main pathways of cancer cell dissemination to distant sites (Figure 3). VI is a morphologically detectable crucial step in tumour progression and spreading, which explains its presence among CRC prognostic factors besides the depth of tumour invasion through the layers of the bowel wall, lymph node involvement and the presence of distant metastases [5]. The presence of blood vessel invasion is an obvious prerequisite for the development of blood-borne metastases. Although VI is an established independent prognostic factor of haematogenous recurrence and survival in CRC, it is not incorporated in the TNM of CRC unlike renal, hepatic, penile and testicular cancer [4].

Liver is the most common site of haematogenous metastasis in CRC. According to the cascade hypothesis proposed by Weiss, lung metastases follow liver involvement and finally arterial metastases may develop to other sites [22]. In rectal carcinoma, there are two possible ways for venous spreading. Upper third rectal tumours metastatize to liver through the portal system, whereas middle and lower third cancers utilize the inferior vena cava to reach the lungs because of the anatomy of venous drainage of the rectum. However, there are a lot of exeptions to these general rules [23].

Figure 3 Main pathways of tumour cell dissemination in CRC



Brown and Warren were the first to highlight the prognostic significance of VI in rectal cancer in 1938 [8, 24, 25, 26]. In an autopsy review, they dissected 170 patients who died of advanced rectal neoplasm. Histological evidence of VI was found in 61% of cases, and 71% of them had visceral metastases, while no case with visceral metastases without VI was detected [24].

To detect VI, the following structures should be recognized in an ideal situation: lumen with tumour cell embolus and blood or thrombus, endothelial lining, circular smooth muscle cells and elastic fibres in vein wall, adjacent artery (Table 9).

Recognizing the luminal tumour cell and blood or thrombus rarely needs special stains. If swollen endothelial cells resemble neoplastic cells, cytokeratin and general endothelial markers can solve the issue. Endothelium, which is specific to vascular structures, can be highlighted by immunohistochemical markers. Factor VIII-related antigen, CD31 and CD34 are used to highlight lymphatic as well as blood vessel endothelial cells [10]. On H&E-stained

slides, no reliable distinction can be made between lymphatic and blood vessels. Immunohistochemical lymphatic endothelial markers such as podoplanin (D2-40), lymphatic endothelial hyaluronan receptor 1 (LYVE-1), vascular endothelial growth factor receptor-3 (VEGFR3), PROX-1 and chemokine scavenging receptor D6 can be applied to differenciate between them [27]. However, it must be stressed that the endothelium of invaded veins is often destroyed beyond recognition.

Table 9 Histological detection of VI

histological structure	**characteristics**	**special stains, immunohistochemistry**
lumen	tumour cells in a round, ovoid space, may contain red blood cells or thrombus	cytokeratin
luminal lining	endothelium, may disappear	CD31, CD34
vein wall	concentric smooth muscle cells, concentric elastic fibres	SMA, desmin, elastic stains
neighbourhood	accompanying artery	elastic stains

Histochemical or immunohistochemical stain for smooth mucle cells and elastic staining for elastin, which stain the smooth muscle and the elastin in the wall of veins respectively, can be used to enhance the detection of VI [25, 26, 28, 29]. Verhoeff's stain, Weigert's stain, Miller'stain and orcein are the most widely used staining methods to detect elastic fibres [26, 30, 31]. Elastic stains also highlight the accompanying artery the presence of which aid the identification of veins.

Some authors classified VI into morphologic types (Table 10) [31-33]. Sato et al. classified VI into embolization (tumour cells filled the lumen) and non-embolization (luminal tumour cells did not adhere to the vein wall or were mainly in the intima) types [32]. Shirouzu and co-workers divided the non-embolization group into floating and intimal types [33]. Sternberg applied similar system with partially different designations [31]. Most of the studies

have found invasion type insignificant [26]. However, Ouchi et al., who categorized VI into filling, floating and occlusive types and defined occlusive type as tumour cells growing in the lumen with marked thickening and inflammation of the vessel wall, stated that the occlusive type is associated with reduced likelihood of distant metastases [16]. After assessment of extramural VI cases, Talbot et al. discovered that loose clumps of tumour cell in veins, direct contact between neoplastic cells and blood, and the invasion of the capillaries in the vein walls means adverse prognostic impact, whereas protective mantle of endothelial cells, aneurysmal dilatation, and inflammation appeared to predict better prognosis [34].

