Vascular Invasion, Perineural Invasion, and Tumour Budding : Predictors of Outcome in Colorectal Cancer

Johannes Betge, Cord Langner

Institute of Pathology, Medical University of Graz, Graz, Austria.

Abstract

Tumour stage reflected by the AJCC/UICC TNM system is currently regarded as the most powerful prognostic parameter in patients with colorectal cancer. However, additional histopathological markers are required to improve clinical decision-making with respect to follow-up scheduling and administration of adjuvant therapy. In this review we summarize the available literature regarding the prognostic impact of venous and lymphatic invasion, perineural invasion and tumour budding in colorectal cancer. Special emphasis was placed on patients with AJCC/UICC stage II disease, the risk of lymph node metastasis in early cancer and the prediction of local recurrence in rectal cancer. For each of the markers, the different methods of evaluation, implications resulting from different definitions used in previous studies as well as future perspectives are discussed in detail. (Acta gastroenterol. belg., **2011**, 74, **516-529**).

Key words : colon cancer, rectal cancer, early colorectal cancer, vascular invasion, lymphatic invasion, venous invasion, lymphovascular invasion, perineural invasion, tumour budding, prognosis, outcome, immunohistochemistry, univariable analysis, multivariable analysis.

Introduction

Tumour resection is the treatment of choice for patients with colorectal cancer. Outcome prediction is pivotal in these patients. An ideal system of classification would only identify two categories – patients cured by surgery and those who will ultimately die of disease (1). The latter might consequently benefit from intensified surveillance strategies and/or adjuvant therapy. Interdisciplinary efforts aim at identifying these patients at risk for failure.

Tumour stage reflected by the AJCC/UICC TNM system is currently regarded as the most powerful prognostic parameter, however, patients with tumours of the same pathologic stage may experience considerably different clinical outcomes (2-4). Patients with stage I cancer (pT1-2, N0, M0) are generally considered to share favourable prognosis. However, a small subgroup of them will die due to local or distant recurrence after curative resection. Chemotherapy primarily based on 5-fluoruracil is usually administered in stage III colon cancer (pT1-4, N1/N2, M0) and has decreased tumour recurrence, while neoadjuvant chemoradiotherapy and total mesorectal excision (TME) have improved local control of rectal cancer (5). Risk estimation of stage II cancer (pT3-4, N0, M0) is particularly critical because it determines whether adjuvant therapy should be offered (6). Currently, parameters to identify high-risk stage II patients who might benefit from adjuvant therapy are not well defined. Moreover, according to a comprehensive study analyzing the SEER database, patients with stage IIIA tumours appear to be associated with a significantly improved survival compared with patients with stage IIB disease (7).

Histopathological markers, such as vascular invasion, perineural invasion and tumour budding have been shown to be of additional prognostic value in affected patients. Thus, these markers could facilitate patient counselling and clinical decision-making with respect to follow-up scheduling, administration of adjuvant therapy, and evidence-based design of clinical trials. However, recording of these parameters has so far only partially been implemented in current practice guidelines (8-11). In this review, we summarize the literature on the prognostic value of the three markers mentioned above, focussing on patients with stage II disease and early colorectal cancer.

Blood and Lymph Vessel Invasion

Definition and nomenclature

The invasion of tumour cells into lymph or blood vessels plays a crucial role in the metastatic process. Lymphatic invasion is diagnosed, when tumour cells are present in vessels with an unequivocal endothelial lining, yet lacking a thick (muscular) wall. Blood vessel invasion refers to the involvement of veins and is characterized by the presence of tumour cells in vessels with a thick (muscular) wall or in vessels containing red blood cells (Fig. 1) (12,13). However, discrimination between lymphatic channels and thin walled post-capillary venules may be difficult. For this reason, the use of the terms “small vessels” instead of lymph vessels and “large vessels” instead of blood vessels has been discussed (14). Moreover, in some studies, both types of vascular invasion have been lumped together and referred to as “lymphovascular invasion” or simply as

Correspondence to : Cord Langner, M.D., Institute of Pathology, Medical University of Graz, Auenbruggerplatz 25, 8036 Graz, Austria.
E-mail : cord.langner@medunigraz.at

Submission date : 30/06/2011
Acceptance date : 31/07/2011

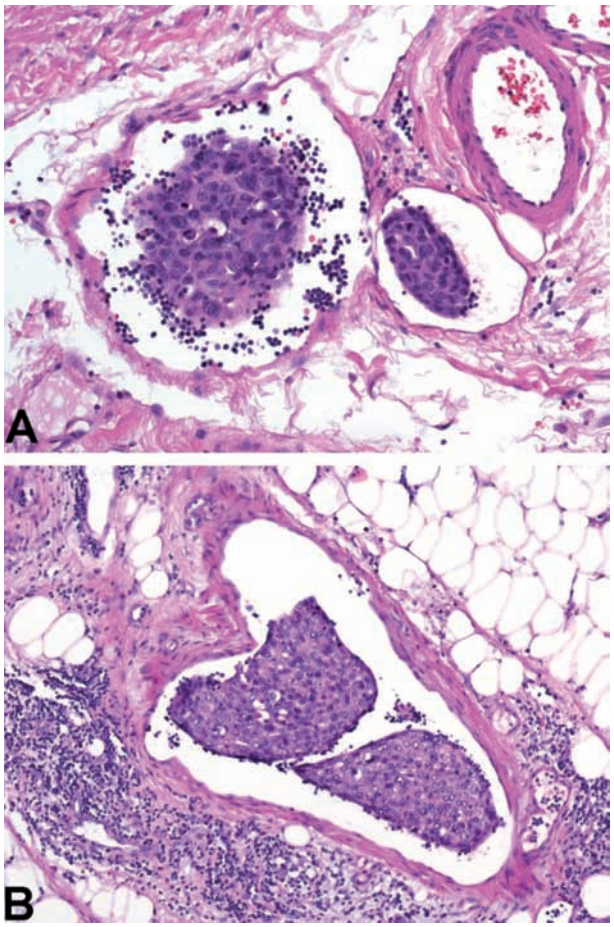

Fig. 1. — Lymphatic (A), and venous (B) invasion in colorectal cancer.

"vascular invasion", which is problematic, since the term "lymphovascular invasion" in some studies only refers to lymphatic invasion, and the term "vascular invasion" is by some authors used for venous invasion only. The site of vessel involvement within the bowel wall is also of importance. Intramural vessel invasion which is limited to vessels in the submucosal and/or muscular layer has been differentiated from extramural vessel invasion which includes vessels located beyond the muscularis propria, i.e. within the pericolic adipose tissue (15). Finally, some authors also took the extent of vascular invasion into consideration (16-18).

Prognostic significance

Venous invasion has been shown to increase the risk of distant metastases (19-23) and also regional lymph node metastasis (24-26) in patients with colorectal cancer. Furthermore, venous invasion has been significantly associated with disease-free, cancer specific and overall survival in univariable analyses (12,16,22,27-35), and in multivariable analyses (15,19,36-43), for details compare Table 1.

For instance, Liang *et al.* (37) reported 5-year overall survival rates of 36% and 84% for patients with and without blood vessel invasion in univariable and a nearly two fold risk for failure in multivariable analysis. According to our own study (15), patients with and without venous invasion had actuarial 5-year cancer-specific survival rates of 30% and 75%, respectively, and the prognostic impact of venous invasion was comparable to that of T classification, stronger than that of tumour grade, yet inferior to nodal status in multivariable analysis.

Some authors reported a stronger prognostic value of extramural venous invasion compared with that of intramural invasion (12,15,27,28,44), or regarded only extramural venous invasion in analysis (45-47). Recent practice guidelines also stress the importance of recording extramural venous invasion in the pathology report (8,9).

Of note, few studies presenting venous invasion as a poor prognostic marker in univariable analysis failed to show independent prognostic value in multivariable analyses regarding colorectal (16,32-34), colon (29) or rectal cancer (44).

Similar to venous invasion, lymphatic invasion has been significantly associated with poor outcome in univariable (33,34,48,49) and multivariable analyses (13, 44,50,51). However, the prognostic value is probably smaller than that of venous invasion, since in most studies analyzing both venous and lymphatic invasion regarding prediction of outcome or recurrence, either both or only venous invasion retained independent prognostic impact (37,40,43,45,49,51), or both factors were negative (33,34). Only Minsky *et al.* (13) and Tang *et al.* (50) presented lymphatic invasion but not venous invasion as independent predictor of dismal prognosis, while another study did not observe prognostic significance of lymphatic invasion despite excluding tumours with venous invasion from analysis (52).

When lymphatic and venous invasion were lumped together and assessed as lymphovascular invasion the majority of studies proved lymphovascular invasion as independent predictor of outcome (53-57) while a few failed to identify independent impact on outcome (58, 59).

Prognostic significance in UICC stage II disease

In UICC stage II colon cancer literature data suggest that venous invasion is an important prognostic factor, while lymphatic invasion seems to play a minor role. Thus, Morris *et al.* (60) noted independent prognostic impact regarding outcome for venous invasion, classification T4 and perineural invasion, but not for lymphatic invasion in their comprehensive study on 1306 stage II patients. In the group of patients under 75 years of age who are more likely to be considered for adjuvant chemotherapy the hazard ratio for venous invasion (HR 2.82) was higher than that of T classification (HR 2.03). Sato *et al.* (17) recently published a large series presenting the extent of venous invasion (in addition to the number of dissected lymph nodes, sex, age, adjuvant

Table 1. — **Selected studies showing vascular invasion as independent prognostic marker in multivariable analysis**

