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ORIGINAL PAPER

Heterogeneity of pT3 Colorectal Carcinomas According to the Depth of Invasion

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Abstract Colorectal carcinomas (CRCs) infiltrating through the muscularis propria layer without infiltration of adjacent structures, organs or the serosa—i.e. the pT3 tumors, compose the largest subset of large intestinal carcinomas treated by surgical resection. They are heterogeneous in terms of prognosis. CRCs treated by surgery in a period of 69 months were prospectively classified as pT3a tumors (invading to a maximum of 5 mm beyond the muscularis propria) and pT3b tumors (invading deeper). Their nodal status, incidence of vascular invasion and the presence or absence of distant metastases were analyzed in relation to the depth of invasion. Of the 593 CRCs primarily treated by surgery 429 were pT3 tumors. CRCs categorized as pT3a had significantly lower rates of nodal involvement (44% vs 75%), massive nodal involvement (pN2) (9% vs 39%), venous invasion (17% vs 30%) and distant metastasis (11% vs 28%) than pT3b tumors. Significant differences in these prognostic variables in pT3a and pT3b cancers were observed both for carcinomas of the colon and those of the rectum. Such differences were not obvious in further 66 ypT3 cases of rectal carcinoma receiving neoadjuvant treatment before surgery. Tumors in the pT3a category are associated with a better prognostic profile than pT3b tumors. This subdivision might be useful in both prognostication and treatment planning.

Keywords Colorectal carcinoma · TNM · Depth of invasion · Metastasis · Venous invasion

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Introduction

The main prognosticators of colorectal carcinomas include the presence or absence of distant metastasis, the depth of invasion through the anatomic layers of the bowel, regional lymph node involvement and vascular (venous) invasion. The first three of these parameters are included in practically all staging systems [1], including the TNM (tumor-node-metastasis) classification of malignant tumors. [2, 3] The presence of both distant and nodal metastases and also that of venous invasion is related to the depth of invasion which is reflected by the pT category of the TNM classification. [4–7] The presence of nodal metastases is also associated with the presence of distant ones. [8]

Most of the cases of colorectal carcinomas treated surgically currently belong to the category of pT3 tumors, i.e. those invading through the muscularis propria, but not crossing the limits of the organ including the peritoneum. The depth of invasion is at least in part a time dependent variable (the disease needs to cross the submucosal and muscularis layers before) and reflects the time span since the tumor was formed. On the other side, it may also be related to tumor aggressivity, as more aggressive tumors are

expected to invade faster. On the basis of the above considerations, tumors just passing through the outer layer of the muscularis propria must be in a less advanced stage than those which have already invaded deeper beyond this anatomical and staging limit.

The aim of the present study was to analyze the heterogeneity of pT3 colorectal carcinomas by differentially evaluating the relation of these tumors to nodal status, vascular invasion and distant metastasis.

Materials and Methods

Patients with colorectal carcinomas undergoing non-laparoscopic surgical resection of their tumors at the Department of Surgery of the Bács-Kiskun County Hospital or the Kiskunfélegyháza City Hospital between April 2002 and December 2007 were the subjects of the present study. All resection specimens were assessed at the Department of Pathology of the Bács-Kiskun County Hospital.

All specimens were fixed in buffered formalin. After taking at least five transverse blocks from the tumor (whenever possible) for the assessment of the depth of invasion, the perivisceral fat was removed and lymph nodes were searched for by palpation with the aim of recovering as many lymph nodes as possible. The same method of lymph node recovery was used for mesorectal excision specimens, although these were first sliced perpendicular to the long axis to assess the completeness of the mesorectal excision. Whenever a lymph node was cut through during the process, both parts were identified and submitted for histology. Smaller lymph nodes were embedded as a whole, whereas larger ones were either halved or sliced with all pieces submitted for histological evaluation. A few cases also underwent lymphatic mapping [9], and the lymph nodes of these patients were also subjected to limited step-sectioning (three levels separated by 250 μm) and cytokeratin (AE1/AE3, Dakocytomation, Glostrup, Denmark) immunostaining on two slides. Isolated tumor cells, as defined by the TNM classification were considered part of the node-negative cases. [2]

