TRANSANAL ENDOSCOPIC MICROSURGERY IN RECTAL CANCER

OUTCOME AND TUMOR SELECTION

P.G. Doornebosch

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The publication of this thesis was financially supported by B-K Medical Benelux NV, Covidien Nederland BV, Johnson & Johnson Medical BV, Roche Nederland BV, IJsselland Hospital

Cover, Layout and Printing: Optima Grafische Communicatie, Rotterdam.

ISBN: 978-90-8559-995-1

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Proefschrift

Ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. P.F. van der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op donderdag 10 juni 2010
klokke 17.15 uur

door

Pascal Gabriël Doornebosch

geboren te Rotterdam
in 1970

PROMOTIECOMMISSIE

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Voor Lien en onze jongens

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Is the increasing role of transanal endoscopic microsurgery in curation for T1 rectal cancer justified?

A systematic review

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Adapted from Acta Oncol. 2009;48(3):343-353

EPIDEMIOLOGY

Colorectal cancer is one of the leading causes of death and accounts for approximately 300.000 new cases in Europe and the USA.[1] In the Netherlands in 2005 over 10.000 patients were diagnosed with colorectal carcinoma.[2] Rectal cancer approximately constitutes 25% of all colorectal carcinomas. Almost half of all patients eventually die from the disease.
Majority of rectal cancers develop from benign pre-neoplastic lesions: the adenomatous polyps or adenomas. Progression from a benign adenoma to a malignant carcinoma passes through a series of well-defined histological stages, which is referred to as the adenoma-carcinoma sequence.[3] Because of the implementation of population-based screening programs, the number of patients with early staged rectal carcinomas is likely to increase in the near future.[4]

There has been an impressive evolution in the therapy for rectal cancer. In 1826 Lisfranc was credited the first person to remove the cancer bearing segment of the rectum; he did so using a transanal approach.[5] In that era, the only feasible treatment of rectal cancer consisted of a colostomy to relieve obstruction, as first described by Amussat in 1839.[6] In 1885, Kraske and colleagues approached rectal cancer using a trans-sacral approach, which is removing the coccyx and distal sacrum, with preservation of the anus and muscles.[7] In 1908 the abdomino-perineal resection (APR) was reported, which can be attributed largely to Ernest Miles.[8] After observing a high incidence of cancer recurrence in patients undergoing local treatment for rectal cancer, he developed the concept of radical rectal excision. Miles postulated the lymphatic spread of rectal cancer was directed superiorly and that this surgery allowed complete resection of the anorectum and draining lymphatics. The APR procedure, which gained acceptance largely because it was oncologically sound and successful, has led to the cure of many patients with rectal tumors. Its feasibility was further enhanced by the availability of blood transfusion, allowing this radical surgery to become the most popular method of dealing with rectal cancer by 1947. In 1923 Hartmann described a two-stage procedure for upper rectal cancer.[9] After an artificial anus was constructed, during the second operation the cancer bearing segment was excised and the closed upper rectum was reperitonealized. Dixon established the safety of the anterior resection in the late 1940`s, but this approach was mainly limited to the treatment of upper rectal cancer until the 1970`s.[10] At that time, the introduction of circular stapling devices facilitated the technical possibility of low rectal anastomosis and even colo-anal anastomosis. This technological advance, along with the recognition that distal margins of < 2 cm did not compromise outcome, dramatically changed the approach to many patients.
The most recent advance was the introduction of the concept of total mesorectal excision (TME). This technique has meanwhile shown, by Heald et al and many others, to minimize local recurrence, to allow even ultralow resections with colo-anal anastomosis to be accepted as appropriate operations, resulting in survival rates comparable with APR, without the need for a permanent colostomy.[11] Nonetheless, these reconstructive operations are associated with a

relatively high rate of complications, including anastomotic leakage, genito-urinary dysfunction, defecation disorders and up to 4% mortality.

More or less parallel with the advent of TME, others focused on the improved possibilities of local excision (LE) for rectal cancer, initially as a palliative procedure, but now even with curative intent in selected tumors. The technique most commonly used is the transanal approach, according to Parks.[12] This however suffers from poor exposure and inadequate access to lesions, especially in the upper rectum, resulting in recurrence rates up to 60 percent.[13, 14] Trans-sacral (Kraske) and trans-sphincteric (Mason) approaches are technically demanding and invasive, resulting in high morbidity (up to 40 percent), often severe and mortality rates of 1-5 percent.[15-22] Moreover, recurrence rates range between 12 to 25 percent.

Transanal endoscopic microsurgery (TEM) is a recently introduced minimal invasive technique for local excision of rectal tumors.[23] In adenomas TEM is superior in safety and local control and tumors in the entire rectum can be treated and therefore TEM is the method of choice.[24-27] In a recent report by You et al, from 1989 to 2003 the rate of LE for T1 rectal carcinomas in the USA increased from 26.6 to 43.7% and from 5.8 to 16.8% for T2 rectal carcinomas.[28] This increasing role is ultimately reflected by several national guidelines, propagating selected tumors suitable for LE.[29] In many studies it is emphasized LE is safe regarding morbidity and mortality, especially compared to (conventional) radical surgery. The main question to be answered however is whether LE is justified from an oncologic point of view. The safety of a local procedure has to be balanced against the higher risk of local recurrences and/or worsened survival. In T2 rectal carcinomas, both local recurrence rates and survival rates after LE are worse compared to radical surgery, and therefore LE is considered a valid option only in palliative procedures.

LOCAL EXCISION OR RADICAL SURGERY

Radical surgery (RS) for T1 rectal carcinomas leads to excellent results.[30] Local recurrence rates are invariably low, ranging from zero to six percent. Five and 10-year survival rates are as high as 82 and 68%, respectively. Can similar results be achieved by applying LE according to Parks for T1 rectal carcinomas? No randomized study has been performed, but several comparative studies have been published upon this issue.[14, 28, 31-34] (Table 1) The earlier mentioned study of You et al. reports upon outcome after LE according to Parks (LE) in comparison to radical surgery (RS). In the LE group patients were older and tumors were smaller and located more distal. LE was significantly safer, as expressed by the lower morbidity rate (5.6% vs. 14.6%, $p < 0.001$). The vast majority of tumors were excised microscopic radical (R0) in both groups (95% vs. 99%). Regarding oncologic outcomes, 5-years local recurrence rates after R0 excision were 12.5% after LE and 6.9% after RS ($p = 0.003$). Overall survival rates were comparable (LE 77.4%, RS 81.7%, $p = 0.09$), however disease specific survival rates were significantly lower after LE (93.2% vs. 97.2%, $p = 0.004$).

Table 1. Comparative series of local excision according to Parks (LE) versus radical surgery (RS) for T1 rectal carcinomas.

Author	LE vs. RS (no.)	R0: LE vs. RS (5-yrs %)	LR: LE vs. RS (5-yrs %)	OR: LE vs. RS (5-yrs %)	OS: LE vs. RS (5-yrs %)	Other survival: LE vs. RS (5-yrs %)
Mellgren (2000)[14]	69 vs 30	100 vs 100	18 vs 0‡	21 vs 9	72 vs 80	DSS: 95 vs. 95
Nascimbeni (2004)[31]	70 vs 74	NS	6.6 vs 2.8‡	21 vs 10‡	72 vs 90‡	DSS: 89 vs. NS DFS: 67 vs. 84‡
Bentrem (2005)[32]	151 vs 168	NS	15 vs 3‡	23 vs 6‡	89 vs. 93*	DSS: 93 vs. 97*
Endreseth (2005)[33]	35 vs 256	83 vs 100‡	12 vs 6‡	12 vs 13	70 vs. 80*‡	DFS: 64 vs. 77*‡
Ptok (2007)[34]	105 vs 312	100 vs 100	6 vs 2‡	10 vs 6	84 vs 92	DFS: 91 vs 92
You (2007)[28]	601 vs 493	95 vs 99	12.5 vs 6.9‡	16 vs 10‡	77 vs. 82	DSS: 93 vs. 97‡

R0= microscopic radical excision, LR= local recurrence, OR= overall recurrence, DSS= disease specific survival, DFS= disease free survival, OS= overall survival

Survival rates are 5-years, unless otherwise specified; ‡ statistically significant ($p < 0.05$), NS= not stated; * patients who received neoadjuvant and/or adjuvant therapy were not excluded.

A prospective multicenter observational study was performed by Ptok et al.[34] In their study, selection was made based on histopathological criteria. In case of a low-risk T1 rectal carcinoma, that is well or moderately differentiated, radically excised, smaller than three centimeters and without lymph vascular invasion, LE is presumed curative. Both LE according to Parks and TEM were performed and not analyzed separately. After LE local recurrence rate was higher (LE 6%, RS 2%; $p = 0.049$), although tumor-free survival was comparable (LE 91%, RS 92%; $p = 0.39$). Mellgren et al. reported upon outcome after LE for 69 T1 rectal carcinomas, in comparison to 30 T1N0 rectal carcinomas treated by RS.[14] Neither group received neoadjuvant chemoradiation. In the LE group, tumors were significantly smaller and located more distally. After LE local recurrence rates were higher (18 versus zero percent; $p = 0.03$), as well as overall recurrence rates, although the latter not significantly (21 versus nine percent; $p = 0.54$). Five-year survival rates were comparable (LE 72%, RS 80%; $p = 0.50$). Another study was performed by Bentrem et al.[32] In their study 319 consecutive patients with T1 rectal carcinomas were treated by LE according to Parks (n=151) or RS (n=168) over a 17-year period. In the RS group 18% of tumors were node-positive; no tumor selection regarding differentiation grade and/or lymph vascular invasion was applied. Again, in the LE group tumors were smaller and located more distally. After LE adjuvant radiotherapy was given in case of close margins (n=11) or high-risk pathology (n=5). None of the patients received adjuvant systemic chemotherapy. After RS, in case of positive lymph nodes adjuvant radiotherapy (n=16) or chemotherapy (n=29) was given. At five years, local recurrence rate after LE was 15% versus three percent after RS ($p = 0.0001$). Overall recurrence rates also differed significantly (LE 23%, RS six percent; $p < 0.001$). Disease-specific

and overall survival rates were similar for LE and RS. Of all recurrences after LE, 77% could be resected radically, compared to 50% of local recurrences after RS. A nationwide, prospective study was performed by Endreseth et al.[33] They analyzed outcome of 291 T1M0 rectal carcinomas treated by LE according to Parks (n=35) or RS (n=256). In the LE group patients were older and tumors were smaller and located more distally and only in the minority of tumors with LE a R0 (microscopic negative) excision margin could be obtained. After excluding R2 (macroscopic irradical) procedures, local recurrence rate after LE was still significantly higher compared to RS (12 versus six percent; p = 0.01). Overall survival (70 versus 80%; p = 0.04) and disease free survival (64 versus 77 percent; p = 0.01) were significantly worse after LE.

Interpretation of all above mentioned studies remains difficult, as a selection bias may have been introduced, as expressed by the smaller, more distal located tumors treated by LE. Nevertheless, in all studies a significant proportion of tumors recurred, although in majority of studies this seems not to influence survival rates.

TEM OR PARKS

Can results be improved by using another technique for local excision? In rectal adenomas it was shown that application of transanal endoscopic microsurgery (TEM) results in lower recurrence rates compared to LE according to Parks.[26, 27] Can these results be extrapolated for T1 rectal carcinomas? Four studies were retrieved in which TEM was compared with another type of surgery (LE according to Parks and/or RS). (Tables 2 and 3)

Only one randomized controlled trial for clinical T1 rectal carcinomas has been performed.[35] This trial included 52 patients with presumed T1 rectal carcinomas, well or moderately differentiated, during an eight-year period. Patients were randomized to TEM or RS. Post-inclusion two patients were excluded because of a later pTNM staging. Twenty-four patients were treated using the TEM technique and 26 patients underwent anterior resection. Both groups were comparable in age and gender distribution. TEM proved to be the safest technique in the early postoperative period and patients required less postoperative analgesics. With median follow-up more than 40 months, local recurrence rate after TEM was 4.1 percent (1/24). In the RS group no local recurrence occurred. Five-year procedure specific survival rates were 96 percent for both groups.

Langer et al. reported (retrospectively) upon outcome after TEM in comparison to LE according to Parks and RS.[26] Overall 182 tumors (58 pT1 rectal carcinomas (G1/2) and 124 benign rectal tumors) were identified. Both local techniques proved to be faster in comparison to RS, resulting in less blood loss and shorter time of hospitalization. Also, complication rates after TEM and LE according to Parks were significantly lower compared to RS. An important outcome in this study was a significant higher rate of irradical excisions after LE according to Parks (TEM R1=19%, Rx=5%; Parks R1=37%, Rx=16%; p = 0.001). Local recurrence rates after RS were only

Table 2. Comparative series of TEM versus LE according to Parks and/or radical surgery.

Author	Type of study	Inclusion criteria	Type of surgery	Number of T1 carcinomas	Level of evidence‡
Winde (1996)[35]	RCT	uT1, G1/2	TEM	26	IIb
			RS	26	
Heintz (1998)[36]	Retrospective	pT1	TEM	58	IIIb
			RS	45	
Lee (2003)[37]	Retrospective	cT1N0, G1/2	TEM	52	IIIb
			RS	17	
Langer (2003)[26]	Retrospective	pT1,G1/2	TEM	20	IIIb
			Parks	20	
			RS	18	

RCT= randomized controlled trial, cT/N= clinical T/N-staging, uT/N= presumed T/N-stage based on endorectal ultrasound, pT= T-stage based on histopathological investigation, G1= well differentiated, G2= moderately differentiated, TEM= transanal endoscopic microsurgery, RS= radical surgery
‡= according to Oxford Centre for Evidence-based Medicine Levels of Evidence.

Table 3. Comparative series of TEM versus LE according to Parks and/or radical surgery.

Authors	TEM vs. other (no.)	R0: (%)	LR: (5-yrs %)	OR: (5-yrs %)	OS: (5-yrs %)	Other survival: (5-yrs %)
Winde (1996)[35]	TEM 24	NS	4	4	NS	DSS: 96
	RS 26		0	4		DSS: 96
Heintz (1998)[36]	TEM low risk 46	78	4	4	79	NS
	RS low risk 34	100	3	6	81	
	TEM high risk 12	58	33	33	62	NS
	RS high risk 11	100	18	18	69	
Lee (2003)[37]	TEM 52	100	4	NS	100	DFS: 96
	RS 17	100	0		93	DFS: 94
Langer (2003)[26]	TEM 20	76	10	NS	NS	100 (2-years)
	Parks 20	47‡	15			100 (2-years)
	RS 18	100	0			93 (2-years)

TEM= Transanal Endoscopic Microsurgery, Parks = LE according to Parks, RS = radical surgery, R0 = microscopic radical excision, LR = local recurrence, OR = overall recurrence, OS = overall survival
DFS = disease free survival, DSS = disease specific survival; survival rates are 5-years, unless otherwise specified ‡ = statistically significant ($p < 0.05$).

3.7%, which was no different after TEM (8.9%). Following LE according to Parks local recurrence rate was 26.3% ($p = 0.0055$ versus TEM). Statistical analysis of risk factors for development of a recurrence, detected only tumor-size ($p = 0.0236$) and recurrent tumor at the time of operation ($p = 0.0231$) to be significant. Tumor grading, tumor dignity (adenoma/carcinoma), distance from the anal verge and residual status (R0, R1, Rx) proved to be non-significant factors. Disease specific survival rates between the three treatment groups were comparable.

Two retrospective studies could be identified comparing TEM to RS. Heintz et al. found in case of a T1 low-risk carcinoma, meaning well to moderately differentiated without lymph

vascular-invasion, TEM resulted in 78% radical excisions (R0).[36] In this subgroup of 46 tumors, after TEM local recurrence rate was four percent compared to three percent after RS for T1 low-risk carcinomas; this difference was not significant. Overall survival rates between both treatment groups were comparable (TEM 79%, RS 81%). In case of a T1 high-risk carcinoma, that is poorly differentiated and/or (lymph-) vessel invasion, using TEM only 58% of tumors could be excised radically (R0). Local recurrence rate after TEM was 33%, compare to 18% after RS. Overall survival rate after TEM was 62%, compared to 69% after RS.

Lee et al. compared TEM with RS for cT1N0 rectal carcinomas, well or moderately differentiated.[37] Local recurrence rates were comparable (TEM four percent, RS zero percent; $p = 0.95$). Also overall and disease-free survival rates were comparable.

There is an abundance of published case series reporting on outcome after TEM for T1 rectal carcinomas.[38-53] (Table 4) Inclusion criteria in these studies are not always clear, and immediate salvage procedures were sometimes performed, thereby possibly introducing a selection bias. In all series TEM is a safe procedure with complication rates varying between 5-26 percent. These complications are almost always minor with re-operation rates between 0-7 percent. Mortality is rare after TEM. All studies have a follow-up duration of more than 24 months and recurrence rates vary between 0-26 percent. If calculated, five years disease specific survival rates after TEM vary between 81-100 percent and overall survival rates range from 73 to100 percent.

PREOPERATIVE TUMOR SELECTION

Although TEM seems to be the method of choice in local excision of T1 rectal carcinomas, local recurrence rates remain high. Can results be further improved by proper tumor selection?

One of the problems encountered is the unexpected finding of a carcinoma in presumed adenomas. This rate can be as high as 34%. A possible solution might be identifying genomic events within the adenoma fraction of a carcinoma, as recently reported by Lips et al.[54, 55] They found specific chromosomal events, gain of 8q22-24, 13q and 20q, and loss of 17p and 18q12-22, to be far more abundant in carcinomas than in adenomas. In adenoma fractions from cases with a carcinoma (infiltrating at least in the submucosa), twice the amount of such 'malignant aberrations' was observed, compared to pure adenomas. Furthermore, combined aberrations such as gain of 13q and loss of 18q were only found in adenomatous fractions of carcinomas and not in benign lesions. Based on these five genomic events associated with carcinoma, a clear distinction between adenoma and carcinoma tissue could be made. Whether these results are clinically relevant, remains to be seen. It seems more relevant to identify tumors suitable for TEM, that is rectal adenomas and T1 rectal carcinomas, which have to be discriminated from T2 or more invasive carcinomas, as these latter have to be treated by radical surgery. Most studies focusing on T-stage, found endorectal ultrasound (ERUS) to be more accurate than conventional

Table 4. Case series of TEM in T1 rectal carcinomas.

Author	Type of study	Inclusion criteria	No.	Comments	LR	OS	DSS
Smith (1996)[50]	retrospective	NS	30	No adjuvant therapy	3/30 (10%)	NS	NS
Mentges (1997)[49]	prospective	G1/2 curative intent (N=60) G3 in selected patients (N=4)	64	No adjuvant therapy	2/52 (4%)	NS	NS
Demartines (2001)[42]	prospective	G1/2, LVI -	9	One pt adjuvant therapy, type NS	1/7 (14%)	NS	NS
De Graaf (2002)[41]	retrospective	NS	21	No adjuvant therapy	2/19 (11%)	NS	NS
Dafnis (2004)[40]	retrospective	NS	10	No adjuvant therapy	1/10 (10%)	NS	NS
Stipa (2004)[52]	retrospective	uT1-T3, < 3 cm	39	Overall 43% of pts pre-/postoperative RT	5/39 (13%)	92%	92%
Duek (2005)[43]	retrospective	G1/2, < 3cm, <10 cm from dentate line, cN0	25	No adjuvant therapy	0/25 (0%)	NS	NS
Endreseth (2005)[44]	retrospective	NS	8	No adjuvant therapy	0/8 (0%)	NS	NS
Floyd (2006)[45]	retrospective	NS	53	No adjuvant therapy	4/53 (8%)	100%	100%
Ganai (2006)[46]	retrospective	NS	21	One pt postoperative CRT	4/21 (19%)	73%	89%
Borschitz (2006)[38]	prospective	pT1	105	21 pts immediate RS No adjuvant therapy	11/84 (13%)	93% (low-risk, R0)	94% (low-risk, R0)
Stipa (2006)[51]	retrospective	uT1/T2, uN0	23	2 pts preoperative CRT 2 pts postoperative RT	2/23 (9%)	91%	91%
Bretagnol (2007)[39]	retrospective	G1/2, < 3cm	31	3 pts immediate RS	3/28 (11%)	79%	81%
Whitehouse (2007)[53]	retrospective	NS	25	2 pts immediate RS Pre-/postoperative CRT not clear	6/23 (26%)	NS	NS
Lezoche (2007)[47]	prospective	uT1N0	51	Pre-/postoperative CRT not mentioned	0/51 (0%)	94%	100%
Maslekar (2007)[48]	prospective	G1/2 en 3	27	No adjuvant therapy	0/27 (0%)	NS	NS

uT/N= presumed T/N-stage based on endorectal ultrasound, pT= T-stage based on histopathological investigation, G1= well differentiated, G2= moderately differentiated, G3= poorly differentiated, NS= not stated, LR= local recurrence, RS= radical surgery, OS= overall survival, DSS= disease specific survival, CRT= chemoradiotherapy, RT= radiotherapy.

computerized tomography (CT) scanning and magnetic resonance imaging (MRI).[56] Whether ERUS has additional value in the preoperative staging of rectal tumors, especially in identifying tumors possibly suitable for TEM, should be addressed properly.

Depth of invasion is not the only criterion in identifying tumors suitable for TEM. Main difference between TEM and radical surgery is the omission of lymph node dissection. In general in T1 rectal carcinomas it is assumed lymph node metastases are present in 4-14% of cases.[57] A more recent study performed by Nascimbeni et al. found invasion in submucosa level 3 (Sm3), lymph vessel invasion and distal rectal carcinomas to be significant contributors to lymph node metastases.[58]

Can we identify, preoperative, tumors already harboring lymph node metastasis? Using single nucleotide polymorphism array analysis of chromosomal instability patterns in rectal tumors, the finding of gain on chromosome 1q might correlate with lymph node metastasis, however validation studies have to be awaited. None of the conventional pre-operative staging methods, ERUS/CT-scan/MRI has yielded satisfactory results upon identifying lymph node metastases. A recent break through was the introduction of MRI-USPIO.[59, 60] Preliminary data show an increased accuracy for nodal status prediction as compared to non-enhanced MRI. However, again further studies have to be awaited.

POSTOPERATIVE TUMOR SELECTION

In most cases based on definite histopathological staging after LE a decision has to be made upon the necessity for immediate salvage surgery. In case additional salvage surgery is performed after LE according to Parks, controversy remains upon outcome.[61, 62] Accepted, although not validated, low-risk criteria in T1 rectal carcinomas, are well to moderate differentiation, carcinomas smaller than three centimetres, without lymph vessel invasion. Above these features, probably excision margin (microscopic radical (R0) versus microscopic irradical (R1)) may be of major importance. Only three studies specifically addressed the outcome after TEM for low- versus high-risk carcinomas. Mentges et al. found recurrence rates after TEM for low-risk carcinomas (n= 52) to be only 3.8 percent; however recurrence rates for high risk carcinomas (n= four) were not given, thereby prohibiting adequate comparison.[49] A retrospective, comparative study was performed by Heintz et al.[36] In low-risk carcinomas (n=46) in 78 percent an R0 excision margin with TEM was obtained, whereas in high-risk carcinomas (n=12) only 58 percent of tumors were excised microscopic radical. Regarding local recurrences, in the low-risk group two carcinomas recurred (four percent) and in the high-risk group four carcinomas (33 percent). All recurrences were after a microscopic irradical (R1) excision. Overall survival rates after TEM for low- and high-risk carcinomas were 79 and 62 percent respectively (p-value not given).

A meticulous evaluation was performed by Borschitz et al, with emphasis on margin of excision.[38] In 105 tumors TEM was performed. Immediate salvage was performed in 21 tumors, for varying reasons. In case a R0 excision was obtained, that is an excision margin of > 1 mm, in low-risk carcinomas recurrence rate was only four percent. In high-risk carcinomas with R0 status, the local recurrence rate was already 20 percent. If the excision margin was < 1 mm, unknown (Rx) or positive (R1), the local recurrence rate after TEM was 46 percent. Immediate radical surgery in case of margin < 1 mm, unknown margin status (Rx) or positive margin (R1), results in local recurrence rates of six percent. Survival rates in low-risk carcinomas, microscopic radically excised are 94 percent and if microscopic irradical excised 57 percent. Immediate radical surgery in irradical excised T1 carcinomas results in survival rates of 93 percent.
In contrast to the above studies, Langer et al. found 24 percent of all TEM specimens to be R1 or Rx, but excision margin status was not of significant influence on developing local recurrences.[26] This unexpected finding was thought to be reflected by inadequate follow up and/or limited patient numbers. All above findings warrant a larger study, specifically addressing the role of histopathological staging in predicting high probability for a local recurrence after TEM for T1 rectal carcinomas.

SALVAGE SURGERY FOR LOCAL RECURRENCES FOLLOWING TEM

Local recurrences in rectal cancer after radical surgery (TME) are considered incurable, with only few patients amenable to salvage surgery. Recurrences after LE seem to be more related to the rectum than to the pelvic wall, as is seen in recurrences after RS. In the literature most series on salvage surgery for local recurrences after LE lack both an adequate number of patients undergoing salvage procedures and adequate follow-up to allow proper analysis. Disease free survival rates following salvage procedures for local recurrences after local excision range between 30-58 percent.[63-66] Moreover, to obtain a R0 resection, extended resections are required, often involving multi-visceral excision. Results after salvage surgery were significantly worse compared to immediate radical surgery in case of adverse histopathological features.[61]
One must realize however that the above series and data are based on local recurrences after LE according to Parks.
In T1 rectal carcinomas local recurrence rates after TEM vary between 0-26 percent. Salvage surgery in case of a local recurrence after TEM seems amenable to most patients, with often a possible R0 resection.[51] However, because of the low number of patients and short duration of follow up, reliable long term results have to be awaited.

