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Original Research Article

Microscopic intramural spread in patients with rectal cancer after neoadjuvant chemoradiation☆

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ABSTRACT

Objective: This study investigates microscopic intramural spread (MIS) after neoadjuvant (chemo)radiotherapy on Total Mesorectal Excision (TME) specimens of rectal cancer patients and explores the necessity of an additional treatment margin for endorectal radiation boosts (for example through contact brachytherapy (CXB)) or local excisions.

Methods: A cohort of patients from Maastricht University Medical Center (MUMC +) treated between 2016 and 2022 was analyzed. Patients underwent MRI, CT scans, and sigmoidoscopy six weeks after radiotherapy, followed by surgery. Pathological analysis of TME specimens, including whole mount macro-cassettes, was performed to measure residual macroscopic tumor and MIS. Fragmented and continuous MIS were recorded parallel and perpendicular to the bowel wall.

Results: Out of 54 patients, 37 (69%) exhibited no MIS. MIS was observed in 4/18 (22%) of patients with ycT1-2 tumors and 13/36 (36%) of patients with ycT3-4 tumors. 4 patients (7%) showed continuous MIS and 15 (28%) showed fragmented MIS. No patients with ypT1-2 had MIS.

Conclusions: 69% of patients do not retain MIS post-neoadjuvant therapy. Knowledge of tumor thickness seems crucial for patient selection for CXB.

Introduction

The introduction of the Total Mesorectal Excision (TME) has significantly decreased the risk of locoregional recurrence for patients with rectal cancer and is currently the cornerstone of potentially curative treatment[1]. In distal rectal cancer, a permanent colostomy is frequently necessary which may impact the patient's quality of life. Furthermore, in restorative procedures, patients may suffer from Low Anterior Resection Syndrome (LARS) or other complications after surgery, and for elderly patients there is a significant mortality risk associated with surgery[2–6]. For these reasons, organ preservation after neoadjuvant therapy has gained momentum in the last years.

In more advanced (often characterized by cT3c-4 tumors or involvement of mesorectal fascia) rectal cancer, neoadjuvant (chemo) radiotherapy is often used to reduce the risk of a locoregional recurrence after TME[7,8]. A pathological complete response (CR) is seen in 15–27 % of patients after neoadjuvant chemoradiation[7,9]. For these patients, surgery might not be necessary or could be postponed. In patients with clinical complete response (cCR), a watch-and-wait strategy has been proposed internationally, in which close follow-up according to strict guidelines is essential[10].

In the past years, a number of studies have explored methods to increase the cCR rate. One such method is called Total Neoadjuvant Treatment (TNT) which includes additional chemotherapy before or after the standard (chemo)radiotherapy[11]. Another method is to take advantage of dose–response relationship in rectal cancer by increasing the radiation dose through a radiotherapy boost. Such a boost can be delivered through external beam radiotherapy (EBRT) or through, for

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example, contact x-ray brachytherapy (CXB). CXB in particular is a promising technology, as the steep dose fall-off allows for a very high dose (i.e. 30 Gy per fraction) to be delivered to a very limited volume. Several studies have illustrated the feasibility and promise of a CXB boost[12,13], and the recently published OPERA trial showed a 97 % organ preservation rate at three years for patients with rectal cancers less than 3 cm in diameter[14].

When giving a local radiotherapy boost, it is essential to treat all tumor cells with a sufficient dose. It has been described that rectal cancers may show concentric (shrinking symmetrically towards a central point) or scattered (fragmented) shrinkage, or a combination of both as a response to (chemo)radiotherapy[15–17]. As a result, microscopically visible intramural and/or perirectal disease, in other words, disease extension parallel (lateral) but also perpendicular (in depth) to the rectal wall, may be more extensive than the macroscopically visible residual cancer. The extent of the microscopic intramural spread (MIS) could imply that aiming for the macroscopic residual cancer may not always be sufficient to eradicate all of the remaining disease. For some cases, a larger safety margin to include MIS may be necessary when giving a radiation boost, as well as for local excision[15]. One *meta*-analysis with individual patient data on MIS showed that 80 % of patients did not show MIS, and that in order to treat all microscopic disease in 95 % of patients, a margin of 5.5 mm would be necessary around the visible tumor/ulcer. However, MIS was usually only measured in one dimension, preventing a clear definition of the microscopic tumor volume[18].