	Year	Patients	Site	Inclusion criteria	Prevalence (%)	Stain	Target value	Significant in multivariable analysis			
								Venous invasion	Lymphatic invasion	Lympho-vascular invasion	Other
Knudsen *et al.* (19)	1983	682	Rectum	Resectable tumors	V1 : 38.9%	H&E	5-year OS	Yes	n.d.	n.d.	Age, Dukes stage, perineural invasion, liver metastasis at operation
Freedman *et al.* (39)	1984	769	Rectum		n. spec	n. spec	5-year OS	Yes	n.d.	n.d.	Dukes stage, grade, age, height of tumour
Chapuis *et al.* (36)	1985	709	Colo-rectum		V1 :19%	n. spec.	5-year OS	Yes	n.d.	n.d.	Stage, grade, level of direct spread, age, sex, obstruction
Minsky *et al.* (13)	1989	462	Colo-rectum	Potentially curative resection	V1 : 44% L1 : 13%	elastic tissue stain	5-year OS	No	Yes	n.d.	Stage, grade
Michelassi *et al.* (55)	1991	603	Colon		n. spec.	n. spec	5-year OS	n.d.	n.d.	Yes	Stage, race, tumour morphology
Harrison *et al.* (47)	1994	348	Rectum		V1 : 21.2% (extramural)	H&E, elastic tissue stain in 64 cases	5-year OS	Yes (extramural)	n.d.	n.d.	Depth of tumour invasion, lymph node metastasis, Crohn's-like lymphoid reaction
Newland *et al.* (38)	1994	597	Colo-rectum	Nodal positive	V1 : 29%	n. spec.	5-year OS	Yes	n.d.	n.d.	Apical lymph node involvement, spread involving a free serosal surface, invasion beyond the muscularis propria, location in the rectum, grade, age, gender
Tang *et al.* (50)	1995	565	Colo-rectum	Stages I-III	V1 : 8% L1 :43%	n. spec.	5-year/10-year CSS	No	Yes (extramural, yet intramural n.s.)	n.d.	Lymph node metastasis, rectal cancer, absence of lymphocytic infiltration, invasion through bowel wall, perineural invasion outside bowel wall, gender
Takahashi *et al.* (40)	1996	610	Colon	Curative operation	V1 : 30% L1 : 60%	n. spec	5-year DFS	Yes	No	n.d.	Size ≥4cm, Dukes stage, elevated CEA level
		644	Rectum	Curative operation	V1 : 34% L1 : 61%	n. spec	5-year DFS	Yes	No	n.d.	Chemotherapy, location in the lower rectum, serosal invasion, Dukes stage, residual tumour, elevated CEA level
Petersen *et al.* (62)	2002	268	Colon	Stage II	V1 : 33%	H&E, elastic stain in equivocal cases	5-year OS	Yes	No	n.d.	Peritoneal involvement, margin involvement, tumour perforation
Hohenberger *et al.* (46)	2005	1067	Rectum		Extramural V1 : 17.8%	n. spec	5-year CSS	Yes (extramural)	n.d.	n.d.	Elevated CEA level, stage, R1/R2, grade

Morris *et al.* (60)	2006	1306	Colon	Stage II	n. spec	n. spec	5-year CSS	Yes	No	n.d.	T4, perineural invasion
Liang *et al.* (37)	2007	419	Colo-rectum		V1 : 41.2%, L1 : 54.2%	Podoplanin, CD34	5-year OS	Yes	No	n.d.	Stage, N
Lin *et al.* (64)	2009	375	Colon	Stage II	LVI : 5.9%		3-year DFS	n.d.	n.d.	Yes	Obstruction at presentation
Desolneux *et al.* (116)	2010	362	Colo-rectum	Stages I-II	V1 : 13% L1 : 4.1%	H&E	5-year OS	Yes	Yes	n.d.	Age, number of lymph nodes removed, perineural invasin, T4
Lim *et al.* (53)	2010	2417	Colo-rectum		LVI : 25.2	H&E	5-year OS	n.d.	n.d.	Yes	T, N, M, gender
							5-year DFS	n.d.	n.d.	Yes	T, N, M
Sato *et al.* (17)	2011	1476	Colon	Stage II	V1 : 16% L1 :15%	n. spec.	Recurrence	Yes (extensive vs. slight)	n.d.	n.d.	Growth pattern (Jass), CA19-9 level, emergency operation, postoperative ileus
							OS	Yes (extensive vs. slight)	No	n.d.	CA 19-9 level, number of dissected lymph nodes, gender, age, postoperative chemotherapy, emergency operation, growth pattern (Jass)
Betge *et al.* (15)	2011	381	Colo-rectum		V1 : 23% L1 : 33%	H&E	5-year CSS	Yes	No	n.d.	Age, T, N
							5-year PFS	Yes	Yes	n.d.	T, N

Abbreviations : CSS, cancer-spe cific survival ; DFS ; disease-free survival ; H&E, haematoxylin and eosin ; L1, lymphatic invasion ; LVI, lymphovascular invasion ; n.d., not determinded ; n.s., not significant ; n. spec, not specified ; OS, overall survival.

chemotherapy, emergency operation, and growth pattern), yet not lymphatic invasion as independent predictor of overall survival. Other studies similarly presented venous invasion (15,61,62) or venous invasion plus lymphatic invasion classified as lymphovascular invasion (63-66) as independent prognostic parameters in stage II disease. Two studies, however, noted an association of vascular invasion with adverse outcome only in rectal cancer (67) or did not find a significant association (68), respectively. Nevertheless, altogether, venous invasion is an important parameter when stratifying patients for adjuvant therapy.

Prognostic significance in early colorectal cancer

Cancer limited to the submucosal layer in its deepest extent (T1 disease), regardless of nodal status, is regarded as early cancer. In this subgroup identification of node-positive cases is of utmost importance since local therapy alone (e.g. polypectomy or submucosal dissection) will not be sufficient and surgical therapy with regional lymph node dissection is necessary. Traditionally, early cancers are classified as either low risk (regional lymph node metastasis in < 2%) or high risk (regional lymph node metastasis in 5-25%, median 14%), based upon depth of invasion, tumour differentiation, resection status and presence of lymphatic invasion (25,69-74). Remarkably, also venous invasion has been related to an increased risk for lymph node metastasis, possibly due to the strong association between presence of lymphatic and presence of venous invasion in tumour tissue (24-26). Finally, venous invasion, yet not lymphatic invasion has been independently associated with a higher risk for distant metastases in early colorectal cancer (24), while lymphovascular invasion has been significantly related to regional lymph node spread and/or decreased local recurrence free survival (75,76).

Prognostic significance for local recurrence in rectal cancer

The significance of vascular invasion regarding the prediction of pelvic failure in rectal cancer patients is less clear. While some studies observed a significant association between lymphovascular invasion and local recurrence (77-79), others demonstrated that only

tumour stage and margin status were predictors of local recurrence (80). Finally, a systematic study by Dresen *et al.* (45) including 277 rectal cancer patients identified both lymphatic and extramural venous invasion (in addition to circumferential resection margin status, serosal involvement and poor tumour differentiation) as independent predictors of local tumour failure. According to data from our group, however, lymphovascular invasion was associated with local recurrence only in univariable, yet not in multivariable analysis (81).

Problems and perspectives

The incidence of lymphovascular invasion is reported to vary between 10% and 89.5% in different series, which is not only due to the selection of advanced cases in some series, but also due to differences and difficulties in diagnosing vascular invasion in cancer tissue (82,83). The definitions given above are simple, but accurate diagnosis may be difficult in selected cases. Likewise, in our recent study (15), we observed marked differences between routine and review pathology, only the latter showing significant association with outcome. Moreover, a systematic interobserver variation study showed only poor to moderate agreement regarding the diagnosis of extramural venous invasion, even amongst gastrointestinal pathologists working together in a single unit (84). Some authors suggested that accuracy of diagnosis of vascular invasion might be improved applying ancillary histochemical and/or immunohistochemical stains (23, 24,85-89). However, immunohistochemical staining is labour intensive, time consuming and expensive and failed to improve interobserver agreement in another study (90,91). Hence, the routine use of histochemical or immunohistochemical stains in all tumours may not be efficient, and is not recommended in current practice guidelines (8,9). Nevertheless, special stains, in particular Elastica van Gieson, CD31 and D2-40, may be helpful in equivocal cases.

Moreover, diagnoses of lymph and blood vessel invasion may depend on the number of analyzed tissue blocks. In the 1999 consensus statement, the College of American Pathologists recommended the analysis of at least three, optimally five blocks of tumour at the point of deepest invasion for microscopic examination (92). However, according to our data (15), embedding of less than five blocks is most probably insufficient. In conclusion, future efforts should aim at standardization of morphological criteria and staining methods, embedded in elaborate quality control setting, in order to improve the significance of the pathology report.

Perineural Invasion

Definition and morphology

The invasion of veins and lymphatic channels represent the classic routes for metastatic cancer spread. However, another increasingly recognized route for neoplastic invasion and cancer spread may be seen along peripheral nerves (Fig. 2). The most commonly used definition for perineural invasion was given by Batsakis, describing it as tumour cell invasion "in, around, and through the nerves" (93). Therefore, it may be observed in all three connective tissue sheaths embracing peripheral nerves, consisting of the edoneurium around individual axons and Schwann cells, the perineurium which is a concentrically multilayered formation enclosing each fascicle, and the epineurium, which envelops the entire nerve (94). Moreover, perineural invasion may be considered positive if tumour cells are seen in the perineural space, not invading through the epineurium, but surrounding at least one third of the nerve (95,96).

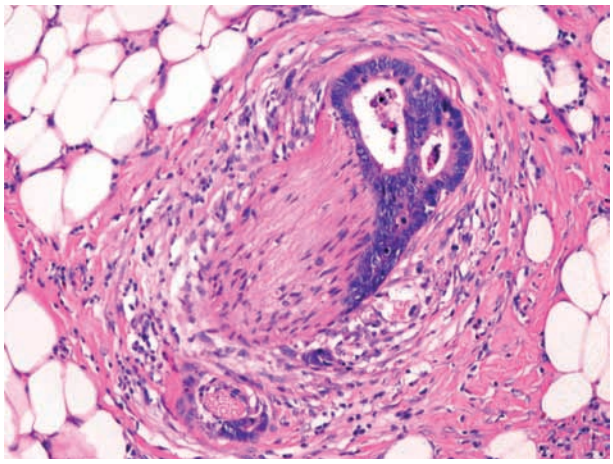

Fig. 2. — Perineural invasion in colorectal cancer

Perineural invasion has first been described in head and neck cancers, and since then has become an important prognostic marker in different types of cancer, including prostate, pancreas, billiary tract, stomach, and also colorectal cancer (94). It is worth mentioning that perineural invasion is not a sub-category of lymphovascular invasion, since no lymphatic channels are present within the perineural space (97,98).

Prognostic significance

Perineural invasion is a strong prognostic indicator in colorectal cancer. It has been significantly associated with decreased survival and high rate of recurrence in univariable (22,31,99,100) and multivariable (19,20,34, 42,50,81,96,101-109) analysis. For details compare Table 2.