Table 10 The corresponding VI types in different VI typing systems

series	**VI types**		
Sato	embolization	non-embolization	
Shirouzu	embolic	floating	intimal
Sternberg	filling	floating	infiltrating

The reported incidence of VI in CRC shows marked variability ranging from 10 to 90% [7, 16, 24, 30, 35, 36, 37]. Assuming that VI had been present in all patients with distant haematogenous metastases, 10.5% and 29.6% false negative rates for VI were calculated by Ouchi and Sternberg [16, 30], respectively. According to Sternberg et al., this variation is due to differences in the characteristics of the tumours of the reported series, technical differences in specimen processing and interobserver variation [30]. In other words, the detection rate of VI is influenced by tumour features (stage and differentiation), the amount of tumour examined (number and size of blocks and sections), staining methods (H&E versus elastic stain or immunostains) and the skill of the reporting pathologist (experience, mindfulness, enthusiasm). To minimize the false negative rate, they suggested increasing the number of blocks and slides, using elastic stains and cutting tangential blocks from the perimeter of the tumour, across the mesentery, and from mesenteric vessels. Their results indicate that there is a direct relationship between VI incidence and tumour stage, while inverse relationship was found between VI incidence and tumour differentiation, and the greater mean number of blocks was examined, the higher rates of VI were detected [31]. According to the calculation

of Talbot el al., 3.9% of cases with extramural VI would be missed if 5 blocks were taken, while 41.3% would be missed if only 2 blocks were processed [34]. The Royal College of Pathologists recommends taking a minimum of 4 blocks to optimise the detection of key prognostic features [38]. In the Consensus Statement of the College of American Pathologists, submitting of 5 or more tumour blocks is considered to be optimal [5].

VI has been defined by Talbot as „tumour present in endothelium-lined space surrounded by a rim of smooth muscle or containing red blood cells" [34]. This definition is used to identify venous involvement on H&E-stained slides, but does not help in identifying all instances of VI. Other signs have been implemented as markers of VI on H&E-stained slides, and these include the „protruding tongue" (smooth-bordered protrusion of tumour tissue into pericolorectal fat usually adjacent to an artery) and the „orphan artery" (a focus of circumscribed tumour with an adjoining artery, but no accompanying vein) signs [11, 39].

Elastic stains have been implemented in the detection of VI, as they result in a higher visualisation. On average, a threefold increase in the VI detection rate was experienced in some studies comparing H&E and elastic stains (Table 11) [10, 12, 25, 30, 40-42]. In this setting, tumour cells surrounded by an elastic lamina are considered VI. In their recent study, Kojima et al. set uniform criteria to identify VI on elastic stained slides: "Presence of elastica-stained internal elastic membrane covering more than half of the circumference surrounding the tumour cluster"[43].

Table 11 VI detection rates with routine and elastic staining in some studies in the same or similar CRC patient groups

Series	VI detection rate	
	H&E	elastic stain
Kingston et al.	10%	48%
Inoue et al.	17%	47%
Roxburgh et al.	18%	58%
Sejben et al.	18%	71%
Bogner et al.	28%	67%
Khan et al.	32%	59%
Sternberg et al.	52%	70%

Sometimes immunohistochemistry is applied to enhance VI detection. Smooth muscle actin immunohistochemistry was reported to improve VI detection relevant for survival prediction [29]. Inoue and colleagues found that FVIII-RA immunohistochemistry is not useful to enhance vascular invasion [40]. The first study to use CD31 and CD34 to detect VI in CRC was conducted by Kingston et al. They found that CD31 was less sensitive in VI detection than elastic staining. CD34 gave better results in Dukes C patients, but worse in Dukes B cases [10]. Bellis et al. demonstrated VI with FVIII-RA and actin immunohistochemistry in 62% in CRC patients, whereas only in 20% with H&E [29]. Van Wyk and co-workers demonstrated enhanced lymphatic invasion with D2-40, while CD31 was less sensitive to detect VI compared with elastic staining [27]. Lapertosa et al. state that vimentin and desmin are helpful to differentiate vascular invasion from lymph node capsule and peritumoural fibrous stroma. Vascular wall is vimentin and desmin positive, but lymph node capsule is vimentin negative, and peritumoural fibrous stroma is desmin negative [28]. Endothelium of tumour invaded veins often disappears preventing its immunohistochemical identification. All in all, immunohistochemistry is labour intensive, time consuming and expensive as a method, and it cannot be recommended for routine use, only in special cases when other methods fail to solve the problem.