Blocks were embedded in paraffin and the pathology assessment was made on hematoxylin eosin (HE) stained slides. Orcein, an elastic stain was also added to HE in the final period, and 117 tumors were also assessed by this staining. All tumors were staged according to the 6th edition of the TNM, and data regarding depth of invasion, venous invasion, nodal or distant metastases were recorded according to the categories defined in the staging books. [2, 3] The maximum depth of invasion of pT3 tumors was measured whenever possible, and cancers with not more than 5 mm invasion beyond the muscularis propria layer were prospectively labeled as pT3a, those extending deeper were labeled as pT3b according to previous suggestions. [10, 11] Cases where the depth of invasion could not be determined (generally due to the destruction and disappearance of the muscular wall, or sometimes to the poor orientation of the tissue blocks) were classified as pT3x.

Data on the synchronous distant metastatic status were available from the multidisciplinary tumor board meetings. As routine staging procedures, patients had chest X-ray and abdominal ultrasound examination. Some of them, at the beginning of the period analyzed, also routinely had bone scans. Laparotomy was also part of the staging for colon tumors. Patients with no data available on the metastatic status were considered Mx for the purpose of this study.

Twenty-three patients with multiple synchronous tumors were also included and their tumor with the deepest invasion was included in the analysis in keeping with the staging rules. [2, 3, 10] Rectal cancer patients who had preoperative radiotherapy or chemoradiotherapy were also included, although their tumors were labeled according to the ypTNM categories (reflecting their post neoadjuvant therapy staging). [2, 3] These latter tumors were analyzed separately. Colon carcinomas and rectal cancers were analyzed together because their rules for the TNM classification are identical, but a subset analysis was performed with the omission of the five tumors with unknown localization.

A written consent was obtained from the hospital's data protection and safety manager and was accepted by the institutional ethical board due to the retrospective nature of the analysis and the lack of patients' identification.

The Pearson's chi-square test was used for the comparison of the categorical data in different T stages using the VassarStats statistical package (R Lowry, VassarStats, Poughkeepsie, NY, USA). The chi-square test could be calculated only if all cell frequencies expected on the basis of the null hypothesis were equal to or greater than 5. As the Fisher exact test (FET) was devised for low case numbers, cases with smaller numbers were analyzed by this test, and this is separately indicated in the results, whenever applicable. [12]

The level of significance was set to <0.05 for both tests.

Results

Data on 701 patients' tumors were analyzed. One hundred eight patients with rectal carcinoma received radiotherapy or chemoradiotherapy preoperatively. The median age of the patients was 69 with a male to female ratio of 393/308. As mentioned in the introduction, the majority of the cases

belonged to the pT3 (429/593, 72%; 327/454, 72% in the colon and 97/134, 72% in the rectum) or the ypT3 (66/108, 61%) categories, respectively. The median number of lymph nodes evaluated was 17.

For tumors treated primarily by surgery, the pT categories of the TNM classification and their corresponding frequencies of pN and M categories and venous invasion are given in Table 1.

There was a gradual increase in the rates of lymph node positive colorectal cancers with higher pT categories: 5%, 18%, 54% and 70% for pT1, pT2, pT3 and pT4 tumors, respectively (Table 1).

The node positivity rate of pT3a tumors was 44%, that of pT3b tumors was 75% and that of pT3x cancers fell in between (48%). The nodal involvement rate of pT3a tumors was significantly different from that of pT3b tumors ($p<0.0001$) and also differed from that of pT2 tumors ($p=0.0003$). Within the node-positive group of tumors, the pN1 and pN2 rates of pT3a and pT3b tumors were also significantly different ($p<0.0001$). To note is that tumors located in the colon or rectum showed differences in their proportion in the T3 subcategories, with more pT3a tumors in the rectum (43%) than in the colon (30%).