In particular, Liebig *et al.* (96) reported upon a significantly improved 5-year disease-free survival for patients with perineural invasion-negative tumours compared with those with perineural invasion-positive tumours (65% vs. 16%), and a similarly improved 5-year overall survival rate (72% vs. 25%). Data from our group (81) are comparable : actuarial 5-year disease-free survival rates for patients with perineural invasion-negative and -positive tumours were 68% and 11%, respectively, and

Table 2. — **Selected studies showing perineural invasion as independent prognostic marker in multivariable analysis**

	Year	Patients	Site	Inclusion criteria	PNI (%)	Target value	Univariable analysis	Significant in multivariable analysis
Bentzen *et al.* (42)	1988	468	Rectum	Stage II	17%	5-year OS	Not specified	Age above 60, PNI, venous invasion, tumour located <10cm from the anal verge, elevated CEA level
				Stage III	38%	5-year OS	Not specified	PNI, venous invasion, tumour located <10cm from the anal verge, elevated CEA level, tumour diameter, resection of neighbouring organs
Shirouzu *et al.* (101)	1992	501	Rectum		20%	8-year OS	Stage B2m+g : 8-yearsurvival rates in patients without PNI and in patients with PNI 88.6 % and 80% (not significant) ; Stage C2m+g : 8-year survival rate without PNI was 71.5%, with PNI 29. 1% (significant)	PNI (only stage C2m+g analyzed)
Bognel *et al.* (102)	1995	339	Rectum	Potentially curative Surgery	34%	5-year OS	PNI negative : 66%, PNI positive 46% ; other significant variables : age, type of surgery, distance from anal verge, tumour penetration, tumour size, location of involved nodes, number of positive nodes, vascular invasion	Age, distance from anal verge, number of positive nodes, PNI, tumour penetration
Mulcahy *et al.* (67)	1997	117	Colo-rectum	Stage II	8.5%	5-year OS	Significant : bowel obstruction, necrosis, PNI	Necrosis, PNI
Fujita *et al.* (34)	2003	341	Colo-rectum		24%	2-year DFS	PNI negative : 94%, PNI positive 63% ; other significant variables : depth of invasion, lymph node status, lymphatic invasion, venous invasion, growth type, streak type, focal dedifferentiation	Lymph node status, depth of invasion, PNI
Law *et al.* (107)	2004	622	Rectum		8.7%	5-year CSS	PNI negative : 78.7%, PNI positive 27.6% ; other significant variables : stage, lymphovascular invasion, adjuvant radiotherapy	Stage, lymphovascular invasion, PNI
Stewart *et al.* (103)	2008	304	Rectum	Curative intent	3.6%	DFS	Significant : stage, T, N, adjuvant chemotherapy, tumour fixation, involvement of the radial margin, presence of mucin, lymphovascular invasion, PNI	Stage, radial margin stats, adjuvant chemotherapy, PNI
Liebig *et al.* (96)	2009	269	Colo-rectum		22%	5-year OS	PNI negative : 72%, PNI positive 25% ; other significant variables : age, T, N, distant metastases, stage, R-status, grade	PNI, stage IV
						5-year DFS	PNI negative : 77.9%, PNI positive 22.1% ; other significant variables : age, T, N, distant metastases, stage, R-status, grade	PNI, stage IV
Oh *et al.* (105)	2009	350	Colon	T3, T4	T3 : 12%, T4 : 19%	5-year OS	Significant : N, lymphovascular invasion, PNI	N, lymphovascular invasion, PNI
Tsai *et al.* (104)	2009	521	Colon	Stages I-III	48.6 %	Post-operative early relapse	Significant : vascular invasion, PNI, high postoperative CEA level, type of surgery	Vascular invasion, PNI, high post-operative CEA level
		257	Rectum	Stages I-III	58.8 %	Post-operative early rela3pse	Significant :vascular invasion, PNI	PNI
Poeschl *et al.* (81)	2010	381	Colon		13%	5-year PFS	5-year PFS rates : PNI negative : 68%, PNI positive 11% ;	PNI, T, R-status
						5-year CSS	5-year CSS rates : PNI negative : 72%, PNI positive 26% ;	PNI, T, R-status, lymphovascular invasion
			Rectum		18%	5-year PFS	Colon vs. rectum comparable (not shown)	PNI, T, R-status, lymphovascular invasion
						5-year CSS		PNI, T, R-status, lymphovascular invasion, grade, age

Abbreviations : CEA, carcinoembryonic antigen ; CSS, cancer-specific survival ; DFS, disease-free survival ; n.d., noty determined ; OS, overall survival ; PNI, perineural invasion ; PDS, progression-free survival.

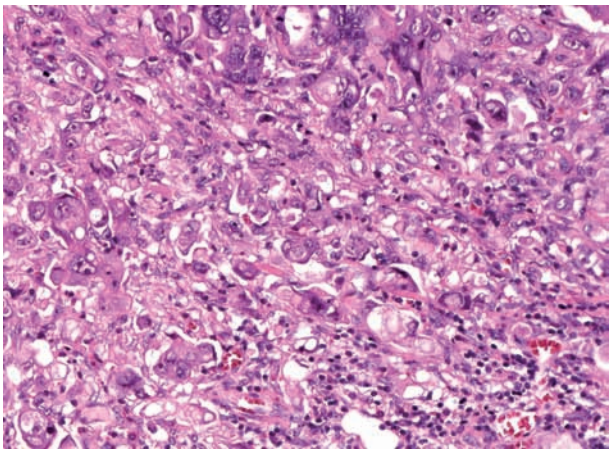

Fig. 3. — Colorectal cancer showing high grade tumour budding characterized by large numbers of isolated single cells or small clusters of cells scattered in the stroma at the invasive tumour margin.

actuarial 5-year cancer-specific survival rates for patients with perineural invasion-negative and -positive tumours were 72% and 26%, respectively. In multivariable analysis, perineural invasion proved to be an independent predictor of both disease progression and cancer related death. However, yet again some studies failed to show prognostic influence of perineural invasion in multivariable analysis (58,110,111).

Prognostic significance in UICC stage II disease

In UICC stage II disease, data suggest that perineural invasion plays a major role for prediction of disease progression and/or recurrence, but a smaller role for prediction of overall and/or cancer-related survival. Several studies observed independent prognostic impact on tumour recurrence, yet not on overall survival (95,112, 113). Another study presented perineural invasion as an independent prognostic variable for disease free survival, but did not analyze perineural and lymphovascular invasion separately (65). However, other authors only noted prognostic significance regarding tumour recurrence, yet failed to substantiate these finding in a multivariable model which identified depth of tumour invasion and vascular invasion as independent predictors of outcome (63,114,115). Regarding studies restricted to node negative patients (Stages I and II) at least two studies noted a prognostic effect of perineural invasion on cancer-related survival (116,117), one only in univariable analysis (118).

Prognostic significance in early colorectal cancer

In early stages of colorectal cancer, perineural invasion is only very rarely observed or simply not existent : Studies from Liebig *et al.* (96) and from our group (81) did not reveal perineural invasion in UICC Stage I disease. Huh *et al.* (119) found perineural invasion in only 4.5% of T1 and T2 cases. These authors, however, noted independent impact on prediction of regional lymph node spread with a ten fold increase of odds ratio. It is, however, unclear in this study, how many perineural invasion-positive cancers were in fact T1. Only Peng *et al.* (75) stated independent prognostic value regarding recurrence free survival in T1 rectal cancer, but they analyzed perineural and lymphovascular invasion lumped together and therefore the presence of perineural invasion and consequently the prognostic effect might have been overestimated in this study. Finally, one other study analyzing risk factors for lymph node metastases in 168 submucosal cancers found only one perineural invasion-positive tumour which did not show lymph node metastases (120). Hence, perineural invasion does not play a role for identifying high risk patients after local therapy of early colorectal cancer.

Prognostic significance for local recurrence in rectal cancer

Perineural invasion is a possibly valuable predictor of local recurrence in rectal cancer and may be used as an indicator to stratify patients for intensive follow-up. Several authors noted an independent prognostic impact of perineural invasion regarding local tumour failure (20,95,106,121,122). However, other studies failed to identify perineural invasion as a predictor of local tumour recurrence in univariable (58,123) or multivariable (107) analysis, rendering positive microscopic resection margin, focal dedifferentiation (i.e. tumour budding) (123), T and N classification (58), or stage and the use of peranal coloanal anastomosis (107) as independent prognostic variables, respectively. According to data of our group (81), incomplete tumour resection and perineural invasion were the only independent predictors of local tumour recurrence, thereby surpassing the prognostic effect of lymphovascular invasion. Data in this regard, however, are conflicting : Peng *et al.* (124) proved perineural invasion to be the only independent prognostic variable regarding local recurrence, while lymphovascular invasion did not predict local tumour failure. Enker *et al.* (121) proved both perineural and lymphatic invasion to be independent predictors of local recurrence. Dresen *et al.* (45) finally noted independent prognostic impact only for lymphovascular, yet not for perineural invasion.

Additional comments and perspectives

Marked differences exist regarding the reported prevalence of perineural invasion, ranging between 3.6 and 58.8 per cent in colorectal cancer tissues (81,102-104) Since perineural invasion has only very recently been recognized as important histopathological variable for routine pathology reporting of colorectal cancer (see the 7th edition of the AJCC/UICC TNM system from 2009), underreporting of this factor may have compromised the results of former studies analyzing its prognostic impact. Additionally, perineural invasion may be difficult to

recognize : Minute foci of perineural invasion may escape detection, and tumour cells around nerves may be obscured by inflammatory cells or mucinous pools (94). Therefore, immunohistochemical staining of S100 protein might facilitate the identification of nerves and consequently perineural invasion. With the help of this technique, perineural infiltration was identified in 70% of 50 colorectal cancer cases, compared with 14% on H&E stained sections (125). Accordingly, using S100 protein immunostaining perineural invasion was observed in 82% of 40 oral cavity squamous cell carcinomas, compared with 30% in the original pathology reports and with 62% on review of original H&E stained slides (126). However, in a study of 238 prostate biopsy cores, S100 protein immunostaining significantly increased the detection of nerves, but not of perineural invasion compared with H&E staining data (127). In conclusion, since data regarding the value of immunohistochemical staining for the detection of perineural invasion in colorectal cancer are rare, and regarding its prognostic significance are in fact lacking, future studies are warranted.

Tumour Budding

Definition and historical overview

Invasive tumour growth is the basis for infiltration of regional lymph or blood vessels and ultimately for metastatic cancer spread. Histological growth characteristics at the invasive front may reflect tumour aggressiveness and have thus been considered as prognostic markers. Jass *et al.* (1,128) categorized the invasive margin of rectal cancer as "infiltrating" if a tumour invaded in a diffuse manner with widespread penetration of normal tissues by a process of seemingly effortless dissection between the normal structures of the bowel wall, often with no recognizable margin of growth and lack of a host response, and as "expanding" if tumours were well circumscribed with a pushing invasive margin. The pattern of growth had prognostic impact and was included in the Jass' prognostic classification of rectal cancer and in his suggestions regarding the grading of rectal cancer (1,128).

Another approach to evaluate the invasive margin and thereby probably tumour aggressiveness is assessing the extent of tumour budding. Specifically, budding has been defined as the presence of isolated single cells or small clusters of cells (composed of fewer than five cells) scattered in the stroma at the invasive tumour margin (129,130). The concept later termed tumour budding has been anticipated by Japanese researchers already in the late forties and fifties and referred to as "sprouting", but then disappeared from the literature and has been rediscovered not until the late eighties (130). Budding is also closely related to the findings of Gabbert *et al.* (131) in murine colon carcinomas, who reported on "a striking dissociation of the organized tumour cell complexes into isolated tumour cells together with a loss of most of the cytological features of differentiation". Moreover, budding can be regarded as histological correlate of epithelial-mesenchymal transition, a process originally known from embryonic development. This term comprises the migration of (tumour) cells via reducing intercellular contacts (down-regulation of E-cadherin), forming cytoplasmatic protrusions (i.e. lamellipodia) through reorganizing the cytoskeleton to make new cell contacts (with integrins) for anchoring, before contraction of the cell body, ultimately leading to relocation of the cells (132,133). Finally, budding cells have been attributed stem cell capacities with the potential of redifferentiation at distant sites (132).