FUTURE PERSPECTIVES

Preoperative chemoradiation in rectal carcinomas results in significant downstaging with complete pathological response in approximately 15 percent of advanced rectal carcinomas.[67-71] These figures might even be improved in earlier stages of rectal cancer.[72] If local control is improved by preoperative radiotherapy and preoperative chemoradiotherapy results in sterilizing lymph node metastases, local excision following preoperative chemoradiotherapy might be a logical step. One randomized controlled trial investigating this treatment strategy was performed.[73] Forty patients with histologic proven adenocarcinomas, staged as uT2-N0-M0, G1/2, within six centimeters from the anal verge, were randomized to TEM or laparoscopic TME. Preoperative chemoradiotherapy was given by means of 5,040 cGy in 28 fractions with concomitant 5-fluorouracil infusion (2000 mg/m^2/day). Restaging was performed and patients went on to the planned operation. Surgery was not influenced by preoperative treatment. Local and distant recurrence rates were 10 percent following TEM and 12 percent following laparoscopic TME. Overall survival rates were 95 percent and 83 percent respectively. All differences were not significant. Because this study has several major methodological shortcomings, one has to be cautious to draw any conclusions from this single study.

Another proposed regimen is a rectal sparing treatment after neoadjuvant treatment with clinical complete response.[74] Definite evidence, ideally by means of a randomized controlled trial, has to be awaited and until then this treatment should be considered experimental.

In the near future, special focus of interest will be on non-surgical therapy or local excision of rectal carcinomas following neo-adjuvant chemoradiotherapy. The only series on TEM following neo-adjuvant chemoradiotherapy showed the procedure to be feasible with promising early results. Again however, before definite conclusions can be drawn, larger, randomized studies have to be initiated.

In conclusion, based upon merely retrospective case series, TEM has been incorporated enthusiastically in the surgical armamentarium. Despite the lack of level I evidence, TEM seems justified in well-selected T1 rectal carcinomas. To avoid unjustified use of TEM in rectal carcinomas, using molecular profiling, combined with improved radiological staging modalities, besides node positive tumors, also tumors with a high chance of a local recurrence have to be diagnosed preoperatively. Further area of investigation should be on neo-adjuvant therapies of rectal carcinomas combined with TEM in a randomized setting.

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CHAPTER 2

Aim of the thesis

AIM OF THE THESIS

Local excision, and in particular transanal endoscopic microsurgery (TEM), is increasingly being applied in the treatment of T1 rectal cancer. In several national guidelines this treatment option is incorporated, however several issues have to be addressed before this is justified.

Chapter 1 contains a review of the relevant literature on TEM for T1 rectal cancer.

The aim of the thesis, outlined here in chapter 2, is to define which T1 rectal cancers are suitable for TEM, in order to improve outcome. Besides oncologic outcome, quality of life (QOL) after TEM is studied and compared to after TME. Also, possible improvements regarding tumor selection are explored.

In chapter 3 oncologic outcome after TEM for T1 rectal cancer is studied and directly compared to after TME.

Chapter 4 studies the possible salvage options and outcome in recurrent tumors following TEM for T1 rectal cancer.

Chapter 5 is a study on QOL following TEM and in chapter 6 QOL after TEM is compared to QOL after TME.

In chapter 7 we investigated whether endorectal ultrasound could identify tumors possibly suitable for TEM.

In chapter 8 we performed a study upon tumor analysis in order to identify features suggestive of rectal cancer in (presumed) rectal adenomas.

Chapter 9 contains an analysis of histopathological features, which may be predictive for a local recurrence in T1 rectal cancer, treated solely with TEM.

Chapter 10 contains a summary, which is also given in Dutch in chapter 11.

CHAPTER 3

Transanal endoscopic microsurgery versus total mesorectal excision of T1 rectal adenocarcinomas with curative intention

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Eur J Surg Oncol. 2009 Dec;35(12):1280-1285

INTRODUCTION

In rectal cancer, total mesorectal excision (TME) is the gold standard. This optimised and standardized surgical technique, combined with preoperative radiotherapy, has improved outcome. [1, 2] Counterbalancing this improvement is the high rate of (severe) morbidity and even mortality. [3-6] Local excision of rectal adenocarcinomas is a much safer procedure and transanal excision (TE) is the technique most commonly used. However, transanal endoscopic microsurgery (TEM) is nowadays considered the method of choice. [7] Only in T1 rectal adenocarcinomas TEM is considered adequate if curation is intended.

Quirke showed that standardized processing of resection specimens for rectal adenocarcinomas revealed a higher percentage of incomplete excision, which significantly correlated to an increased risk on both local and distant recurrences and on decreased survival. [8] This resulted in the concept of TME and adjustment of histological examination of the specimen. Although TEM is being implemented in several national guidelines for T1 rectal adenocarcinomas, the role of pathological assessment of the specimen has been limited mainly to basic histopathologic criteria. [9] Excision margin status after both TE and TEM, has been demonstrated to be a predictor for local recurrence, however, this has only been shown in case studies. [10-12] Most studies comparing outcome after local excision for T1 rectal adenocarcinomas with TME do not focus on excision margin status. Moreover, standardized pathological assessment lacks, and this may have caused the varying outcome. [13-18]

As the incidence of T1 and T2 rectal cancer will most likely increase in the near future, because of introduction of population-based screening programs, this warrants a thorough analysis of oncologic outcome following TEM for T1 rectal adenocarcinomas. [19] The aim of this prospective study was to compare the impact of margin status, assessed with standardized pathology after TEM and TME for T1 rectal cancer.

PATIENTS AND METHODS

The Dutch TME trial started in 1996, and 1530 Dutch patients with mobile rectal adenocarcinomas were randomly assigned either to short term preoperative radiotherapy followed by TME or to TME alone. The study protocol included standardized processing of the specimen, described in detail elsewhere. [20] Only T1 rectal adenocarcinomas were considered eligible for this study. In the IJsselland hospital, a tertiary referral centre for TEM and participating in the Dutch TME trial, patients with T1 rectal adenocarcinomas were also deemed feasible for TEM. Selection was based upon the same study protocol, with complementary rigid rectoscopy and endorectal ultrasound (ERUS). Eligibility for the current study was in accordance with the Dutch TME trial protocol with some exceptions. Patients who underwent TME and had synchronous distant metastases, only discovered at laparotomy, were not excluded, because if TEM had

been therapy of choice, metastases would not have been disclosed. Furthermore, patients who previously underwent pelvic operations or resections of left-sided large bowel or rectum were not excluded. For TEM patients World Health Organisation Performance Score (WPS) was not a criterion (in the Dutch TME trial WPS limited to 2 or less was an inclusion criterion). TEM patients were only eligible if there were no signs of lymph node metastases on MRI and/or ERUS and excision margins were negative.

If T1 rectal cancer only emerged at histology of the excised specimen following TEM, patients were offered follow-up only or immediate additional TME. In case excision margins were positive following TEM, patients also were offered immediate TME or intensive follow up after repeat TEM, in order to obtain negative excision margins. The TEM technique is described in detail elsewhere. [21] Tumor size after TEM as well as TME was assigned as the largest diameter. TEM specimens were pinned on a corkboard before fixation. Fixation, serial transverse slicing, embedding, staining, sectioning and examination of the specimens were done according to descriptions detailed elsewhere. [8, 20]

Both groups were followed according to the Dutch TME trial protocol. Moreover, rigid rectoscopy and endorectal ultrasound were performed at every visit except for the colonoscopy visit in the TEM patients. Endpoints studied were morbidity, mortality, margin status, local recurrence, distant recurrence, overall survival and cancer specific survival. Local recurrence was defined as evidence of a tumor within the lesser pelvis. Distant recurrence was defined as evidence of a tumor in any other area. In all patients in this study informed consent had been obtained.

Data were analyzed with SPSS statistical software (version 14.0 for Windows, SPSS, Chicago). Chi-square tests were used to compare proportions. Mann-Whitney tests were used to compare continue variables. Univariate analyses of cumulative probability of local and distal recurrence, as well as overall and cancer-specific survival were carried out by the Kaplan-Meier method, and the evaluation of differences between the two groups was performed with the log-rank test. The Cox proportional hazards model was used to calculate hazard ratios and 95% confidence intervals in the univariate and multivariate analyses. A two-sided p-value of 0.05 or less indicated statistical significance.

RESULTS

Of the 1530 Dutch patients entered in the TME trial, a total of 76 patients with T1 rectal adenocarcinomas were present (5%). One patient was excluded because of a second malignancy. Seventy-five patients were eligible for this study. In 1 patient excision margin was positive (1.3%). In 86 patients TEM was performed for T1 rectal adenocarcinomas. In 5 patients excision margins were positive (5.8%). Six patients, including 2 patients with incomplete margins, chose for additional TME and were excluded. Eighty patients were entered in the study, including the remaining 3 patients with initial positive excision margins. TEM was repeated in these patients,

no residual tumor tissue was found and excision margin was considered negative. Patient, tumor and operation characteristics are depicted in Table 1. Both groups were comparable, except that TEM patients had higher WPS pre-operatively ($p < 0.001$).

TEM proved to be safer compared to TME reflected by operating time, blood loss, hospital stay, morbidity, re-operations and stoma formation (all $p < 0.001$). Complications after TEM were present in 5 patients (5.8%). In three patients a urinary tract infection occurred, and one patient with a cardiac history suffered from cardiac pain and dysrythmia leading to medical treatment on the coronary care unit. In one patient, following a segmental resection, anastomotic stenosis with disabling complaints occurred. Hegar dilation proved unsuccesful, necessitating renewed TEM for correction. Histopathologic evaluation only showed fibrosis. After TME, 48 patients suffered from 72 complications (64%). The majority was severe, necessitating re-operations in 13.3% of all patients (anastomotic leakage 6.9%, re-bleeding 9.3% and ileus 6.7%). In 58 patients a primary anastomosis was constructed, with a diverting ileostomy in 44. In two patients a Hartmann's procedure was performed and in 15 patients an abdomino-perineal excision. A stoma was constructed during re-operation in another 2 patients. Ten out of 44 diverting ileostomies have never been reversed and in 5 patients after reversal again a stoma was constructed resulting in 43% of the TME patients having a definite stoma at the time of evaluation. Following TEM, five (6%) patients had a colostomy, because of a local recurrence necessitating salvage surgery. (Table 2) There was no mortality after TEM, and after TME 4% of patients died ($p = 0.07$). Median follow-up after TEM was 42 months (range, 1-127) and after TME 84 months (range, 30-115). Local recurrence rate was 24% after TEM compared to 0% after TME patients ($p < 0.0001$; Figure 1). Details of local and distant recurrences following TEM and TME are given in Table 2. After TEM 15 local recurrences were observed of which 13 were diagnosed within the first 18 months (86.7%). Median time to local recurrence was 10 months (range, 5-50). In 12 patients (80%) salvage surgery was performed, limited to TME, without mortality and without renewed local recurrences.

Distant metastases developed in 6 patients. None of the TEM patients without local recurrence developed distant metastases or died cancer-related. After TME 6 patients developed distant recurrences. Overall survival was 75% after TEM and 77% after TME ($p = 0.9$; Figure 2). Cancer specific survival was 90% after TEM and 87% after TME ($p = 0.5$; Figure 3). In regard to both overall survival and cancer-specific survival, neither surgical technique used, age, gender or WPS were risk factors.

DISCUSSION

After TME for rectal adenocarcinomas, morbidity varies from 10 to 62%, and mortality varies from 3.3 to 25.8%. [1-6] Morbidity is often severe, especially if preoperative radiotherapy is added. Long-term functional outcome is poor, having major impact on quality of life. Reduced

Table 1. Patient-, tumor- and operation characteristics of the patients enrolled in the study.

	TEM	TME	
Number of patients	80	75	
Age (yrs)	71 (44-92)	67 (48-83)	ns
Female: male	32: 48	27: 48	ns
WPS 0: 1: 2/3	42: 18: 20	60: 14: 0	p < 0.001
Tumor diameter (cm)	3.0 (0.5-13)	2.5 (0.5-7.5)	ns
Tumor distance from dentate line (cm)	8.0 (0-15)	7.0 (0-15)	
0-5	17	14	ns
5-10	44	34	
10-15	18	25	
Operating time (min)	40 (10-125)	180 (70-360)	p < 0.001
Blood loss (ml)	0 (0-250)	1000 (50-15000)	p < 0.001
Hospital stay (days)	3 (2-13)	14 (7-121)	p < 0.001
Morbidity (%)	5 (5.1)	48 (64)	
-surgical complications			
-abdominal wound dehiscence	0	1 (1.3)	
-perineal wound dehiscence	0	1 (1.3)	
-intestinal necrosis	0	1 (1.3)	
-ileus	0	5 (6.7)	
-anastomotic leakage	0	4 (6.9)	
-re-bleeding	0	7 (9.3)	
-other	1 (1.2)	3 (4)	
-infections			
-abdominal wound	0	8 (10.7)	
-perineal wound	0	2 (2.7)	
-urinary tract	3 (3.4)	10 (13.3)	All p < 0.001
-intra-abdominal abscess	0	2 (2.6)	
-sepsis	0	4 (5.3)	
-other	0	2 (2.6)	
-febris e causa ignota	0	1 (1.3)	
-general complications			
-venous thrombosis	0	1 (1.3)	
-pulmonary	0	6 (8)	
-embolism	0	3 (4)	
-cardiac	1 (1.2)	2 (2.6)	
-other	0	7 (9.3)	
-delirium	0	1 (1.3)	
-multi organ failure	0	1 (1.3)	
Re-operations (%)	1 (1.2)	10 (13.3)	p < 0.001
Stoma formation (%)	0	61 (81.3)	
-at first operation	0	59 (78.7)	p < 0.001
-at re-operation	0	2 (2.6)	
Mortality (%)	0	3 (4.0)	P = 0.07

WPS = World Health Organization Performance Score; data given are numbers or medians with ranges between parentheses. Morbidity = number of patients with one or more complications.

morbidity and mortality is often the motive for local excision in rectal adenocarcinomas. Morbidity is predominantly minor, occasionally leading to re-operation and formation of a stoma and without functional disorders having impact on quality of life. [22] Morbidity and mortality in

Table 2. Characteristics of local and distant recurrences after TEM or TME for T1 rectal cancer.

Primary surgery	LR	LR-free interval (months)	Salvage therapy	pTNM (salvage surgery)	R0 vs. other	DR	Interval (months)	FU (months)	Survival status
TEM	Yes	5	LAR	pT3N0	R0	-	-	16	Alive
TEM	Yes	5	APR	pT2N0	R0	-	-	34	DNCR
TEM	Yes	6	APR	pT2N0	R0	-	-	33	DNCR
TEM	Yes	7	LAR	pT2N0	R0	-	-	69	Alive
TEM	Yes	10	APR	pT3N0	R0	-	-	69	Alive
TEM	Yes	10	LAR	pT3N0	R0	-	-	16	Alive
TEM	Yes	11	LAR	pT3N1	R0	-	-	19	Alive
TEM	Yes	12	LAR	pT3N0	R0	-	-	20	Alive
TEM	Yes	40	CTh,APR	pT0N0	R0	-	-	49	Alive
TEM	Yes	5	LAR	pT3N0	R0	Liver,lung	5	13	DCR
TEM	Yes	12	LAR, CTh	pT3N2	R1	Liver	27	39	DCR
TEM	Yes	19	Hp	pT2N0	R0	Liver	19	40	DCR
TEM	Yes	5	None	cT3	-	Liver	5	15	DCR
TEM	Yes	20	CTh	cT4	-	Liver	22	30	DCR
TEM	Yes	50	CTh	cT4	-	Lung	50	52	Alive
TME	No	-	-	-	-	Skin	5	7	DCR
TME	No	-	-	-	-	Peritoneal	0	20	DCR
TME	No	-	-	-	-	Liver, bone	28	29	DCR
TME	No	-	-	-	-	Liver, lung, brain	29	34	DCR
TME	No	-	-	-	-	Liver	23	39	DCR
TME	No	-	-	-	-	Lung	16	57	DCR

APR= abdomino-perineal resection; AR= anterior resection; Cth= chemotherapy; Hp= Hartmann's procedure; - = not applicable; p= pathological; c= clinical; R0= microscopic radical; R1= microscopic irradical; DCR= died cancer-related; DNCR= died not cancer-related.

this study are in line with literature and again demonstrate the safety of TEM and the consequences of TME. This is even reinforced by the fact TEM patients had worse WPS compared to TME patients.

However, morbidity should not be the main endpoint measured when choosing between two operation techniques for rectal adenocarcinomas. After local excision of rectal adenocarcinomas, outcome varies strikingly, even when limited to T1 rectal adenocarcinomas. As a result, it is looked at with caution and most authors emphasize its adoption only in carefully selected patients. [23]

Microscopic radical excision is a prerequisite to diminish recurrences after TME for rectal cancer. [8] Standardized histological examination revealed a higher percentage of incomplete resection with significant correlation to an increased risk on both local and distant recurrences and on decreased survival. This resulted in the concept of TME and adjustment of histological examination of the TME specimen. Excision margin status after local excision is also a significant prognostic factor. In 1990 Graham concluded that after local excision positive excision margins were

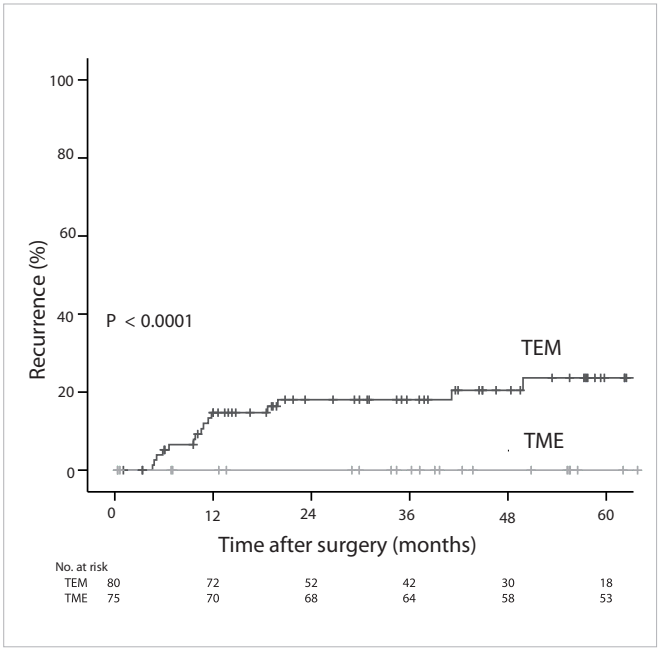

Figure 1. Local recurrence rates after TEM and TME for T1 rectal cancer.

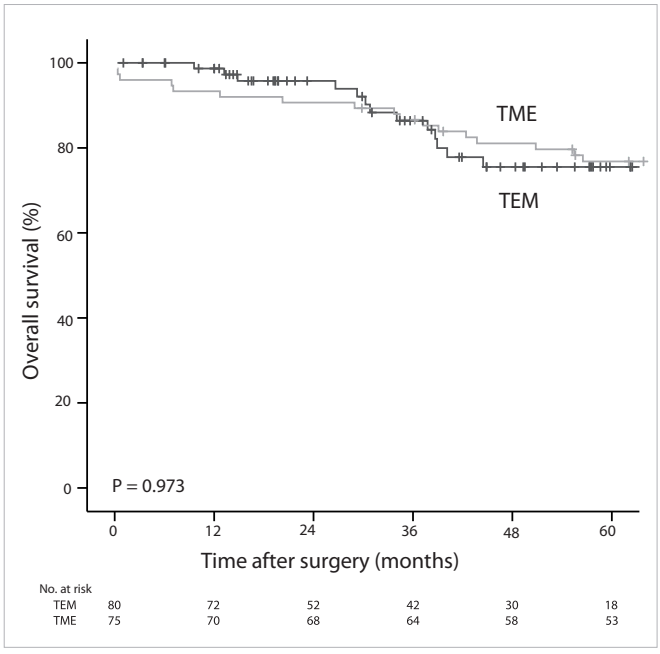

Figure 2. Overall survival after TEM and TME for T1 rectal cancer.

associated with increased local recurrence rates and decreased survival. [11] Also in case studies on TEM, excision margin status has proven to be a predictor for recurrence. [10, 12] However, comparative studies, focusing on TE or TEM and TME for T1 rectal adenocarcinomas, are subject to possible bias as patient selection criteria and (neo-) adjuvant strategies are not elucidated. Furthermore, the method of histological investigation remains unclear and the presence of incomplete or doubtful margins was not an exclusion criterion.

Unprotocollized histopathologic evaluation leads to underestimation of positive excision margins. [12, 24] With TEM, even with standardized histopathologic evaluation, negative excision margins can be obtained in over 90% of specimens. [25] This may be one of the most contributing factors to improved oncologic outcome following TEM, compared to after TE. [16] This hypothesis warranted the current study.

Regarding survival, we found that if negative excision margins are confirmed by thorough, protocollized histopathologic evaluation, no differences between TEM or TME occurred. This is in line with all other comparative studies of TEM and TME. [16-18] Following TME never a local recurrence occurred, and after TEM, despite a 100% negative excision margin status, local recurrence rate was 24%. This is higher than the 4.1 to 10% observed by other TEM centres and even higher to the 4 to 18% after TE. A possible explanation for this result has yet to be clarified.

Focussing on prevention of local recurrence after local excision of rectal cancer is caused by the fact that local recurrences after radical excision are difficult to treat with many renewed local recurrences and poor prognosis. [26] Literature on salvage surgery for local recurrence after local excision is limited. Most series lack both an adequate number of patients undergoing salvage procedures and adequate follow-up to allow proper analysis. It only concerns local recurrences following TE as technique used. [27, 28] Disease free survival rates following salvage procedures range between 30-58%. Moreover, to obtain a R0 resection, extended resections are required, often involving multivisceral excision. Results after salvage surgery are worse compared to after immediate salvage surgery in case of adverse histopathologic features. [10, 24, 29] Salvage surgery in case of a local recurrence following TEM seems amenable to most patients, with often a possible R0 resection. In this study, of 15 local recurrences 12 were amenable to salvage surgery (80%), of which in 11 (92%) a R0 resection could be obtained by performing a TME. Maybe the elegant and precise technique of TEM is the key element for these results. Or perhaps it was the early detection due to the intensive follow-up. About 90 per cent of recurrences were diagnosed within 18 months. Moreover, about 25% of the local recurrences were diagnosed only with endorectal ultrasound as described by others. [30]

In conclusion, TEM is a safer procedure than TME for T1 rectal adenocarcinomas. Despite obtaining a negative excision margin status, local recurrence rate is still unacceptably high and efforts should be made to investigate prognostic factors. Survival rates are comparable after TEM and TME, although long-term results have to be awaited. Salvage surgery for local recurrences is possible, however future studies are needed to spare as many patients as possible from the adverse effects of TME.

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CHAPTER 4

Treatment of recurrences after transanal endoscopic microsurgery for T1 rectal cancer

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In press Dis Colon Rectum

INTRODUCTION

Total mesorectal excision (TME) is the gold standard for rectal cancer, because this treatment modality offers the highest chance of cure. This standardised and optimised surgical technique has lowered the recurrence rates and improved survival.[1,2] In an attempt to avoid the substantial morbidity and mortality of TME, local excision has been suggested a therapeutic option in the treatment of well-selected patients with early rectal cancer.[3,4] But, after transanal excision unacceptable high rates of incomplete tumor removal in up to 39 percent have been observed, proven to be a key predictor for recurrence.[5-9]

Transanal endoscopic microsurgery (TEM), introduced by Buess et al.[10], is an optimised technique for the local excision of rectal tumors, which enables excellent access and visualization of the surgical field and allows precise and full-thickness excision of the tumor. Using TEM, the rate of microscopic negative excision margins (R0), even with standardised pathology, for T1 tumors exceeds 90%.[11,12] Because of this latter, in combination with the very low mortality and morbidity rates, TEM is nowadays considered a potential curative treatment of T1 rectal cancer.[5,13,14]

However, even after TEM local recurrence rates range from 0 to 24%, and the results of salvage surgery for recurrent tumors are matters of concern. [15-17] In literature, only salvage surgery after transanal excision of T1 rectal cancer is addressed. [18-20] To our knowledge no data exist on patients treated for a recurrence after TEM surgery and in this study we present the treatment possibilities and outcome of patients with a local recurrence after TEM for T1 rectal cancer.

PATIENTS AND METHODS

From 1996, in the IJsselland hospital, a referral centre for TEM, 88 consecutive patients underwent TEM for pT1 rectal cancer and were followed as part of a prospective, comparative study. As described previously all patients were screened according to a standard protocol.[21] The initial TEM procedure was performed by two surgeons. A full-thickness excision was performed in all lesions, and in all tumors a microscopic negative excision margin of 2 millimetres or more was obtained (R0), as shown by protocollized pathology. None of the patients received any form of (neo-) adjuvant treatment. Follow-up was according to the Dutch guidelines on rectal cancer with additional rigid rectoscopy and endorectal ultrasound (ERUS) every 3 months the first 2 years, and every 6 months thereafter for the detection of local recurrences. During the last two years of the study period magnetic resonance imaging (MRI) of the lesser pelvis was introduced as a part of the follow-up as well, and nowadays is routinely performed at 12, 24 and 36 months. A local recurrence was defined as recurrent tumorous tissue within the lesser pelvis and endoluminal, if present, within the proximity of the scar tissue of the initial operation. All recurrences were histologically proven and when appropriate, salvage surgery was performed. Initially patients were treated without neo-adjuvant treatment (five patients), later on with

preoperative short-course radiotherapy (six patients) and nowadays with preoperative long-course chemoradiotherapy (five patients).
Following salvage surgery, patients were followed according to the Dutch guidelines for rectal cancer. Patient data were collected in a central, digital database. Patient survival was assessed using the Kaplan–Meier life-table method.