There was a gradual increase in the rates of vascular (venous) invasion with higher pT categories: 2%, 22% and 30% for pT2, pT3 and pT4 tumors, respectively (Table 1). The differences in rates were significant only for pT2 vs pT3 ($p=0.0003$).

The venous invasion rate of pT3a was significantly different from that of pT3b tumors ($p=0.0004$) and also differed from that of pT2 tumors ($p=0.03$). On the other hand, the vascular invasion rate of pT3b tumors was nearly similar to that of pT4 cancers ($p=0.92$). The rate of venous invasion in pT3x tumors fell in between that of pT3a and pT3b tumors.

Only 342 patients primarily treated by surgery had data on metastatic status (Table 1). As expected the pT4 tumors had significantly higher rates of distant metastasis than the pT3 tumors ($p<0.0001$) or the pT3b subset ($p=0.03$). Of interest to the study is the significant difference in the distant metastasis rate of the pT3a and pT3b subsets ($p=0.001$). To note is the fact that the M1 rate of pT3x cases was intermediate between the rates of pT3a and pT3b tumors.

The subset of node-negative patients was also analyzed separately (Table 2). Obviously the rates of vascular invasion and distant metastases were smaller in this subset than in the whole series or in node-positive tumors. The difference in the rate of venous invasion was significantly different between pT3a and pT3b tumors (FET, $p=0.03$) but there was no significant difference in the rates of distant metastasis between the two groups (FET).

When carcinomas primarily treated by surgery were grouped according to their site, i.e. those arising in the rectum ($n=134$) and those developing in the colon ($n=454$), the differences between pT3a and pT3b tumors were found to be significant for the proportion of node-positive disease, of pN2 disease, of distant metastases and of venous invasion in both localizations (Table 3).

The TNM categories and the frequencies of ypN and M stage categories and vascular invasion of tumors resected after neoadjuvant treatment are given in Table 4.

For patients primarily treated with neoadjuvant therapy, there was also a gradual increase in the rates of lymph node positive colorectal cancers with higher ypT categories: 0% 4%, 51% and 100% for ypT0, ypT2, ypT3 and ypT4 tumors, respectively (Table 4). Carcinomas of categories ypT0, ypT1 and ypT4 were too few for statistical conclusions. There was a significant difference in the proportions of involved lymph nodes between categories ypT2 and ypT3 or ypT3a ($p<0.0001$ for both), whereas the proportion of positive nodes was not significantly different between ypT3a (54%) and ypT3b (64%) tumors ($p=0.52$). Interestingly ypT3x tumors had a lower proportion of node-positivity (35%).

Table 1 The pN, V an M categories according to the different pT categories of tumors treated by primary surgery

	pN0	pN1	pN2	V0	V1	Mx	M0	M1 ([a])	All
pT1	18	1 (5%)	0	17	2 (11%)	10	9	0	**19**
pT2	47	8 (14%)	2 (4%)	56	1 (2%)	26	31	0	**57**
pT3	196	138 (32%)	95 (22%)	336	93 (22%)	182	204	43 (17%)	**429**
pT3a	113	70 (35%)	17 (9%)	166	34 (17%)	78	109	13 (11%)	**200**
pT3b	33	48 (36%)	51 (39%)	92	40 (30%)	51	58	23 (28%)	**132**
pT3x	50	20 (21%)	27 (28%)	78	19 (20%)	53	37	7 (16%)	**97**
pT4	26	28 (32%)	34 (39%)	62	26 (30%)	34	29	25 (46%)	**88**
All T	287	175 (30%)	131 (22%)	471	122 (21%)	252	273	68 (20%)	593

Values in parentheses represent the proportion of tumors in the same pT category having the feature in question—expressed as percentages

[a] Only cases with known metastatic status (M0 and M1) considered for the percentages

Table 2 The V an M categories according to the different pT categories of the pN0 tumors treated by primary surgery