Prognostic significance

Tumour budding has been presented as prognostic variable in colorectal cancer, independently predicting poor survival (129,134-137) and high risk of recurrence (138-140). For details compare Table 3. For instance, Ueno *et al.* (136) analyzed two cohorts of rectal cancer patients, one consisting of 638 patients previously used for the Jass' prognostic classification (1) and one with 476 patients. They observed significantly lower 5-year survival rates of patients with tumours showing high grade budding (41% and 43%) compared with those characterized by low grade budding (84% and 83%). Moreover, budding was significantly related to survival in stepwise regression analysis and had the second highest hazard ratios (HR 2.21 and HR 2.35) only surpassed by extramural spread (HR 2.74 and HR 3.10) in both cohorts, yet demonstrating higher risk ratios than tumour differentiation, extramural venous invasion, the number of metastatic lymph nodes, and apical nodal involvement.

However, Sy *et al.* (141) did not identify additional independent prognostic information beyond that given by routine pathology reporting, but they only analyzed stage III tumours. Regarding the risk for distant metastasis, literature data are conflicting : Two studies stated independent prognostic significance regarding extrahepatic metastases or recurrence, respectively (142,143), while other authors only stated prognostic significance regarding distant metastases in univariable, yet not in multivariable analyses (144-146).

Prognostic significance in UICC stage II disease

The value of tumour budding for prediction of long term prognostic outcome of stage II colorectal cancer patients has not been extensively studied. Nakamura *et al.* (147) reported survival rates of patients with high grade budding in stage II tumours not significantly different from patients with stage III disease analyzing 200 colon cancer patients. In Cox regression, budding was the most powerful independent prognosticator regarding survival (HR 4.89), stronger than serosal surface involvement (HR 2.56), while venous and lymphatic invasion did not show independent prognostic influence. Two

Table 3. — **Selected studies showing independent prognostic significance of tumour budding in multivariable analysis**

	Year	Patients	Site	Inclusion criteria	Stain	Budding (%)	Target value	Univariable analysis	Significant in multivariable analysis
Ueno *et al.* (129)	2002	638	Rectum	Potentially curative resection	H&E	30.1% high grade (>10)	5-year OS	Five-year survival rate 84.0% in patients with budding intensity ≤10, but only 40.7% in patients with budding intensity >10	Budding, number of involved lymph nodes, extramural spread, lymphocytic infiltration, apical nodal involvement, grade
Okuyama *et al.* (137)	2003	196	Colon	T3, G1/G2	H&E	43,3%	5-year OS	No significant difference in survival curves observed between patients with budding-positive Stage II lesions and patients with Stage III lesions	Budding
Ueno *et al.* (136)	2004	476 (2nd dataset)	Rectum	n. spec.	H&E	53.1% high grade (>10)	5-year CSS	5-year survival rates of patients with low-grade budding 82.5%, 5-year survival rates of patients with high-grade budding 42.8%	Budding, number of involved lymph nodes, grade, extramural venous invasion, extramural spread, apical nodal involvement
Park *et al.* (139)	2005	174	Colon	≥T2, well or moderately dif	Keratin	89%	5-year DFS	5-year DFS 85.2% (budding 0-3), 76.1% (Budding 4-5), 73.1% (budding 6-9), and 49% (budding 10-38)	Budding, perineural invasion
Prall *et al.* (150)	2005	182	Colorectum	Stages I-II ; R0	Keratin	32% high degree (>25 buds/field of vision	OS	Significant : budding, infiltrating growth pattern, venous invasion	Budding, venous invasion
Shinto *et al.* (151)	2006	136	Colorectum	T3	H&E	40% high grade (>10)	5-year OS	Significant : age, distant metatstasis, nodal metastasis, venous invasion, budding, cytoplasmic podia	Budding, age, distant metastases, cytoplasmic podia
Choi *et al.* (140)	2007	244	Rectum	≥T2, G1/G2, no distant metastasis	H&E	92%	5-year DFS	5-year DFS for patients with budding intensity ≤10: 76.7%, 5-year DFS for patients with budding intensity >10: 47.2%	Budding, N, stage, perineural invasion, T
Kanazawa *et al.* (134)	2008	159	Colorectum		H&E	Mild 34%, moderate 37%, marked 29%	5-year CSS	Significant : depth of invasion, lymph node metastasis, stage, grade	Budding, stage
Nakamura *et al.* (147)	2008	200	Colon	Stage II	H&E	35% high grade (budding at >1/3 of the entire invasive margin)	5-year/10-year OS	Significant : serosal surface involvement, budding	Budding, serosal surface involvement
Zlobec *et al.* (135)	2008	1420	Colorectum		n. spec.	n. spec.	5-year OS	n. spec.	Budding, T, N, vascular invasion, grade, RHAMM, EGFR, Tumor infiltrating lymphocytes, urokinase plasminogen activator, RKIP, MST-1
Ohtsuki *et al.* (138)	2008	149	Colorectum	T2-T4, no neoadjuvant therapy	H&E, Keratin	63% H&E, 73% IHC	DFS	Significant : wall penetration, lymph node metastasis, lymphatic invasion, venous invasion, liver metastasis, budding	IHC : Budding, N ; H&E : Budding not significant (significant : N, wall penetration)
Wang *et al.* (149)	2008	128	Colorectum	T3 N0 M0	H&E	38-50% high	5-year CSS	Significant : growth pattern (Jass), lymphovascular invasion, perineural invasion, budding	Budding (others not shown)

CSS, specific survival ; DFS, disease-free survival ; IHC, immunohistochemistry ; N.D., not determined ; n. spec, not specified ; OS, overall ; PFS, free survival.

other studies analyzing stage II patients with T3 depth of invasion also identified tumour budding as independent prognostic factor regarding cancer-specific survival in univariable (148) and multivariable (149) analyses. Other studies, lumping all node-negative tumours together (stage I and II disease), also stated prognostic impact regarding survival (150) and, yet only in univariable analysis, regarding distant metastasis (145). Moreover, there are studies identifying tumour budding as independent prognostic variable in T3 colorectal cancer, but those did not exclude node positive cases and have therefore only limited significance with respect to stage II disease (137,151-153).

Prognostic significance in early colorectal cancer

A major role of tumour budding for prediction of lymph node metastases in early colorectal cancer has been confirmed in a number of studies, the majority of these including regression analysis (24,71,74,76,120, 154-162). Moreover, budding has been associated with locoregional recurrence (163,164). However, impact on overall survival was not be proven by Wang *et al.* (74), in a retrospective study of 159 T1 colorectal cancer patients.

Prognostic significance for local recurrence in rectal cancer

Tumour budding has been significantly associated with local recurrence in univariable (153) and multivariable (123,165) analysis. Moreover, tumour budding independently predicted locoregional failure (including both local recurrence and lymph node metastasis) in early colorectal cancer (163,164). Akasu *et al.* (123) identified focal dedifferentiation (i.e. tumour budding) and positive resection margin as independent predictors of local recurrence in rectal cancer, while perineural invasion and lymphovascular invasion did not predict local tumour failure. Further studies, however, comparing the prognostic yield of the three histopathological variables in this regard are warranted.

Comments and Perspectives

Lack of internationally accepted standards and guidelines for the assessment of tumour budding may compromise its evaluation in the routine setting. Thus, although most authors refer to the criteria of Ueno *et al.* (129) or Hase *et al.* (130), (i) budding foci have been counted in the area where the intensity of budding is maximal or in several areas of the tumour border in different slides, generating average bud counts or indexes, (ii) different cut of values for the classification of budding intensity have been used, and (iii) budding has been evaluated at different levels of magnification and/or visual fields of varying size (120,134,147,149,166,167). Moreover, budding foci have been defined as compromising of five cells or less, however some authors have set the limit to 4 tumour cells (133,151).

Of note, Morodomi *et al.* (168) introduced more broadly settled criteria for the evaluation of tumour budding : They summarised budding as ether the occurrence of *microtubular cancer nests*, defined as "bundles of five or more cancer cells occurring in a well differentiated region (mainly the active invasive area) which showed a tubular structure", or the occurrence of *undifferentiated cells*, defined as "isolated cancer cells without a distinct structure". Several subsequent studies assessed budding according to Morodomi's criteria or modifications of this system (74,134,137,148,153,169).

Finally, the use of immunohistochemical stains, e.g. applying antibodies directed against pan-keratin, the intermediate filament of epithelial cells, may improve the detection of budding cancer cells at the invasive tumour margin and has accordingly been used by several authors (138,144,150,151,155,166). In particular, Ohtsuki *et al.* (138) observed prognostic significance of budding regarding disease free survival in T2-4 patients only evaluating immuohistochemically stained, but not evaluating H&E stained slides. However, as stated above regarding the assessment of vascular invasion, immunohistochemical staining is labour intensive, time consuming and expensive. Hence, the routine use of Immunohistochemistry may not be efficient and is not recommended in current practice guidelines (8,9). Nevertheless, special stains, in particular keratin immunostaining, may be helpful in equivocal cases and the authors recommend its use for early cancer cases.

In conclusion, although tumour budding is increasingly recognized as an important predictor of tumour progression and cancer-related death, as well as lymph node metastasis in early cancer cases, the establishment of standardized criteria for the evaluation of tumour budding is inevitable in order to improve the quality of assessment and the comparability of scientific studies. Future prospective studies should compare the prognostic power of tumour budding related to vascular and perineural invasion since studies in this regard are currently lacking.

Conclusion

In conclusion, the presented histopathological parameters are central prognostic factors in colorectal cancer and should be used to facilitate patient counselling and clinical decision-making with respect to follow-up scheduling, administration of adjuvant therapy, and evidence-based design of clinical trials. New prognostic markers, in particular on the molecular level, have to surpass the prognostic power of the three parameters discussed above before consideration in routine practice. Nevertheless, international standardization of morphological criteria embedded in a quality control setting need to be established in order to improve and/or guarantee performance status of risk stratification for affected patients and comparability of scientific studies.