In terms of endorectal contact brachytherapy (CXB), delivered by the Papillon+™ (Ariane™ Medical Systems, Alfreton, United Kingdom), the applied dose is reported at the exit surface of the rectal applicator. Due to the steep dose fall-off, it is important to realize that only 50–60 % of this applied dose remains at 5 mm depth, whereas only 30–40 % reaches a 10 mm depth[19]. This seems to suggest that only tumors with a limited thickness can be adequately treated. However, to the best of our knowledge, there is little data on the extrapolation of these doses on actual tumor thicknesses in the bowel (rectal) wall.

The aim of this cohort analysis is to investigate MIS in patients with residual rectal cancer after (chemo)radiotherapy by measuring the extent of MIS parallel as well as perpendicular to the bowel wall irrespective of the tumor regression pattern. This information will hopefully allow for more insight into whether a safety margin should be included in a radiation boost to eradicate all disease, and if so, how extensive this margin should be. In addition, the aim is to explore potential limitations of CXB with regard to residual macroscopic and microscopic tumor thickness.

Methods

In accordance with the Dutch Central Committee on Research involving Human Subjects (CCMO), a declaration was received by the local Medical Ethics committee (METC MUMC+, number 2019–0750) stating that this research does not fall under the Medical Research Involving Human Subjects Act (WMO). Patients diagnosed with rectal adenocarcinoma at the Maastricht University Medical Center (MUMC +) who received (chemo)radiotherapy and TME surgery between 2016 and 2022 with an interval of at least 6 weeks and for whom macro-cassettes (slides in which the whole tumor bed was embedded to allow for measurements parallel and perpendicular to the bowel wall) were available were included.

Clinicopathological data, including treatment data (radiation dose, time between (chemo)radiotherapy and surgery) were extracted from the electronic medical files.

As part of the standard MUMC + protocol, around six weeks after (chemo)radiotherapy, all patients underwent a Magnetic Resonance Imaging(MRI), a thoraco-abdominal computer tomography (CT) scan, and sigmoidoscopy for restaging. For the purpose of this study, ycT-stage was defined as the T-stage following neoadjuvant (chemo)radiotherapy, as determined on MRI. Since ycT1 or ycT2 cannot be differentiated on MRI, a stated 'good response' by the radiologist in the absence of ycT3 characteristics was classified as ycT1-2 for the purpose of this study. A poor response retained original cT-stage for the purpose of this study. All included patients were classified as having residual tumor (as determined on MRI and/or endoscopy) at restaging and thus had a surgical resection.

TME rectal cancer resection specimens were prepared and treated according to standard operating procedure in MUMC + . This pathology protocol for rectal cancer resection specimens dictates 48-hour formalin fixation of the specimen and the use of whole mount macro-cassettes, allowing for visualization of a complete transection of the TME specimen. Detailed pathology analysis was performed by dedicated gastrointestinal (GI)-pathologists with TNM classification according to the UICC TNM 8th edition[20]. On these hematoxylin-eosin-stained slides of the macro-cassettes, measurements were made for the macroscopic tumor, as seen on the slides with the naked eye without magnification as well as microscopically for continuous as well as fragmented MIS, both parallel and perpendicular to the bowel wall (in depth). Tumor fragments were considered as the presence of clusters of tumor cells distanced from the lateral or the deepest border of the residual ulcer/cancer by $\geq$ 1 mm of normal/fibrotic tissue. Fig. 1 shows the different types of MIS and how the measurements were done. Maximal macroscopic depth and diameter was determined on the available macro-cassettes, otherwise correlation with the corresponding microscopic measurements would not be possible. The measurements for this study were made by the first author after training by a dedicated GI-pathologist. The measurements were checked randomly by the dedicated GI-pathologist. All measurements were later re-done by the first author to double-check.

Statistical Analyses

Descriptive statistics were performed to generally characterize the patient and tumor data. The mean MIS was calculated for the entire group and subdivided into the types and directions of MIS (continuous or fragmented; lateral or in depth respectively).