	V0	V1	Mx	M0	M1[a]	All
pT1	16	2 (11%)	10	8	0	**18**
pT2	46	1 (2%)	20	27	0	**47**
pT3	168	28 (14%)	83	106	7 (6%)	**196**
pT3a	100	13 (12%)	42	66	5 (7%)	**113**
pT3b	24	9 (28%)	14	17	2 (11%)	**33**
pT3x	44	6 (12%)	27	23	0	**50**
pT4	22	4 (15%)	10	14	2 (13%)	**26**
All	252	35 (12%)	123	155	9 (5%)	287

Values in parentheses represent the proportion of tumors in the same pT category having the feature in question—expressed in percentages
[a] Only cases with known metastatic status (M0 and M1) considered for the percentages

The overall venous invasion rate of ypT tumors (Table 4) was somewhat lower than that of the pT tumors. The FET revealed no significant differences between progressive pT categories. The highest rate of venous invasion was seen in the ypT3a subset.

The metastasis rates of the tumors treated by neo-adjuvant therapy (Table 4) are not suitable for analysis, because their rate of M1 was low, in keeping with the idea that the treatments were in general given to patients without recognized distant metastases.

Discussion

Our study also supports the previously described phenomenon that increasing invasion depth as reflected by the higher pT categories of the TNM classification [2, 3] is associated with the worsening of other recognized prognostic markers: a higher rate of nodal involvement, of greater nodal involvement (pN2), of venous invasion and of distant metastasis. As most of the tumors treated surgically belong to the pT3 category we looked at the possibility of subdividing this large group into subgroups of different expected prognosis on the basis of other prognostic markers. By choosing an arbitrary inclusive limit of 5 mm beyond the muscularis propria layer, we were able to distinguish between to subsets labeled as pT3a and pT3b tumors which had significantly different rates of nodal involvement, massive nodal involvement, vascular invasion and distant metastasis. As expected, the evaluated prognostic markers of pT3a tumors fell closer to those of pT2 tumors, and those of pT3b tumors more closely resembled the prognostic data of pT4 tumors, than the prognostic parameters of pT3 tumors as a single group. Tumors belonging in the pT3 category in which no subcategorization into pT3a or pT3b could be done (pT3x cases) had rates of nodal involvement, venous invasion and distant metastasis falling between that of pT3a and pT3b cancers.

As nodal involvement is generally considered a major prognostic disadvantage, the subset of node-negative cancers was also analyzed separately. The somewhat higher rate of distant metastases in the pT3b category was not found significantly different from that of pT3a tumors, but there was a significantly higher proportion of cases presenting with venous invasion in the more advanced pT3b carcinomas.

According to these findings, it makes sense to divide the pT3 category into subsets of pT3a and pT3b according to the depth of invasion beyond the muscular layer of the large bowel as on the basis of other prognostic factors, the two subcategories refer to tumors with different prognosis.

Due to the lack of survival data, the ultimate outcome of the tumors could not be assessed directly, but a series from the United Kingdom [13], one from New England [14] and two from Germany [15] documented significantly worse survival rates for rectal cancer patients with deeper infiltration of the mesorectum than those with a rather superficial infiltration.

All of the 514 cases from the Erlangen colorectal cancer registry could be segregated into pT3a (n=220) and pT3b

Table 3 Differential distribution of the pN, M and V categories in the pT3a and pT3b tumors according to their localization in the colon or the rectum

Colon	pN+/pN0	p	pN2/pN1	p	M1/M0[a]	P	V1/V0	p
pT3a	58/79 (42%)	<0.0001	12/46 (9%)	0.0003	10/77 (11%)	0.01	21/116 (15%)	0.03
pT3b	82/31 (73%)		42/40 (37%)		19/51 (27%)		30/83 (27%)	
Rectum								
pT3a	26/32 (44%)	0.0007	5/21 (36%)	0.02	2/30 (6%)	0.03	11/47 (19%)	0.0004
pT3b	17/2 (89%)		9/8 (42%)		4/7 (36%)		10/9 (53%)	