References

1. JASS J.R., LOVE S.B., NORTHOVER J.M. A new prognostic classification of rectal cancer. *Lancet*, 1987, **1** : 1303-1306.
2. COMPTON C.C. Optimal pathologic staging : defining stage II disease. *Clin. Cancer Res.,* 2007, **13** : 6862-6870.
3. MCLEOD H.L., MURRAY G.I. Tumour markers of prognosis in colorectal cancer. *Br. J. Cancer*., 1999, **79** : 191-203.
4. LYALL M.S., DUNDAS S.R., CURRAN S., MURRAY G.I. Profiling markers of prognosis in colorectal cancer. *Clin. Cancer Res.,* 2006, **12** : 1184-1191.
5. NAGTEGAAL I.D,. VAN DE VELDE C.J., MARIJNEN C.A., VAN KRIEKEN J.H., QUIRKE P., DUTCH COLORECTAL CANCER GROUP *et al*. Low rectal cancer : a call for a change of approach in abdominoperineal resection. *J. Clin. Oncol.,* 2005, **23** : 9257-9264.
6. ROSEN L.S, BILCHIK A.J., BEART R.W., JR, BENSON A.B., 3RD, CHANG K.J., COMPTON C.C. *et al*. New approaches to assessing and treating early-stage colon and rectal cancer : summary statement from 2007 Santa Monica Conference. *Clin. Cancer Res.,* 2007, **13** : 6853-6856.
7. O'CONNELL J.B., MAGGARD M.A., KO C.Y. Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging. *J. Natl. Cancer. Inst.,* 2004, **96** : 1420-1425.
8. WASHINGTON M.K., BERLIN J., BRANTON P., BURGART L.J., CARTER D.K., FITZGIBBONS P.L. *et al*. Protocol for the examination of specimens from patients with primary carcinoma of the colon and rectum. *Arch. Pathol. Lab. Med.,* 2009, **133** : 1539-1551.
9. JASS J.R., O'BRIEN J., RIDDELL R.H., SNOVER D.C., Association of Directors of Anatomic and Surgical Pathology. Recommendations for the reporting of surgically resected specimens of colorectal carcinoma : Association of Directors of Anatomic and Surgical Pathology. *Am. J. Clin. Pathol.,* 2008, **129** : 13-23.
10. ENGSTROM P.F., ARNOLETTI J.P., BENSON A.B., 3RD, CHEN Y.J., CHOTI M.A., COOPER H.S. *et al*. NCCN Clinical Practice Guidelines in Oncology : colon cancer. *J. Natl. Compr. Canc. Netw.,* 2009, **7** : 778-831.
11. ENGSTROM .PF., ARNOLETTI J.P., BENSON A.B., 3RD, CHEN Y.J., CHOTI M.A., COOPER H.S. *et al*. NCCN Clinical Practice Guidelines in Oncology : rectal cancer. *J. Natl. Compr. Canc. Netw.,* 2009, **7** : 838-881.
12. TALBOT I.C., RITCHIE S., LEIGHTON M., HUGHES A.O., BUSSEY H.J., MORSON B.C. Invasion of veins by carcinoma of rectum : method of detection, histological features and significance. *Histopathology*., 1981, **5** : 141-163.
13. MINSKY B.D., MIES C., RICH T.A., RECHT A. Lymphatic vessel invasion is an independent prognostic factor for survival in colorectal cancer. *Int. J. Radiat. Oncol. Biol. Phys.* 1989, **17** : 311-318.
14. WASHINGTON M.K. Colorectal carcinoma : selected issues in pathologic examination and staging and determination of prognostic factors. *Arch. Pathol. Lab. Med.,* 2008, **132** : 1600-1607.
15. BETGE J., POLLHEIMER M.J., LINDTNER R.A., KORNPRAT P., SCHLEMMER A., REHAK P. *et al*. Intra- and extramural vascular invasion in colorectal cancer - prognostic significance & quality of pathology reporting. *Cancer*, 2011 (in press).
16. TSUCHIYA A., ANDO Y., KIKUCHI Y., KANAZAWA M., SATO H., ABE R. Venous invasion as a prognostic factor in colorectal cancer. *Surg. Today,* 1995, **25** : 950-953.
17. SATO H., MAEDA K., SUGIHARA K., MOCHIZUKI H., KOTAKE K., TERAMOTO T. *et al*. High-risk stage II colon cancer after curative resection. *J. Surg. Oncol.,* 2011, **104** : 45-52.
18. SHIROUZU K., ISOMOTO H., KAKEGAWA T., MORIMATSU M. A prospective clinicopathologic study of venous invasion in colorectal cancer. *Am. J. Surg.,* 1991, **162** : 216-222.
19. KNUDSEN J.B., NILSSON T., SPRECHLER M., JOHANSEN A., CHRISTENSEN N. Venous and nerve invasion as prognostic factors in postoperative survival of patients with resectable cancer of the rectum. *Dis. Colon. Rectum*., 1983, **26** : 613-617.
20. HORN A., DAHL O., MORILD I. Venous and neural invasion as predictors of recurrence in rectal adenocarcinoma. *Dis. Colon. Rectum.,* 1991, **34** : 798-804.
21. OUCHI K., SUGAWARA T., ONO H., FUJIYA T., KAMIYAMA Y., KAKUGAWA Y. *et al*. Histologic features and clinical significance of venous invasion in colorectal carcinoma with hepatic metastasis. *Cancer,* 1996, **78** : 2313-2317.
22. KRASNA M.J., FLANCBAUM L., CODY R.P., SHNEIBAUM S., BEN ARI G. Vascular and neural invasion in colorectal carcinoma. Incidence and prognostic significance. *Cancer*, 1988, **61** : 1018-1023.
23. INOUE T., MORI M., SHIMONO R., KUWANO H., SUGIMACHI K. Vascular invasion of colorectal carcinoma readily visible with certain stains. *Dis. Colon. Rectum,*. 1992, **35** : 34-39.
24. SUZUKI A., TOGASHI K., NOKUBI M., KOINUMA K., MIYAKURA Y., HORIE H. *et al*. Evaluation of venous invasion by Elastica van Gieson stain and tumor budding predicts local and distant metastases in patients with T1 stage colorectal cancer. *Am. J. Surg. Pathol*., 2009, **33** : 1601-1607.
25. EGASHIRA Y., YOSHIDA T., HIRATA I., HAMAMOTO N., AKUTAGAWA H., TAKESHITA A. *et al*. Analysis of pathological risk factors for lymph node metastasis of submucosal invasive colon cancer. *Mod. Pathol*., 2004, **17** : 503-511.
26. BAYAR S., SAXENA R., EMIR B., SALEM R.R. Venous invasion may predict lymph node metastasis in early rectal cancer. *Eur. J. Surg. Oncol.,* 2002, **28** : 413-417.
27. TALBOT I.C., RITCHIE S., LEIGHTON M.H., HUGHES A.O., BUSSEY H.J., MORSON B.C. Spread of rectal cancer within veins. Histologic features and clinical significance. *Am. J. Surg.,* 1981, **141** : 15-17.
28. TALBOT I.C., RITCHIE S., LEIGHTON M.H., HUGHES A.O., BUSSEY H.J., MORSON B.C. The clinical significance of invasion of veins by rectal cancer. *Br. J. Surg.,* 1980, **67** : 439-442.
29. MINSKY B.D., MIES C., RICH T.A., RECHT A., CHAFFEY J.T. Potentially curative surgery of colon cancer : the influence of blood vessel invasion. *J. Clin. Oncol*., 1988, **6** : 119-127.
30. HARRISON J.C., DEAN P.J., EL-ZEKY F., VANDER ZWAAG R. Impact of the Crohn's-like lymphoid reaction on staging of right-sided colon cancer : results of multivariate analysis. *Hum. Pathol.,* 1995, **26** : 31-38.
31. HORN A., DAHL O., MORILD I. The role of venous and neural invasion on survival in rectal adenocarcinoma. *Dis. Colon. Rectum.,* 1990, : 598-601.
32. DEANS G.T., PATTERSON C.C., PARKS T.G., SPENCE R.A., HEATLEY M., MOOREHEAD R.J. *et al*. Colorectal carcinoma : importance of clinical and pathological factors in survival. *Ann. R. Coll. Surg. Engl.,* 1994, **76** : 59-64.
33. TAKEBAYASHI Y., AKLYAMA S., YAMADA K., AKIBA S., AIKOU T. Angiogenesis as an unfavorable prognostic factor in human colorectal carcinoma. *Cancer*., 1996, **78** : 226-231.
34. FUJITA S., SHIMODA T., YOSHIMURA K., YAMAMOTO S., AKASU T., MORIYA Y. Prospective evaluation of prognostic factors in patients with colorectal cancer undergoing curative resection. *J. Surg. Oncol*., 2003, **84** : 127-131.
35. BURTON S., NORMAN A.R., BROWN G., ABULAFI A.M., SWIFT R.I. Predictive poor prognostic factors in colonic carcinoma. *Surg. Oncol*., 2006, **15** : 71-78.
36. CHAPUIS P.H., DENT O., FISHER R., NEWLAND R.C., PHEILS M.T., SMYTH E. *et al*. A multivariate analysis of clinical and pathological variables in prognosis *after resection of large bowel cancer. Br. J. Surg*., 1985, **72** : 698-702.
37. LIANG P., NAKADA I., HONG J.W., TABUCHI T., MOTOHASHI G., TAKEMURA A. *et al*. Prognostic significance of immunohistochemically detected blood and lymphatic vessel invasion in colorectal carcinoma : its impact on prognosis. *Ann. Surg. Oncol.,* 2007, **14** : 470-477.
38. NEWLAND R.C., DENT O.F., LYTTLE M.N., CHAPUIS P.H., BOKEY E.L. Pathologic determinants of survival associated with colorectal cancer with lymph node metastases. A multivariate analysis of 579 patients. *Cancer*., 1994, **73** : 2076-2082.
39. FREEDMAN L.S., MACASKILL P., SMITH A.N. Multivariate analysis of *prognostic factors for operable rectal cancer. Lancet*., 1984, **2** : 733-736.
40. TAKAHASHI T., KATO T., KODAIRA S., KOYAMA Y., SAKABE T., TOMINAGA T. *et al*. Prognostic factors of colorectal cancer. Results of multivariate analysis of curative resection cases with or without adjuvant chemotherapy. *Am. J. Clin. Oncol.,* 1996, **19** : 408-415.
41. TOMINAGA T., SAKABE T., KOYAMA Y., HAMANO K., YASUTOMI M., TAKAHASHI T. *et al*. Prognostic factors for patients with colon or rectal carcinoma treated with resection only. Five-year follow-up report. *Cancer*., 1996, **78** : 403-408.
42. BENTZEN S.M., BALSLEV I., PEDERSEN M., TEGLBJAERG P.S., HANBERG-SOERENSEN F., BONE J. *et al*. A regression analysis of prognostic factors after resection of Dukes' B and C carcinoma of the rectum and rectosigmoid. Does post-operative radiotherapy change the prognosis ? *Br. J. Cancer.,* 1988, **58** : 195-201.
43. CHAPUIS P.H., DENT O.F., BOKEY E.L., NEWLAND R.C., SINCLAIR G. Adverse histopathological findings as a guide to patient management after curative resection of node-positive colonic cancer. *Br. J. Surg*., 2004, **91** : 349-354.
44. MINSKY B.D., MIES C., RECHT A., RICH T.A., CHAFFEY J.T. Resectable adenocarcinoma of the rectosigmoid and rectum. II. The influence of blood vessel invasion. *Cancer*, 1988, **61** : 1417-1424.
45. DRESEN R.C., PETERS E.E., RUTTEN H.J., NIEUWENHUIJZEN G.A., DEMEYERE T.B., VAN DEN BRULE A.J. *et al*. Local recurrence in rectal cancer can be predicted by histopathological factors. *Eur. J. Surg. Oncol*., 2009, **35** : 1071-1077.