Exploratory analyses related to the percentual dose depth (PDD) curve of CXB and tumor thickness were performed.

Results

Macro-cassettes were available for 54 patients who received surgery at MUMC + after having undergone neoadjuvant (chemo)radiation with a minimum interval of 9 weeks between neoadjuvant therapy and surgery (range 9–80, median 14).

Patient characteristics

Table 1 shows the descriptive patient characteristics. The majority of patients (56 %) had a cT3 tumor, and 72 % had a cN2 status. Notably, 6 patients (11 %) had a cM1 status. The majority (60 %) of patients had well- to moderately differentiated tumors. Seven patients showed a regrowth, meaning a reappearance of the rectal tumor at the location of the primary tumor after an initial clinical complete response in patients who subsequently did not receive surgery.

Treatment characteristics

Most patients (62 %) received a dose of 25 x 2 Gray (Gy), while 28 x 1.8 Gy and 5 x 5 Gy were also used (22 % and 15 % respectively). 85 % of patients received concurrent chemotherapy in the form of capecitabine concurrently with 25 or 28 fractions. Of the patients having received 5 x 5 Gy, five (63 %) patients had an M1 status and received chemotherapy after radiotherapy and preceding surgery.

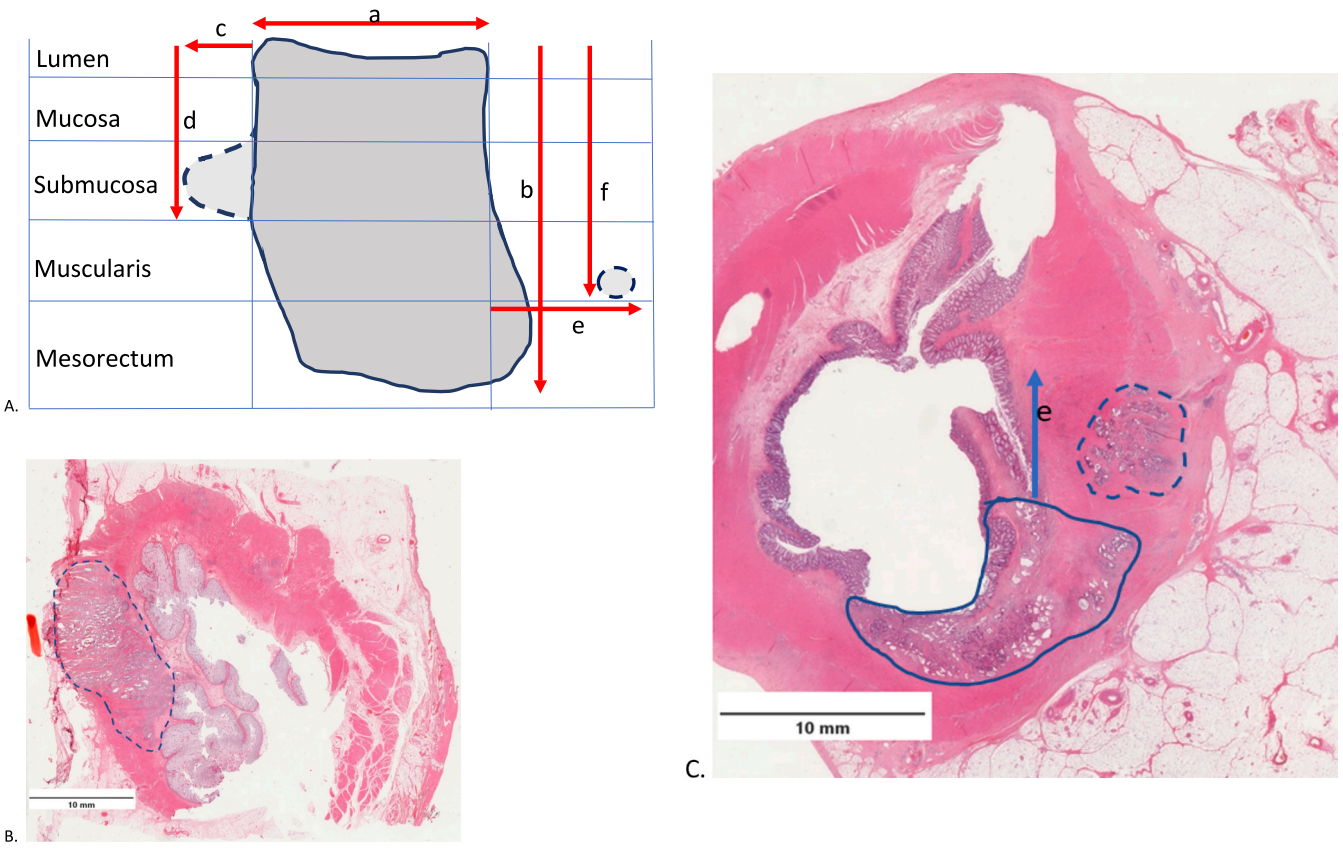

Fig. 1. A. Diagram showing an exemplary tumor in the rectal wall with lateral microscopic intramural spread (MIS) as well as fragmented MIS (F). The dark grey shape is the macroscopic tumor as seen endoluminally and the naked eye in the pathology specimen, while the light grey structures depict MIS. The arrows illustrate the measurements made. (a) Macroscopic maximal diameter of the residual tumor corresponding to the endoluminal diameter of the tumor. (b) Macroscopic maximal depth of the residual tumor, as measured in the pathology specimen without microscope. (c) Lateral extent of the lateral continuous MIS. (d) Maximal depth of the lateral continuous MIS. (e) Maximal lateral extent of the tumor fragment (fragmented MIS) measured from the edge of the macroscopic tumor. (f) Maximal depth of continuous MIS. B. Haematoxylin-eosin stained macroslide of TME specimen. An area of continuous MIS (dotted line) in the muscularis propria is seen which stems from the more proximal macroscopic tumor (not shown on this slide). The arrow depicts the extent of the depth of the continuous MIS (d) as demonstrated in 1A. C. Haematoxylin-eosin stained macroslide of TME specimen. The macroscopic tumor (the endoluminally visible residual tumor (solid line) as well as fragmented MIS (dotted line) are shown with measurement corresponding to 1A(e).