RESULTS

Out of 88 patients followed, in 18 patients a local recurrence occurred. Patient and primary tumor characteristics are depicted in Table 1 and 2. Only three tumors primarily harboured accepted high-risk features, which are poor differentiation and/or (lymph-) vessel invasion. All others were so-called low-risk tumors. Besides these features, of 16 tumors with known submucosal invasion depth, six invaded the deep part of the submucosa (Sm3).
Median age of patients at the time of recurrence was 74 years (range, 56-84), 50% of the patients were male. Median time to a local recurrence after the initial TEM procedure was 10 months (range, 4-50). At regular follow-up visits, ten recurrences were found intra-luminal and six patients extra-luminal, only visible with ERUS. In two patients recurrences were detected only with MRI. The first patient (patient number 13) refused intensive follow-up, and presented one year later with lower back pain. MRI showed a locally advanced (cT4) recurrence. The

Table 1. Patient- and initial tumor characteristics.

Patient number	Age (years)	Sex	ASA-classification	Interval between TEM and local recurrence (months)
1	74	female	2	10
2	83	female	3	6
3	79	male	3	19
4	82	male	3	5
5	77	female	3	7
6	72	female	1	20
7	68	male	3	5
8	61	male	3	12
9	84	female	2	11
10	56	male	1	6
11	80	male	2	11
12	71	female	1	12
13	75	male	3	41
14	72	female	1	10
15	64	male	1	50
16	80	female	1	24
17	73	female	2	4
18	59	male	1	7

ASA= American Society of Anaesthesiologists.

Table 2. Initial tumor characteristics at TEM operation.

Patient number	Entire tumor area (cm^2)	Invasive carcinoma size (mm)	Differentiation grade	LVI	BVI	Sm classification
1	52.00	0.3	Moderate	No	No	Superficial
2	7.50	6	Moderate	No	No	Deep
3	36.00	17	Moderate	No	Yes	Superficial
4	56.25	1.8	Moderate	No	No	Deep
5	2.25	8	Good	No	No	Deep
6	12.00	5	Moderate	No	No	Superficial
7	14.00	17	Moderate	No	Yes	Superficial
8	49.00	6	Poor	Yes	Yes	Superficial
9	63.00	5	Moderate	No	No	Superficial
10	49.00	15	Moderate	No	No	Deep
11	42.00	10	Moderate	No	No	Superficial
12	7.50	6	Moderate	No	No	Deep
13	17.50	10	Moderate	No	No	Deep
14	52.00	15	Moderate	No	No	Superficial
15	10.00	10	Moderate	No	No	Superficial
16	3.00	10	Moderate	No	No	Superficial
17	5.00	16	Moderate	No	No	Unknown
18	27.50	5	Moderate	No	No	Unknown

LVI= lymph vessel invasion; BVI= blood vessel invasion; Sm= submucosal invasion depth; superficial= Sm 1+2; deep= Sm3.

second patients (patients number 15) had complaints in between two (intensive) follow-up visits and MRI showed as well a locally advanced local recurrence pre-sacral. MRI was not part of the intensive follow-up protocol at that time. Most probably the recurrence was missed at rectoscopy and ERUS. Following neo-adjuvant chemoradiotherapy a microscopic negative excision margin was obtained in both.

Salvage surgery characteristics are given in Table 3.Two patients were not operated. Patient number 6 withdrew from intensive follow-up and presented elsewhere with low back pain 20 months after the TEM procedure. A clinical T4 local recurrence was found with synchronous metastatic disease in the liver. Palliative chemotherapy was started, and the patient died ten months later.

In patient number 9, preoperative work-up failed to diagnose a T1 rectal cancer. For unclear reasons, postoperative additional investigations, focusing on metastatic disease, were not performed. Six months after the TEM procedure already a local recurrence was suspected, which could only be confirmed half a year later, after repeated biopsies. At the time of diagnosis, massive hepatic metastases causing liver failure were found and she died three months later.

Salvage surgery was performed in 16 patients. In two patients (patient number 3 and 7) synchronous liver metastases, initially deemed resectable, were found. Despite obtaining a

Table 3. Salvage and survival characteristics.

Patient number	Type of salvage surgery	Neoadjuvant therapy	TNM	R0 vs R1	DM/other	Adjuvant therapy	FU duration (months)	Survival status
1	APR	none	pT3N0M0	R0	-	-	112	Alive
2	APR	none	pT2N1M0	R0	-	-	32	DNCR
3	HP	5x5	pT3N2M1	R0	Liver	-	22	DCR
4	APR	none	pT2N0M0	R0	-	-	28	DNCR
5	LAR	5x5	pT3N0M0	R0	-	-	92	Alive
6	None	none	cT4NxM1	-	Liver	Palliative ChT	10	DCR
7	APR	5x5	pT3N0M1	R0	Liver, lung	-	7	DCR
8	LAR	5x5	pT3N2M0	R1	Liver	ChT	13	DCR
9	None	none	cT3NxM1	-	Liver	Palliative ChT	3	DCR
10	LAR	5x5	pT3N0M0	R0	-	-	31	Alive
11	LAR	5x5	pT3N1M0	R0	Lung, re-LR	-	27	DCR
12	LAR	none	pT3N0M0	R0	-	-	25	Alive
13	APR	CRT	pT0N0M0	R0	-	-	27	Alive
14	LAR	none	pT3N0M0	R0	-	-	20	Alive
15	APR	CRT	pT3N0M0	R0	-	-	18	Alive
16	LAR	CRT	pT3N2M0	R0	Liver	-	8	DCR
17	LAR	CRT	pTisN1M0	R0	-	-	6	Alive
18	LAR	CRT	pT0N1M0	R0	-	-	2	Alive

APR= abdomino-perineal resection; LAR= low anterior resection; HP= Hartmann's procedure; TNM= tumor node metastasis classification; R0= microscopic negative excision margin, R1= microscopic positive excision margin; DM= distant metastasis; FU= follow-up; 5x5= short-course radiotherapy, 5 times 5 Gray; CRT= chemoradiotherapy; ChT= chemotherapy; DNCR= died non-cancer related; DCR= died cancer related; re-LR= renewed local recurrence.

microscopic negative excision margin in both, rapidly progressive metastatic disease developed and patients were treated with palliative chemotherapy. They died seven and respectively 22 months following the salvage procedure.

In 15 out of 16 salvage procedures, a microscopic negative excision margin was obtained, without the need for multivisceral resections. In one patient a microscopic positive excision margin (R1) was obtained, and adjuvant chemotherapy was administered. There was no postoperative mortality. Median follow up after salvage treatment was 20 months (range, 2 – 112). One of the operated patients developed a local re-recurrence and 7 patients developed distant metastases and died because of progressive disease.

The overall survival after salvage surgery at 1 year was 79% and at 3 years only 31%. (Figure 1) Since there were two non-cancer related deaths (patient 2 and 4) the cancer related survival at one year was 79% and at 3 years 58%. (Figure 2)

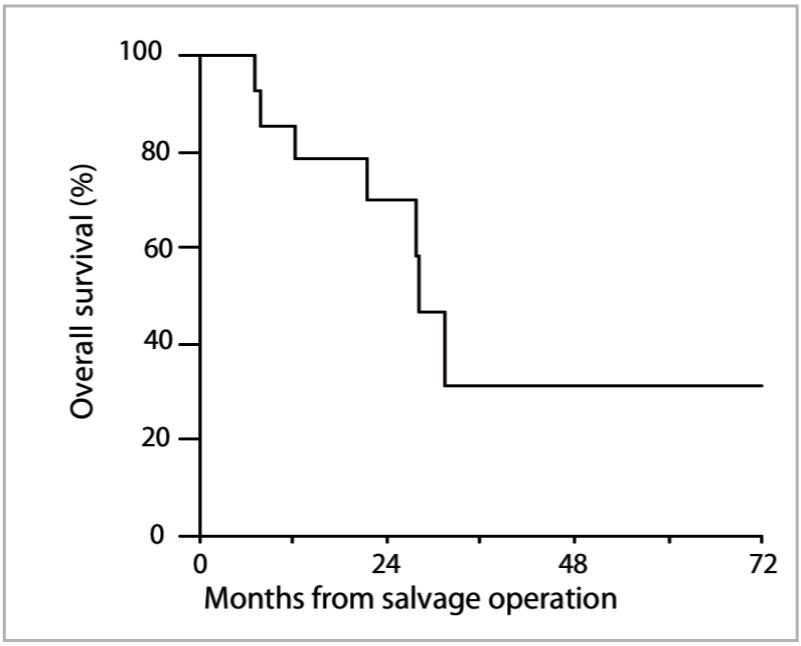

Figure 1. Overall survival following salvage operation.

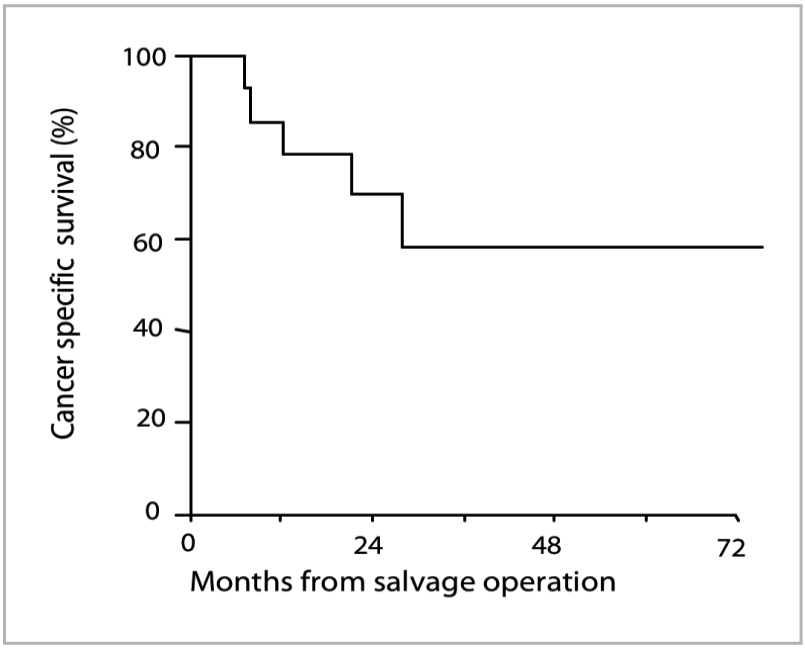

Figure 2. Cancer-related survival following salvage operation.

DISCUSSION

Transanal endoscopic microsurgery (TEM) is the method of choice in the treatment of rectal adenomas. Morbidity as well as mortality is extremely low compared to total mesorectal excision (TME). [21] However, when considering local excision a curative option in rectal cancer for fit patients, surgeons face a dilemma. Although, a large majority (70-85%) of patients can be cured by TEM, the risk of cancer recurrence is substantially high, varying between 10% and 28% for pT1 rectal cancer.[2,22-24] Low recurrence rates of only 0.4 to 1.7% are reported after TME.[2,25,26] In most studies reporting on local recurrences following local excision for T1 rectal cancers, there is a bias in patient and tumor selection. This is a major confounding factor when interpreting outcome. In the present series, all T1 rectal cancers excised with TEM were included, provided a microscopic negative excision margin (R0) was confirmed at pathological examination. In our series obtaining a R0 excision did not prevent from a local recurrence. Therefore, improving tumor selection is of major importance. Whether basic histopathologic criteria, differentiating high- and low-risk T1 rectal cancers, are able to perform this, is subject of debate. [27-30] In our series only three T1 cancers initially exhibited so-called high-risk features (poor differentiation and/or (lymph-)vascular invasion). Furthermore, five tumors deeply invaded the submucosa (Sm3), which may also be a predictive feature for lymph node metastasis. In those nine presumed high-risk tumors, always salvage surgery was possible with only in one a microscopic positive excision margin (patient 8), whereas in nine primarily low-risk tumors in seven a salvage procedure was performed. The fifty percent rate of high-risk tumors seems high, however when reviewing our TEM specimens, of all T1 rectal cancers treated solely with TEM that did not recur, also 50 percent of tumors exhibited one or more of the accepted high-risk features. Because of the low number of patients, conclusions regarding high-risk features and the biological behaviour of those tumors are inappropriate. Larger studies focusing on adequate tumor selection are therefore urgently needed.

Local recurrences may present as an intra-luminal or extra-luminal rectal mass. Therefore, next to rectoscopy, additional diagnostic tools seem mandatory in the follow-up regimen in patients treated with TEM for T1 rectal cancer. In our series, six out of 18 local recurrences were solely found with ERUS, which otherwise may have been missed. This finding is confirmed by other series focusing on the role of ERUS in the follow-up regimen of locally excised rectal cancers.[31] However, ERUS still has its limitations. In one of the two patients with a late recurrence in our series, one was missed with rectoscopy and ERUS. Therefore, since then MRI of the lesser pelvis is added as well in the follow-up protocol in our hospital. By applying this intensive follow-up regimen in our patients, only one out of 16 patients who adhered to this protocol, was diagnosed at an advanced, incurable stage. In the remaining patients, almost always a R0 resection was possible (94% R0).

This is the first series, to our knowledge, reporting on outcome of local recurrent disease following TEM for T1 rectal cancer. However, comparison of the results of salvage surgery after TEM

and transanal excision is extremely difficult. For instance, in the studies by Friel et al. and Weiser et al, both T1 and T2 rectal cancers were initially included, whereas in the present series only T1 rectal cancers were deemed feasible for TEM.[27, 28] In the study from Minnesota salvage surgery was considered curative in 79 percent of cases. With a mean follow-up of 39 months disease free survival rate was 59%, with 17 percent renewed local recurrences. In the study reported by the group of MSKCC in 98 percent of patients a potential curative resection was possible, however in 55 percent of procedures a multivisceral resection was necessary. Five-year disease specific survival in this series was 53 percent. In our series of 16 salvage procedures, in 15 it was potentially curative (94 percent), and never a multivisceral resection was necessary. With a median follow-up of 20 months following salvage surgery, only one renewed local recurrence occurred. Overall survival at three years was only 31 percent and disease specific survival 58 percent. These results do not seem better to those after failed transanal excision, however in the present series also two patients with incurable disease at the time of diagnosis are included, whereas in the other series only results after salvage procedures are given and they may have excluded patients with incurable disease, which may worsen results. Therefore, a clear comparison between outcome after failed TEM and transanal excision for T1 rectal cancer is impossible. Obtaining a microscopic negative excision margin is a prerequisite, however does not seem to be the main problem in the present series. The substantial proportion of patients (39%) that eventually was diagnosed with metastatic disease after the salvage operation is striking. Of the original 88 patients never metastatic disease occurred in the absence of a local recurrence. An explanation could be that local recurrences after TEM for T1 rectal cancer represent a different biological group, in which salvage treatment should be intensified. Besides neo-adjuvant treatment, adding adjuvant treatment in patients with a local recurrent tumour might improve outcome.

In conclusion, recurrent disease after TEM for T1 rectal cancer is a major problem. Salvage surgery for achieving local control is feasible in most of the patients, without the need for multivisceral resections. This may be attributable to intensive follow-up. However, survival is limited, mainly due to distant metastases. Tailoring selection of T1 rectal cancers and exploring possible adjuvant treatment strategies following salvage procedures should be the next steps, in order to improve survival.

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CHAPTER 5

Impact of transanal endoscopic microsurgery on quality of life and functional outcome

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Int J Colorectal Disease 2008 (Jul);23:709-713

INTRODUCTION

Local excision of benign rectal tumors is safer compared to radical surgery and considered treatment of choice. [1-3] Several techniques have been developed for local excision, with the transanal technique according to Parks as the most frequently used. [1, 4] Other techniques used are the dorsal trans-sacral approach (Kraske) and the dorsal trans-sphincteric approach (York-Mason). [5-9] Each procedure has its own (dis-) advantages, and none of the procedures mentioned is able to achieve local excision of tumors throughout the entire rectum. Transanal endoscopic microsurgery (TEM) demonstrated to be a safe procedure capable of overcoming this shortcoming. In early publications even distal sigmoid tumors could be locally excised with excellent results. Moreover, recurrence rates are minimal compared to other local techniques. As a result the indication for local excision of rectal tumors has expanded dramatically. [10-13]

Few studies have addressed functional outcome following TEM, and with the operation rectoscope with a length of 12 or 20 centimetres and a diameter of 40 mm, scepticism towards postoperative faecal continence remains. In manometric studies after TEM there seems to be a temporary detrimental impact on internal sphincter functioning, although without clinical significance. [14-16]

Cataldo et al. recently performed a prospective study on faecal continence and incontinence-specific quality of life after TEM, using standardized surveys. [17] They stated TEM does not result in significant alterations. These results are promising, especially with a relative short duration of follow-up of six weeks in this study. As known from other types of rectal surgery, incidence of faecal incontinence diminishes with time. [18] This could imply results after TEM may even improve with longer follow-up.

Quality of life is increasingly recognised as the ultimate endpoint when assessing clinical outcomes after different surgical interventions because it measures the patient's perspective. The precise impact of the TEM procedure on quality of life has not been well studied. This prospective study was set out to provide a comprehensive insight into the impact of TEM on functional outcome and quality of life.

PATIENTS AND METHODS

Between January 2004 and January 2006, a consecutive series of fifty patients were referred for a TEM procedure. All patients were evaluated preoperatively according to a standard protocol including rigid rectoscopy, tumor biopsy and endorectal ultrasound. If TEM was considered feasible patients were eligible for this study. Informed consent had to be given before inclusion. Local medical ethical committees approved this study. Always a full-thickness excision was performed. The portion of the tumor located within the sphincter musculature was excised partial thickness. Before and at least six months after the TEM procedure patients were asked to fill out

a questionnaire to assess anorectal functioning and quality of life. All data were collected by an independent research coordinator, not previously involved in the patients' care. We recorded the demographics, operative details, postoperative length of stay, postoperative complications and functional outcome for each participant. We evaluated functional outcome by means of a detailed questionnaire based on the Faecal Incontinence Severity Index (FISI) [19]. This system, developed by Rockwood, uses two basic components: the type of incontinence and its frequency. FISI scores range from zero (total continence) to 61 (complete incontinence to solid stool on a daily basis). We used the validated weighting scores that are based on patients input. Quality of life was evaluated using both the EuroQol EQ-5D and the Faecal Incontinence Quality of Life (FIQL) score. The EuroQol EQ-5D consists of a so-called Index score representing the societal value of the health state, and has a scale ranging from 0 (no quality of life) to 100 (optimal quality of life). The EuroQol EQ-5D also uses a visual analogue scale, the EQ-VAS, representing the patient perspective. This scale ranges from 0 (no quality of life) to 100 (optimal quality of life). The EuroQoL EQ-5D scores were compared with a sex- and age-matched, community based sample of healthy persons without co-morbidity.[20] The FIQL score as described by Rockwood et al. measures specific quality of life issues, expected to affect patients with faecal incontinence. [21] This instrument is composed of 29 questions within 4 domains: lifestyle issues, coping/behavior, depression and self-perception, and embarrassment. The scores in the FIQL range from a minimum score of 1 to a maximum of 4, for all of the scales (1= quality of life alteration present most of the time, 4= none of the time). Data are presented as medians and standard deviations. Changes within groups were evaluated using the nonparametric one-sample Wilcoxon's signed-rank test. Comparison of these changes between groups was conducted using the Mann-Witney U test. The Spearman`s correlation coefficient was used for correlation between the different findings. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

Fifty patients were eligible and informed consent was obtained. Three patients were excluded. In two patients TEM could not be performed because of bulky tumor or technical problems. An additional patient underwent low anterior resection for locally recurrent disease within six months of the TEM. The remaining forty-seven patients completed both questionnaires and were included for analysis. All of these patients were alive without evidence of recurrent disease. The group consisted of 22 males and 25 females. Median age was 67 years (range 40-84). Preoperative diagnosis was villous adenoma in all patients. Median distance from the distal tumor margin to the dentate line was 7 (range 0–15) cm and median tumor size 20 (range 4–53) cm^2. The median rate of captured circumference of the rectal wall was 40 percent (range 5-80). (Table 1) Median operative time, defined as beginning when the rectoscope was inserted and ending when it was removed, was 55 minutes (range 10–140). Complications developed in 4 of 47 (8.5 percent)

Table 1. Patient, tumor and procedure related characteristics.

Number of patients	47
Male/female	22/25
Median age in years (range)	67 (40-84)
Median distance from dentate line in cm`s (range)	7 (0-15)
Median tumor size in cm^2 (range)	20 (4-53)
Median capture of circumference of rectal wall in % (range)	40 (5-80)
Median duration of operation in minutes (range)	55 (10-140)
Complications	4/47 (8,5%)
Urinary retention	2
Urinary tract infection	1
Bloodtransfusion	1
Reoperations	None
Length of hospital stay in days (range)	4 (3-9)

patients. Two patients had urinary retention, one patient a urinary tract infection and one suffered from a low hemoglobin rate requiring blood transfusion. No reoperations were necessary and mortality rate was zero. Median length of stay was 4 days (range 3-9). (Table 1)

Definite histopathological examination of the resected specimens revealed an adenoma in 44 patients and an invasive carcinoma in 3 patients (pT1 in two patients and pT2 in one patient). These three patients were reluctant to major surgery and were observed with rectoscopy and endorectal ultrasound every three months without signs of recurrence at six months after TEM. In three adenomas excisional margin was considered microscopic irradical, resulting in 94% of tumors being radically excised. Six months after surgery, mean FISI scores were found to be decreased (pre-operative: 10, post-operative: 7, $p < 0.01$; Figure 1), depicting an improvement in faecal continence. Overall when preoperative and postoperative FISI scores were compared, 24 patients improved, 16 patients were unchanged and seven deteriorated. Operation time or tumor size did not influence the change in FISI score. There was a significant correlation between the decrease in FISI score and tumor height ($p = 0.02$). Reduction of FISI was significantly greater in patients with a tumor location within seven centimetres from the dentate line ($p = 0.01$). (Table 2) Mean scores and ranges of the EuroQol EQ-5D are presented in Table 3. Mean general quality of life score from the patients` perspective (EQ-VAS) was found to be significantly higher six months after TEM ($p < 0.02$). The observed changes in EQ-VAS showed no correlation with the postoperative alterations in FISI scores or tumor characteristics. Mean pre-operative EQ-VAS score in our group was lower compared to the mean EQ-VAS score of the sex- and age-matched general population ($p = 0.02$). Postoperative EQ-VAS score was comparable with the general population. Mean Index score (social perspective) remained the same ($p = 0.09$). Both pre- and postoperative EQ-5D index scores were similar to those of the sex-age matched general population. Comparing the change from baseline in FIQL scores, a statistically significant improvement was observed in two of the four domains (embarrassment; $p = 0.03$

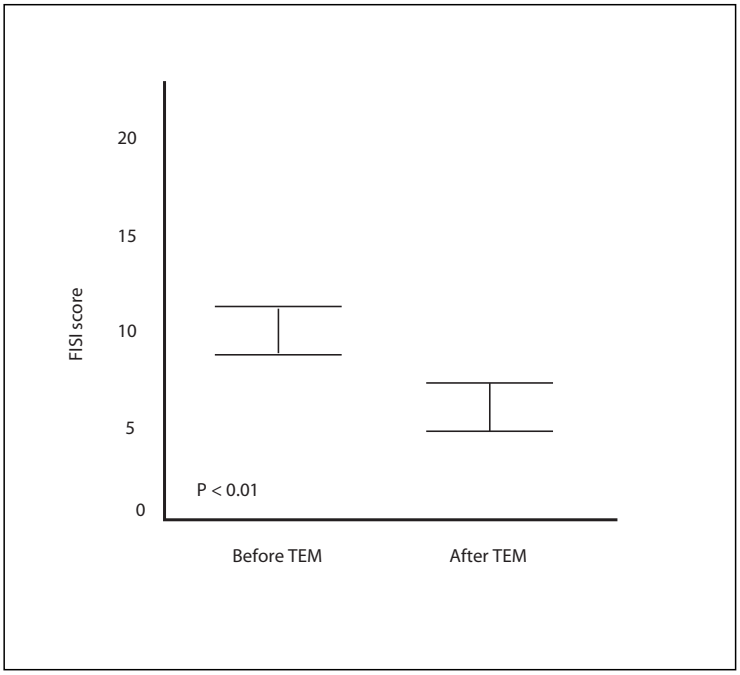

Figure 1. Mean Faecal Incontinence Severity Index (FISI) scores (±SEM) before and after transanal endoscopic microsurgery (TEM)

Table 2. Mean Faecal Incontinence Severity Index (FISI)-scores.