46. HOHENBERGER W., BITTORF B., PAPADOPOULOS T., MERKEL S. Survival after surgical treatment of cancer of the rectum. *Langenbecks Arch. Surg*., 2005, **390** : 363-372.
47. HARRISON J.C., DEAN P.J., EL-ZEKY F., VANDER ZWAAG R. From Dukes through Jass : pathological prognostic indicators in rectal cancer. *Hum. Pathol.,* 1994, **25** : 498-505.
48. LIN M., MA S.P., LIN H.Z., JI P., XIE D., YU J.X. Intratumoral as well as peritumoral lymphatic vessel invasion correlates with lymph node metastasis and unfavourable outcome in colorectal cancer. *Clin. Exp. Metastasis.,* 2010, **27** : 123-132.
49. OGATA Y., TORIGOE S., MATONO K., SASATOMI T., ISHIBASHI N., SHIDA S. *et al*. Prognostic factors after potentially curative resection in stage II or III colon cancer. *Kurume Med. J.,* 2005, **52** : 67-71.
50. TANG R., HO Y.S., YOU Y.T., HSU K.C., CHEN J.S., CHANGCHIEN C.R. *et al*. Prognostic evaluation of DNA flow cytometric and histopathologic parameters of colorectal cancer. *Cancer*, 1995, **76** : 1724-1730.
51. MEYER A., MERKEL S., BRUCKL W., SCHELLERER V., SCHILDBERG C., CAMPEAN V. *et al*. Cdc2 as prognostic marker in stage UICC II colon carcinomas. *Eur. J. Cancer*, 2009, **45** : 1466-1473.
52. PTOK H., MEYER F., STEINERT R., VIETH M., RIDWELSKI K., LIPPERT H. *et al*. No prognostic impact of isolated lymphovascular invasion after radical resection of rectal cancer--results of a multicenter observational study. I*nt. J. Colorectal Dis.,* 2007, **22** : 749-756.
53. LIM S.B., YU C.S., JANG S.J., KIM T.W., KIM J.H., KIM J.C. Prognostic significance of lymphovascular invasion in sporadic colorectal cancer. *Dis. Colon. Rectum*, 2010, **53** : 377-384.
54. MICHELASSI F., BLOCK G.E., VANNUCCI L., MONTAG A., CHAPPELL R. A 5- to 21-year follow-up and analysis of 250 patients with rectal adenocarcinoma. *Ann. Surg*., 1988, **208** : 379-389.
55. MICHELASSI F., AYALA J.J., BALESTRACCI T., GOLDBERG R., CHAPPELL R., BLOCK G.E. Verification of a new clinicopathologic staging system for colorectal adenocarcinoma. *Ann. Surg,.* 1991, **214** : 11-18.
56. MEGUERDITCHIAN A.N., BAIRATI I., LAGACE R., HAREL F., KIBRITE A. Prognostic significance of lymphovascular invasion in surgically cured rectal carcinoma. *Am. J. Surg*., 2005, **189** : 707-713.
57. DU C.Z., XUE W.C., CAI Y., LI M., GU J. Lymphovascular invasion in rectal cancer following neoadjuvant radiotherapy : a retrospective cohort study. *World J. Gastroenterol.*, 2009, **15** : 3793-3798.
58. DAS P., SKIBBER J.M., RODRIGUEZ-BIGAS M.A., FEIG B.W., CHANG G.J., HOFF P.M. *et al*. Clinical and pathologic predictors of locoregional recurrence, distant metastasis, and overall survival in patients treated with chemoradiation and mesorectal excision for rectal cancer. *Am. J. Clin. Oncol*., 2006, **29** : 219-224.
59. MEHRKHANI F, NASIRI S, DONBOLI K, MEYSAMIE A, HEDAYAT A. Prognostic factors in survival of colorectal cancer patients after surgery. *Colorectal Dis.,* 2009, **11** : 157-161.
60. MORRIS M., PLATELL C., DE BOER B., MCCAUL K., IACOPETTA B. Population-based study of prognostic factors in stage II colonic cancer. *Br. J. Surg*., 2006, **93** : 866-871.
61. TSAI H.L., CHENG K.I., LU C.Y., KUO C.H., MA C.J., WU J.Y. *et al*. Prognostic significance of depth of invasion, vascular invasion and numbers of lymph node retrievals in combination for patients with stage II colorectal cancer undergoing radical resection. *J. Surg. Oncol.,* 2008, **97** : 383-387.
62. PETERSEN V.C., BAXTER K.J., LOVE S.B., SHEPHERD N.A. Identification of objective pathological prognostic determinants and models of prognosis in Dukes' B colon cancer. *Gut*, 2002, **51** : 65-69.
63. UEN Y.H., LIN S.R., WU D.C., SU Y.C., WU J.Y., CHENG T.L. *et al*. Prognostic significance of multiple molecular markers for patients with stage II colorectal cancer undergoing curative resection. *Ann. Surg*., 2007, **246** : 1040-1046.
64. LIN C.C., LIN J.K., CHANG S.C., WANG H.S., YANG S.H., JIANG J.K. *et al*. Is adjuvant chemotherapy beneficial to high risk stage II colon cancer ? Analysis in a single institute. *Int. J. Colorectal Dis*., 2009, **24** : 665-676.
65. QUAH H.M., CHOU J.F., GONEN M., SHIA J., SCHRAG D., LANDMANN R.G. *et al*. Identification of patients with high-risk stage II colon cancer for adjuvant therapy. *Dis. Colon. Rectum*, 2008, **51** : 503-507.
66. LENNON A.M., MULCAHY H.E., HYLAND J.M., LOWRY C., WHITE A., FENNELLy D. *et al*. Peritoneal involvement in stage II colon cancer. *Am. J. Clin. Pathol.,* 2003, **119** : 108-113.
67. MULCAHY H.E., TONER M., PATCHETT S.E., DALY L., O'DONOGHUE D.P. Identifying stage B colorectal cancer patients at high risk of tumor recurrence and death. *Dis. Colon. Rectum*, 1997, **40** : 326-331.
68. KHANKHANIAN N., MAVLIGIT G.M., RUSSELL W.O., SCHIMEK M. Prognostic significance of vascular invasion in colorectal cancer of Dukes' *B class. Cancer*, 1977, **39** : 1195-1200.
69. TYTHERLEIGH M.G., WARREN B.F., MORTENSEN N.J. Management of early rectal cancer. *Br. J. Surg*., 2008, **95** : 409-423.
70. DEINLEIN P., REULBACH U., STOLTE M., VIETH M. Risk factors for lymphatic metastasis from pT1 colorectal adenocarcinoma. *Pathologe*, 2003, **24** : 387-393.
71. TATEISHI Y., NAKANISHI Y., TANIGUCHI H., SHIMODA T., UMEMURA S. Pathological prognostic factors predicting lymph node metastasis in submucosal invasive (T1) colorectal carcinoma. *Mod. Pathol.,* 2010, **23** : 1068-1072.
72. ISHII M., OTA M., SAITO S., KINUGASA Y., AKAMOTO S., ITO I.. Lymphatic vessel invasion detected by monoclonal antibody D2-40 as a predictor of lymph node metastasis in T1 colorectal cancer. *Int. J. Colorectal Dis.,* 2009, **24** : 1069-1074.
73. TOMINAGA K., NAKANISHI Y., NIMURA S., YOSHIMURA K., SAKAI Y, SHIMODA T. Predictive histopathologic factors for lymph node metastasis in patients with nonpedunculated submucosal invasive colorectal carcinoma. *Dis. Colon. Rectum*., 2005, **48** : 92-100.
74. WANG H.S., LIANG W.Y., LIN T.C., CHEN W.S., JIANG J.K., YANG S.H. *et al*. Curative resection of T1 colorectal carcinoma : risk of lymph node metastasis and long-term prognosis. *Dis. Colon. Rectum*., 2005, **48** : 1182-1192.
75. PENG J., CHEN W., SHENG W., XU Y., CAI G., HUANG D. *et al*. Oncological outcome of T1 rectal cancer undergoing standard resection and local excision. *Colorectal Dis*., 2011, **13** : e14-9.
76. YASUDA K., INOMATA M., SHIROMIZU A., SHIRAISHI N., HIGASHI H., KITANO S. Risk factors for occult lymph node metastasis of colorectal cancer invading the submucosa and indications for endoscopic mucosal resection. *Dis. Colon. Rectum.,* 2007, **50** : 1370-1376.
77. NISSAN A., STOJADINOVIC A., SHIA J., HOOS A., GUILLEM J.G., KLIMSTRA D. *et al*. Predictors of recurrence in patients with T2 and early T3, N0 adenocarcinoma of the rectum treated by surgery alone. *J. Clin. Oncol.*, 2006, **24** : 4078-4084.
78. MERCHANT N.B., GUILLEM J.G., PATY P.B., ENKER W.E., MINSKY B.D., QUAN S.H. *et al*. T3N0 rectal cancer : results following sharp mesorectal excision and no adjuvant therapy. *J. Gastrointest. Surg*., 1999, **3** : 642-647.
79. YUN H.R., LEE L.J., PARK J.H., CHO Y.K., CHO Y.B., LEE W.Y. *et al*. Local recurrence after curative resection in patients with colon and rectal cancers. *Int. J. Colorectal Dis.,* 2008, **23** : 1081-1087.
80. BOUVET M., MILAS M., GIACCO G.G., CLEARY K.R., JANJAN N.A., SKIBBER J.M. Predictors of recurrence after local excision and postoperative chemoradiation therapy of adenocarcinoma of the rectum. *Ann. Surg. Oncol.,* 1999, **6** : 26-32.
81. POESCHL E.M., POLLHEIMER M.J., KORNPRAT P., LINDTNER R.A., SCHLEMMER A., REHAK P. *et al*. Perineural invasion : correlation with aggressive phenotype and independent prognostic variable in both colon and rectum cancer. *J. Clin. Oncol.,* 2010, **28** : e358-360.
82. SATO T., UENO H., MOCHIZUKI H., SHINTO E., HASHIGUCHI Y., KAJIWARA Y. *et al*. Objective criteria for the grading of venous invasion in colorectal cancer. A*m. J. Surg. Pathol.,* 2010, **34** : 454-462.
83. STERNBERG A., MIZRAHI A., AMAR M., GROISMAN G. Detection of venous invasion in surgical specimens of colorectal carcinoma : the efficacy of various types of tissue blocks. *J. Clin. Pathol.,* 2006, **59** : 207-210.
84. LITTLEFORD S.E., BAIRD A., ROTIMI O., VERBEKE C.S., SCOTT N. Interobserver variation in the reporting of local peritoneal involvement and extramural venous invasion in colonic cancer. *Histopathology*, 2009, **55** : 407-413.
85. VASS D.G., AINSWORTH R., ANDERSON J.H., MURRAY D., FOULIS A.K. The value of an elastic tissue stain in detecting venous invasion in colorectal cancer. *J. Clin. Pathol.,* 2004, **57** : 769-772.
86. STERNBERG A., AMAR M., ALFICI R., GROISMAN G. Conclusions from a study of venous invasion in stage IV colorectal adenocarcinoma. *J. Clin. Pathol*., 2002, **55** : 17-21.
87. KINGSTON E.F., GOULDING H., BATEMAN A.C. Vascular invasion is underrecognized in colorectal cancer using conventional hematoxylin and eosin staining. *Dis. Colon. Rectum*., 2007, **50** : 1867-1872.
88. ABDULKADER M., ABDULLA K., RAKHA E., KAYE P. Routine elastic staining assists detection of vascular invasion in colorectal cancer. *Histopathology*, 2006, **49** : 487-492.
89. ROXBURGH C.S., MCMILLAN D.C., ANDERSON J.H., MCKEE R.F., HORGAN P.G., FOULIs A.K. Elastica staining for venous invasion results in superior prediction of cancer-specific survival in colorectal cancer. *Ann. Surg.,* 2010, **252** : 989-997.
90. COMPTON C.C. Colorectal carcinoma : diagnostic, prognostic, and molecular features. *Mod. Pathol*., 2003, **16** : 376-388.
91. HARRIS E.I., LEWIN D.N., WANG H.L., LAUWERS G.Y., SRIVASTAVA A., SHYR Y. *et al*. Lymphovascular invasion in colorectal