Pathological analysis

8 (15 %) patients showed a pathological complete response for the primary tumor (Tumor Regression Grade (TRG) 1 according to Mandard)[21]. These were all patients without a clinical complete response on MRI and/or endoscopy and were therefore included in the analysis below. 31 (57 %) patients showed a good response (TRG 1—3).

Percentages were calculated with regards to the entire cohort. 37 patients (69 %) showed no MIS. 4 patients (7 %) showed continuous MIS and 15 (28 %) showed fragmented MIS. In 12 patients (22 %) lateral MIS was seen of which 3 (6 %) was continuous and 9 (17 %) was fragmented. 16 patients (30 %) showed MIS in depth of which 2 (4 %) was continuous and 14 (26 %) fragmented.

4/18 (22 %) of patients with ycT1-2 tumors showed MIS, while 13/36 (36 %) of patients with ycT3-4 tumors showed MIS. None of the patients with ypT1-2 tumors showed MIS.

Table 2 shows MIS frequencies and percentages.

Table 3 shows the depths of residual tumor (macroscopic and microscopic combined) in relation to ypT stage.

Patients (15 %) had a regrowth after initial clinical complete response. Of these patients, 2 patients (25 %) showed MIS

Fig. 2 illustrates the relationship between macroscopic depth to the combined macroscopic and microscopic depth, as well as its correlation to percentual dose depth for a standard contact brachytherapy applicator for ypT1-2 and ypT3 tumors.

Discussion

For this research, macro-cassettes in which the entire TME plane was included were analyzed for the presence of MIS. The results suggest that, in accordance with a previously published *meta*-analysis by Verrijssen et al. based on individual patient data, the majority of patients will not have MIS beyond the macroscopic tumor after neoadjuvant (chemo) radiotherapy[18].

The previously published *meta*-analysis showed a percentage of 80 % of patients without MIS. The measurements for included papers were all performed parallel to the bowel wall, with some limited to the 'distal' parallel direction due to the surgical nature of the paper. It seems logical that measuring in all directions including perpendicular to the bowel wall will result in discovering more MIS, as suggested in this study. In addition, not all studies of the mentioned *meta*-analysis included tumor fragmentation in their analysis, therefore it is possible that 80 % is a slight overestimation. Keeping in mind the results from the current paper, it may be safe to say that 69–80 % of patients will not show MIS after (chemo)radiotherapy.