Values in parentheses represent the proportion of all tumors in the same pT3 subcategory having the feature in question (i.e. the numerator of the column heading)—expressed in percentages
[a] Only cases with known metastatic status (M0 and M1) considered for the percentages

Table 4 The ypN, V an M categories according to the different ypT categories of tumors treated by surgery following neoadjuvant treatment

	ypN0	ypN1	ypN2	V0	V1	Mx	M0	M1	All
ypT0	5	0	0	5	0	1	4	0	**5**
ypT1	8	0	1	9	0	2	6	1	**9**
ypT2	26	0	1	24	3	4	23	0	**27**
ypT3	32	25	9	52	13				**66**
ypT3a	16	15	4	28	7	10	24	1	**35**
ypT3b	5	6	3	12	2	3	9	2	**14**
ypT3x	11	4	2	14	3	6	11	0	**17**
ypT4	0	0	1	1	0	0	0	1	**1**
All yT	71	25	12	93	15	26	77	5	108

(n=294) tumors, with different rates of nodal involvement (43% vs 60%), venous invasion (28% vs 50%), locoregional recurrence (10% vs 26%) and cancer-related 5-year-survival (85% vs 54%) between the two subsets. [15] These data are in keeping with our present findings in mixed colon and rectal cancers. A notable difference between the two studies seems to be the relative overlap of pT3a tumors with pT2 tumors and pT3b tumors with pT4 tumors in the Erlangen series, both in terms of locoregional recurrence free and cancer related survival. Although we did not investigate survival, just surrogate prognostic markers associated with worse survival, our series demonstrated significant differences between pT2 and pT3a tumors in terms of nodal involvement and venous invasion and also suggested a relevant difference in distant metastasis between these two categories. The difference between pT3b and pT4 tumors was significant only for the distant metastasis rate. Our data further support the notion that pT2 and pT3a tumors or pT3b and pT4 tumors should not be merged.

Another interesting difference between the German series [15] and ours is the relatively high proportion (nearly 23%) of the cases which could not reliably be classified as either pT3a or pT3b in our series. This inability was generally derived from the destruction of the muscularis propria layer or the poor orientation of some tissue blocks preventing adequate measurements of invasion. Since the deepest invasion was considered for the classification, and this could not be measured for these tumors, it was felt, that such cases are better put into a separate (pT3x) category. The intermediate rates of the variables assessed suggest that part of the cases labeled as pT3x belonged to the pT3a category, whereas the rest belonged to the pT3b tumors. Nevertheless, for cases where the classification can be done, our data and the inference from survival data associated with different depths of invasion of pT3 rectal carcinomas suggest that segregation of these tumors into pT3a and pT3 categories might be useful for prognostication and treatment recommendations, especially in the node-negative subset of patients.

The depth of tumor invasion in rectal carcinomas may also be relevant when assessed by imaging methods, therefore it may be useful in the preoperative setting too. [16]

We are not aware of studies comparing pT3 colon carcinomas invading the subserosal layer or non-peritonealized pericolonic fat to a minimum extent with those invading deeper. However, our subset analysis suggests that the subdivision between pT3a and pT3b tumors might be important even in the cases of colon carcinomas, in keeping with the common staging rules for cancers arising in the whole length of the large intestine.