The wide range of VI detection rates in CRC in different series indicates that the recognition of this phenomenon is not free of interobserver variability. Littleford et al. reported only poor-to-moderate agreement in the diagnosis of extramural VI on H&E-stained sections [7]. Harris and colleagues investigated the impact of application of CD31 and D2-40 immunostains on interobserver variation, on the reporting of VI and lymphatic invasion in stage II CRC. They found that interobserver agreement was poor irrespective of implementation of immunohistochemistry [44]. The Royal College of Pathologists suggests that the detection rate of extramural VI in CRC should be at least 30% in colorectal resection specimens. This figure could be seen as an audit standard to which pathology departments can compare their results [38]. In a survey conducted in Ontario, Canada, more than 70% of participating pathologists detected VI in less than 10% of CRC resection specimens [45]. A study comparing VI detection rates in CRC examined by specialized gastrointestinal pathologists and general pathologists found a 30% VI detection rate among specialists and 13% among non-specialists [39]. Magnetic resonance imaging (MRI) can detect extramural

VI with a sensitivity of 62% and a specificity of 88%. Preoperative MRI findings should be available for pathologist to aid the pathological examination [26].

The rate of elastica stain detected VI being around 60-70% is higher than the proportion of CRC cases developing distant metastases. Therefore, VI has a false positive rate as a predictor of haematogenous metastases. Some phenomena called pseudo-VI predispose to overdiagnosing VI with special stains: arterial invasion, tangentially sectioned subserosal elastic lamina, mucosal protrusion into the submucosa, periganglionic, perineural, perinodal and perifollicular elastic fibres or periglandular or perimuscular elastosis [46].

Veins can have a substantial thickening of their internal elastic lamina, and therefore, distinction from arterial invasion must be borne in mind. Arteries have a more regular, sharp, wavy elastic lamina of rather even thickness localised at the luminal surface of the muscular media layer, whereas veins may have a usually thinner internal elastic lamina-like layer in the intima, which is irregular, wavy, but of uneven thickness, and often multiplied rather than single. Of note is the fact that the muscular media layer of arteries has sparse elastic fibres, by contrast with veins which have abundant elastic fibres at this location. Although these basic principles may be helpful, they may be altered in diseased arteries, and it is not exceptional to see multiplication of the internal elastic lamina and/or the accumulation of elastic fibres in the muscular layer. Arterial involvement can cause ischemic type necrosis in the tumour and does generally not cause metastatic disease. The reported incidence of arterial invasion ranges from 0-1% in different series [47].

The presence of vascular structure and tumour cells or clusters within a circular structure may sometimes represent vascular wall invasion rather than real large vessel invasion with tumour cell in the lumen. These features can be mistaken for VI on H&E-stained slides. The identification of the internal elastic lamina surrounded by tumour cell clusters rather than the internal elastic lamina surrounding tumour cells may be a clue to identify these lesions as vascular wall invasion. The presence of a single endothelium-lined lumen in the central unaffected part of the lesion may further substantiate this. Some layers of the bowel wall may be bordered by elastic fibres, although these are not consistently present everywhere, and may range from virtually lacking to prominent, their prominence being possibly related to previous injury (e.g. neoadjuvant treatment). Their presence may sometimes take a circular form and

therefore mimic the internal elastic lamina of a vein. Of these elastic fibre networks, the peritoneal, or subserosal elastic lamina, is probably the most important. It can be very prominent at some segments of the colon, and is particularly prone to form circular shapes in some planes of section. Elastic fibres may also occur beneath the muscularis mucosae, where diverticular protrusions of the mucosa into the submucosa may also give ground to pseudo-VI if such structures are involved by neoplastic glands. The presence of elastic fibre networks at specific anatomic/histologic layers should always be considered when dealing with elastica stain-detected vascular invasion, although they represent differential diagnostic problems only when the tumour involves the tissues around them, and there are misleading planes of section (Figure 4). To avoid misdiagnosis, it must be remembered that relatively large vessels are not expected to be present at very close proximity to the serosa, and especially, they do not run perpendicular to its surface.

The intermuscular elastic layer is generally not prominent, but can be accentuated around the ganglia of the myenteric plexus, which is not uncommonly involved by CRC infiltrating the muscularis propria layer (Figure 5).