As expected, the maximum depth of tumor in this study increased with increasing ypT stage. The recently published GEC-ESTRO guidelines for contact x-ray brachytherapy (CXB) express cT1-T3a tumors as well as cT3b with good downstaging following radiotherapy under inclusion criteria. This is due to the risk of node-positive disease in T3 tumors as well as the idea that T3 tumors generally have too great of a

Table 1
Patient characteristics.

Characteristics		N (%)
Total patiënts		54
Initial cT stage	1	0
	2	1 (2)
	3	32 (58)
	4	19 (35)
	Unknown	2 (4)
Initial cN stage	0	6 (11)
	1	8 (15)
	2	39 (72)
	Unknown	1 (2)
Neoadjuvant treatment given		46 (85)
	Chemoradiotherapy*	
	5 x 5 Gy	8 (15)
ypT stage	0	8 (15)
	1	4 (7)
	2	11 (20)
	3	26 (48)
	4	4 (7)
	Unknown	1 (2)
ypN stage	0	41 (76)
	1	9 (17)
	2	3 (6)
	Unknown	1 (2)
ycT stage	1–2**	18 (33)
	3	28 (52)
	4	8 (15)
ycN stage	0	29 (54)
	1	17 (31)
	2	8 (15)
Patients with pathological complete response (%)		7 (13)
Number of regrowths		7 (13)
Median time between neoadjuvant therapy and surgery (weeks)		14 (range 9–80)

*Fractionation included 25 x 1.8 Gy (2 %), 25 x 2 Gy (61 %) and 28 x 1.8 Gy (22 %), all in combination with Capecitabine chemotherapy.

Table 2
MIS frequencies and percentages *.

Type of MIS	Total	Orientation	
		Parallel to bowel wall	Perpendicular to bowel wall (depth)
Continuous	4 (7 %)	3 (6 %)	2 (4 %)
Fragmented	15 (28 %)	9 (17 %)	14 (26 %)
Total	17 (31 %)	12 (22 %)	16 (30 %)

*Of the total group including the pCR patients (all of which being non-cCR).

Table 3
Depth and diameter of residual tumor according to ypT stage*.

ypT-stage	No. of patients	Mean depth residual macroscopic tumor (range) mm	Total mean depth (range)** mm	Mean lateral diameter (range) mm	Total mean lateral diameter (range) * mm
1	4	3.3 (1.4–6.3)	3.3 (1.4–6.3)	7.7 (1.9–12)	7.7 (1.9–12)
2	11	5.6 (1.2–19)	5.6 (1.2–19)	14.6 (4.4–26.7)	15.7 (4.4–26.7)
3	26	9.0 (3.5–19.7)	9.5 (3.5–19.7)	19.5 (3.9–62.7)	20.3 (3.9–62.7)
4	4	14.5 (8–17.3)	18.0 (8–33)	37.8 (19.8–63.4)	37.8 (19.8–63.4)

* Of the total group including the pCR patients (all of which being non-cCR).
**sum of macroscopic and microscopic residual tumor.

thickness for adequate dose coverage in depth. The PDD graphs show the swift dose fall-off that occurs when going deeper into the tissue. For example, at 5 mm depth, only 50–60 % of the CXB dose remains. A 20 mm-thick tumor will receive less than 20 % of the delivered dose, which could explain a possible higher re-growth/relapse rate for these thicker tumors. On the other hand, due to the high dose given and the waiting time between each fraction (generally 2 weeks), tumor regression is often apparent between fractions, possibly correlating to better dose coverage of the remaining macroscopic tumor in the second and third fractions. In addition, fast regression of a transmurally growing tumor may result in an increased risk of ulceration or necrosis. Data on the risks of rectal wall perforation after treatment of higher T-stages (cT3c and onwards) is scarce, although extreme cases such as perforation have not been described after CXB. Another factor to keep in mind is the shrinkage of tumor in between CXB fractions. The possibility of the presence of MIS may lightly suggest the advice to retain the same applicator size regardless of tumor shrinkage in between fractions. However, as the majority of tumors do not retain MIS, more research is warranted on this subject as the increase in irradiated volume may also increase the risk of toxicity to the bowel wall.