Although the number of cases analyzed after neoadjuvant therapy was smaller, and therefore the power of the conclusion is weaker, it seemed that the subdivision of ypT3 tumors into ypT3a and ypT3b is less useful. There was no significant difference between the two subgroups in terms of nodal involvement or venous invasion, and no stepwise increasing proportions of these parameters could be established for ypT3a, ypT3x and ypT3b tumors. This phenomenon could be related to the fact that neoadjuvant therapy destroys the tumor and the bowel wall too, and the reliable determination of the post-treatment extent of invasion of the residual tumor may be more difficult. In addition, venous invasion may be less easily identified in these cancers, and lymph node retrieval is also less fruitful. [17]

In summary, we demonstrated that pT3 colorectal carcinomas invading 0.5 mm or less beyond the muscularis propria layer (pT3a tumors) are associated with a better prognostic profile in terms of nodal involvement, vascular invasion and distant metastases than those infiltrating deeper (pT3b tumors). This observation was true for both carcinomas of the rectum and those of the colon. However it seemed that a similar subclassification was of no use after neoadjuvant treatment. The subdivision of the largest colorectal cancer category, the pT3 cases might be useful in both prognostication and treatment planning. It might be inferred that patients with pT3b tumors might require more aggressive systemic treatment than those with pT3a, although this issue needs further exploration, with adequate stratification in clinical trials.

References

1. Cserni G (2003) Nodal staging of colorectal carcinomas and sentinel nodes. J Clin Pathol 56:327–335
2. Sobin LH, Wittekind C (eds) (2002) UICC TNM classification of malignant tumours, 6th edition. John Wiley and Sons Inc., New York
3. Greene FL, Page DL, Fleming ID, et al (eds) (2002) AJCC Cancer Staging Handbook—TNM Classification of Malignant Tumors, 6th edition. Springer Verlag, New York
4. Heinzerling JH, Anthony T, Livingston EH (et al) (2007) Predictors of distant metastasis and mortality in patients with stage II colorectal cancer. Am Surg 73:230–238
5. Ricciardi R, Madoff RD, Rothenberger DA (et al) (2006) Population-based analyses of lymph node metastases in colorectal cancer. Clin Gastroenterol Hepatol 4:1522–1527
6. Abdulkader M, Abdulla K, Rakha E (et al) (2006) Routine elastic staining assists detection of vascular invasion in colorectal cancer. Histopathology 49:487–492
7. Wong SK, Jalaludin BB, Henderson CJ (2008) Direct tumor invasion in colon cancer: correlation with tumor spread and survival. Dis Colon Rectum 51:1331–1338
8. Adachi Y, Inomata M, Kakisako K (1999) Histopathologic characteristics of colorectal cancer with liver metastasis. Dis Colon Rectum 42:1053–1056
9. Cserni G, Vajda K, Tarján M (et al) (1999) Nodal staging of colorectal carcinomas from quantitative and qualitative aspects. Can lymphatic mapping help staging? Pathol Oncol Res 5:291–296
10. Wittekind C, Greene FL, Henson DE (eds) (2003) TNM Supplement: a Commentary on Uniform Use. 3rd edn. Wiley-Liss, New York
11. Compton CC, Greene FL (2004) The staging of colorectal cancer: 2004 and beyond. CA Cancer J Clin 54:295–308
12. Lowry R (2008) VassarStats. http://faculty.vassar.edu/lowry/VassarStats.html Cited 11 Jul 2008
13. Cawthorn SJ, Parums DV, Gibbs NM (et al) (1990) Extent of mesorectal spread and involvement of lateral resection margin as prognostic factors after surgery for rectal cancer. Lancet 335 (8697):1055–1059
14. Willett CG, Badizadegan K, Ancukiewicz M (et al) (1999) Prognostic factors in stage T3N0 rectal cancer: do all patients require postoperative pelvic irradiation and chemotherapy? Dis Colon Rectum 42:167–173
15. Merkel S, Mansmann U, Siassi M (2001) The prognostic inhomogeneity of pT3 rectal carcinomas. Int J Colorectal Dis 16:298–304
16. Harewood GC, Kumar KS, Clain JE (2004) Clinical implications of quantification of mesorectal tumor invasion by endoscopic ultrasound: All T3 rectal cancers are not equal. J Gastroenterol Hepatol 19:750–755
17. Scott N, Thorne C, Jayne D (2004) Lymph node retrieval after neoadjuvant radiotherapy for rectal adenocarcinoma. J Clin Pathol 57:335–336