The use of neoadjuvant radiotherapy or chemoradiotherapy in rectal cancer may lead to elastosis, the accumulation of elastic fibres at uncommon locations. In such conditions, periglandular fibres obviously create the situation of rounded structures that may mimic an internal elastic lamina. Muscle bundles may also be surrounded by elastic fibres in such situations, and this may also create a background for a pseudo-VI pattern if the tumour infiltrates these bundles. Although more common after neoadjuvant irradiation, similar elastosis may develop in other noxious settings, for instance, hypoxia or tears resulting from herniation.

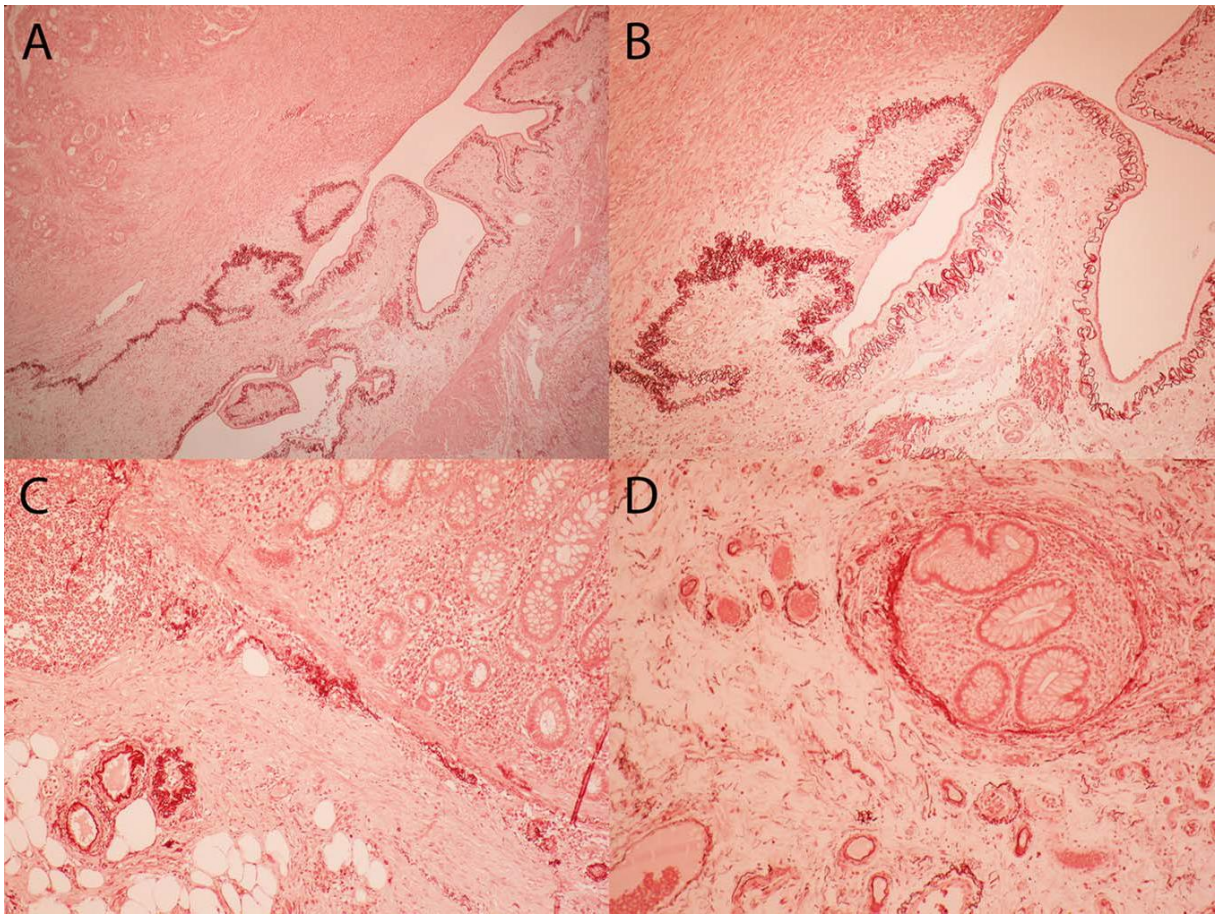


Figure 4 Elastic layers at the boundaries of the bowel wall layers. A and B: Prominent subserosal elastic layer demonstrating circular and semicircular shapes in this plane of section. C: Discontinuous elastic fibres beneath the muscularis mucosae. D: Diverticulum-like outpouching of the mucosa with a more prominent, although still incomplete circumferential submucosal elastic layer, following preoperative radiotherapy. Should the mucosa be involved by carcinoma in such an anatomic situation, the criterion of having an elastic lamina around at least half the periphery of a tumour cluster could be realised without true VI (Orcein ×40, A,; ×100, B–D).