Limited data exists in terms of tumor control probability for rectal cancer. Appelt et al. illustrated in 2013 that there seems to be a dose–response relationship for rectal cancer[22], and extrapolating the delivered dose of recent studies such as OPERA to this curve seem to underline this relationship. In this illustrated dose–response relationship, local control at 2 years is related to the theoretical doses given in several studies combining external beam radiotherapy boost with a form of boost radiotherapy[23].

As a theoretical exercise, keeping in mind the data from Fig. 2, a ypT1-2 tumor showing a macroscopic thickness of 11 mm, which would also, according to this study, include any microscopic disease, would receive 25 % of the CXB dose which is prescribed at the surface of the applicator. Keeping in mind that the total prescribed dose is usually 3 x 30 Gy = 90 Gy in three fractions, the dose given at this depth would be 22.5 Gy (EQD2 = 32.8 Gy using α/β of 10 Gy), meaning that combined with, for example 50 Gy EBRT, the total dose given to the rectal tumor would be 88.8 Gy. Correlating this to the curve by Appelt et al., one can see that a local control of approximately 70 % could theoretically be obtained and that this approaches the plateau of the curve.

Using the same calculating principles, an ypT3 tumor with a macroscopic thickness of almost 17 mm and a total macroscopic and microscopic thickness of 24 mm would translate to 8 % of the prescribed dose given, meaning that the total EQD2 including EBRT would only equal 57.4 Gy, theoretically corresponding with a much lower local control rate of 25 % at 2 years.

Equally, the results from this study illustrate that some T3 tumors can very well be treated with an adequate dose, and that some T2 tumors may not adequately be treated by CXB. Endorectal ultrasound can very help in determining the thickness (depth) of macroscopic tumor invasion, aiding in patient selection for CXB. Clearly, more research is needed pertaining to the effect of different tumor thicknesses as well as the dose actually delivered to the tumor and surrounding rectal wall. Interestingly, it was Papillon himself who described that CXB diminishes the tumor thickness layer by layer with each fraction. This raises the question of whether it is absolutely necessary to treat the entire thickness of the tumor from the first fraction[24].

The recently published OPERA trial confirmed CXB as a promising treatment for patients with early rectal cancer showed promising results for patients with early rectal cancer (cT1-3abN0-1) receiving CXB in addition to chemoradiotherapy[14]. Particularly for patients with tumors < 3 cm, administering CXB before chemoradiotherapy resulted in a 3-year organ preservation rate of 97 %. For patients with tumors > 3 cm, response assessment took place after chemoradiotherapy and CXB was administered regardless of clinical response, although in some instances of very good response after chemoradiotherapy the dose of the final fraction was decreased.