cancer : an interobserver variability study. *Am. J. Surg. Pathol.,* 2008, **32** : 1816-1821.
92. COMPTON C.C., FIELDING L.P., BURGART L.J., CONLEY B., COOPER H.S., HAMILTON S.R. *et al.* Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. *Arch. Pathol. Lab. Med.,* 2000, **124** : 979-994.
93. BATSAKIS J.G. Nerves and neurotropic carcinomas. *Ann. Otol. Rhinol. Laryngol*., 1985, **94** : 426-427.
94. LIEBIG C., AYALA G., WILKS J.A., BERGER D.H., ALBO D. Perineural invasion in cancer : a review of the literature. *Cancer*, 2009, **115** : 3379-3391.
95. PENG J., SHENG W., HUANG D., VENOOK A.P., XU Y., GUAN Z. *et al*. Perineural invasion in pT3N0 rectal cancer : the incidence and its prognostic effect. *Cancer*, 2011, **117** : 1415-1421.
96. LiEBIG C., AYALA G., WILKS J., VERSTOVSEK G., LIU H., AGARWAL N. *et al*. Perineural invasion is an independent predictor of outcome in colorectal cancer. *J. Clin. Oncol*., 2009, **27** : 5131-5137.
97. RODIN A.E., LARSON D.L., ROBERTS D.K. Nature of the perineural space invaded by prostatic carcinoma. *Cancer*, 1967, **20** : 1772-1779.
98. HASSAN M.O., MAKSEM J. The prostatic perineural space and its relation to tumor spread : an ultrastructural study. *Am. J. Surg. Pathol.,* 1980, **4** : 143-148.
99. MATSUSHIMA T., MORI M., KIDO A., ADACHI Y., SUGIMACHI K. Preoperative estimation of neural invasion in rectal carcinoma. *Oncol. Rep*., 1998, **5** : 73-76.
100. SHIROUZU K., ISOMOTO H., KAKEGAWA T. Prognostic evaluation of perineural invasion in rectal cancer. *Am. J. Surg.*, 1993, **165** : 233-237.
101. SHIROUZU K., ISOMOTO H., MORODOMI T., ARAKI Y., HOSHIKO M., AKAGI Y. *et al*. Clinicopathologic study of perineural invasion in rectal cancer. *Kurume Med. J.*, 1992, **39** : 41-49.
102. BOGNEL C., REKACEWICZ C., MANKARIOS H., PIGNON J.P., ELIAS D., DUVILLARD P. *et al*. Prognostic value of neural invasion in rectal carcinoma : a multivariate analysis on 339 patients with curative resection. *Eur. J. Cancer,* 1995, **31A** : 894-898.
103. STEWART D., YAN Y., MUTCH M., KODNER I., HUNT S., LOWNEY J. *et al*. Predictors of disease-free survival in rectal cancer patients undergoing curative proctectomy. *Colorectal Dis.*, 2008, **10** : 879-886.
104. TSAI H.L., CHU K.S., HUANG Y.H., SU Y.C., WU J.Y., KUO C.H. *et al*. Predictive factors of early relapse in UICC stage I-III colorectal cancer patients after curative resection. *J. Surg. Oncol*., 2009, **100** : 736-743.
105. OH S.Y., KIM Y.B., PAEK O.J., SUH K.W. Contiguous invasion per se does not affect prognosis in colon cancer. *J. Surg. Oncol*., 2009, **99** : 71-74.
106. UENO H., HASE K., MOCHIZUKI H. Criteria for extramural perineural invasion as a prognostic factor in rectal cancer. *Br. J. Surg.*, 2001, **88** : 994-1000.
107. LAW W.L., CHU K.W. Anterior resection for rectal cancer with mesorectal excision : a prospective evaluation of 622 patients. *Ann. Surg*., 2004, **240** : 260-268.
108. UEHARA K., NAKANISHI Y., SHIMODA T., TANIGUCHI H., AKASU T., MORIYA Y. Clinicopathological significance of microscopic abscess formation at the invasive margin of advanced low rectal cancer. *Br. J. Surg*., 2007, **94** : 239-243.
109. KANG H., MIN B.S., LEE K.Y., KIM N.K., KIM S.N., CHOI J. *et al.* Loss of E-cadherin and MUC2 expressions correlated with poor survival in patients with stages II and III colorectal carcinoma. *Ann. Surg. Oncol*., 2011, **18** : 711-719.
110. CHUANG S.C., SU Y.C., LU C.Y., HSU H.T., SUN L.C., SHIH Y.L. *et al*. Risk factors for the development of metachronous liver metastasis in colorectal cancer patients after curative resection. *World. J. Surg.*, 2011, **35** : 424-429.
111. DERICI H., UNALP H.R., KAMER E., BOZDAG A.D., TANSUG T., NAZLI O. *et al*. Multivisceral resections for locally advanced rectal cancer. Colorectal Dis., 2008, **10** : 453-459.
112. HUH J.W., KIM H.R., KIM Y.J. Prognostic value of perineural invasion in patients with stage II colorectal cancer. *Ann. Surg. Oncol.*, 2010, **17** : 2066-2072.
113. LAW W.L., HO J.W., CHAN R., AU G., CHU K.W. Outcome of anterior resection for stage II rectal cancer without radiation : the role of adjuvant chemotherapy. *Dis. Colon. Rectum*, 2005, **48** : 218-226.
114. TSAI H.L., YEH Y.S., YU F.J., LU C.Y., CHEN C.F., CHEN C.W. *et al.* Predicting factors of postoperative relapse in T2-4N0M0 colorectal cancer patients via harvesting a minimum of 12 lymph nodes. *Int. J. Colorectal. Dis*., 2009, **24** : 177-183.
115. BURDY G., PANIS Y., ALVES A., NEMETH J., LAVERGNE-SLOVE A., VALLEUR P. Identifying patients with T3-T4 node-negative colon cancer at high risk of recurrence. *Dis. Colon. Rectum*, 2001, **44** : 1682-1688.
116. DESOLNEUX G., BURTIN P., LERMITE E., BERGAMASCHI R., HAMY A., ARNAUD J.P. Prognostic factors in node-negative colorectal cancer : a retrospective study from a prospective database. *Int. J. Colorectal. Dis.*, 2010, **25** : 829-834.
117. ONATE-OCANA L.F., MONTESDEOCA R., LOPEZ-GRANIEL C.M., AIELLO-CROCIFOGLIO V., MONDRAGON-SANCHEZ R.,CORTINA-BORJA M. *et al*. Identification of patients with high-risk lymph node-negative colorectal cancer and potential benefit from adjuvant chemotherapy. Jpn. J. Clin. Oncol., 2004, **34** : 323-328.
118. DI FABIO F., NASCIMBENI R., VILLANACCI V., BARONCHELLI C., BIANCHI D., FABBRETTI G. *et al*. Prognostic variables for cancer-related survival in node-negative colorectal carcinomas. *Dig. Surg.*, 2004, **21** : 128-133.
119. HUH J.W., KIM H.R., KIM Y.J. Lymphovascular or perineural invasion may predict lymph node metastasis in patients with T1 and T2 colorectal cancer. *J. Gastrointest. Surg.*, 2010, **14** : 1074-1080.
120. CHOI D.H., SOHN D.K., CHANG H.J., LIM S.B., CHOI H.S., JEONG S.Y. Indications for subsequent surgery after endoscopic resection of submucosally invasive colorectal carcinomas : a prospective cohort study. *Di.s Colon. Rectum.*, 2009, **52** : 438-445.
121. ENKER W.E., HAVENGA K., POLYAK T., THALER H., CRANOR M. Abdominoperineal resection via total mesorectal excision and autonomic nerve preservation for low rectal cancer. *World J. Surg*., 1997, **21** : 715-720.
122. BENTZEN S.M., BALSLEV I., PEDERSEN M., TEGLBJAERG P.S., HANBERG-SORENSEN F., BONE J. *et al*. Time to loco-regional recurrence after resection of Dukes' B and C colorectal cancer with or without adjuvant postoperative radiotherapy. A multivariate regression analysis. *Br. J. Cancer*, 1992, **65** : 102-107.
123. AKASU T., TAKAWA M., YAMAMOTO S., ISHIGURO S., YAMAGUCHI T., FUJITA S. *et al*. Intersphincteric resection for very low rectal adenocarcinoma : univariate and multivariate analyses of risk factors for recurrence. *Ann. Surg. Oncol.*, 2008, **15** : 2668-2676.
124. PENG .J, SHENG W., HUANG D., VENOOK A.P., XU Y., GUAN Z. *et al*. Perineural invasion in pT3N0 rectal cancer : the incidence and its prognostic effect. *Cancer*, 2010, **117** : 1415-1421.
125. BELLIS D., MARCI V., MONGA G. Light microscopic and immunohistochemical evaluation of vascular and neural invasion in colorectal cancer. *Pathol. Res. Pract.*, 1993, **189** : 443-447.
126. KURTZ K.A., HOFFMAN H.T., ZIMMERMAN M.B., ROBINSON R.A. Perineural and vascular invasion in oral cavity squamous carcinoma : increased incidence on re-review of slides and by using immunohistochemical enhancement. *Arch. Pathol. Lab. Med.*, 2005, **129** : 354-359.
127. ZHOU M., PATEL A., RUBIN M.A. Prevalence and location of peripheral nerve found on prostate needle biopsy. *Am. J. Clin. Pathol.*, 2001, **115** : 39-43.
128. JASS J.R., ATKIN W.S., CUZICK J., BUSSEY H., MORSON B.C., NORTHOVER J.M. *et al*. The grading of rectal cancer : historical perspectives and a multivariate analysis of 447 cases. *Histopathology*, 1986, **10** : 437-459.
129. UENO H., MURPHY J., JASS J.R., MOCHIZUKI H., TALBOT I.C. Tumour 'budding' as an index to estimate the potential of aggressiveness in rectal cancer. *Histopathology*, 2002, **40** : 127-132.
130. HASE K., SHATNEY C., JOHNSON D., TROLLOPE M., VIERRA M. Prognostic value of tumor “budding” in patients with colorectal cancer. *Dis. Colon. Rectum*, 1993, **36** : 627-635.
131. GABBERT H., WAGNER R., MOLL R., GERHARZ C.D. Tumor dedifferentiation : an important step in tumor invasion. *Clin. Exp. Metastasis*. 1985, **3** : 257-279.
132. ZLOBEC I., LUGLI A.. Invasive front of colorectal cancer : dynamic interface of pro-/anti-tumor factors. *World J. Gastroenterol.*, 2009, **15** : 5898-5906.
133. PRALL F. Tumour budding in colorectal carcinoma. *Histopathology*. 2007, **50** : 151-162.
134. KANAZAWA H., MITOMI H., NISHIYAMA Y., KISHIMOTO I., FUKUI N., NAKAMURA T. *et al*. Tumour budding at invasive margins and outcome in colorectal cancer. *Colorectal Dis*., 2008, **10** : 41-47.
135. ZLOBEC I., TERRACCIANO L., TORNILLO L., GUNTHERT U., VUONG T., JASS J.R. *et al*. Role of RHAMM within the hierarchy of well-established prognostic factors in colorectal cancer. *Gut*., 2008, **57** : 1413-1419.
136. UENO H., PRICE A.B., WILKINSON K.H., JASS J.R., MOCHIZUKI H., TALBOT I.C. A new prognostic staging system for rectal cancer. *Ann. Surg.*, 2004, **240** : 832-839.
137. OKUYAMA T., NAKAMURA T., YAMAGUCHI M. Budding is useful to select high-risk patients in stage II well-differentiated or moderately differentiated colon adenocarcinoma. *Dis. Colon. Rectum*, 2003, **46** : 1400-1406.
138. OHTSUKI K., KOYAMA F., TAMURA T., ENOMOTO Y., FUJII H., MUKOGAWA T. *et al*. Prognostic value of immunohistochemical analysis