FISI-score	Preoperative	Postoperative	Statistical Significance
Overall	10 (2)	6 (2)	p < 0.01
Duration of operation < 55 minutes (N=24)	9 (4)	7 (3)	p = 0.24
Duration of operation > 55 minutes (N=23)	12 (3)	4 (2)	p = 0.17
Tumors < 7 cm from dentate line (N=21)	16 (5)	5 (2)	p = 0.01
Tumors > 7 cm from dentate line (N=26)	6 (2)	7 (3)	p = 0.43
Median tumor size < 20 cm^2 (N=27)	12 (4)	6 (3)	p = 0.12
Median tumor size > 20 cm^2 (N=20)	8 (3)	6 (3)	p = 0.32

Numbers in parentheses are standard deviations. Lower values indicate better anorectal functioning.

Table 3. Mean EuroQoL EQ-5D scores.

	Control group	Preoperative	Postoperative	Statistical Significance
EQ-VAS	82 (7)	77 (14)	82 (11)	p = 0.02
Index score	86 (6)	84 (11)	89 (9)	p = 0.09

Numbers in parentheses are standard deviations. EQ-VAS represents the patients` perspective on quality of life, Index score represents the societal value on quality of life. Higher scores indicate higher quality of life. Both scores are compared with a healthy sex- and age matched control group.

Table 4. Mean Faecal Incontinence Quality of Life (FIQL) scores.

	Preoperative	Postoperative	Statistical Significance
FIQLS			
Lifestyle	3.7 (0.3)	3.9 (0.3)	p = 0.05
Coping	3.6 (0.5)	3.8 (0.4)	p = 0.10
Depression	3.7 (0.3)	3.9 (0.4)	p = 0.08
Embarrassment	3.1 (0.3)	3.7 (0.4)	p = 0.03

Numbers in parentheses are standard deviations. Higher scores indicate higher quality of life.

and lifestyle; p = 0.05). The domains of lifestyle, coping and behaviour, and embarrassment were correlated with the FISI (all p < 0.05). (Table 4) Overall, EQ-5D and FIQL scores were not affected by age and gender of the patients. Surgical aspects and tumor characteristics did not influence the outcome.

DISCUSSION

In rectal adenomas, TEM has emerged as the procedure of choice, because of its safety and low local recurrence rates. Especially compared to radical surgery TEM has proven its safety.[22, 23] However, possible adverse effects of TEM have to be addressed. The use of a rectoscope with a four centimetres diameter, introduced transanal, has lead to substantial scepticism regarding impact on anorectal functioning. In earlier studies we already showed TEM to be superior to total mesorectal excision regarding postoperative defecation disorders, although this did not result in improved quality of life. [24] In the present study TEM resulted in improved faecal continence as measured by the Faecal Incontinence Severity Index (FISI). This apparent paradox may be attributed to preoperative tumor symptoms as mucinous or bloody discharge, prolapse, tenesmi and/or urge, giving rise to incontinence-like symptoms. Postoperative improvement of continence was most significant in tumors within seven centimetres from the dentate line but disappeared in our study in tumors above seven centimetres from the dentate line. Kreis et al. performed manometric studies after TEM and found a significant reduction in anal resting pressure one year postoperative and a temporary reduction in anal squeezing pressure, resulting in a temporary rise in urge-incontinence. [25] Kennedy et al. found a significant reduction in anal resting pressure six weeks after TEM. [26] This reduction was significantly correlated with duration of the procedure, but mean continence score was not changed after TEM. Both of the above studies however did not use validated questionnaires on faecal continence, and therefore comparison with our study is difficult. Cataldo et al. reported on the impact of TEM on functional outcome and incontinence specific quality of life, using the same questionnaires. [17] No significant alteration was found in faecal continence after TEM. The discrepancy between both studies may be explained by the relative short interval between the TEM procedure and postoperative questioning of six weeks in the Cataldo series. Also in his study, indications for

TEM were heterogeneous which may have influenced results. The positive effect of TEM on faecal continence in our series may be explained by the differences in preoperative FISI score between both studies (10 versus 2.4), depicting more continence problems among the patients in our series. Another explanation may be the differences in tumor distance from the dentate line (present series median seven centimetres, Cataldo series 11 centimetres). Also in our series tumors were larger (median 20 cm^2 versus 8.75 cm^2). Because tumors were larger in our series more extensive resections were performed, not seldom in tumors located within the sphincter apparatus. These latter were already shown to influence recto-inhibitory reflex, reflex sphincter contraction, rectal sensitivity and compliance. [16] Further analysis within our series upon this issue showed only tumor distance from the dentate line less than seven centimetres to be a significant contributing factor. These results however are based upon low number of patients and therefore solid conclusions cannot be drawn. Although in our study TEM resulted in a significant improve in continence, the postoperative FISI was still worse compared to the Cataldo series (7 versus 2.4). Regarding quality of life, Cataldo found TEM was of no significant influence. In our series mean general quality of life score from the patients' perspective, EQ-VAS, was significantly higher after TEM. This improvement could not be explained by improved FISI-scores, but probably to lower pre-operative EQ-VAS scores as compared to healthy controls. Another explanation may be the rejoice phenomena, that is patients are relieved the tumor has been excised, and in most cases an adenoma was found. [27] However, because of the low number of invasive carcinomas in our series this is purely theoretical. The societal value of general quality of life, EQ-5D, remained unchanged. Measuring quality of life using the Faecal Incontinence Quality of Life (FIQL) questionnaires, resulted in a significant improve in two of the four FIQL domains (embarrassment and lifestyle). Moreover the domains of lifestyle, coping and behaviour, and embarrassment were all significantly correlated with the FISI.In conclusion, how are these results to be interpreted? This study supports the hypothesis rectal tumors give rise to incontinence-like symptoms, especially in low-lying rectal tumors. After the tumor is excised using the TEM technique, faecal continence improves. TEM itself does not improve continence, but also does not deteriorate faecal continence. Mean quality of life from the patients` perspective following TEM is improved.

Based on, as we know, the only two studies addressing anorectal functioning and quality of life after TEM in one study, it can be concluded TEM does not impair faecal continence. Also, quality of life is not negatively influenced by the TEM procedure itself, and therefore TEM is the procedure of choice in all rectal adenomas.

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CHAPTER 6

Quality of life after transanal endoscopic microsurgery and total mesorectal excision in early rectal cancer

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Colorectal Disease 2007 (Jul);9:553-558

INTRODUCTION

Surgery for rectal cancer remains the only treatment modality offering a chance of cure. From the oncologic point of view total mesorectal excision (TME) is the gold standard. This standardized and optimized surgical technique has lowered the recurrence rates and probably improved survival. [1-5] Sphincter saving procedures are preferred, even in very distal rectal carcinomas, in which low colo-anal anastomosis or inter-sphincteric techniques are used. [6-8]

Unfortunately, most patients suffer adverse consequences from such radical surgery. The operative dissection of the rectum may damage the pelvic autonomic nerves, disturbing bladder and sexual function. [9-11] The closer the anastomosis to the anal canal, the worse the surgical and functional outcome. [12, 13] Furthermore, construction of a permanent colostomy following abdomino-perineal resection may be associated with clinically significant psychological problems. [14] Finally, especially in the elderly mortality after TME is substantial.[15, 16]

In a strive to avoid the morbidity and mortality after TME, local excision is considered a therapeutic option in the treatment of well-selected patients with early rectal cancer. Several techniques have been developed of which transanal excision according to Parks, trans-sphincteric (or York-Mason) excision, trans-sacral (or Kraske) excision, and transanal endoscopic microsurgery (TEM), are the techniques most described. [17-23] TEM seems to be the method of choice, because it is safe and offers complete resection, is also possible in larger and more proximal tumors and comes with the lowest recurrence rates in adenomas. Points of discussion after local excision for early rectal cancer are the wide range of local recurrence rates from 0 to 24%, its impact on survival and the results of salvage surgery. [24-27] In the studies regarding TEM in T1 rectal cancer local recurrence rates seem limited and survival comparable to radical surgery. [28-31] However, definite evidence is lacking.

Performing TEM, a rectoscope is used with a diameter of four centimetres. This may attribute to sphincter dysfunction after TEM. The effect of the TEM procedure by means of quantitative studies using manometry is anecdotic, showing temporary internal sphincter dysfunction. However, never long-term clinical relevance could be shown. [32]

Quality of life is increasingly recognised as a crucial factor when assessing clinical outcomes after different surgical interventions because it measures the patient's perspective. [33-35] If oncologic outcome is the same in early rectal cancer after TEM and TME, QOL could be the real key outcome in clinical decision-making. Quality of life after TEM is sparsely studied. A recent study of Cataldo et al. found no significant alterations in faecal continence or disease specific QOL after TEM. [36]

In this study we present a retrospective analysis of QOL after TEM for T1 carcinomas compared with a sex- and age-matched sample of patients with T+N0 rectal cancer after sphincter saving surgery with TME and a sex- and age-matched sample of healthy persons.

PATIENTS AND METHODS

To determine the quality of life after TEM for T1 carcinomas, a consecutive series of 54 patients were studied. These patients were operated in one hospital (IJsselland Hospital) between 1996 and 2003. Patients were analysed according to a standard protocol. The TEM technique has been extensively described in an earlier report. [37] Patients who underwent immediate radical surgery and patients with proven local or distant recurrences were excluded. Validated questionnaires were sent to eligible patients. All results were compared to the results from a sex- and age-matched sample of patients obtained from a consecutive series of 111 patients who had undergone curative (R0) sphincter saving surgery for stage I and II rectal cancer by TME between 1997 and 2002 at a university centre and two district hospitals. None of these patients had a diverting ileostomy and all were disease-free at the time of evaluation. Both groups were compared to a sex- and age-matched community-based sample of healthy persons.

We used the EuroQol EQ-5D, EQ-VAS and the European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 and QLQ-CR38 cancer specific questionnaires. The EuroQol EQ-5D consisted of a so-called "index score" representing "the societal value" of the health state, and a visual analogue scale, the EQ-VAS, representing the patient perspective. Regarding QOL from patients` and social perspective, both groups were compared with a sex- and age-matched control group of healthy persons. [38] Disease specific quality of life after TEM and TME was measured according to the official scoring procedures for the EORTC QLQ-C30 and EORTC QLQ-CR38 questionnaires. The EORTC QLQ-C30 was developed to assess the quality of life of cancer patients. It contains 30 items that can be computed in five functional scales (physical, role, emotional, cognitive and social functioning), three symptom scales, and six single items (fatigue, nausea and vomiting, pain, dyspnoea, insomnia, loss of appetite, constipation, diarrhoea and financial difficulties). [39] EORTC QLQ-CR38 was designed especially for the evaluation of colon cancer therapy from a patient perspective. [40] It is subdivided into two functional scales (i.e. body image and sexual functioning), seven symptom scales (micturition problems, gastrointestinal tract symptoms, chemotherapy side effects, defecation problems, stoma related problems and male and female sexual problems), and three single-item measures (sexual enjoyment, weight loss and future perspective). The validity and reliability of these questionnaires have been established in Dutch patients with colorectal cancer. In both QLQ-C30 and the QLQ-CR38 scores are summed within scales and rescaled from 0 to 100. A higher score indicates better functioning for all functioning scales and for two of the single items, sexual enjoyment and future perspective. A higher scale on all symptom scales and the remaining single item (weight loss) indicate a lower level of symptoms. [41]

When appropriate, patient groups were compared using the chi-square test or Fisher`s exact test. Continuous variables were compared using the Mann-Whitney test. Comparisons between groups were also performed, using ANOVA, allowing for gender, age and time of follow-up. A p-value < 0.05 was considered statistically significant.

RESULTS

TEM was performed in 54 patients. Of the original group 18 patients could not be included. Eleven patients died during follow up, three of them due to disease related causes (all local recurrence and distant metastasis). Three patients were excluded because of local recurrences, one patient because of a distant recurrence. One patient was excluded because during the same session a right hemicolectomy was performed. Two patients could not be contacted as they had moved abroad and their new address was not available. The questionnaires were sent to the remaining 36 patients. 31 questionnaires were returned, resulting in an overall response rate of 86%. Of the responders, 18 were male, with a median age of 71 years (range 46-90). In the TME group 31 patients were included, 18 male and 13 female with a median age of 70 years (range 51-87 years).

Patient and tumor characteristics of both groups are listed in Table 1. Regarding clinical characteristics, the patients after TEM did not differ from the TME group. The median duration of time interval between the operation and the mailing was 28 months (range: 5 - 91 months).

From the patient perspective, mean general quality of life score (EQ-VAS) was similar after TEM, TME and controls (Table 2). Also from the social perspective, the mean EQ-5D index score did not differ between the three groups. Scores of the EORTC QLQ-CR30 and the QLQ-CR38 for the patient groups are presented in Table 3 and 4. Univariate analysis showed a significant difference between the two groups regarding defecation problems. TEM patients had less defecation problems than after TME patients ($p < 0.05$). A trend towards better sexual functioning after TEM was seen, especially in male patients, although it did not reach statistical significance.

Table 1. Baseline characteristics of the responders.

	TEM	TME
Numbers of patients	31	31
Median age	71 (46 - 90)	71 (51 - 87)
Median Length of follow-up in months	31 (5-91)	39 (9 - 62)
Male / female (%)	58 / 42	58 / 42
Tumor (T-)stage (%)	T1=31 (100)	T1=3 (10) T2=8 (26) T3=20 (64%)
Location tumor (0-5/ 5-15 cm from dentate line)	29/71	29 / 71
Preoperative radiotherapy (%)	0	18
Co morbidity (%)	19	19

Data are percentages or median numbers with ranges in parentheses. TEM = transanal endoscopic microsurgery, TME = total mesorectal excision.

Table 2. General quality of life scores.

	TEM	TME	Population
EQ-VAS	76 (20 - 100)	70 (30 - 100)	76 (68 - 84)
EQ-5D	81 (-18 - 100)	76 (26 - 100)	76 (67 -86)

Data are mean scores with ranges in parentheses. EQ-VAS = Quality of life from the patient perspective, EQ-5D = Quality of life from the social perspective. TEM = transanal endoscopic microsurgery, TME = total mesorectal excision, Population = a sex- and age-matched, community-based sample of healthy persons without co-morbidity.

Table 3. Disease specific quality of life scores (EORTC QLQ-C30).

	TEM		TME	
	Mean	Median (range)	Mean	Median (range)
Physical function	78	87 (0 - 100)	83	90 (20 - 100)
Role function	81	100 (0 - 100)	80	83 (0 - 100)
Emotional function	82	92 (0 - 100)	82	92 (17 - 100)
Cognitive function	84	100 (0 - 100)	86	100 (17 - 100)
Social function	60	67 (0 - 100)	69	67 (0 - 100)
Global health status	73	83 (33 - 100)	74	75 (17 - 100)
Fatigue	76	89 (0 - 100)	80	81 (11 - 100)
Nausea/vomiting	90	100 (0 - 100)	95	100 (17 - 100)
Pain	80	100 (0 -100)	89	100 (0 -100)
Dyspnoea	87	100 (0 - 100)	87	100 (0 - 100)
Sleep disturbance	76	100 (0 - 100)	82	100 (0 - 100)
Appetite loss	93	100 (33 - 100)	97	100 (33 - 100)
Constipation	93	100 (33 - 100)	85	100 (0 - 100)
Diarrhoea	86	100 (0 - 100)	89	100 (0 - 100)
Financial worries	94	100 (33 - 100)	94	100 (0 - 100)

A high subscale score indicates low distress and good functioning. TEM = transanal endoscopic microsurgery, TME = total mesorectal excision.

DISCUSSION

The major axiom of surgical treatment of rectal cancer has historically been to remove the primary lesion with adequate margins and as much of the attendant lymphatic drainage as possible. The risk of lymph node metastases and therefore the prognosis for rectal cancer depends on certain histopathologic criteria as depth of tumor infiltration and histological grading. According to this, when the tumor only invades submucosa (pT1), lymph nodes are involved with metastasis in 3-14 percent of patients, depending on the presence of certain unfavourable histopathologic criteria. [42, 43] Thus, patients with minimal invasive, histological favourable lesions without evidence of spread would be well served with local excision alone. Concern has been made on oncologic outcome after local excision for early rectal cancer. [25, 26, 44] After transanal excision local recurrence rates are infrequently high and the role of salvage surgery is

Table 4. Disease specific quality of life scores (EORTC QLQ-CR38).

	TEM		TME	
	Mean	Median (range)	Mean	Median (range)
Micturition problems	79	77 (22 - 100)	81	78 (44 - 100)
Gastrointestinal problems	81	87 (33 - 100)	80	80 (40 - 100)
Weight loss	92	100 (33 - 100)	94	100 (33 - 100)
Body image	90	100 (44 - 100)	88	100 (0 - 100)
Defecation problems	91	90 (57 - 100) *	77	80 (47 - 100) *
Stoma problems	-	-	-	-
Chemo side-effects	89	100 (22 - 100)	90	89 (22 - 100)
Sexual function	27	17 (0 - 100)	24	17 (0 - 83)
Sexual enjoyment	61	67 (0 - 100)	53	67 (0 - 100)
Male sex problems	62	83 (0 - 100)	46	42 (0 - 100)
Female sex problems	89	92 (33 - 100)	81	83 (33 - 100)
Future perspective	71	67 (0 - 100)	72	67 (0 - 100)

A high subscale score indicates low distress and good functioning. TEM = transanal endoscopic microsurgery, TME = total mesorectal excision. * $p < 0.05$ versus TME.

uncertain.[27] The main problem when reviewing the literature on local excision for early rectal cancer is the diversity of used techniques and varying patient and tumor selection. Compared to other local techniques TEM has emerged as the method of choice in T1 early rectal cancer as it yields lower recurrence rates. [45] Moreover, comparable results to radical surgery can be achieved with TEM. [28, 29] Nevertheless, definite evidence for TEM in T1 early rectal cancer is still lacking. When the TEM procedure is considered a therapeutic option, this latter aspect should be discussed in detail with every patient before obtaining informed consent.

It seems reasonable to assume that quality of life after local excision using the TEM technique is better than after radical resection. However, no prospective trial has been initiated to investigate this assumption. As for radical surgery, several studies have shown that functional results, especially bladder and sexual functioning, are bad. [9-11]

In the present study QOL after TEM is compared to QOL after radical resection, and to our knowledge is the first study to address this subject. Although being retrospective and hence limited, several remarkable findings have come forward. Both after TEM and TME patients rank their quality of life as high as that in the population-based reference group. Moreover, QOL was no different between TEM and TME patients. This finding might be due to methodological shortcomings of our study design: its retrospective nature, the relatively small number of patients and the lack of control measurements before treatment limit the present study. Another plausible explanation could be the fact that several patients were only diagnosed to have a carcinoma after the TEM procedure. At that point patients are told to have rectal cancer and TME is the gold standard. They are offered the choice between an additional TME and follow-up only. When the patient chooses for follow-up the rectum is re-examined every three

months by means of digital rectal examination, rigid rectoscopy and endorectal ultrasound. This may burden them to the feeling of being at risk of developing a local recurrence with its impact on QOL. Furthermore, the relatively high QOL, observed among our patients after TME, might be explained by the fact that the measurement followed their earlier diagnosis of a life-threatening disease, which changed their perceptions of the length of life, thereby shifting their expectations and priorities with regard to life fulfilment. Successful treatment therefore might result in a higher quality of life as reported by the patient. This effect, known as 'rejoice', has been noted from the beginning of quality-of-life research. [46]

Functional outcome after rectal surgery is frequently impaired. Most studies report sustained reduction in resting sphincter pressures after sphincter saving surgery with TME. This decrease has been attributed to the dilatation performed when the circular stapler is inserted. However, there is strong evidence that direct sphincter trauma is not a major cause for dysfunction. Several manometric studies have suggested neurogenic injury rather than morphologic damage as the explanation for postoperative functional disorder. [47] Hallgren et al. investigated the changes in resting sphincter pressure during the different stages of restorative proctocolectomy and either hand sewn or stapled pouch-anal anastomosis. [48] In both techniques the resting pressure was reduced in a sequential manner during the surgical procedure, with an immediate decrease in pressure after division of the superior rectal artery, a further reduction after full mobilization of the rectum, followed by another equally large drop at the final stage after construction of the anastomosis by either technique.

Because of the 4 cm diameter of the rectoscope, the prospect of continence following TEM was of concern. Although a significant decrease in both anal resting pressure and squeeze pressures occurs initially, these pressures return to pre-operative values at a mean of four months after TEM. [32, 36, 49] A possible explanation might be the fact that TEM keeps the neural autonomic pathways regulating sphincter tone intact. In our study, after TEM, patients had significant less defecation problems, as found with the EORTC QLQ-CR38 questionnaire. In a recent study a correlation between alterations of the anal sphincters and the functional outcome could not be demonstrated. [50] Therefore the interesting question arises whether the postoperative compliance and sensory perception are the determining functional factors. It is well known that the functional outcome after low anterior resection improves with time. It has been shown that this improvement is associated with an increase of compliance. [51-53] The better functional outcome in TEM patients might be due to the fact that the original rectum remains unaffected. Several authors have suggested that radiation to soft tissues of the pelvis worsens postoperative neorectal function. [54] However, in the present study only 18 percent of TME patients had preoperative radiotherapy. This low percentage might mitigate the differences in functional outcome in this study.

In a recent report it was stated that sexual problems after radical surgery for rectal cancer are common, and efforts to prevent and treat it should be increased. [9] In our study there was a

trend towards better sexual functioning after TEM, especially in male patients, although it never reached statistical significance.

On the basis of this study, despite the methodological shortcomings, it might be concluded that there is no difference in impact on QOL from the patients` and social perspective after TEM and TME. Defecation problems after TEM are less encountered than after TME. This difference could play a role in the choice of surgical therapy in early rectal cancer. One should keep in mind the retrospective nature of the study and future prospective studies are needed to answer the question whether TEM for low risk T1 carcinoma is superior to TME regarding oncologic outcome and postoperative QOL.

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CHAPTER 7

The role of endorectal ultrasound in therapeutic decision-making for local versus transabdominal resection of rectal tumors

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Dis Colon Rectum. 2008 Jan;51(1):38-42

INTRODUCTION

Local excision of rectal adenomas (TVA) is a validated treatment modality. Concern has been made regarding local recurrences after local excision, but with the introduction of transanal endoscopic microsurgery (TEM) this risk has become minimal and even larger and more proximal located TVA can be excised. [1-3] In these larger presumed benign rectal lesions, based on preoperative biopsy, definite histopathology reveals a carcinoma in up to 34% of tumors. [4, 5] As a result patients with missed carcinoma need to undergo additional radical surgery by means of total mesorectal excision (TME). Although evidence is lacking, prior TEM procedures may burden immediate radical surgery with possible higher morbidity, including increased risk on a (permanent) stoma. Moreover, this unexpected histopathological finding and the need for additional surgery impede patients' satisfaction. Finally, oncologic outcome in this subgroup of patients is questionable. [6, 7]

Extensive efforts have been made to improve preoperative diagnosis of rectal tumors, with computerized tomography (CT), magnetic resonance imaging (MRI) and endorectal ultrasound (ERUS) as the techniques most commonly used. Each adjunct has its limits, but ERUS seems the most adequate with accuracy rates for tumor infiltration (T-stage) ranging from 64-95%. [8-13]

If ERUS, however, is to be considered essential in preoperative staging, accuracy may not be the only relevant issue. Feasibility of ERUS in all rectal tumors referred for local excision and the additional value of ERUS in therapeutic decision-making are equally important. In this prospective study we investigated the feasibility of ERUS in all TVA referred to our hospital for TEM. Also the additional value of ERUS in diagnosing invasive carcinomas and its role in therapeutic decision making are studied.

PATIENTS AND METHODS

From April 2000 to May 2006 in 264 consecutive patients with 268 tumors preoperative biopsy revealed a TVA. In all tumors ERUS was intended. The group consisted of 128 males and 136 females. Median age was 70 years (range 29-91 years) and median distance from the dentate line to the distal tumor margin was 8 cm (0-20 cm). Fifty-six tumors were located in the lower third of the rectum (0-5 cm), 133 in the middle third (5-10 cm) and 79 in the upper third (10-15 cm). Median tumor area was 12.3 cm^2 (0.25-156 cm^2). In 69 tumors it concerned residual tumor tissue after recent endoscopic treatment or a recurrent tumor.

Two hours prior to ERUS a cleansing enema was given. Patients were placed in lithotomy position and digital rectal examination and rigid rectoscopy were performed. After inspection of the tumor, the tip of the rectoscope was positioned proximal to the upper margin of the tumor and the ERUS–probe was introduced via the rectoscope with the tip of the probe outside the rectoscope. Both were pulled back simultaneously manually. Ultrasound examinations were

documented on videotape. The ultrasound equipment used was a B&K Medical Scanner (Naerum, Denmark) Type 2101 with a type 1850 rotating endosonic probe with a 10 MHz crystal.
If the tumor could not be reached or passed completely during rectoscopy, or if technical problems occurred, such as inability of cleansing the rectum or equipment failure, the tumor was considered not assessable. In assessable tumors all rectal wall layers had to be visualized without artefacts before considering ERUS conclusive. Ultrasound staging was compared with definite histopathological findings.
Statistical analysis was performed using the SPSS® version 11.0 (SPSS, Chicago, Illinois, USA). Continuous variables and percentages were compared between groups using the Mann-Whitney test or Chi-Square test, respectively. A p-value of 0.05 (two-sided) was considered as the limit of significance and 95% confidence intervals (95% CI) are calculated for various percentages. Kappa statistics were calculated to quantify the agreement between ERUS- and histopathological stages.