Intraneural spread with perineural elastic fibres, very uncommonly perinodal elastic fibres (lymph node capsules are generally devoid of elastic fibres), an in anorectal carcinomas the perifollicular elastic sheet may give ground to the morphology of tumour cells surrounded by elastica positive layers, that is, pseudo-VI [46].

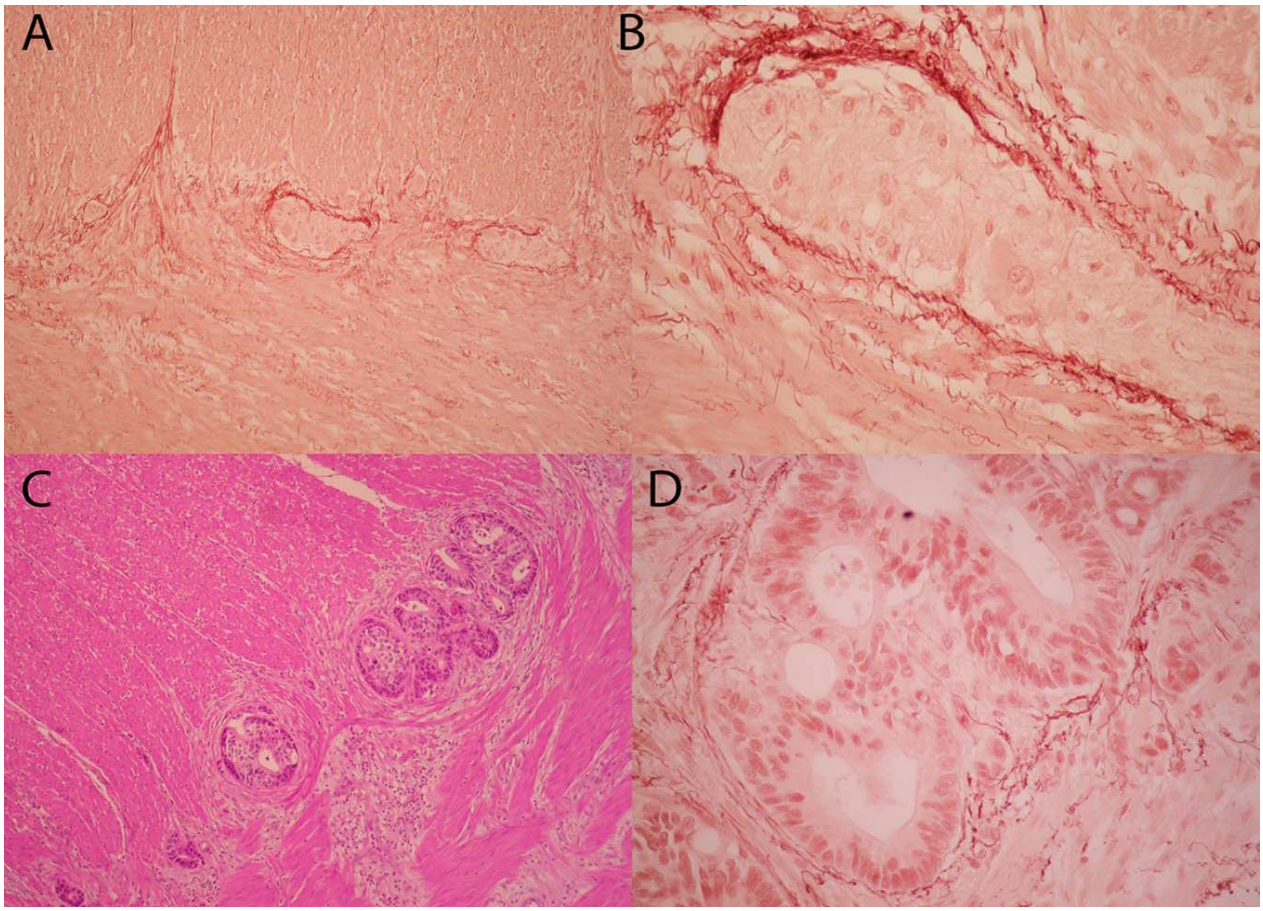


Figure 5 Periganglionic elastic fibres as a potential mimic of VI. A: Accentuated periganglionic elastic fibres seen around two ganglia. A few elastic fibres are weakly highlighted between the inner and outer layers of the muscularis propria (intermuscular layer). B: Ganglion cells surrounded by elastic fibres. C: Ganglion involvement by CRC that seems discontinuous from the main tumour mass probably as a result of perineural invasion along the myenteric plexus. D: An elastic layer around the destroyed ganglion; although the staining is weaker than in examples seen on parts A and B, it is definitely there.

In conclusion, VI in CRC is believed to be a commonly underreported phenomenon. Taking at least 4-5 blocks, looking for orphan artery and protruding tongue signs, and application of an elastic stain on every tumour block are practical and easily introducible measures to reduce false negativity. However, the implementation of routine elastica staining