of tumor budding in colorectal carcinoma. *Anticancer Res*., 2008, **28** : 1831-1836.
139. PARK K.J., CHOI H.J., ROH M.S., KWON H.C., KIM C. Intensity of tumor budding and its prognostic implications in invasive colon carcinoma. *Dis. Colon. Rectum*, 2005, **48** : 1597-1602.
140. CHOI H.J., PARK K.J., SHIN J.S., ROH M.S., KWON H.C., LEE H.S. Tumor budding as a prognostic marker in stage-III rectal carcinoma. *Int. J. Colorectal Dis*., **2007, 22** : 863-868.
141. SY J., FUNG C.L., DENT O.F., CHAPUIS P.H., BOKEY L., CHAN C. Tumor budding and survival after potentially curative resection of node-positive colon cancer. *Dis. Colon. Rectum*, 2010, **53** : 301-307.
142. NAKAMURA T., MITOMI H., KIKUCHI S., OHTANI Y., SATO K. Evaluation of the usefulness of tumor budding on the prediction of metastasis to the lung and liver after curative excision of colorectal cancer. *Hepatogastroenterology*, 2005, **52** : 1432-1435.
143. UENO H., MOCHIZUKI H., HASHIGUCHI Y., HATSUSE K., FUJIMOTO H., HASE K. Predictors of extrahepatic recurrence after resection of colorectal liver metastases. *Br. J. Surg.*, 2004, **91** : 327-333.
144. MARKL B., RENK I., ORUZIO D.V., JAHNIG H., SCHENKIRSCH G., SCHOLER C. *et al*. Tumour budding, uPA and PAI-1 are associated with aggressive behaviour in colon cancer. *J. Surg. Oncol.*, 2010, 102 : 235-241.
145. ZLOBEC I., BAKER K., MINOO P., JASS J.R., TERRACCIANO L., LUGLI A. Node-negative colorectal cancer at high risk of distant metastasis identified by combined analysis of lymph node status, vascular invasion, and Raf-1 kinase inhibitor protein expression. *Clin. Cancer Res*., 2008, **14** : 143-148.
146. IWAYA K., OIKAWA K., SEMBA S., TSUCHIYA B., MUKAI Y., OTSUBO T. *et al*. Correlation between liver metastasis of the colocalization of actin-related protein 2 and 3 complex and WAVE2 in colorectal carcinoma. *Cancer Sci.*, 2007, **98** : 992-999.
147. NAKAMURA T., MITOMI H., KANAZAWA H., OHKURA Y., WATANABE M. Tumor budding as an index to identify high-risk patients with stage II colon cancer. *Dis. Colon. Rectum*, **2008, 51** : 568-572.
148. TANAKA M., HASHIGUCHI Y., UENO H., HASE K., MOCHIZUKI H. Tumor budding at the invasive margin can predict patients at high risk of recurrence after curative surgery for stage II, T3 colon cancer. *Dis. Colon. Rectum*, 2003, **46** : 1054-1059.
149. WANG L.M., KEVANS D., MULCAHY H., O'SULLIVAN J., FENNELLY D., HYLAND J. *et al*. Tumor budding is a strong and reproducible prognostic marker in T3N0 colorectal cancer. *Am. J. Surg. Pathol.*, 2009, 33 : 134-141.
150. PRALL F., NIZZE H., BARTEN M. Tumour budding as prognostic factor in stage I/II colorectal carcinoma. *Histopathology*, 2005, **47** : 17-24.
151. SHINTO E., JASS J.R., TSUDA H., SATO T., UENO H., HASE K. *et al*. Differential prognostic significance of morphologic invasive markers in colorectal cancer : tumor budding and cytoplasmic podia. *Dis. Colon. Rectum*., 2006, **49** : 1422-1430.
152. YOSHIDA K., YOSHIMATSU K., OTANI T., YOKOMIZO H., OGAWA K. The depth of tumor invasion beyond the outer border of the muscularis propria as a prognostic factor for T3 rectal/rectosigmoid cancer. *Anticancer Res*., 2008, **28** : 1773-1778.
153. OKUYAMA T., OYA M., ISHIKAWA H. Budding as a useful prognostic marker in pT3 well- or moderately-differentiated rectal adenocarcinoma. *J. Surg. Oncol.,* 2003, **83** : 42-47.
154. OGAWA T., YOSHIDA T., TSURUTA T., TOKUYAMA W., ADACHI S., KIKUCHI M. *et al*. Tumor budding is predictive of lymphatic involvement and lymph node metastases in submucosal invasive colorectal adenocarcinomas and in non-polypoid compared with polypoid growths. *Scand. J. Gastroenterol*., 2009, **44** : 605-614.
155. ISHIKAWA Y., AKISHIMA-FUKASAWA Y., ITO K., AKASAKA Y., YOKOO T., ISHII T. *et al*. Histopathologic determinants of regional lymph node metastasis in early colorectal cancer. *Cancer*, 2008, **112** : 924-933.
156. YAMAUCHI H., TOGASHI K., KAWAMURA Y.J., HORIE H., SASAKI J., TSUJINAKA S. *et al*. Pathological predictors for lymph node metastasis in T1 colorectal cancer. *Surg. Today*, 2008, **38** : 905-910.
157. KANEKO I., TANAKA S., OKA S., YOSHIDA S., HIYAMA T., ARIHIRO K. *et al*. Immunohistochemical molecular markers as predictors of curability of endoscopically resected submucosal colorectal cancer. *World J. Gastroenterol.,* 2007, **13** : 3829-3835.
158. MASAKI T., MATSUOKA H., SUGIYAMA M., ABE N., SAKAMOTO A., ATOMI Y. Actual number of tumor budding as a new tool for the individualization of treatment of T1 colorectal carcinomas. *J. Gastroenterol. Hepatol*., 2006, **21** : 1115-1121.
159. HASE K., SHATNEY Ch., MOCHIZUKI H., JOHNSON D.L., TAMAKUMA S., VIERRA M. *et al*. Long-term results of curative resection of "minimally invasive" colorectal cancer. *Dis. Colon. Rectum*, 1995, **38** : 19-26.
160. UENO H., MOCHIZUKI H., HASHIGUCHI Y., SHIMAZAKI H., AIDA S., HASE K. *et al*. Risk factors for an adverse outcome in early invasive colorectal carcinoma. *Gastroenterology*, 2004, **127** : 385-394.
161. KOMORI K., HIRAI T., KANEMITSU Y., SHIMIZU Y., SANO T., ITO S. *et al*. Is "depth of submucosal invasion > or = 1,000 microm" an important predictive factor for lymph node metastases in early invasive colorectal cancer (pT1) ? *Hepatogastroenterology*, 2010, **57** : 1123-1127.
162. KAZAMA S., WATANABE T., AJIOKA Y., KANAZAWA T., NAGAWA H. Tumour budding at the deepest invasive margin correlates with lymph node metastasis in submucosal colorectal cancer detected by anticytokeratin antibody CAM5.2. *Br. J. Cancer*, 2006, **94** : 293-298.
163. MASAKI T., SUGIYAMA M., MATSUOKA H., ABE N., IZUMISATO Y., GOTO A. *et al*. Clinical utility of grading criteria for submucosal invasion in the prognosis of T1 colorectal carcinomas. *J. Gastroenterol.,* 2003, **38** : 37-44.
164. MASAKI T., GOTO A., SUGIYAMA M., MATSUOKA H., ABE N., SAKAMOTO A. *et al*. Possible contribution of CD44 variant 6 and nuclear beta-catenin expression to the formation of budding tumor cells in patients with T1 colorectal carcinoma. *Cancer*, 2001, **92 :** 2539-2546.
165. SYK E., LENANDER C., NILSSON P.J., RUBIO C.A., GLIMELIUS B. Tumour budding correlates with local recurrence of rectal cancer. *Colorectal Dis.,* 2011, **13** : 255-262.
166. ZLOBEC I., MOLINARI F., MARTIN V., MAZZUCCHELLI L., SALETTI P., TREZZI R. *et al*. Tumor budding predicts response to anti-EGFR therapies in metastatic colorectal cancer patients. *World J. Gastroenterol*., 2010, **16** : 4823-4831.
167. KAJIWARA Y., UENO H., HASHIGUCHI Y., MOCHIZUKI H., HASE K. Risk factors of nodal involvement in T2 colorectal cancer. *Dis. Colon. Rectum*, 2010, **53** : 1393-1399.
168. MORODOMI T., ISOMOTO H., SHIROUZU K., KAKEGAWA K., IRIE K., MORIMATSU M. An index for estimating the probability of lymph node metastasis in rectal cancers. Lymph node metastasis and the histopathology of actively invasive regions of cancer. *Cancer*, 1989, **63** : 539-543.
169. OKUYAMA T., OYA M., ISHIKAWA H. Budding as a risk factor for lymph node metastasis in pT1 or pT2 well-differentiated colorectal adenocarcinoma. *Dis. Colon. Rectum*., 2002, **45** : 628-634.