RESULTS

Of the 268 tumors in this study 231 (86%) were assessable for ERUS. Median distance from the dentate line in not assessable tumors was 11 cm, which was significantly different from assessable tumors, with a median distance of 7 cm ($p < 0.001$). Of the not assessable tumors 62% were located more than 10 cm proximal from the dentate line. There was no difference in median tumor area between assessable and not assessable tumors. Also the percentage of recurrent/residual tumors was not different. (Table 1)
In 16 tumors ERUS was not performed because of a technical failure (patient incontinence n= 8, inability of cleansing the rectum n=4, equipment failure n=4). Twenty-one tumors were not assessable because they could not be reached or passed due to stenosis and angulation in the rectosigmoid, or because the tumor was too voluminous.
In 210 of 231 assessable tumors (91 %) ERUS was considered conclusive. (Table 2) Fifteen of the 21 tumors in which ERUS was not conclusive were residual or recurred after prior endoscopic treatment (71%). Compared to the tumors in which ultrasound staging was considered conclusive, this proportion was significantly higher ($p < 0.001$).
Definite histopathological staging revealed TVA in 166 tumors (79%), T1 rectal carcinoma in 30 (14.3%), T2 rectal carcinoma in 13 (6.2%) and T3 rectal carcinoma in 1 (0.5%). Overall accuracy of ERUS is 84%. (Table 3) ERUS correctly staged 147 tumors as TVA, with a corresponding sensitivity of 89%. (Table 4) ERUS correctly staged 38 tumors as invasive with a corresponding sensitivity of 86%. Positive and negative predictive values were 96% and 67% respectively.
If only TVA are considered indications for local excision, based on preoperative biopsy 44 tumors would have been undertreated with TEM, as definite histopathology revealed a carcinoma (21%; 95% CI: 15-26%). Based on ERUS 6 tumors would have been undertreated (3%;

Table 1. Tumor characteristics regarding feasibility of ERUS.

	Assessable tumors N=231	Not assessable tumors N=37
Distance from dentate line in cm§	7 (0-20)	11 (3-18)*
Tumor distribution from dentate line		
0-5 cm	55 (24%)	1 (3%)
5-10 cm	120 (52%)	13 (35%)
10-15 cm	56 (24%)	23 (62%)*
Tumor surface (cm^2)§	14 (0.25-156)	8 (0.25-130)
Number of recurrent/residual tumors at referral (%)	63 (27%)	6 (16%)

§ Values are median (range); * $p < 0.001$.

Table 2. Tumor characteristics regarding possibility of staging with ERUS.

	ERUS conclusive N=210	ERUS not conclusive N=21
Distance from dentate line in cm§	7 (0-15)	8 (0-13)
Tumor distribution from dentate line		
0-5 cm	48	7
5-10 cm	112	8
10-15 cm	50	6
Tumor surface (cm^2)§	12 (0.25-156)	14 (0.25-80)
Number of recurrent/residual tumors at referral (%)	48 (23%)	15 (71%)*

§ Values are median (range); * $p < 0.001$.

95% CI: 1-6%). This reduction in undertreatment is statistically significant ($p < 0.01$). Based on preoperative biopsy no tumor would have been overtreated with TME, whereas based on ERUS 19 ultrasonically presumed T2/T3 carcinomas would have been overtreated (9%; 95% CI: 5-13%). This increase in overtreatment is statistically significant ($p < 0.01$).

If TVA and T1 carcinomas are both considered indications for local excision, based on preoperative biopsy 14 tumors would have been undertreated with TEM (7%; 95% CI: 4-11%). Based on ERUS, 6 ultrasonically presumed tumors suitable for TEM would have been undertreated (3%; 95% CI: 1-6%). This reduction in undertreatment is significant ($p < 0.01$). Based on preoperative biopsy no tumor would have been overtreated with TME, whereas based on ERUS 9 ultrasonically presumed T2/T3 carcinomas would have been overtreated (4%; 95% CI: 2-8%). This increase in overtreatment is significant ($p < 0.01$).

Table 3. Agreement of preoperative ERUS with definite histopathological T-staging.

	Histopathological T-staging				
ERUS T-staging	pTVA	pT1	pT2	pT3	Total
uTVA	147	4	1	1	153
uT1	14	22	4	0	40
uT2	4	2	7	0	13
uT3	1	2	1	0	4
Total	166	30	13	1	210

Overall accuracy 84% (176/210), Kappa coefficient 0.59, sensitivity in diagnosing: TVA 89% (147/166), T1 carcinomas 73% (22/30), T2 carcinomas 54% (7/13).

Table 4. Agreement of ERUS and histopathology in diagnosing tubulovillous adenomas.

	Histopathological staging		
ERUS T-staging	pTVA	pT1/T2/T3	Total
uTVA	147	6	153
uT1/T2/T3	19	38	57
Total	166	44	210

Sensitivity 89% (147/166), Kappa-coefficient 0.68, specificity 86% (38/44), positive predictive value 96% (147/153), negative predictive value 67% (38/57).

Table 5. Agreement of ERUS and histopathological staging in diagnosing TVA and T1 carcinomas.

	Histopathological T-staging		
ERUS T-staging	pTVA/T1	pT2/T3	Total
uTVA/T1	187	6	193
uT2/uT3	9	8	17
Total	196	14	210

Sensitivity 95% (187/196), Kappa-coefficient 0.48, specificity 57% (8/14), positive predictive value 97% (187/193), negative predictive value 47% (8/17).

DISCUSSION

Local excision of rectal TVA is the method of choice. As a tertiary referral centre for TEM, we are frequently encountered with tumors considered suitable for local excision using the TEM technique. TEM has proven to be a safe procedure for TVA, with the possibility to excise larger and more proximal located tumors. [1, 2] In presumed rectal TVA, especially in larger tumors, definite histopathology may reveal a carcinoma. In case a carcinoma was missed with biopsy, immediate radical surgery after local excision might be more difficult with possibly increased morbidity. Moreover, in distal located tumors prior local excision could decrease the possibility on sphincter saving surgery. Finally, oncologic outcome in this subgroup of patients is questionable. [6, 7] For these reasons adequate preoperative staging is of major importance.

Of all frequently used staging modalities in rectal tumors ERUS is the most promising, as accuracy concerning tumor invasion depth is higher compared to CT scanning and at least as

accurate as MRI. [8-10] The additional value of ERUS in preoperative staging, compared to other modalities, is expressed by the power to discriminate TVA from invasive carcinomas. ERUS was already shown to be able to correctly establish a cancer diagnosis in 81% of the missed carcinomas at biopsy. [14] These results are of major importance as treatment options, local excision versus radical surgery, are to be discussed with every patient. In rectal carcinomas TME is the gold standard. Although evidence is sparse, in T1 rectal carcinomas the role of TEM has been re-appraised. [15, 16] If both TVA and T1 rectal carcinomas are considered suitable candidates for local excision, ERUS might be of additional value in discriminating these two from more invasive carcinomas. However, especially in larger and more proximal tumors ERUS may be more difficult and if ERUS is considered a useful preoperative adjunct, feasibility in all rectal tumors has to be investigated. One study suggested that in 13% of all rectal tumors ERUS was not feasible. The percentage of not assessable tumors increased from 11% in distal tumors (0-4 cm above anocutaneous line) to 34% in proximal located tumors (12-16 cm above anocutaneous line). However, uniformity regarding the technique of ERUS lacked. Moreover, physicians were allowed not to perform ERUS if considered without additional value. This resulted in 63% of all rectal tumors in which ERUS was not performed. Moreover, only 10% of all tumors in which ERUS was performed were located proximal in the rectum. In our series in all tumors referred for local excision by means of TEM, ERUS was intended. In 86% of all tumors ERUS was technically feasible. If not feasible, distance from the dentate line proved to be a significant contributing factor. Proper interpretation of ERUS imaging was possible in 78% of all tumors. The only significant factor negatively influencing interpretation of ERUS imaging was residual or recurrent disease, especially after recent (endoscopic) manipulation ($p < 0.001$). Several authors already stated that tumor biopsy or (endoscopic) manipulation prior to ERUS should be avoided if on clinical grounds local excision is considered suitable. [11] This could lower the proportion of patients in which ERUS is not conclusive.

Because of the possibility to excise larger tumors with TEM in a large proportion of presumed adenomas a carcinoma is found. In our series in 21% of tumors an unexpected carcinoma was found, which is comparable to other series. [4, 5] The role of ERUS in preoperative evaluation of presumed TVA is significant. In a relatively large review ERUS correctly established a cancer diagnosis in 81% of preoperative (biopsy) misdiagnosed TVA. The need for additional surgery and other associated problems caused by misdiagnosis could be decreased from 24 to five percent. [14] These results are confirmed in our study, with 86% of missed carcinomas on biopsy corrected with ERUS. In TVA sensitivity rates of 89% and specificity rates of 86% can be achieved. The main advantage of ERUS in presumed adenomas is the high positive predictive value of 96%, meaning if ERUS confirms the tumor as TVA only in four percent an invasive carcinoma is found at definite histopathological staging.

The question which rectal tumors are suitable for local excision using the TEM technique is still unanswered. In rectal cancer TME is the gold standard, but evidence, although anecdotic, is growing that T1 rectal carcinomas may be candidates for TEM. [15-18] This means distinction

between TVA and T1 carcinomas may be of less priority but the difference between T1 and more invasive carcinomas is essential. Sailer et al. stated if TVA and T1 carcinomas are considered one ultrasonic entity, ERUS reaches a sensitivity of 81% and a specificity of 98%.[11] They concluded ERUS is helpful in therapeutic decision-making between local excision and radical surgery. In our series sensitivity in diagnosing TVA and T1 carcinomas was 95%. In 14 tumors (7%) a T2 or more invasive carcinoma was found at definite histopathology. Preoperative biopsy found none of these carcinomas, whereas ERUS correctly classified eight of these tumors as uT2/uT3. If ERUS findings would have been used as adjunct in therapeutic decision-making, 57% of missed T2 and T3 carcinomas on biopsy could have been spared prior local excision. This absolute risk reduction in undertreatment, 7 versus 3 per cent, was statistically significant ($p < 0.01$). However, ERUS classified nine tumors as not suitable for local excision (uT2 or higher), which proved to be adenomas (five) or T1 carcinomas (four). This increase in possible overtreatment, 0 versus 4 per cent, was also statistically significant ($p < 0.01$). This overstaging is also found in other series and is a major drawback of ERUS. [11]

In conclusion, based upon this study ERUS is technically feasible in almost all rectal tumors in which preoperative biopsy shows tubulovillous adenoma. Proper ERUS interpretation is possible in 78% of all presumed rectal TVA. ERUS can discriminate between adenomas and invasive carcinomas and has, next to biopsy findings, a substantial additional value in recognizing TVA suitable for local excision. If a carcinoma is suggested with ERUS, one has to discuss treatment options, local excision versus radical surgery, with every patient. This study has shown that if T1 rectal carcinomas are considered suitable candidates for TEM, ERUS has a major additional value in preoperative staging.

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CHAPTER 8

Progression and tumor heterogeneity analysis in early rectal cancer

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Clin Cancer Res. 2008 Feb 1;14(3):772-781

INTRODUCTION

Colorectal cancer is one of the leading causes of mortality and accounts for ~300,000 new cases per year in Europe and the United States. [1] Approximately 25% of these cases are rectal cancers, and the incidence of its benign precursor lesion, adenomas, is far higher. Total mesorectal excision is the gold standard to treat carcinomas, transanal endoscopic microsurgery (TEM) is the method of choice to treat sessile adenomas. [2-4] Although it has not yet been proven, T1 rectal carcinomas may be good candidates for TEM without compromising oncologic outcome. [5-7] On the other hand, an invasive carcinoma (beyond the muscularis mucosae) is found after local excision in a large proportion of presumed benign tumors, which shows the need for more precise staging. [5, 8] Several possible imaging techniques have additional value, and endorectal ultrasound seems most promising; however, not all cases are eligible for endorectal ultrasound, and overstaging is a serious problem. [9] It should be noted that TEM-treated cases of early rectal cancer mostly consist of adenoma tissue. Thus, there is a need for additional preoperative staging methods that can accurately facilitate therapeutic decision-making in the treatment of rectal tumors. Ideally, a combination of methods should be able to reliably discern benign adenomas from adenomas containing a carcinoma focus, as well as predict lymph node metastasis.

Chromosomal instability is the main characteristic of many different tumor types, including rectal cancer. To date, many studies have been done in colorectal cancer to assess chromosomal gains, losses or LOH. Commonly involved regions in (colo-) rectal cancer are 5q, 8, 13q, 17p, 18q and 20q, as established by different groups. [10-14] Other studies specifically analyzed rectal cancer precursor lesions and found that commonly involved chromosomal aberrations are already frequent in adenomas or are correlated with high-grade dysplasia. [15-18] Several studies identified intratumor heterogeneity, which is characterized by patterns of different chromosomal aberrations in different tumor areas of the same lesion. [19, 20]

In a previous study, we used single nucleotide polymorphism (SNP) arrays to detect copy number aberrations and LOH in rectal adenomas and carcinomas at different clinical stages. [12] Considering the frequent malignant events, gain of 8q, 13q and 20q, and loss of 17p and 18q, we have built a rectal cancer progression model. In addition, we found that (combinations of) these "malignant" events were increasingly found in adenoma fractions of carcinoma cases in comparison with pure adenomas. We now did a systematic comparison of chromosomal instability patterns in adenoma and carcinoma fractions in the same lesion of early rectal cancer cases that were treated by TEM. The effect of intratumor heterogeneity in a partly overlapping set of tumors was assessed by chromosomal instability analysis of three different ex vivo core biopsies per tumor, which were taken postoperatively.

MATERIAL AND METHODS

Samples

Material from 36 rectal carcinomas was obtained. These tumors were preoperatively classified as adenomas, but in all cases, definite histopathology revealed the presence of a carcinoma. All patients were treated using the TEM technique at the IJsselland Hospital (Capelle a/d IJssel, the Netherlands) or Reinier de Graaf Hospital (Delft, the Netherlands). None of the patients received (neo-) adjuvant radiotherapy or chemotherapy. All samples were reviewed by a pathologist (H.M.), dysplasia was scored, and tumor cell percentage was assessed (50-80%). From these tumors, we analyzed an adenoma (also indicated as A/C), a carcinoma (also indicated as C/C), and a normal tissue fraction. For comparison, we used data from 21 pure rectal adenomas (also indicated as A/A) from a previous study. [12]

For intratumor heterogeneity analysis, three core biopsies were taken postoperatively, ex vivo, from 13 of the carcinoma cases and 5 of the pure adenoma cases at the surface of the tumor. These biopsies were randomly taken and snap frozen in liquid nitrogen. Biopsies contained either adenoma or carcinoma tissue.

The local medical ethical committee approved the study (protocol number P04.124). Table 1 shows all sample characteristics.

DNA isolation

Formalin-fixed, paraffin-embedded (FFPE) tissue from the adenoma and carcinoma fractions was analyzed. DNA was extracted as previously described. [21] Briefly, three tissue punches (0.6 mm diameter) were obtained using a tissue microarrayer (Beecher Instruments, Sun Prairie, WI), and DNA was isolated with proteinase K. Formalin-fixed, paraffin-embedded DNA was subsequently cleaned up using the Genomic Wizard kit (Promega).

DNA from the frozen tumor biopsies was extracted as previously described using the Genomic Wizard kit. [12]

All DNA concentrations were measured with the PicoGreen method (Invitrogen-Molecular Probes, Breda, The Netherlands), and DNA quality was checked on a 1% agarose gel.

Array analysis

The use of SNP arrays is a well-established method for copy number and LOH analysis. Therefore data were not validated with cytogenetics in the present study. Validation studies are well documented by us and others. [22-24]

For each cell isolate, 1 µg of DNA was used for the BeadArrays. Illumina BeadArrays, in combination with the linkage mapping panel version 4_v3 or version 4_v4B (Illumina, San Diego, CA), were used and respectively contained 5,861 or 6,008 SNP markers distributed evenly over the genome with an average physical distance of 482 kb. Samples were prepared according to the

Table 1. Patient characteristics.

ID	Sex	Age	Size	Fraction analyzed†	Carcinoma		Biopsy	Recurrence	Distant metastasis
					T-stage	N-stage			
1	M	63	8	LC	2			x	
2	F	70	3.5	LC	1			x	x
3	F	89		HC	1				
4	F	77	1.5	HC	1	1		x	
5	F	56	7.5	LC	3	1	aaa		
6	M	59	4.5	LC	2				
7	M	77	7	LC	2				
8	M	55	3	LH	1		aaa		
9	F	79		HC	2				
10	M	61	7	LC	1		aaa	x	x
11	M	74	2.5	HC	1				
12	M	60	5	HC	1				
13	F	56	3.8	HC	1				
14	F	77	4	LHC	2				
15	F	73	3	HC	1				
16	M	79	4	HC	1		ccc		
17	M	61	10	LH	1				
18	F	56	8.6	LH	1		aaa	x	
19	M	45	5	LC	1				
20	M	60	2.5	LC	1				
21	F	49	2	LC	1				
22	M	68		HC	2				
23	F	70	1.5	LC	1		aac		
24	F	58	1	LHC	1				
25	M	46	5	HC	2	1	acc		
26	M	53	3.5	HC	2		ccc		
27	F	47		HC	2				
28	F	83	9	HC	1			x	x
29	F	73	2	HC	1		aaa		
30	F	65		HC	1		aaa		
31	M	64	5	HC	2				
32	M	71	6.5	LC	1				
33	M	80	1	HC	1				
34	F	58	3	HC	1				
35	M	70	11	HC	1			x	
36	F	73	8	LH	1			x	
37*	M	82	13.5	L	0				
38	M	75	7.5	H	0				
39	F	72	5	H	0				
40	M	62	7.5	H	0				
41	M	75	8	H	0				
42	M	78	4	H	0				
43	F	87	2	L	0		aaa		
44	M	61	5	H	0				
45	F	87	5	H	0				
46	M	67	9	L	0				
47	F	74	2	L	0		aaa		
48	F	68	2	L	0				
49	F	52	6	L	0		aaa		
50	M	53	9	L	0		aaa		
51	F	52	6.5	L	0				
52	F	63	7.3	L	0				
53	M	60	6	L	0				
54	M	79	5	L	0		aaa		
55	M	73	6.5	H	0				
56	F	40	11	L	0				
57	F	81	4.5	H	0				
58	M	69	7.5		1		aaa		
59	M	73	3.5		1		aac		
60	F	83	2		2		ccc		

NOTE: x= a recurrence or distant metastasis. Abbreviations: L= adenoma with low-grade dysplasia; H= adenoma with high-grade dysplasia; C= carcinoma; a= adenoma tissue; c= carcinoma tissue.

* Case 37 to 57 are pure adenomas from the previous study.

Goldengate assay. [25] Gene calls were extracted using the gene calling programs GeneCall and GTS Reports (Illumina, San Diego, CA).

Copy number and LOH analysis

Copy numbers were determined based on intensity of the individual SNPs. [23] LOH was analyzed by comparing the genotypes from paired normal and tumor DNA. Analyses were done using the R-package beadarraySNP. In addition, chromosome visualization of LOH was done in Spotfire DecisionSite (Spotfire, Somerville, MA). [26] LOH was calculated as described[1]. Briefly, LOH was computed from the gene call score and the gene train score output of GeneCall and GTS Reports (Illumina, San Diego, CA). LOH was called for high quality heterozygous SNPs in the normal tissue (gene call score/gene train score ratio > 0.8) that were, in the paired tumor, homozygous or showed a gene call score/gene train score ratio of <0.8. Only LOH at a stretch of two or more SNPs was scored. [26] When both physical loss and LOH were detected at a specific region, the LOH detected is an additional indication of physical loss. In the case where no copy number change was detected, LOH was interpreted as copy neutral LOH.

APC and KRAS mutation screening

APC and KRAS mutation detection were performed as described. [27] PCR product (5-10 ng) was sequenced with 6 pmol of M13 forward or reverse primer on an ABI 3700 DNA Analyzer using Big Dye Terminator Chemistry (Applied Biosystems, Forster City, CA). Sequences were analyzed with Mutation Surveyor™ DNA variant analysis software (version 2.61 Softgenetics, State College, PA).

p53 and SMAD4 immunohistochemical analysis

Triplicate tissue cores from tumor areas, selected by a pathologist (H.M.) based on (H&E)-stained slides, were taken from each specimen (Beecher Instruments, Silver Springs, MD, USA). These punches, which had a diameter of 0.6 mm, were arrayed on a recipient paraffin wax block using standard procedures. [28, 29] A paraffin sectioning aid system (Instrumedics Inc., Hackensack, NJ) was used to facilitate cutting 5-µm sections of the tissue micro-array. After antigen retrieval (microwave oven treatment for 10 minutes in 10 mmol/L citrate buffer pH 6.0 (p53) or Tris-EDTA pH 8.0 (SMAD4)), endogenous peroxidases were inactivated by 1% H_2O_2/PBS. Sections were incubated overnight at room temperature with mouse anti-human monoclonal antibodies directed against p53 (clone D0-7, 1:1000 dilution; NeoMarkes) or SMAD4 (clone B-8, 1:100 dilution; Santa Cruz Biotechnology). The sections were then incubated and stained with a biotinylated secondary antibody in PBS/bovine serum albumin 1% (p53) or Envision HRP-ChemMate

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kit (SMAD4; DAKO). Diaminobenzidine tetrahydrochloride was used as a chromogen for p53 staining. The slides were counterstained with hematoxylin. p53 was scored in four different categories based on any level of nuclear staining: 1% to 25% positive nuclei (indicative for a wildtype status), 25% to 75% positive nuclei, > 75% positive nuclei (the latter two mostly indicative for a mutation) or completely negative (uninformative). SMAD4 was scored in the following categories: no nuclear staining with a positive internal control (total loss), weak nuclear staining (down regulation) and moderate to strong nuclear staining (positive).

Statistics

Student's t-test was used to compare means of continuous variables between two groups. χ^2 tests were done to test significance between groups for specific loss and gain events. Physical loss and copy neutral LOH were considered as identical events in these analyses. Correlations between two tumor fractions were computed using Pearson's correlation coefficients. For all analyses, p-values of < 0.05 were considered as significant. All these analyses were done using Statistical Package for the Social Sciences 12 (SPSS).

RESULTS

Chromosomal aberrations

In a previous study, we typed copy number profiles using SNP arrays in 77 fresh frozen tumors of different stages. [12] We subdivided the adenoma tissue into pure adenomas (A/A) and adenoma fractions of cases with a carcinoma focus (A/C). The carcinoma tissue was subdivided in tumor samples consisting of a mixture of adenoma and carcinoma tissue (AC/C), carcinoma tissue alone (C/C) and primary tumors in cases with lymph node metastasis (C/C (N+)). Importantly, the latter two contained no or only minimal adenoma tissue, whereas the A/C cases consisted predominantly of adenoma tissue. We found five specific chromosomal aberrations (gain of 8q, 13q and 20q and loss of 17p and 18q), which could discriminate adenomas from carcinomas.
With the aim of studying the early aberrations already present in the adenoma fraction of carcinoma cases, we assessed copy number alterations and LOH in paired adenoma (A/C) and carcinoma (C/C) formalin-fixed, paraffin-embedded tissues of 36 TEM treated rectal carcinomas. In two cases, two different adenoma fractions were identified, and for four cases, the carcinoma fraction was too small to be analyzed; therefore, both the adenoma fraction with low and high grade dysplasia were analyzed, finally leading to a total number of 32 C/C fractions and 42 A/C fractions. Table 2 shows the most frequent chromosomal changes per sample group; in supplementary Table 1, all genomic and genetic abnormalities are shown for each case. The A/C and C/C fractions were compared with each other and with the pure adenomas (A/A) from the previous study. [12] From that study, we learned that only specific adenoma events (loss of 1p36, 4q32-pter and 5q and gain of 7p15-11 and 12q13) were frequently involved in the A/A

Table 2. Common aberrations (%) in different tumor fractions.

	AA n=21	A/C L n=18	A/C H n=24	A/C n=42	C/C n=32	p-value * A/C H vs. L	A/C vs. A/A	C/C vs. A/A	C/C vs. A/C
Adenoma events									
loss 1p36	19	39	29	33	38	n.s.	n.s.	n.s.	n.s.
loss 4q32-pter	29	11	13	12	22	n.s.	n.s.	n.s.	n.s.
LOH/loss 5q	29	50	38	43	38	n.s.	n.s.	n.s.	n.s.
gain 7p15-11	29	17	13	14	25	n.s.	n.s.	n.s.	n.s.
gain 12q13	19	22	8	14	12	n.s.	n.s.	n.s.	n.s.
Carcinoma events									
gain 8q22-24	10	17	21	19	41	n.s.	n.s.	0.01	0.042
gain 13q	5	17	33	26	59	n.s.	0.049	< 0.001	0.005
loss 17p	14	28	33	31	44	n.s.	n.s.	0.02	n.s.
loss 18q12-22	14	33	46	40	66	n.s.	0.028	< 0.001	0.031
gain 20q	10	33	46	40	47	n.s.	0.007	0.003	n.s.
gain 13q combined with loss 18q12-22	0	12	13	12	41	n.s.	0.037	< 0.001	0.005
Lymph node metastasis									
gain 1q23	0	0	0	0	9	n.d.	n.d.	n.s.	0.023
Other progression events									
8p loss	5	6	8	7	34	n.s.	n.s.	0.007	0.003
14q loss	10	0	8	5	22	n.s.	n.s.	n.s.	0.024
15q loss	0	6	8	7	25	n.s.	n.s.	0.003	0.032
19q gain	5	0	4	2	16	n.s.	n.s.	n.s.	0.028
Mutations †									
KRAS	53 (9/17)	67 (10/15)	67 (12/18)	67 (22/33)	50 (12/24)	n.s.	n.s.	n.s.	n.s.
APC	61 (11/18)	76 (13/17)	50 (10/20)	62 (23/37)	46 (13/28)	n.s.	n.s.	n.s.	n.s.
KRAS & APC	28 (5/18)	47 (7/15)	32 (6/19)	38 (13/34)	16 (4/25)	n.s.	n.s.	n.s.	n.s.
Immunohistochemistry †									
P53	5 (1/20)	0 (0/13)	46 (10/22)	29 (10/35)	63 (17/27)	0.001	0.022	< 0.001	0.006
SMAD4-faint ‡	48 (10/21)	88 (15/17)	64 (14/22)	74 (29/39)	81 (22/27)	n.s.	0.04	0.13	n.s.
SMAD4-neg ‡	0 (0/21)	18 (3/17)	18 (4/22)	18 (7/39)	41 (11/27)	n.s.	0.011	< 0.001	n.s.