increases the risk of false positivity. Therefore VI mimics should be excuded to achieve optimal results (Figure 6).

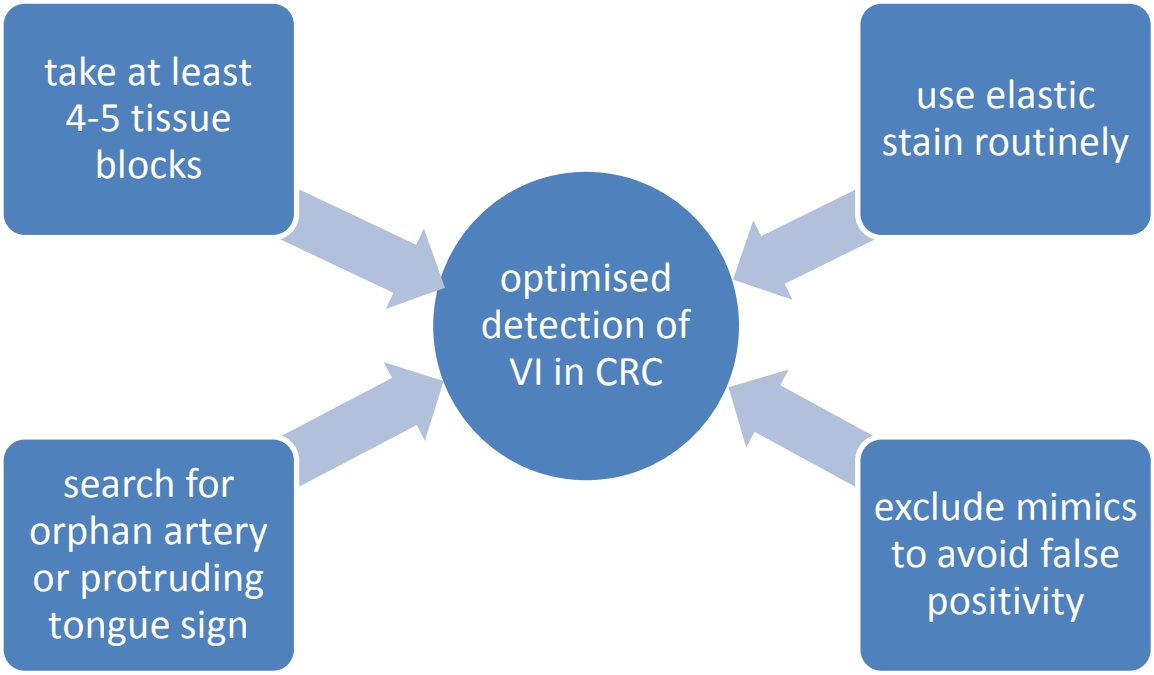


Figure 6 Basic steps to improve VI detection in CRC

Although the frequency of VI is substantially increased by the use of an elastic stain, and this is of prognostic relevance, it is clear that not all tumours with identified VI develop distant metastasis. The sensitivity of VI for the development of synchronous or future metastases seemed idealistically perfect (100%) in our preliminary study with a rather short median follow-up of 17 months, but its specificity was much less optimal (39%) [42]. The association between the development of distant metastasis and orcein-detected VI (but not H&E-detected VI) was significant. After a longer follow-up, this association was confirmed, and again H&E-detected VI did not show a similar association. The sensitivity to identify patients with distant metastasis any time in the course of their disease was still reasonably good (92%) after longer follow-up, its specificity remained low. From the clinical point of view, identifying a feature with higher specificity and positive predictive value would be ideal. In this respect lymph node involvement seems better, although its specificity of 56% is still not ideal. VI may be a logical source of non-peritoneal distant metastasis developing by