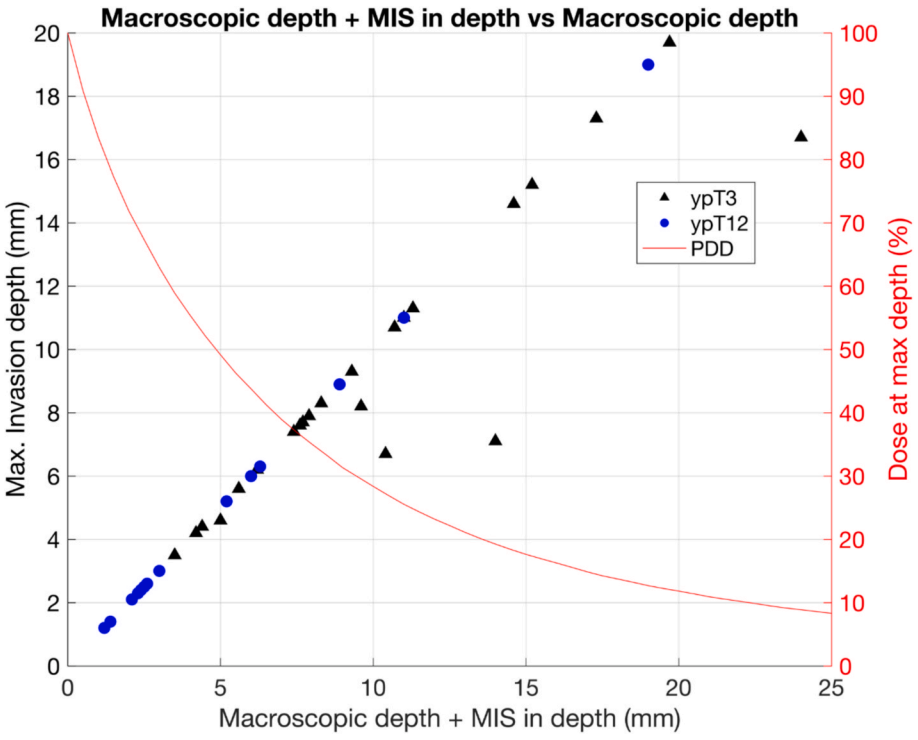

Fig. 2. Graph showing the relationship between macroscopic depth ((b)in Fig. 1A) to the combined macroscopic and microscopic depth ((b) and/or (f) in Fig. 1), as well as its correlation to percentual dose depth for a standard contact brachytherapy applicator for ypT1-2 and ypT3 tumors.

An interesting observation of this study is that ypT1-2 tumors portrayed no MIS in this study. This seems to imply that generally, tumors that are limited in size or show a good response after neoadjuvant treatment seem to portray less MIS. More advanced tumors or tumors that show a worse clinical response may be more prone to retaining MIS. The ongoing OPAXX trial (clinicaltrials.gov NCT05772923) in the Netherlands aims to investigate the organ preservation rate for patients treated with neoadjuvant (chemo)radiotherapy[25]. Patients with lower to mid-rectal cancer who have a near complete response or a small residual tumor mass < 3 cm as well as ycN0 at initial response assessment 6–8 weeks following short- or long-course radiotherapy are eligible for inclusion. Included patients are then randomized between a contact x-ray brachytherapy boost and an extended waiting interval, after which a new response assessment will take place. In the case of a remaining small lesion after the extended waiting interval, a transanal local excision will be offered. The primary endpoint of this trial reflects the efficacy of both additional treatment options. Secondary endpoints are related to toxicity and morbidity, as well as to oncological and functional outcomes[25]. Hopefully, the results will reveal more insights into the efficacy of CXB for initially more advanced tumors.

Limitations of this paper include the fact that not all specimens included macro-cassettes of the entire tumor. Due to this, overestimation of tumor fragmentation is possible as a result of missing in-between slides. Therefore, maximal macroscopic depth and diameter was determined on the available macro-cassettes, otherwise correlation with the corresponding microscopic measurements would not be possible.

Conclusion

In this study, 69 % of patients did not retain MIS during their response to neoadjuvant (chemo)radiotherapy. Determining tumor thickness, for example by using endorectal ultrasound, seems to be a valuable aid in patient selection as well as personalizing treatment in the future for CXB.

CRediT authorship contribution statement

A.E. Verrijssen: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. **E.J. Van Limbergen:** Conceptualization, Formal analysis, Supervision, Methodology, Visualization, Writing – review & editing. **M. Bellezzo:** Formal analysis, Software, Visualization, Writing – review & editing. **H. I. Grabsch:** Conceptualization, Data curation, Investigation, Supervision, Methodology, Project administration, Resources, Visualization, Writing – review & editing. **R. Houben:** Data curation, Formal analysis, Software, Validation, Visualization, Writing – review & editing. **D. Goudkade:** Data curation, Formal analysis, Supervision, Validation, Writing – review & editing. **J. Melenhorst:** Formal analysis, Methodology, Resources, Visualization, Writing – review & editing. **I. Samarska:** Data curation, Formal analysis, Supervision, Validation, Writing – review & editing. **G. Paiva Fonseca:** Data curation, Formal analysis, Supervision, Validation, Writing – review & editing. **F. Verhaegen:** Conceptualization, Data curation, Investigation, Supervision, Methodology, Project administration, Resources, Visualization, Writing – review & editing. **M. Berbee:** Conceptualization, Formal analysis, Investigation, Supervision, Methodology, Visualization, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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