Abbreviations: n.s.= not significant; n.d.= not determined. *p-values were computed by χ^2 test. † For both mutational analysis and immunohistochemistry, not all cases could be typed, due to technical limitations. For each group the number of typed individuals with a mutation/staining and the total number typed are indicated in brackets. ‡ Reduced expression of SMAD4 protein expression (SMAD4-faint); completely negative for SMAD4 protein expression (SMAD4-neg).

cases. In the current study, we observed that the carcinoma or "malignant" events were all significantly different between the C/C and A/A groups (Table 2). Three of the five events were also significantly different between the A/C and A/A groups (13q gain, 20q gain, and 18q12-22 loss) and between the C/C and A/C groups (8q22-24 gain, 13q gain, and 18q12-22 loss). In addition, 13q gain combined with 18q loss was significantly different between the groups. Moreover, additional carcinoma progression events were identified in this study: loss of 8p, 14q and 15q and gain of 19q were all increased in carcinoma fractions (C/C) in comparison with their adenoma counterparts (A/C).

Mutations of APC and KRAS

To supplement chromosomal instability data, mutational status of colorectal cancer genes APC and KRAS was studied. A major function of the APC protein is β-catenin degradation. Mutations in APC result in the loss of β-catenin binding sites; however, when the mutation is in the mutation cluster region, one or two active β-catenin binding sites are retained. Albuquerque et al. posed that the position and type of the second hit on APC depends on the localization of the first hit. [30] Patients with the first mutation around codon 1300 acquire the second hit by allelic loss, whereas patients with a first mutation elsewhere acquire truncating mutations within the mutation cluster region rather than loss/LOH. The amount of remaining β-catenin binding sites might lead to a different biological behavior of the tumors. For KRAS, the type of mutation was also suggested to be of significance. In a large data set, the valine alteration was correlated with shorter survival in relation to other mutations. [31]

In the pure adenomas, we observed high percentages of APC and KRAS mutations (61% and 53% respectively), comparable with frequencies in the A/C (62% and 67%) and C/C tumor fractions (50% and 46%, Table 2). For APC, we examined whether patients with 5q retention had other types of APC mutations compared with cases with 5q LOH/loss. In the cases with 5q LOH/loss, we observed that 64% had an APC mutation, whereas cases with 5q retention showed a frequency of 52% (not significant). There was no difference in the type of mutation and, consequently, in the amount of remaining β-catenin binding sites, among A/A, A/C and C/C samples. For KRAS, we examined if we could detect any difference in type of mutation. Glycine to valine and glycine to aspartic acid were the most frequent alterations (n=11 and n=13, respectively). However, no difference in type of mutation was observed among A/A, A/C, and C/C samples. The A/C group had the most double mutations; 38% had a mutation in both APC and KRAS, compared with 28% for the A/A cases and 16% for the C/C cases. However, this difference was not significant.

p53 and SMAD4 immunohistochemistry

Not many target genes on chromosomes 8q, 13q, 17p, 18q and 20q have been unequivocally identified. However, the role of p53 on 17p and SMAD4 on 18q has been amply documented in the tumorigenesis of CRC. [32-35] Nevertheless we cannot rule out completely that other genes

are targeted by these chromosomal aberrations as well. Because reliable immunohistochemistry was available, we did p53 and SMAD4 immunohistochemical staining on tissue microarrays and correlated the findings to allelic loss status. Although 17p loss frequency was only significantly different between the A/A and C/C tumors, aberrant p53 staining (25-100% positive nuclei, indicative for a mutation) was significantly increased in the A/C group compared with the A/A group (29% versus 5%, $p = 0.022$) and in the C/C group compared with both the A/C and A/A groups (63% versus 29%, $p = 0.006$ and 63% versus 5%, $p < 0.001$, respectively; Table 2). In addition, significantly more aberrant staining was observed in the A/C group with high-grade dysplasia compared with low-grade dysplasia (46% versus 0%, $p < 0.001$). For SMAD4, we assessed both the percentage of down-regulation and complete loss of protein expression. Down-regulation of SMAD4, as well as complete loss of SMAD4, were both significantly increased in the A/C cases when compared with the A/A cases (74% versus 48%, $p = 0.04$ and 18% versus 0%, $p = 0.011$, respectively), and complete loss of SMAD4 was different between C/C and A/A cases (41% versus 0%, $p < 0.001$; Table 2). Both down-regulation and complete loss of SMAD4 expression were correlated with 18q loss ($p = 0.018$, $p = 0.011$, respectively).

Association of chromosomal aberrations to clinicopathologic features

We investigated whether several clinicopathologic markers were associated with chromosomal aberrations. The malignant tumors were significantly smaller than the pure adenomas (mean diameter 4.6 versus 6.3 cm, $p = 0.032$); however, the total number of aberrations, or the amount of the five malignant aberrations, did not correlate with tumor size. Furthermore, samples from different T stages were compared. We compared 10 T2 carcinomas with 25 T1 carcinomas. No significant differences were observed between these groups in total chromosomal instability or malignant aberrations. Nine cases with local recurrences were compared with those without recurrences. However, no significant differences were observed. Three samples had lymph node metastasis, but this number was too small to make any comparisons.

Systematic comparison of adenoma and carcinoma tissue in the same lesion

Figure 1 shows a systematic comparison between the adenoma and carcinoma fraction of single cases for all genomic aberrations. Most data points are slightly above the x=y line, indicating that carcinoma fractions have slightly more aberrations than the corresponding adenoma fractions. Correlation coefficients between adenoma and carcinoma fractions were 0.229 ($p = 0.180$) and 0.516 ($p = 0.001$) for the total number of aberrations and the five "malignant" aberrations, respectively. The adenoma fractions with low-grade dysplasia showed fewer aberrations than the adenoma fractions with high-grade dysplasia; however, this difference was not significant. Four carcinoma fractions (11%) showed the same number of aberrations as their corresponding adenoma fraction, whereas 47% showed one to five extra events, and 28% showed more than five extra events in the carcinoma fraction. (Figure 1A) For five cases (14%), the adenoma fraction contained more aberrations than its corresponding carcinoma fraction.

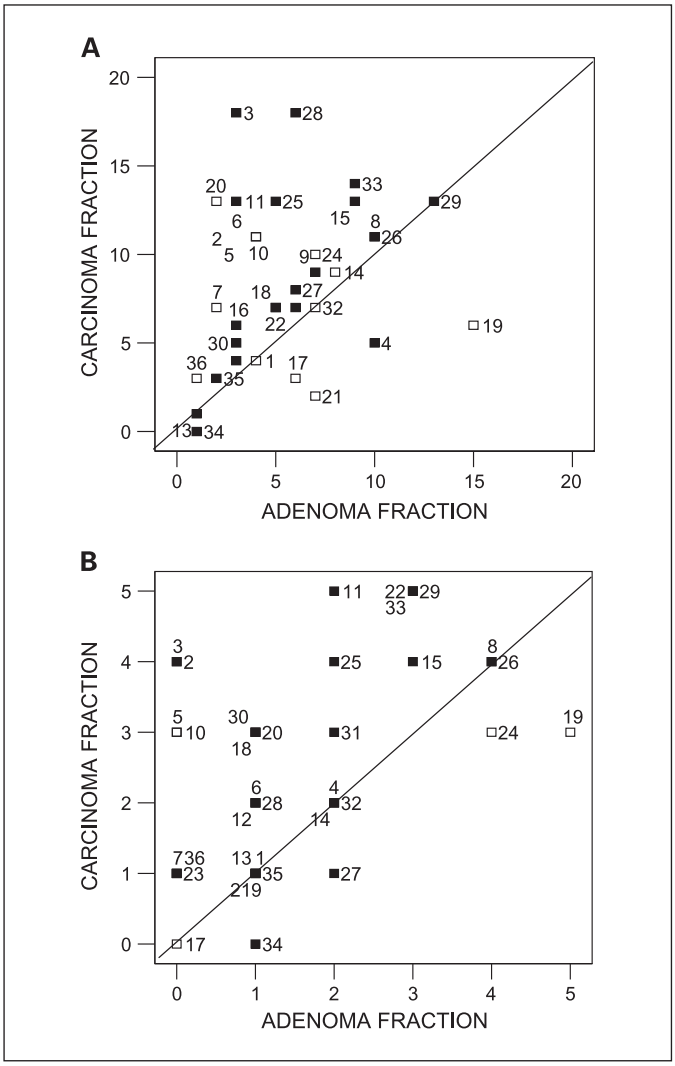

Figure 1. A and B, all 36 adenoma-carcinoma pairs are plotted against each other. X axis, adenoma fraction; Y axis, matching carcinoma fraction. Respectively, the amount of all aberrations (A) and the five malignant events (B) are shown. A, the degree of dysplasia for the adenoma fraction is indicated (white, low-grade dysplasia; black, high-grade dysplasia). Numbers in the plot indicate the sample ID. B, several pairs coincide in the same data point. For cases 8, 17, 18 and 36, no carcinoma fraction was analyzed (see Table 1), and for these samples, we compared the adenoma with low- versus high-grade dysplasia. For samples 14 and 24, the adenoma fraction with low-grade dysplasia was plotted.

Figure 1B compares the occurrence of the five malignant aberrations between the adenoma and carcinoma fraction in the same lesion. In 42% of the adenoma fractions, two or more malignant events were identified. In 11 cases (31%), the amount of malignant events was identical in the adenoma and carcinoma fraction of one tumor. In 25% of all cases, one extra

Table 3. Distribution of genomic alterations over the chromosomes in adenoma and carcinoma fractions of single lesions (n=36).

Chromosome	Aberrations present in both fractions	Aberations present in carcinoma fraction, not in adenoma fraction	Aberrations present in adenoma fraction, not in carcinoma fraction
1p	10	6	5
1q	4	0	1
2p	1	1	0
2q	2	1	0
3p	1	3	2
3q	0	1	0
4p	3	4	3
4q	4	5	1
5p	0	4	2
5q	12	4	4
6p	5	7	2
6q	3	4	1
7p	8	3	0
7q	7	4	1
8p	4	9	2
8q	5	8	2
9p	2	4	1
9q	3	3	2
10p	1	5	1
10q	2	5	2
11p	0	2	0
11q	1	2	1
12p	7	4	2
12q	7	4	1
13q	9	13	1
14q	2	6	1
15q	3	7	1
16p	2	4	1
16q	3	3	2
17p	10	5	3
17q	5	0	5
18p	10	10	2
18q	11	14	2
19p	3	3	0
19q	1	5	0
20p	7	7	2
20q	9	9	2
21q	4	4	1
22q	6	3	2

malignant event was detected in the carcinoma fraction, whereas in 33% two or more extra malignant events were detected. In four cases (11%), the adenoma fraction contained more malignant aberrations than the carcinoma fraction. For cases with more aberrations in the adenoma than in their carcinoma counterparts, we determined if data were in accordance with immunohistochemistry. For instance, in case 24, the adenoma fraction showed loss of 18q and reduced SMAD4 protein expression, whereas the carcinoma fraction showed 18q retention and a normal SMAD4 staining pattern. In the other samples, immunohistochemistry also confirmed chromosomal aberrations.

Table 3 shows the distribution of the genomic changes over the chromosomes. As expected, the malignant aberrations were the most common "progression" events, as these had the highest frequency in the carcinoma fractions, while the corresponding adenoma fractions did not show this event. 13q and 18q were especially strongly increased; in 13 and 14 cases, respectively, the carcinoma fraction contained this event in contrast to the adenoma fraction. The other extra events in the carcinoma fractions did not involve specific chromosomes, as the random distribution of events over the chromosomes shows.

Intratumor heterogeneity analysis in tissue biopsies

For the clinical application of chromosomal instability profiling, accurate analysis of preoperative tissue biopsies is essential. To mimic these biopsies, we investigated three postoperative biopsies for each tumor and estimated how representative these biopsies are for the tissue sample because intratumor heterogeneity is a well-known phenomenon in colorectal cancer.

Three different biopsies were postoperatively taken ex vivo from five pure adenomas and 13 carcinomas at random positions from the surface of the tumor and analyzed with SNP arrays (Table 1). Figure 2A shows genome wide chromosomal aberrations in the different biopsies and their corresponding adenoma or carcinoma fraction.

Roughly the same pattern of aberrations is seen in the different biopsies and the corresponding tumor fraction of the same patient. The number of "malignant" aberrations for all three biopsies, and the adenoma and carcinoma fractions per patient, is comparable for most cases. (Figure 2B) In 3 out of 18 (17%) tumors (cases 16, 30 and 54), the amount of "malignant" aberrations differed considerably between the biopsies and the tumor fractions, whereas in the majority of cases (15 out of 18, 83%) the biopsies showed one different "malignant" aberration at most.

We hypothesized that the biopsy with the largest number of chromosomal aberrations is representative for the tumor. Correlation coefficients for the number of total aberrations and for the number of "malignant" aberrations between that biopsy and the corresponding tissue fraction were 0.660 ($p = 0.003$) and 0.807 ($p < 0.001$; Figure 2C), respectively (biopsies containing adenoma tissue were compared with adenoma fractions, and carcinoma biopsies were compared with carcinoma fractions). We simulated the effect of taking, at random, one or two biopsies (instead of three). Taking only one biopsy resulted in a lower correlation, whereas the effect of two biopsies was nearly comparable with that of three biopsies (data not shown).

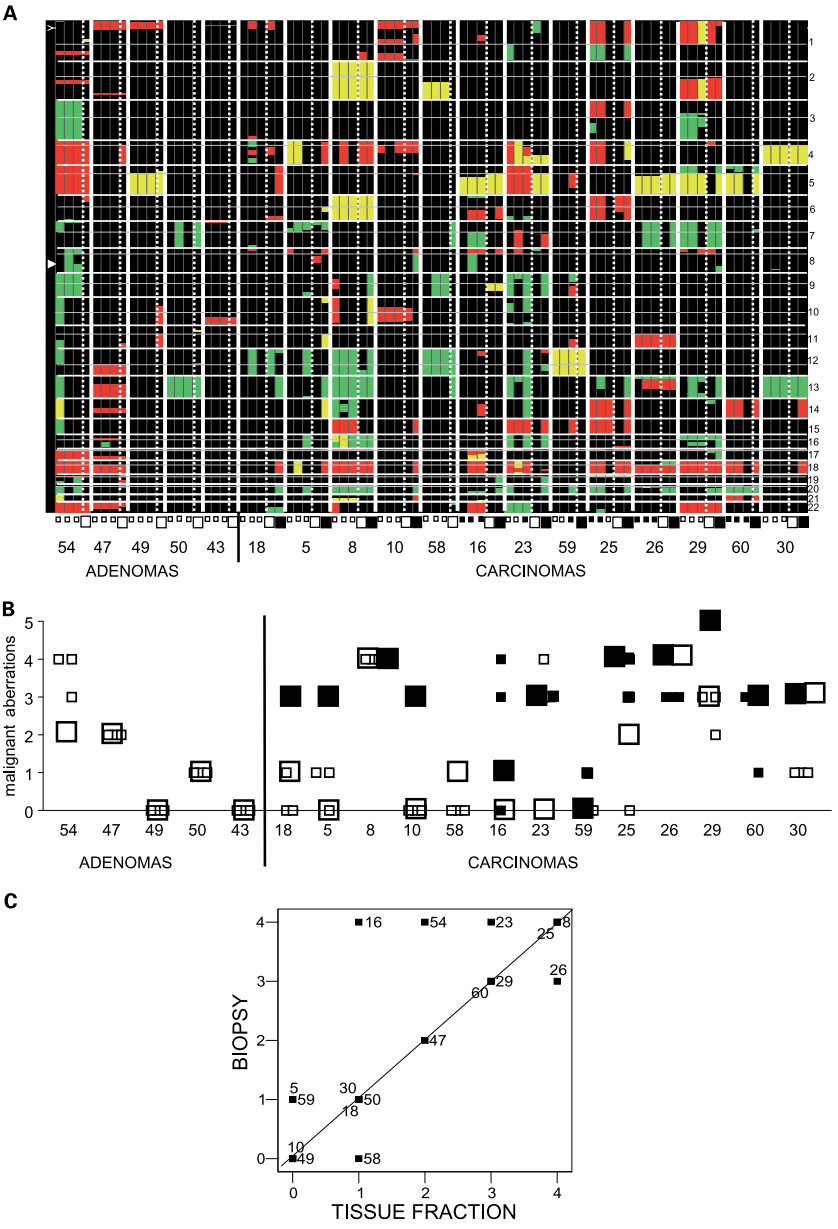

Figure 2. A to C, overview of the 5 pure adenomas and the 13 adenoma-carcinoma pairs from which three biopsies per tumor were analyzed. We show the three biopsies per tumor, the adenoma fraction, and the carcinoma fraction, respectively. Numbers on the X axis indicate the sample ID, whole tumor fractions are indicated by large squares, and biopsies are indicated by small squares (white, adenoma tissue; black, carcinoma tissue). A, all different aberrations are shown for every sample and all chromosomes. Green, gain; red, loss; yellow, copy number neutral LOH. B, amount of five malignant aberrations per tissue sample. C, the amount of five malignant aberrations for the whole tissue fraction (X axis) against the biopsy with the most aberrations (Y axis) was plotted per tumor sample. Labels in the plot indicate the sample ID.

DISCUSSION

For correct preoperative staging of rectal tumors, especially large sessile adenomas eligible for TEM resection, it is necessary to identify those adenomas already containing an invasive focus. In a previous study, we found that five specific chromosomal aberrations could clearly discriminate sessile adenomas from carcinomas. [12] Moreover, in adenoma fractions from cases with a carcinoma, twice the amount of such "malignant" aberrations was observed, as compared with pure adenomas. In the present study, we analyzed the adenoma and carcinoma fractions of 36 rectal tumors and found that two or more malignant events are present in 46% of the adenoma fractions and that the increase in malignant aberrations in adenoma to carcinoma progression was relatively small. Intratumor heterogeneity analysis showed that it is essential to analyze multiple biopsies for a correct assessment of chromosomal instability patterns. The Vogelstein progression model for colorectal tumorigenesis, proposed in 1990 and adapted in the years after, has been addressed by many other studies. [20, 36-38] We now seek to use such data for clinical decision making. Our study showed that three of the five malignant events (gain of 13q and 20q and loss of 18q) were already abundant and significantly increased in rectal adenoma fractions of carcinoma cases compared with pure adenomas. The two other malignant events (8q gain and 17p loss) were not significantly changed, but percentages were increased. Furthermore, 17p loss was related to aberrant nuclear staining for p53 using immunohistochemistry, which was significantly different in adenomas with a carcinoma focus versus pure adenomas. Loss of 18q and SMAD4 immunohistochemistry showed an identical relationship. The relative additional amount of chromosomal aberrations in the transition from adenoma to carcinoma was most often equal in cases with a limited amount of adenomatous aberrations to those with a high amount of such events.

Hermsen et al. described seven cancer-associated events (loss of 8p, 15q, 17p and 18q and gain of 8q, 13q and 20q) that were associated with both carcinomas and adenoma fractions of carcinomas. [15] In addition, they found that these chromosomal abnormalities occurred in specific combinations of a few abnormalities rather than as a mere accumulation of events. We did not identify a specific combination of events but found that most carcinomas have at least two of the five malignant events. In addition, we identified gain of 19q and loss of 8p, 14q, and 15q as later events in carcinoma progression, as these were increased in the carcinoma fractions (C/C) compared with the adenoma fractions (A/C). These regions are, in part, similar to the results of Diep et al, who reported deletion of 8p and 14q and gain of 1q and 19q as late events that correlated with metastasis in a meta-analysis of 859 colorectal cancers. [11]

SNP array analysis of three different ex vivo core biopsies per tumor showed a large degree of intratumor heterogeneity. Hence, it is essential to analyze several tumor fractions per patient for an accurate assessment of genetic changes. Athough intratumor heterogeneity is a well-studied phenomenon in CRC, our study is the first to assess genome wide heterogeneity through SNP array analysis in a series of rectal tumors. [19, 20, 39, 40] Losi et al. found intratumor

heterogeneity in 90% of early colorectal cancers, a percentage that corresponds to our data. [20] In addition, Baisse found heterogeneity in 67% of colorectal cancer. [40] Studies in colorectal and other cancers showed that accumulation of clonal diversity is a fundamental principle in cancer progression. [41-43] In our study, less heterogeneity was present when only the five malignant aberrations were tested. Moreover, a good correlation was established between the biopsy with the most aberrations per patient and the corresponding adenoma or carcinoma fraction. In spite of the observed heterogeneity, it seems that three biopsies per tumor can reliably assess the chromosomal aberrations in rectal tumors.

Surprisingly, some adenoma fractions showed more aberrations than their carcinoma counterparts. Likewise, several biopsies contained other or more aberrations than their corresponding tumor fraction. This interesting finding can be explained by different factors. First, tumor heterogeneity might be a reason; the carcinoma fraction of such a case might have arisen from a different tumor clone than the adenoma fraction studied. The fact that four cases showed either APC or KRAS mutations in the adenoma fraction and not in the carcinoma fraction also suggests that the carcinoma did not arise from the adenoma clone. Consistent with our findings, Zauber et al. found a difference between the adenoma and carcinoma portion of tumors with regard to the KRAS gene in 24% of 37 neoplasms. [44] Second, it was frequently observed that a carcinoma fraction had a larger stromal involvement and thus a somewhat lower tumor cell percentage than the adenoma fraction. Although a lower tumor cell percentage might make it more difficult to depict chromosomal aberrations, most aberrations seemed very reproducible. However, with too many contaminating stromal cells, a certain chromosomal aberration might be present in too few cells to be detected by current techniques. Laser capture microdissection might offer a solution for research, but is not feasible for a clinical application.

A recent study showed that retention of chromosome 5q correlated with liver metastasis in colorectal cancer. [45] The authors found that tumors with 5q deletion (loss or LOH) have a different type of APC mutation than cases with 5q retention. Cases with 5q deletion usually have one APC allele affected by a mutation, usually leaving one or two β-catenin binding sites, whereas cases with retention usually have two different APC mutations. [30] This can lead to differences in residual β-catenin activity, which in turn can show an effect on the neoplastic process. We did not detect any significant difference between cases with 5q retention versus 5q LOH/loss regarding APC mutations in the mutation cluster region. However, we analyzed only the mutation cluster region of APC, starting at codon 1284. If mutations occurred before the mutation cluster region, this would lead to loss of all β-catenin binding sites in one allele, as is probably the case in the samples with 5q retention.

As a large proportion of presumed sessile rectal adenomas seem to identify postoperatively as carcinomas, there is a need for additional preoperative tests. Most carcinomas in this study were preoperatively classified as adenomas; thus, a TEM was done. In the majority of carcinoma cases, preoperative and ex vivo core biopsies contained adenoma tissue, indicating that it is difficult to obtain a correct preoperative diagnosis using standard histopathology. Interestingly,

15 out of 36 (42 %) adenoma fractions of carcinoma cases had two or more malignant aberrations, indicative of malignancy. Aberrant p53 and SMAD4 immunohistochemical staining correlated with 17p and 18q loss, respectively, and were both increased in adenoma fractions of carcinomas in contrast to pure adenomas. Such p53 immunohistochemistry showed an even better discrimination between pure adenomas and adenoma fractions of carcinoma cases than 17p loss, indicating that some cases might have two somatic mutations in the p53 gene, instead of one mutation combined with chromosomal loss. However, we cannot exclude that other genes might be targeted by the loss. For chromosome 8q, 13q and 20q gain, the target genes are largely unknown, although a prime target on 8q might be the cMyc gene, for example. BRCA2, Rb and other tumor suppressor genes locate on chromosome 13q. Although 13q loss is observed in most cancer types, this chromosome usually shows gain in colorectal cancer. Earlier observations indeed showed increased copy numbers of one Rb1 allele, and increased levels of Rb mRNA and protein expression in CRC. [46-48] The role of Rb in colorectal cancer development is thus not clear. Currently we are integrating gene expression analysis with the obtained SNP data in order to study the effect of chromosomal aberrations on the transcriptional level.

Our ex vivo biopsy analysis showed that the analysis of small biopsies is feasible because the chromosomal aberrations were reliably identified. Additionally, biopsies were taken at the surface of the tumor, just as in the preoperative situation. The five chromosomal regions and immunohistochemistry for p53 and SMAD4 should now be evaluated on a large series of multiple preoperative biopsies. However, reservations may exist to the application of the above approach, given that some adenomas tend to harbor more aberrations than their carcinoma counterparts. After validation studies, these methods can hopefully be added to future histological analysis and imaging methods, possibly leading to improved rectal tumor staging.