haematogeneous spread, whereas lymph node metastasis may just be a reflection of a more advanced disease stage, although it may also lead to haematogenous spread on the longer term as lymph is finally drained to the venous system. This study tried to identify qualitative and/or quantitative features that may be different between patients who have VI but did not develop distant metastases and those who did.

Of the variables analysed, only three showed significant differences between patients in the V1M1 and V1M0 groups. The median follow-up of the first group was shorter, more people died in this group, in keeping with the fact that metastases confer a poor prognosis. If it had been the other way round, the follow-up of the V1M0 group being shorter, this would have reduced the credibility of the data. The longer period of follow-up of the second group also implies that these patients were really less likely to develop distant metastatic spread. Although there have been suggestions that both intramural and extramural VI have the same prognostic impact by entering the same vessels at different anatomic layers of the bowels, and we were of the same opinion, our findings suggest that extramural VI may carry a higher risk for metastasis than isolated intramural VI. The superiority of extramural VI over intramural VI as a prognostic factor is acknowledged by several guidelines, and our results are also in keeping with findings of previous and recent works in which both the presence of intramural VI and extramural VI had prognostic impact, but the latter had higher prognostic value [26]. There seems to be no logical explanation for this difference in metastasis rate according to the location of the invaded veins. Hypothetically, there must be a functional difference between tumoural invasion of intramural veins and the extramural part of the same veins; i.e. blood flow from the intramural parts and therefore the metastatic process may be at least partially blocked by the compression of the vessels by the tumour infiltrating deeper and/or increased pressure in these segments of the veins also due to mural and extramural spread of pT3 and pT4 cancers.

Importantly, there seems to be a quantitative difference between patients with VI identified on orcein stained slides developing metastases during the course of their disease and those not developing them. This quantitative difference is reflected by the VI density in this study which was more than double on average in the M1 group as compared with the M0 group. Using an arbitrary cut-off of one VI per slide on average resulted in higher specificity

for metastatic dissemination. Higher VI density values were also found to be associated with greater metastatic incidence in two Japanese studies using elastica van Gieson staining to identify VI [32, 33]. Sato et al. tried to find objective criteria for the grading of VI in CRC. The total number of invaded veins found on elastic staining was divided by the number of sections to determine the average number of VIs on one slide as we did in our present study. With this method, the cases were divided into three grades: G0 if no VI was discovered, G1 if less than 4 VIs per slide were identified, and G2 if the number of VIs per slide was above 4. The number of VIs (grade) showed a positive association with recurrence, and a negative association with relapse free survival [32]. Calculating the average number of VIs per section like Sato, Shirouzu and co-workers used a four-tiered grading (V0: no VI, V1: VI≤3, V2: 3<VI ≤6, V3: VI>6) to quantify VI. They concluded that the higher grade of VI was associated with a higher risk of hepatic metastasis, local recurrence and a decreased survival [33].

It seems obvious that one prognostic factor e.g. VI, however important it is, cannot predict the outcome of CRC. A combination of prognostic indicators is necessary to prognosticate more precisely and to determine patient management. For example, the Petersen prognostic index was created for stage II colon cancer patients, and is calculated on the basis of VI, peritoneal involvement, surgical margin involvement, and perforation through the tumour [48]. A score of 1 was assigned to intramural or extramural VI, peritoneal involvement and margin involvement, whereas a score of 2 was allocated to tumour perforation. The total score was calculated by adding these scores. A total score of 0-1 was associated with a five-year survival of 85.7% and defined the low risk group. Those having a total score of 2-5 belonged to the high risk group with a five-year survival of 49.8% [48]. This prognostic score has been validated in patients with Dukes B and C colon and rectal cancer too [49]. In a collaborative study of our group, the performance of the Petersen index was compared in two centres. Although VI detection rate was significantly higher in one centre, and tumour perforation was more commonly detected in the other, the results confirmed the usefulness of this prognostic index [50]. This also suggests that a single adverse parameter (unless it is transtumoural perforation considered with more weight) has less effect on overall prognosis, and with that the tumour is still placed in the good prognostic category. This is in

keeping with our present results, which also point to the fact that quantifying VI may make sense. A higher amount is associated with more frequent distant metastasis.