In conclusion, adenoma fractions of rectal carcinoma cases show a high degree of chromosomal instability and have a relatively small increase in genomic alterations in their transition to carcinomas. The occurrence of specific chromosomal events could possibly be used to predict the malignant behavior of sessile rectal adenomas. The analysis of several biopsies per patient revealed a large degree of intra-tumor heterogeneity, but when three biopsies per tumor are analyzed, most aberrations are reliably identified.

ACKNOWLEDGMENTS

We thank Stichting Laboratorium Pathologie en Cytologie (Rotterdam, the Netherlands) and Stichting Samenwerking Delftse Ziekenhuizen (Delft, the Netherlands) for providing the tissue samples and Hans Halfwerk for technical assistance. This project was supported by Dutch Cancer Society grant RUL 2003-2807.

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CHAPTER 9

Predictive value of histopathologic criteria for locoregional failure after transanal endoscopic microsurgery for T1 rectal cancer

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Submitted

INTRODUCTION

Rectal cancer affects over 10 000 new patients and causes 4700 deaths each year in the United Kingdom. Introduction of screening programs will increase the incidence of T1-2 rectal cancers.[1] To avoid the morbidity of radical surgery (RS), local excision (LE) of rectal cancer is being applied increasingly [2], but controversy remains in which stages LE is justified. In general, in T2 or more invasive rectal cancers, LE is only considered a valid option in palliative settings because of the high rate of local recurrences (LR) and reduced survival compared to RS.[2] In T1 rectal cancer only, there might be a role for LE with curative intent. Nevertheless, oncologic outcome is conflicting, with LR rates ranging from 6 to 18 per cent and varying survival. [3-7]

Nowadays, transanal endoscopic microsurgery (TEM) is considered method of choice when treating rectal tumors.[8] It is a modification of local excision that greatly improves accessibility, visibility and precision of resection thereby enabling microscopic radical excision of tumors located throughout the entire rectum.[9] After RS for rectal cancer, microscopic positive excision margins (R1) are negative predictors of outcome.[10] In contrast, if with TEM T1 rectal cancers are excised with a microscopic negative excision margin of 2 mm or more (R0), survival is comparable to RS but LR rates up to 24% have still been reported.[11] Several authors therefore questioned the role of LE, including TEM, for all T1 rectal cancers, as survival in recurrent tumors is diminished.[12-14]

A distinction between low- versus high risk T1 rectal cancer has been proposed, to predict which tumors are likely to recur or not following TEM.[15] The distinction is based on basic histopathological criteria, which are differentiation grade, lymph vessel invasion and blood vessel invasion. [16] Also depth of invasion into the submucosa and tumor budding were identified as independent prognostic features. [17-19] However, these features have been challenged and consensus regarding low- versus high-risk criteria in T1 rectal adenocarcinomas is still lacking.[20, 21]

To expand evidence on low- versus high-risk T1 rectal cancer, with respect to LR, in this study we try to identify predictive histopathological features in a selected group of T1 rectal cancers treated with TEM only. Ultimately this may lead to tailor treatment selection in individual rectal cancer patients.

PATIENTS AND METHODS

From a prospective database, containing over 700 patients treated with TEM in a teaching hospital, a subset of 84 eligible patients was identified. Patients with T1 rectal cancer, treated with TEM between January 1996 and December 2008, without (neo-) adjuvant treatment, in which no completion RS was performed, were considered eligible. An excision margin of 2 mm or more was a prerequisite, and only those patients were considered suitable for intensive follow-up. Preoperative evaluation, surgical technique and outcome of the entire group have

already been published.[11] Our hospital acts as a tertiary referral center for TEM. A substantial proportion of patients was referred from other hospitals following snare polypectomy, in whom excision margin was uncertain, for removal of the scar with TEM. A total of 62 patients of whom the specimens of the primary tumor could be re-evaluated, containing an invasive T1 carcinoma, were included in the present study. The group consisted of 27 females and 35 males with a mean age of 69 years (range 44-92). Follow up was according to the Dutch guidelines on rectal cancer with additional rigid rectoscopy and endorectal ultrasound (ERUS) every 3 months the first 2 years, and every 6 months thereafter for the detection of a LR. Magnetic resonance imaging (MRI) of the lesser pelvis was introduced as a part of the follow-up protocol during the study period and is routinely performed at 12, 24 and 36 months following TEM. Mean follow up of the entire group was 53 months (range 6-126). In case a LR was suspected a histological confirmation was obtained by biopsies.

In all patients a renewed histopathological evaluation was performed by two independent pathologists (EZ, JM), blinded to clinical outcome. All tumor features were scored according to predefined criteria (Table 1). Features assessed were specimen- and tumor area, maximum tumor size, size of invasive carcinoma and ratio of invasive carcinoma. Also tumors were scored as high- or low-risk, according to accepted criteria (differentiation grade, lymph vessel invasion (LVI) and blood vessel invasion (BVI)). Furthermore, distance from the deepest invasive front to the muscularis propria was measured in mm and submucosal invasion depth, differentiating between deep and superficial submucosal invasion was scored. We scored tumors as superficial if only the upper two thirds of the submucosa was invaded (Sm1 and 2 according to Kikuchi) and we scored the tumor as deep if the lower one third of the submucosa was invaded (Sm3). The reason for this simplification was that in our series the exact measurement of Sm1-3 was not possible due to secondary tissue changes, such as exophytic tumor growth that could affect normal tissue dimensions of the submucosa. Finally we scored for the presence of so-called tumor budding. Tumor budding is defined as isolated cancer cells or small cell clusters (< five cells) at the advancing edges of the invasive front of the cancer. [22] Positivity for budding was scored when there were > five buds per 20x power field.

All statistical analyses were performed with the Number Cruncher Statistical System 2001 (NCSS Statistical Software, Kaysville, UT, USA). Statistical analysis of categorical variables was performed on cross-tables using the Pearson χ^2 test. The Kaplan-Meier method was used to estimate survival probabilities and these were compared using the log rank test. A p-value of < 0.05 was considered significant.

Table 1. Definitions of the criteria used for the histopathological evaluation of the H&E stained slides of the TEM resection specimens. These criteria were applied to the slide that showed the deepest infiltration of the tumor.

Tumor feature	Predefined criteria
Specimen area	Maximum length x maximum width of specimen, measured after fixation on a cork board
Tumor area	Maximum length x maximum width of tumor, measured after fixation of the specimen on a cork board
Size of invasive carcinoma	Maximum size of part that is truly of carcinogenic differentiation either with invasion in the tunica propria and a cribriform growth pattern (C1) or with invasion through the muscularis mucosae (C2)
Percentage carcinoma	The percentage of the entire lesion removed by TEM that is truly of carcinogenic differentiation either with invasion in the tunica propria and a cribriform growth pattern (C1) or with invasion through the muscularis mucosae (C2).
Tumor grade	Tumor grade is determined by the percentage of the lesions that shows formation of gland-like structures. -Well differentiated (grade I): glandular structures in >95% of the lesion -Moderate differentiated (grade II): glandular structures in 50-95% of the lesion -Poor differentiated (grade III): glandular structures in 5-50% of the lesion -Undifferentiated: glandular structures in <5% of the lesion
Lymph vessel invasion	Invasion in lymph vessel-like structures outside the primary lesion
Blood vessel invasion	Invasion in blood vessel-like structures outside the primary lesion
Invasion depth (mm)	The invasion depth is measured as the distance between the deepest infiltrating part of lesion and the muscularis propria in millimeters
Invasion classification	The invasion depth is classified as: - Deep: when the lesion infiltrates more than 2/3 of the distance between muscularis mucosae and the muscularis propria. - Superficial: when lesion infiltrates less than 1/3 of the distance between muscularis mucosae and the muscularis propria
Budding	Budding is defined as an isolated single cancer cell and a cluster composed of fewer than five cancer cells. These scattered foci are observed in the stroma of the actively invasive frontal region

RESULTS

Patient and tumor characteristics are depicted in Table 2. Overall recurrence rate at three years was 28%. (Figure 1a) Mean maximum tumor size in non-recurrent tumors was 3 cm (range 0.5-8.5), compared to 5.1 cm in recurrent tumors ($p < 0.001$). Mean size of the invasive focus was comparable in both groups (nine mm). LR rates at 1, 2 and 3 years, according to maximum tumor size, are shown in Table 3. A cut-off value of 3 cm proved to be of predictive value, with LR-rates at three years in tumors larger than 3 cm of 39%, versus 16% in tumors of 3 cm and smaller ($p < 0.03$; Figure 1b).

Of nine high-risk tumors, according to accepted criteria (poor differentiation and/or LVI and/or BVI), three recurred (33%), whereas of 53 low-risk tumors 16 recurred (30%; Table 2). This proved

Table 2. Patient and tumor characteristics.

	Non-recurrent	Recurrent	p-value
Number of T1 rectal carcinomas	43	19	
Age (range)	68 (44-92)	69 (50-84)	NS
Female: Male	18:25	9:10	NS
Post snare coagulation	10 (23%)	3 (16%)	NS
Tumor location (%)			
Upper rectum (10-15 cm)	8 (19%)	2 (11%)	NS
Mid rectum (5-10 cm)	21 (49%)	13 (68%)	NS
Lower rectum (0-5 cm)	14 (33%)	4 (21%)	NS
Mean specimen area in cm^2 (range)	19 (2.25-63)	38 (5-84)	$p < 0.001$
Mean tumor area in cm^2 (range)	11 (0.5-56)	34 (2.25-156)	$p < 0.001$
Mean maximum tumor size in cm (range)	3 (0.5-8.5)	5.1 (1.5-9)	$p < 0.001$
Mean invasive carcinoma diameter in mm (range)	9 (1-22)	9 (0.3-17)	NS
Mean invasive carcinoma ratio (%)	46	46	NS
Differentiation grade			
Well	0	1	
Moderate	40	17	
Poor	3	1	NS
Lymph vessel invasion			
Yes	4	1	
No	39	18	NS
Blood vessel invasion			
Yes	4	3	
No	39	16	NS
High-risk	6	3	
Low-risk	37	16	NS
Invasion depth from proper muscle (mm)	1.4 (0.1-6)	1.8 (0.1-10)	NS
Invasion classification			
Superficial	25	11	NS
Deep	18	8	
Tumor budding			
Yes	11	8	P = 0.16
No	32	11	

Numbers are absolute unless otherwise specified. NS= not significant; Low-risk= well or moderately differentiated, no LVI or BVI; High-risk= poorly differentiated and/or LVI and/or BVI.

to differ non-significantly. Also when combining tumor size with accepted high-risk-criteria, there were no significant differences in LR rates between these combination groups.
Submucosal invasion depth and budding were of no significant predictive value either, although the latter almost reached statistical significance. To incorporate all potentially important predictive variables in further analysis, we combined tumor size with submucosal invasion depth, budding and a combination of both. The combination of maximum tumor size and submucosal

Table 3. Local recurrence (LR) rates according to maximum tumor size at 1, 2 and 3 years.

Maximum tumor size	LR-rate at 1 year	LR-rate at 2 years	LR-rate at 3 years	p-value
≤ 4 cm	5%	5%	10%	p = 0.01
> 4 cm	27%	35%	38%	
≤ 3 cm	13%	13%	16%	p < 0.03
> 3 cm	26%	35%	39%	
≤ 2 cm	10%	10%	15%	p = 0.1
> 2 cm	24%	31%	33%	

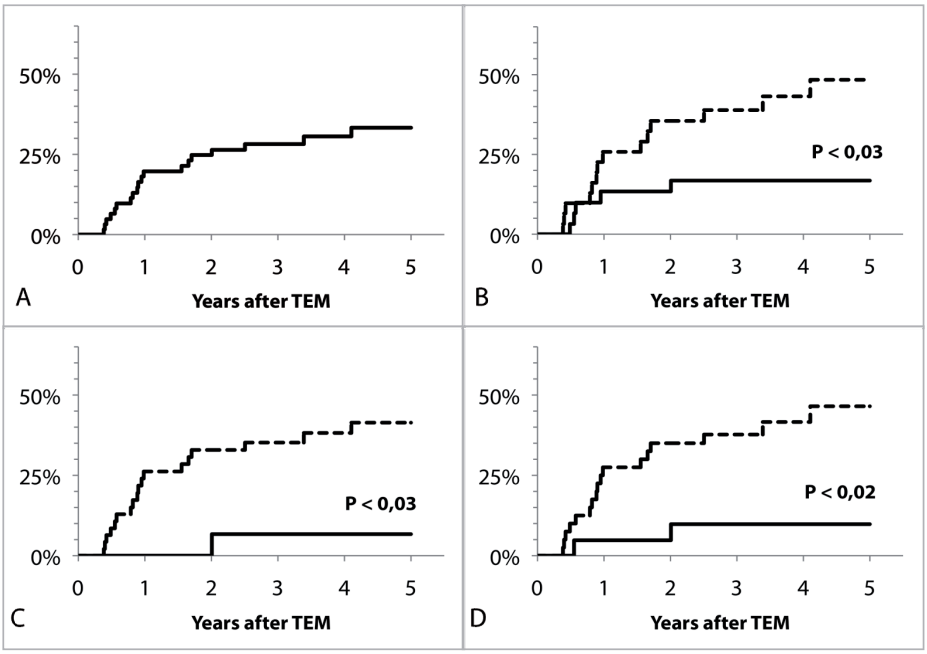

Figure 1. Local recurrence rates according to tumor features.
A (top left): LR rates of all tumors
B (top right): LR rates of tumors ≤ 3 cm (solid line) and tumors > 3 cm (dotted line)
C (bottom left): LR rates of tumors ≤ 3 cm without deep submucosal invasion (solid line) and tumors ≤ 3 cm with deep submucosal invasion or tumors > 3 cm (dotted line)
D (bottom right): LR rates of tumors ≤ 3 cm without budding (solid line) and tumors ≤ 3 cm with budding or tumors > 3 cm (dotted line)

invasion depth appeared to improve results. Patients with a tumor of 3 cm and smaller without deep submucosal invasion had a LR rate at three years of 7%, compared to 35% if submucosal invasion was deep, or maximum tumor size exceeded 3 cm ($p < 0.03$; Figure 1c). Combining tumor size and budding also improved results, with a 3-year LR-rate of 10% in tumors of 3 cm and smaller without budding, compared to 38% if budding was present, or maximum tumor size exceeded 3 cm ($p < 0.02$; Figure 1d).

However, when combining tumor size with both submucosal invasion depth and budding, the differences between the LR rates of the combination groups were not significant ($p < 0.1$).

DISCUSSION

Transanal endoscopic microsurgery (TEM) is being incorporated more and more in the surgical armamentarium for the removal of rectal tumors. Mainly because of worsened functional results after total mesorectal excision and the low rate of lymph node metastases, TEM is adapted in several national guidelines as a curative option in the treatment of selected T1 rectal cancers. [23] However, despite a microscopic radical excision margin in most cases, LR rates remain as high as 24%. [11] As survival is limited in locally recurrent tumors following TEM, tumor selection is of utmost importance.[14]

In our series, maximum tumor size proved to be a highly predictive feature for locoregional failure. This is in accordance with a review by Graham et al, in which local recurrence rates following LE of tumors smaller than 3 cm was 11% versus 33% in larger tumors. [24] However in their review this difference was not significant. In our series dividing between tumors of 3 cm and smaller and tumors larger than 3 cm resulted in LR rates at three years of 16% and 39% respectively, which was a significant difference ($p<0.03$). Dividing between tumors of 2 cm and smaller versus larger tumors, was of no additional value.

Surprisingly, the size of the invasive focus had no influence on the LR rates after TEM. This unexpected finding warrants further investigation on whether spillage of viable tumor cells during the TEM procedure is responsible for the outgrowth of a local recurrence. Another possible explanation could be the outgrowth of untreated lymph node metastases. Other studies already showed that even with ERUS nodal staging in rectal cancer is difficult and probably inadequate. [25] Further studies should focus on these issues and the role of pre- or postoperative radiotherapy should be evaluated.

In the present series of 62 patients, accepted low- and high-risk criteria were of no predictive value. Even combining maximum tumor size with these criteria was of no value. Accepted low-risk tumors are well to moderately differentiated T1 rectal cancers, without (lymph-) vessel invasion. [26] However, evidence is not abundant and inter- and intra-observer variability in scoring each of those items is not to be underestimated. [27-29] This study again questions the reproducibility and predictive value of basic histopathological staging.

Although submucosal invasion depth is also considered a predictive factor in T1 rectal cancer, [18, 30] others questioned the utility of grading criteria for submucosal invasion in T1 colorectal carcinomas. [20, 21] In the present series submucosal invasion depth was not predictive for the development of LR. We also measured absolute distance from the invasive front to the muscularis propria, and again this was of no influence on LR rates.

However, when combining submucosal invasion depth with maximum tumor size the identification of low-risk tumors was possible. In tumors smaller than 3 cm without deep submucosal invasion, LR rate at three years of only 7% was found, compared to a LR rate of 35% in case submucosal invasion was deep or tumor size exceeded 3 cm.

Recently, several other features were added as possible risk factors. Many researchers have already reported that dedifferentiated histology at the invasive margin (tumor budding) is significantly associated with tumor aggressiveness in many types of cancer, including tongue [31], lung [32] and colorectum [33]. Again however, in most series focusing on rectal cancer, the number of studied patients is low and results should be interpreted with caution. [19] In the present series, positivity for budding proved to show a trend towards significance ($p = 0.16$).

Combining size and budding proved to be an accurate predictive combination. In tumors of 3 cm and smaller, without budding, at three years LR rate was 10%, whereas if budding was present or tumor size exceeded 3 cm LR rate was 38% was found.

Finally, in tumors of 3 cm and smaller without budding and without deep submucosal invasion, LR rates differed not significantly (3-years 9% versus 38%; $p < 0.1$), due to the low number of tumors in this subgroup.

How are these results to be translated into daily practice? First of all it seems obvious that in tumors over 3 cm, containing a T1 invasive carcinoma, although TEM is capable of obtaining a microscopic radical excision margin, it is questionable whether TEM is justified, with a three year LR rate of 39%. However, as over 60% of patients will not develop a LR, treating all these tumors with RS seems overtreatment. Nevertheless, it seems we can identify tumors that will not likely recur. In tumors of 3 cm and smaller, without deep submucosal invasion or without tumor budding, LR rates at three years of 7% and 10% respectively were found. These figures may be well accepted as a trade-off when discussing treatment options with patients, as mortality after total mesorectal excision, especially in the elderly, should not be neglected. [34]

Based on our results further studies should be initiated in which more specialized histopathological evaluation by means of immunohistochemistry is incorporated. But also more tumors are to be analyzed to obtain more reliable results. National databases, such as in the UK and Scandinavia, are to be encouraged, because this may be the way to accomplish this.

In conclusion, therapeutic decision making in T1 rectal cancer is tailor made, however the real solution in identifying patients suitable for TEM is not present yet. When discussing all treatment options in T1 rectal cancer with patients, one has to mention the realistic chances on developing a local recurrence following TEM. We found that tumor size alone, or in combination with submucosal invasion depth or tumor budding, appeared to be a significant predictive feature for locoregional failure following TEM for T1 rectal cancer.

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CHAPTER 10

Summary

SUMMARY

In rectal cancer total mesorectal excision (TME) is the gold standard. However, driven by the aim to avoid a permanent colostomy and the morbidity and mortality of TME, the proportion of patients with rectal cancer treated by local excision (LE) has increased the last two decades. In T1 carcinomas, LE is considered a curative option. The introduction of transanal endoscopic microsurgery (TEM) in 1984 was of major influence to this treatment shift. In **Chapter 1** a general introduction and a review on TEM in T1 rectal cancer are given. It is concluded that TEM is used with enthusiasm and with promising results, but the scientific base upon which this treatment regimen is build is limited.

In **chapter 2** the aim of the thesis is presented.

ONCOLOGIC OUTCOME

As described in chapter 1, TEM is increasingly embraced as a curative alternative in T1 rectal cancer. In chapter 3 and 4 we studied the oncologic outcome of the world largest series of patients treated with TEM for T1 rectal cancer.

After TME for rectal cancer, processing and handling of the resection specimen is standardized, with margin status as a predictor for recurrence. This has yet to be implemented for TEM and was studied prospectively in **chapter 3**. Eighty patients after TEM for T1 rectal cancer were compared to 75 patients after TME for T1 rectal cancer. Standardized processing and handling of the excised specimen was mandatory after both TEM and TME. Patients were only considered eligible for follow-up after TEM when excision margins were negative. TEM was safer than TME as reflected by operating time, blood loss, hospital stay, morbidity, re-operation rate and stoma formation (all $P < 0.001$). Mortality after TEM was 0% and after TME 4%. At 5 years, both overall survival (TEM 75% versus TME 77%) and cancer specific survival (TEM 90% versus TME 87%) were comparable. Local recurrence rate after TEM was 24% and after TME 0% ($P < 0.0001$). It is concluded that, although for T1 rectal cancer TEM is a much safer technique than TME and survival is comparable, the local recurrence rate is substantial after TEM, despite negative excision margins after standardized pathology.

In **chapter 4** the management and outcome of local recurrences following TEM for T1 rectal cancer is studied. During intensive follow-up, 18 patients developed a local recurrence and were analyzed with special emphasis on salvage surgery and survival. Median time to local recurrence was 10 months (range, 4 – 50). Median age at diagnosis of the recurrence was 74 years (range, 56 - 84). Two patients were not operated because of concomitant metastatic disease. Sixteen patients underwent salvage surgery, without the need for extensive surgical procedures. In 44% of patients a permanent colostomy was created. There was no postoperative

mortality. Fifteen patients had a microscopic radical resection and one patient a microscopic irradical resection. Median follow-up of all patients was 20 months (range, 2 - 112). One patient had a re-recurrence and seven patients developed distant metastases. At three years, the overall survival was 31% and the cancer-specific survival 58%. It is concluded that for recurrent disease after TEM for T1 rectal cancer, salvage surgery is feasible in most patients, without the need for extensive surgical procedures. This may be attributable to intensive follow-up. Survival however is limited, mainly due to distant metastases. Tailoring selection of T1 rectal cancers and exploring possible adjuvant treatment strategies following salvage procedures should be the next steps, in order to improve survival.

QUALITY OF LIFE OUTCOME

Following TME**,** functional outcome is often poor with subsequent decreased quality of life. Besides oncologic outcome, differences between TEM and TME in functional outcome and quality of life may be important in therapeutic decision-making. TEM is performed via a rectoscope with a diameter of four centimetres, leading to scepticism regarding postoperative functional outcome. In **Chapter 5** functional outcome and quality of life before and after TEM are investigated. Between 2004 and 2006, 47 patients were studied prior to and at least six months after TEM. Functional outcome was determined using the Faecal Incontinence Severity Index (FISI). Quality of life was measured using the EuroQol EQ-5D questionnaire and the Faecal Incontinence Quality of Life (FIQL) score. Six months after surgery, median FISI score was found to be decreased ($p < 0.01$), depicting an improvement in faecal continence. This improvement was most significant in tumors within seven centimetres from the dentate line ($p = 0.01$). From the patient's perspective postoperative quality of life was found to be higher ($p < 0.02$). A significant improvement was observed in two of the four FIQLS domains (embarrassment; $p = 0.03$, lifestyle; $p = 0.05$). The domains of lifestyle, coping and behaviour, and embarrassment were correlated with the FISI (all $p < 0.05$). It is concluded that TEM has no deteriorating effect on faecal continence. Moreover, once the tumor has been excised using TEM, quality of life is improved.

Impact of both TEM and TME on quality of life has never been compared. In **chapter 6** functional outcome and quality of life following TME and TEM were studied. Fifty-four patients underwent TEM for T1 carcinomas. Only patients without known locoregional or distant recurrences were included, resulting in 36 eligible patients in whom quality of life after TEM was studied. The questionnaires used were the EuroQol EQ-5D, EQ-VAS, EORTC QLQ-C30 and EORTC QLQ-CR38. The results were compared to a sex-and age-matched sample of T+N0 rectal cancer patients who had undergone sphincter saving surgery by TME and a sex- and age matched community-based sample of healthy persons. Thirty-one patients after TEM returned

completed questionnaires (overall response rate 86%). Results were compared to 31 TME patients and 31 healthy controls. From the patients' and social perspective quality of life did not differ between the three groups. Compared to TEM, significant more defaecation problems were seen after TME ($p < 0.05$). A trend towards better sexual functioning after TEM, compared to TME, was seen, especially in male patients, although it did not reach statistical significance. It is concluded that quality of life does not seem to differ following TEM and TME, but defecation disorders are more frequently encountered after TME. If oncologic outcome following TEM and TME is comparable, this difference could play a role in the choice of surgical therapy in T1 rectal cancer.

TUMOR SELECTION

Proper preoperative staging in rectal tumors is essential for therapeutic decision-making as several treatment options are at our disposal. Preoperative biopsies frequently fail to diagnose an invasive carcinoma and early rectal cancer is difficult to assess using CT and MRI. Endorectal ultrasound (ERUS) is considered a useful adjunct in preoperative staging of rectal tumors. However, feasibility of ERUS and its role in therapeutic decision-making in presumed rectal adenomas is sparsely studied. In **Chapter 7** this was investigated. In patients referred for TEM, based on benign pathology in preoperative biopsies, ERUS was performed (N=268) and ERUS staging was compared to postoperative histopathological staging. In 231 tumors (86%) ERUS was technically feasible. Median distance from the dentate line was 11 cm in non-assessable tumors and 7 cm in assessable tumors ($p < 0.001$). In 21 tumors (9%)**,** ERUS was not conclusive, mainly in recurrent tumors or after recent endoscopic manipulation ($p < 0.001$). With ERUS**,** in the remaining 210 tumors the rate of preoperative missed invasive carcinomas could be reduced from 21% to 3% ($p < 0.01$). If T1 carcinomas are considered suitable for TEM, with ERUS the proportion of undertreated tumors could be reduced from 7% to 3% ($p < 0.01$). However, based on ERUS 4% of tumors would have been overtreated, since they were overstaged as uT2/T3. This increase in overtreatment was also significant ($p < 0.01$). We concluded ERUS is technically feasible in almost all tumors referred for TEM and ERUS is very reliable in selecting tumors suitable for TEM. Therapeutic decision-making regarding local excision versus radical surgery based on ERUS seems valid.

In **chapter 8** we investigated whether genomic analysis of biopsies could lead to the proper identification of a rectal carcinoma in presumed adenomas. For that purpose, chromosomal instability patterns were systematically compared in adenoma and carcinoma fractions of the same tumor to assess specific steps in rectal tumor progression. We analyzed 36 formalin-fixed, paraffin-embedded invasive carcinomas of which preoperative biopsies only showed adenomatous tissue. Both the adenoma and carcinoma fractions were typed with single nucleotide

polymorphism arrays and compared with 21 previously described pure adenomas. Eighteen cases were included in an intratumor heterogeneity analysis. Five specific "malignant" events (gain of 8q, 13q and 20q and loss of 17p and 18q) and aberrant staining for p53 and SMAD4 were all increased in the adenoma fractions of carcinoma cases compared with pure adenomas. Paired analysis revealed that 31% of the samples had an equal amount of malignant aberrations in their adenoma and carcinoma fractions, whereas 25% had one and 33% had two or more extra malignant events in the carcinoma fraction. Analysis of three core biopsies per tumor showed a large degree of intratumor heterogeneity. However, the number of malignant aberrations in the biopsy with the most aberrations per tumor correlated with the corresponding adenoma or carcinoma fraction ($r = 0.807$; $P < 0.001$). In conclusion, five specific chromosomal aberrations, combined with aberrant staining for p53 and SMAD4, can predict possible progression of sessile rectal adenomas to rectal cancer and might, after validation studies, be added to preoperative staging. Preferably, three biopsies should be taken from the tumor to address intratumor heterogeneity.

In T1 rectal cancer, discussion on high-risk criteria regarding locoregional failure following TEM is ongoing. Outcome may be improved if predictive tumor features for locoregional failure are identified. For that purpose, in **chapter 9,** a histopathological analysis of T1 rectal cancer specimens, excised with TEM, is performed. In 62 specimens, two independent pathologists, blinded for outcome, scored tumors according to predefined criteria. We were able to identify maximum tumor size as a negative predictive feature, as 39% of tumors larger than 3 cm developed a local recurrence, versus 16% of tumors smaller than 3 cm ($p < 0.03$). Accepted high-risk criteria as differentiation grade, lymph vessel invasion and blood vessel invasion were of no predictive value. Only when combining tumor size with submucosal invasion depth and tumor budding, a subgroup of low-risk tumors could be identified. In tumors of 3 cm and smaller without deep submucosal invasion or without budding local recurrence rates at 5 years were only 7% and 10% respectively. It is concluded, that tumor size alone, or in combination with submucosal invasion depth or tumor budding, appeared to be of significant predictive failure of a LR after TEM for T1 rectal cancer.

FUTURE PERSPECTIVES

With the introduction of nationwide screening regimens for colorectal cancer, the incidence of advanced rectal adenomas and early staged rectal cancer is expected to increase substantially. However, as shown in this thesis, the puzzle on TEM for T1 rectal cancer has not been solved yet. For obvious reasons, expansion of evidence is urgently needed.
Though survival after TEM is comparable to TME, a striking feature is the substantial rate of local recurrences if unselected T1 rectal cancer is treated solely with TEM. A microscopic radical

excision margin, confirmed with standardized handling and processing of the specimen, does not improve results. What is the impact of these local recurrences? Salvage surgery is possible with hardly any re-recurrences. From that respect, one could argue that majority of patients is saved the adverse effects of primary TME. On the other hand, patients with a local recurrence after TEM for T1 rectal cancer have impaired survival. Therefore, future research should focus on improving patient selection preoperative or on combining TEM with (neo-) adjuvant treatment, in order to decrease the local recurrence rate.

Obviously, it is essential upfront to identify rectal cancers, even if a biopsy is suggestive for an adenoma. Our newly developed genomic analysis might be useful. However, clinical validation studies are mandatory. This may be achieved on retrospective paraffin-embedded material, but eventually it has to be shown that genomic analysis of in-vivo biopsies is capable of identifying invasive rectal cancer. Further research is also to be initiated on identifying those rectal cancers already harbouring lymph node metastases. In our study on chromosomal instability patterns, one of the striking findings was the identification of node positive rectal cancers expressing a gain on chromosome 1q23 ($p = 0.023$). As with TEM a lymph node dissection is omitted, and local recurrences may be considered outgrowth of lymph node metastases already present at the time of operation, identifying those tumors already harbouring lymph node metastases is of additional value in therapeutic decision-making. In the end this could mean all node-negative mobile rectal cancers can be excised with TEM, although further studies have to be awaited. Another way of identifying node positive rectal cancers is by means of magnetic resonance imaging (MRI), making use of uptake of ultrasmall superparamagnetic iron oxide (USPIO) particles. Early results were promising, however future results have to be awaited. Hopefully in the near future, with genomic analysis of biopsies in combination with improved diagnostic modalities as ERUS, CT-scan and MRI, proper selection of tumors suitable for TEM will be enabled.

In case a carcinoma was missed pre-operatively and excised microscopically radical with TEM, we are not able yet to predict whether this tumor is likely to recur or not. In our series generally accepted risk-criteria, based on basic histological features, are too robust, although the limited number of TEM-treated tumors may also have contributed to our negative results. Efforts should be made to expand the number of tumors analyzed, perhaps by means of nationwide databases, as in the UK and Scandinavia. If in this way the number of TEM treated tumors is expanded, maybe we can finally identify predictive features, not only using basic histology but also by using immunohistochemical staining.
Another strategy could be to consider adjuvant treatment. Maybe the adding of radiotherapy to TEM will decrease the number of local recurrences. However, if all T1 rectal cancers are to be irradiated, based on local recurrence rates of approximately 20 percent, 80 percent of patients will be irradiated without additional value. This seems unethical and therefore we have to focus

on individual tumors. Ideally, based on (excision) biopsy material radiosensitive tumors are recognized and in those tumors (neo-) adjuvant radiotherapy may be added. Another strategy could be to consider adjuvant radiotherapy in presumed high-risk tumors, and in particular those larger than three centimetres.

Finally, exploring alternative treatment regimens is of major interest. Neo-adjuvant chemo-radiotherapy is able in obtaining a complete pathological response in approximately 15% of rectal cancers. In early staged tumors this number may even be higher. However, one of the major concerns is the discrepancy between a clinical and pathological complete response. In one quarter to one third of cases with a clinical complete response, the excised TME specimen proved to harbour vital tumor cells. Therefore, solely relying on clinical response is inadequate and quantification of the pathological response is a prerequisite. TEM could act as an excellence tool for excision of the original tumor area to objectivate the actual pathological response. Currently a multicenter trial is initiated in which mobile rectal cancer (cT1-3N0M0) is treated with neo-adjuvant chemoradiotherapy. In case clinical response is complete or near complete, the pre-treatment tattooed original tumor area is excised using TEM and pathological response is evaluated. If pathological response is complete (ypT0) or if remnant vital tumor cells, limited to the submucosa (ypT1), are present, a wait and see policy is advocated. In case of ypT2 or higher a completion TME is advised. Although experimental and therefore only to be done within the context of a trial, this regimen may identify those patients in whom rectal sparing surgery can be performed.

Combining all above-mentioned issues should lead us to our ultimate goal, tailor-made, rectum-sparing treatment in rectal cancer patients.

CHAPTER 11

Samenvatting

SAMENVATTING

Totale mesorectale excisie (TME) is de gouden standaard voor de behandeling van het rectumcarcinoom. Deze geoptimaliseerde techniek heeft de resultaten sterk verbeterd. Echter, in een poging om de nadelige functionele stoornissen na TME te voorkomen, en om een eventueel (definitief) stoma te voorkomen, neemt de proportie van patiënten die wordt behandeld middels lokale excisie (LE) het afgelopen decennium toe. De introductie van transanale endoscopische microchirurgie (TEM) heeft hier een belangrijke bijdrage aan geleverd. Bij geselecteerde T1 rectumcarcinomen wordt LE als curatief beschouwd. Echter de wetenschappelijke basis voor deze verschuiving in behandeling blijft controversieel.
In **hoofdstuk 1** is middels een systematisch literatuuronderzoek de rol van LE en TEM voor het T1 rectumcarcinoom geëvalueerd. We moeten concluderen dat het bewijs niet geleverd is dat TEM een valide alternatief is voor TME.

In **hoofdstuk 2** wordt het doel van het proefschrift beschreven.

ONCOLOGISCHE UITKOMST

Zoals beschreven in hoofdstuk 1, wordt TEM als alternatief voor de curatieve behandeling van het T1 rectumcarcinoom beschouwd. In hoofdstuk 3 en 4 hebben we de oncologische uitkomst bestudeerd van werelds grootste serie van patiënten behandeld met TEM voor een T1 rectumcarcinoom.
Een van de problemen na TEM voor T1 rectumcarcinomen is het lokaal recidief. Na TME wordt het preparaat gestandaardiseerd bewerkt en onderzocht, waarbij met name excisie marge een prognostisch belangrijke factor is voor het ontwikkelen van een lokaal recidief. Of dit ook van predictieve waarde is bij TEM werd prospectief onderzocht en beschreven in **hoofdstuk 3.** Tachtig patiënten na TEM voor T1 rectumcarcinoom werden vergeleken met 75 patiënten na TME voor een T1 rectumcarcinoom. Het studieprotocol hield onder andere gestandaardiseerd pathologisch onderzoek in en intensieve follow-up controles na de operatie. TEM patiënten werden alleen geschikt geacht voor follow-up als de excisie marge microscopisch radicaal was. TEM was een veiliger operatie dan TME, hetgeen werd uitgedrukt in een kortere operatieduur, minder bloedverlies, kortere opnameduur, minder complicaties, minder her-operaties en minder vaak aanleggen van een stoma (allen $p < 0,01$). Sterfte na TEM was 0% tegen 4% na TME. Vijf jaar na TEM en TME was de totale overleving (75% versus 77%) en kanker specifieke overleving (90% versus 87%) vergelijkbaar. Het lokaal recidief percentage was wel significant hoger na TEM (24% versus 0%). Ondanks het feit dat alle tumoren microscopisch radicaal verwijderd werden, is er dus een substantieel deel van de patiënten die een lokaal recidief na TEM ontwikkelt. In **hoofdstuk 4** worden de behandelingsmogelijkheden en uitkomsten voor het lokaal recidief

na TEM voor T1 rectumcarcinomen bestudeerd. Een totaal van 88 opeenvolgende patiënten die een TEM ondergingen voor een T1 rectumcarcinoom werden prospectief geregistreerd in een database. Achttien patiënten ontwikkelden een lokaal recidief gedurende de follow-up. Deze 18 patiënten werden bestudeerd met nadruk op resultaten na chirurgische behandeling middels TME voor het lokaal recidief, en overleving. De mediane tijd tot aan het ontwikkelen van een lokaal recidief bedroeg 10 maanden (spreiding 4-50 maanden). De mediane leeftijd ten tijde van diagnose van het lokaal recidief was 74 jaar (56-84 jaar). Twee patiënten werden niet geopereerd in verband met de aanwezigheid van uitzaaiingen welke niet genezen konden worden. Zestien patiënten ondergingen een TME, zonder dat er uitgebreide (multiviscerale) resecties noodzakelijk waren. In 44% van de patiënten werd een blijvend colostoma aangelegd. Geen van de patiënten overleed door de operatie. Bij 15 patiënten bleek het lokaal recidief microscopisch radicaal verwijderd en bij een patiënt bleek er sprake van een microscopisch niet radicale resectie. Mediane follow-up duur na de TME bedroeg 20 maanden (2-112 maanden). Een patiënt had een hernieuwd lokaal recidief en zeven patiënten ontwikkelden uitzaaiingen. De totale overleving 3 jaar na de TME bedroeg 31% en de kanker specifieke overleving 58%. Geconcludeerd werd dat het lokaal recidief na TEM voor een T1 rectumcarcinoom een belangrijk probleem is. Weliswaar kan met een TME lokale controle bereikt worden bij de meeste patiënten, wellicht toe te schrijven aan de intensieve follow-up die patiënten ondergaan na TEM, echter de overleving is beperkt, voornamelijk ten gevolge van uitzaaiingen. We bediscussiëren dat op maat gesneden selectie van T1 rectumcarcinomen en eventueel aanvullende behandelingsstrategieën na TME voor een lokaal recidief verder onderzocht dienen te worden om de resultaten te verbeteren.

KWALITEIT VAN LEVEN

Na TME kan de functionele uitkomst slecht zijn met dien ten gevolge verminderde kwaliteit van leven. Naast oncologische uitkomst, kan anorectale functie en kwaliteit van leven na TEM van belang zijn bij de keuze van behandeling. Bij TEM wordt de operatie uitgevoerd via een rectoscoop met een diameter van vier centimeter, hetgeen leidt tot scepsis ten aanzien van postoperatieve anorectale functie. In **hoofdstuk 5** worden faecale continentie en kwaliteit van leven voor en na TEM onderzocht. Tussen 2004 en 2006 werden 47 patiënten voorafgaand en tenminste 6 maanden na TEM onderzocht. De faecale continentie werd vastgesteld met behulp van de "Faecal Incontinence Severity Index" (FISI). De kwaliteit van leven werd gemeten met behulp van de EuroQoL EQ-5D vragenlijst en de "Faecal Incontinence Quality of Life" (FIQL) score. Zes maanden na TEM bleek de mediane FISI-score afgenomen ($p < 0,01$), hetgeen een verbetering in faecale continentie impliceert. Deze verbetering trad met name op bij tumoren binnen een afstand van 7 cm van de linea dentata ($p = 0,01$). Vanuit het perspectief van de patiënt, was de kwaliteit van leven postoperatief hoger ($p < 0,02$). Een significante verbetering

werd waargenomen in 2 van de 4 FIQL-score domeinen: gegeneerdheid ($p = 0,03$) en levensstijl ($p = 0,05$). De domeinen levensstijl, het hoofd kunnen bieden/gedrag en gegeneerdheid correleerden met de FISI (allen $p < 0,05$). De conclusie is dat TEM geen verslechtering van de faecale continentie geeft. Bovendien, nadat de tumor met behulp van TEM is geëxcideerd, neemt de kwaliteit van leven toe.

In **hoofdstuk 6** is de faecale continentie en de kwaliteit van leven zowel na TEM als ook na TME voor het rectumcarcinoom onderzocht. De impact van beide procedures op de kwaliteit van leven is nog nooit vergeleken. In totaal ondergingen 54 patiënten TEM voor het T1 rectumcarcinoom. Alleen patiënten zonder lokaal recidief of afstandsmetastasen werden geïncludeerd, resulterend in 36 geschikte patiënten. De EuroQol EQ-5D, EQ-VAS, EORTC QLQ-C30 en EORTC QLQ-CR38 waren de vragenlijsten die werden gebruikt. De resultaten werden vergeleken met een qua geslacht en leeftijd vergelijkbare steekproef van patiënten met een kliernegatief rectumcarcinoom (T+N0), die sfincter sparende chirurgie met behulp van TME hadden ondergaan, en een qua geslacht en leeftijd vergelijkbare steekproef van gezonde personen uit de bevolking. Door 31 patiënten na TEM werden de ingevulde vragenlijsten terug gestuurd (antwoord percentage 86%). De kwaliteit van leven werd vergeleken met 31 patiënten na TME en 31 gezonde controle personen. Vanuit het perspectief van de patiënt en het sociale perspectief verschilde de kwaliteit van leven niet tussen de 3 groepen. Vergeleken met TEM werden meer defaecatie problemen gezien na TME ($p < 0,05$). Na TME werd, in vergelijking met TEM, een trend richting slechter seksueel functioneren waargenomen, in het bijzonder bij mannelijke patiënten. De conclusie kan zijn dat TEM en TME niet verschillen in kwaliteit van leven postoperatief. Wel worden vaker defaecatie problemen gezien na TME. Dit verschil kan een rol spleen in de keuze van chirurgische behandeling voor het T1 rectumcarcinoom.

TUMOR SELECTIE

Het is van groot belang de resultaten van TEM voor het T1 rectumcarcinoom te verbeteren. Een van de te volgen strategieën zou het verbeteren van de preoperatieve stadiering kunnen zijn. Bij rectumtumoren is het niet zelden dat preoperatieve biopten een carcinoom missen. Endorectale echografie (ERE) wordt als een waardevol instrument gezien bij de preoperatieve stadiering. Echter, technische haalbaarheid van ERE en de rol bij de keuze van chirurgische behandeling is zelden bestudeerd. In **hoofdstuk 7** wordt dit nader onderzocht. ERE werd uitgevoerd bij 268 tumoren verwezen voor TEM, daar middels biopten de diagnose tubulovilleus adenoom (TVA) was gesteld. ERE bleek technisch haalbaar bij 231 tumoren (86%). Mediane afstand vanaf de linea dentata van tumoren waarbij ERE niet haalbaar bleek was 11 centimeter en 7 centimeter bij tumoren waarbij ERE wel technisch haalbaar bleek ($p < 0,001$). Bij 21 tumoren was ERE niet conclusief, voornamelijk bij recidief tumoren of bij tumoren waar recent

endoscopisch was gemanipuleerd ($p < 0,001$). Met behulp van ERE kon het percentage gemiste carcinomen worden gereduceerd van 21 naar 3 procent ($p < 0,01$). Als ook T1 carcinomen geschikt worden geacht voor TEM, kon met behulp van ERE het aandeel van onderbehandelde tumoren worden gereduceerd van 7% naar 3% ($p < 0,01$). Echter, op grond van ERE zou 4% van alle tumoren worden overbehandeld, daar deze onterecht als uT2/T3 waren geïnterpreteerd. Deze toename in overbehandeling was ook significant. We concludeerden dat bij vrijwel alle tumoren verwezen voor TEM, ERE technisch haalbaar is en dat ERE in staat is tumoren te herkennen die geschikt zijn voor TEM. De keuze van chirurgische behandeling, TEM versus TME, kan mede worden bepaald door ERE.

In **hoofdstuk 8** hebben we onderzocht of, naast ERE, biopten een invasief carcinoom kunnen identificeren. Hiertoe hebben we chromosomale veranderingen in paraffinemateriaal van de adenoom- en carcinoomdelen van tumoren, verwijderd met behulp van TEM, gepaard vergeleken. Vroege afwijkingen die al aanwezig zijn in zuivere adenomen, progressie gerelateerde veranderingen (de "kwaadaardige" veranderingen) en late afwijkingen, mogelijk bepalend voor het verdere gedrag van carcinomen, werden achtereenvolgens geïdentificeerd. Er werden significant meer "kwaadaardige" afwijkingen waargenomen in de adenoom delen van carcinomen dan in de zuivere adenomen. Zogenaamde immunohistochemische kleuringen voor twee kandidaat genen, p53 (gelegen op chromosoom 17p) en SMAD4 (gelegen op chromosoom 18q) waren afwijkend in de adenoom delen van carcinomen, veelal in tegenstelling tot de zuivere adenomen. We konden ook specifieke progressie patronen zien in individuele tumoren, door systematisch de adenoom fractie met de bijbehorende carcinoom fractie te vergelijken. Als laatste hebben we nog gekeken naar tumorheterogeniteit, door drie biopten per patiënt te analyseren. Deze analyse toonde aan dat rectumtumoren in grote mate heterogeen zijn, wat betekent dat in een tumor te onderscheiden fracties aanwezig zijn met verschillende biologische kenmerken en vaak een ander aantal chromosomale veranderingen. Er werd echter wel een goede overeenkomst gevonden tussen het biopt met het grootste aantal afwijkingen per patiënt en het bijbehorende tumordeel. Hieruit werd de conclusie getrokken dat deze kleine biopten een goede afspiegeling geven van de gehele tumor, maar dat voor een precieze vaststelling van chromosomale afwijkingen het wel noodzakelijk is om meerdere biopten per tumor te analyseren.

Er wordt aangenomen dat het T1 rectumcarcinoom onderverdeeld kan worden in zogenaamde laag- en hoogrisico tumoren. Echter, in de literatuur bestaat geen eenduidigheid ten aanzien van deze criteria. Resultaten na TEM voor het T1 rectumcarcinoom kunnen wellicht verbeteren als we histopathologische criteria kunnen identificeren die voorspellende waarde hebben ten aanzien van het ontstaan van een lokaal recidief. In **hoofdstuk 9** hebben we dit nader onderzocht. Van 62 met TEM behandelde T1 carcinomen, werd door twee onafhankelijke pathologen het histopathologisch onderzoek herhaald en gescoord op vooraf bepaalde criteria. We konden

maximale tumor grootte als onafhankelijk voorspeller voor een locoregionaal recidief aantonen, daar bij 39% van de tumoren groter dan 3 cm een lokaal recidief ontstond, tegen 16% bij tumoren van 3 cm en kleiner ($p < 0,03$). Geaccepteerde hoogrisico criteria als differentiatiegraad, lymfbaan invasie en bloedvat invasie, hadden geen voorspellende waarde in onze serie. Alleen door tumor grootte te combineren met submucosale invasie diepte of zogenaamde tumor budding, kon een subgroep van laagrisico tumoren worden geïdentificeerd. Bij tumoren van 3 cm en kleiner zonder diepe submucosale invasie of zonder tumor budding, ontstond in 7% respectievelijk 10% van de tumoren een lokaal recidief. Geconcludeerd wordt dat maximale tumor grootte alleen of in combinatie met submucosale invasie diepte of budding, significant van predictieve waarde is ten aanzien van het ontstaan van een lokaal recidief na TEM voor het T1 rectumcarcinoom.

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CURRICULUM VITAE

Pascal Gabriël Doornebosch was born on September 12th 1970 in Rotterdam, the Netherlands. He graduated from Professor Casimir Scholengemeenschap (VWO) in 1989. The same year he started medical school at the Erasmus University in Rotterdam. After completion in 1996 he started as a surgical resident in IJsselland Hospital (Capelle aan den IJssel) until 1997 and in the Leyenburg Hospital (The Hague) from January 1998 till December 1999. In 2000 he started surgical training in the Rijnland Hospital (Leiderdorp, dr. J.W.F.B. Rijksen). In 2002 he continued his training in the Leiden University Medical Center (prof. dr. O.T. Terpstra) after which he returned for his last two years of training to the Rijnland Hospital (dr. S.A. DaCosta). In 2006 and 2007 he received advanced training in surgical oncology in the Erasmus Medical Center (dr. J.H.W. de Wilt and dr. C.J.H. van Eijck). In January 2008 he started as a staff surgeon gastro-intestinal surgery in the Leiden University Medical Center and from January 2009 he works as a consultant surgeon in the IJsselland Hospital (Capelle aan den IJssel). His fields of interest are gastro-intestinal surgery, minimal invasive surgery and proctology. Currently he lives in Moerkapelle with his wife Lien Van Eeghem and their three sons Lucas, Mathijs and Ralph.

NAWOORD

Een proefschrift kan nooit tot stand komen zonder de hulp van anderen. Het is in feite de samensmelting van meerdere wetenschappelijke artikelen, en hier werken zeer veel mensen aan mee. Het is echter onmogelijk iedereen te noemen die een bijdrage heeft geleverd aan dit proefschrift. Toch wil ik een aantal mensen in het bijzonder bedanken.

Uiteraard dank ik mijn promotor, Prof. Dr. R.A.E.M. Tollenaar. Dank voor het in mij gestelde vertrouwen en de hulp die ik onderweg mocht ontvangen. Zeer veel dank ben ik ook verschuldigd aan mijn co-promotor, Dr. E.J.R. de Graaf. Aan jouw hand de eerste voorzichtige wetenschappelijke stappen gezet, en zie waar het toe heeft geleid. Wellicht zullen anderen uit onze omgeving ons voorbeeld volgen.
Prof. Dr. C.J.H. van de Velde, Prof. Dr. J.H.W. de Wilt en Prof. Dr. H. Morreau, dank ik om hun kritische blik op dit proefschrift en ik ben dan ook zeer vereerd met zulke gedreven mensen in mijn promotiecommisie.
Alle co-auteurs dank ik voor hun bijdrage gedurende de afgelopen jaren, evenals een ieder die mij op welke wijze dan ook heeft ondersteund om tot dit resultaat te komen. Het zij nogmaals gezegd, zonder jullie steun zou dit proefschrift niet zijn zoals het nu is.
Tenslotte is dit dankwoord niet compleet zonder het noemen van mijn lieve familie, kinderen en Lien, mijn vrouw. Dank dat je mij de vrijheid gaf om dit werk te voltooien.