Although the results are rather logical, the relatively low number of CRCs and patients in this cohort is a limitation that would require validation of the findings in a larger series.

As a conclusion, it may be reinforced that elastic stains increase the detection rate of VI in CRCs both in the context of frequency in patients with the disease and of quantity in patients with VI. Despite the association of orcein-detected VI and distant metastasis, the usefulness of orcein (or any other elastic stain) may be questioned as it identifies more patients CRC and VI than the ones who will develop metastasis at a certain time of their disease, and advocating changes in therapy on this basis might result in overtreatment. By finding meaningful quantitative (VI density) and qualitative (only intramural versus extramural VI) differences between VI-positive CRCs of patients with and without distant metastasis during follow-up, clinically meaningful VI could hopefully be better defined.

Malignant melanoma is notorious for its capricious behaviour. Tumour thickness is the most important histopathologic prognostic factor [51-57]. The studies on the significance of vascular invasion in melanoma came to different conclusions. Some reports suggest that vascular invasion is an independent prognostic factor [58-60], others hold the opposite view [55-57]. Vascular invasion can be detected on standard slides, however, immunohistochemistry to demonstrate endothelial cells might be useful. The use of immunohistochemistry enhances the identification of vessel invasion in melanoma [52, 56, 58, 59]. To the best of our knowledge, there are no studies on the value of using elastic stains to identify vascular invasion in this tumour type, although these staining methods are reported to be useful in the evaluation of vascular invasion in colorectal, gastric and oesophageal cancer [2 ,8-10, 42, 59, 61, 62]. The use of elastic stains can provide a more precise identification of vascular invasion in CRC compared to routine H&E stain. And the demonstration of venous involvement by an elastic stain in CRC specimens is associated with a higher risk of distant metastasis [42]. Along the same lines, we tried to visualize VI in sections from cutaneous melanoma specimens using orcein staining. Five cases of definite VI were found with this method, while ten cases of vascular invasion were detected with H&E. That is, orcein did not augment the identification of vessel involvement. The cause of our

results could be that melanomas invade lymphatic channels more frequently than blood vessels, and the blood vessels invaded are more often of the capillary type. Orcein stains the elastic fibres in the walls of relatively large veins and small blood and lymphatic vessels cannot be highlighted with it [46]. Another reason lies within the histological structure of the skin containing a lot of elastic fibres in the dermis, which may sometimes be very disturbing as background, although some melanomas may lack this elastic debris, and push downward the elastotic layer associated with solar elastosis as they grow vertically. Elastic fibers are often arranged in circular lines, such as in periadnexal, perineural connective tissue, and can give rise to false positive results and make the distinction between VI and pseudo-VI difficult [46, 62].

6. CONCLUSIONS

Our results suggest that VI is demonstrated with increased frequency in CRC with the orcein stain when compared with H&E staining. VI detected by orcein is associated with synchronous or metachronous distant metastasis similarly to lymph node metastasis. Comparison of VI versus nodal status shows that although both are predictive for the development of distant metastases, the better sensitivity of the first is compensated by the better specificity of the other.

The localisation (intramural versus extramural) and the density/grade of VI demonstrated by orcein in CRC specimens determine its prognostic relevance. Although both intramural and extramural VI is associated with the development of distant metastases, the latter has greater prognostic value. A VI density of at least one VI per slide showed higher specificity for haematogenous dissemination.

On the basis of our investigations and the review of the literature, it is suggested that orcein or other elastica staining should be routinely used for the evaluation of VI in CRC, at least for the node-negative and H&E-based VI-negative cases.

The optimal detection of VI would therefore imply the evaluation of 4-5 tumour blocks, a search for morphological signs of VI (the “protruding tongue” and the “orphan artery” signs) on H&E stained slides, the routine use of an elastica staining and a distinction between mimics of VI (pseudo-VI) and true VI.

Investigating into the applicability of elastic stain to detect VI in cutaneous melanoma, we conclude that orcein stain is not useful for improving the detection of vessel involvement in these tumours.

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APPENDIX

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II.

III.