Timing of surgery following neoadjuvant chemoradiotherapy in locally advanced rectal cancer – A comparison of magnetic resonance imaging at two time points and histopathological responses

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Short title – Association between MRI staging and histopathology
**Purpose:** There is inter-institutional variation in the interval between neoadjuvant chemoradiotherapy (NACRT) and surgery for locally advanced rectal cancer. We aimed to assess the association of magnetic resonance imaging (MRI) at 9 and 14 weeks post-NACRT T-staging (ymrT) and post-NACRT tumour regression grading (ymrTRG) with histopathological outcomes, namely histopathological T-stage (ypT) and histopathological tumour regression grading (ypTRG), in order to inform decision-making about timing of surgery.

**Materials and Methods:** We prospectively studied 35 consecutive patients (26 males) with MRI-defined resection margin threatened rectal cancer who had completed standardized NACRT. Patients underwent a MRI at Weeks 9 and 14 post-NACRT, followed by surgery at Week 15. Two readers independently assessed MRIs for ymrT, ymrTRG and volume change. Each outcome was categorised as a favourable or unfavourable response. ymrT and ymrTRG were analysed against histopathological ypT and ypTRG as predictors by logistic regression modelling and receiver operating characteristic (ROC) curve analyses.

**Results:** Thirty patients had evaluable imaging and pathology. Inter-observer agreement was good for all MR variables (Kappa >0.61). Considering ypT as an outcome variable, a greater association of favourable ymrT, ymrTRG and volume change at Week 14 compared to Week 9 was found (ymrT - OR 31.7; 95%CI 2.7-373.7 vs. OR 17.8; 95%CI 1.7-165.0; ymrTRG - OR 20.0; 95%CI 2.1–186.9 vs. OR 6.8; 95%CI 0.9-48.7; Volume change – OR 22.5; 95%CI 2.0-249.2 vs. N/A). Similarly, considering ypTRG as an outcome variable, a greater association of favourable ymrT, ymrTRG and volume change at Week 14 compared to Week 9 was found (ymrT - OR 16.3; 95%CI 1.6-163.4 vs. OR 9.0; 95%CI 1.6-50.7; ymrTRG - OR 14.0; 95%CI 1.5–134.2 vs. OR 7.6; 95%CI 1.2-48.0; Volume change – OR 22.4; 95%CI 2.2-227 vs. OR 6.3; 95%CI 1.0-38.7).
**Conclusion:** Following NACRT, greater tumour down-staging and volume reduction was observed at Week 14. Timing of surgery, in relation to NACRT, merits further investigation.

**Keywords**

Magnetic resonance imaging, surgery, rectal cancer, tumour regression, time for surgery

**Trial Registration Number**

NCT: 01325909
INTRODUCTION

In the UK colorectal cancer is the third commonest cause of cancer death (1,2) and ~5000 patients underwent surgery for rectal cancer (71% aged > 65 years) during 2014. In 25% of these patients, major resection was preceded by neoadjuvant chemoradiotherapy (NACRT) (3), with the aim of controlling local disease and achieving tumour downsizing and negative resection margins, with marginal gains in overall survival (4–8). High-resolution pelvic magnetic resonance imaging (MRI) is now the gold-standard in preoperative rectal cancer staging (9). The decision to administer NACRT is based on identifying MRI-defined circumferential resection margin (CRM) threatened cancers.

Histopathologists grade tumour response in three ways: firstly assessment of the status of the CRM, secondly the depth of tumor spread and nodal status (ypT and ypN stage), and thirdly by evaluating tumor regression grade (ypTRG) (10,11). A number of studies have shown that both ypT and ypN stage are independent predictors of outcome, and several retrospective studies report a link between outcome and histopathology assessment of final stage or tumor regression after NACRT (12,13). Accurate preoperative assessment of response to therapy may permit the clinical teams to modify definitive treatment (14). A number of different methods have been proposed for assessing response of rectal cancer to CRT on MRI. These include post-treatment T staging (ymrT), volume reduction between baseline and post-treatment,(15) and the modified Response Evaluation Criteria in Solid Tumors (RECIST) measurement (16). In addition to these assessment criteria, the MERCURY study group has developed an MRI-based tumor regression grading (ymrTRG) system by applying the principles of histopathology ypTRG (17,18) and showed that MRI assessment of ypTRG following preoperative therapy predicted survival (17).
It has been suggested that there may be benefits in prolonging the interval between end of NACRT and surgery beyond the common 6-8 weeks (19–21), but evidence is limited.

The aim of this study was to assess MRI-defined *favourable* versus *unfavourable* responders (ymrT, ymrTRG and change in volume) at two time-points post-NACRT and to compare these evaluations with histopathological ypT and ypTRG, in an attempt to inform decisions about optimal timing of surgery with respect to NACRT. We also explored the level of interobserver agreements between central and local MR reviewers for ymrT, mrTRG and volume change at both time points.
METHODS

Patients and Study Design

This prospective pilot trial was performed as a nested sub-study within a larger trial (22) approved by the North West – Liverpool East Research and Ethics Committee (11/H1002/12) and registered with ClinicalTrials.gov (NCT01325909). Written informed consent was obtained from all patients. We recruited consecutive patients between August 2012 and August 2014 referred to the Colorectal Multi-Disciplinary Team (MDT), age ≥18 years, with locally advanced (circumferential resection margin threatened – defined as tumour within 2 mm of the mesorectal fascia or if any T3/4 tumour was arising at <5 cm from the anal verge) resectable rectal cancer, scheduled for standardized NACRT on the basis of Tumour, Node, Metastasis (TNM) classification >T2/N+ with no distant metastasis (23) and WHO Performance Status < 2 (24). Exclusion criteria were: inability to give informed consent, non-resectable disease, and patients who declined surgery or NACRT, or who received non-standard NACRT.

All patients underwent TNM staging involving flexible sigmoidoscopy to obtain tissue for histological diagnosis, completion colonoscopy, chest, abdomen and pelvis computer-aided tomography (CT) and 1.5 T pelvic magnetic resonance imaging (MRI) at baseline. All patients completed 5 weeks NACRT. Standardized radiotherapy consisted of 45 Gy in 25 fractions on weekdays using a 3D conformal technique with CT guidance. A boost dose was given (5.4 Gy in 3 fractions) to the primary tumour only. Oral capecitabine (825 mg.m⁻²) was given twice daily on radiotherapy days. No patient received brachytherapy. At 9 weeks post-NACRT, patients were restaged using chest, abdomen and pelvic CT and pelvic MRI. At 14 weeks post-NACRT, patients were restaged using pelvic MRI, prior to surgery at Week 15.
MRI Technique

MRI technique was performed as described by Patel and colleagues (25,26). MR image analysis was carried out, using the terms ymrT (T stage on MRI images obtained after NACRT), ymrTRG (tumor regression grade on MRI images obtained after NACRT), ypT (T stage on post-treatment histopathological examination of the resection specimen), and ypTRG (tumor regression grade on post-treatment histopathological examination of the resection specimen) to describe the data (25,27). The MRI scans were anonymised and separately reviewed by two radiologists (Central reviewer; GB and Local reviewer; DW) with >35 years of experience in MRI assessment of rectal cancers, in two tertiary referral colorectal cancer centres, using previously defined criteria.

MRI Image Analyses

Images were analysed for ymrTRG, ymrT and percentage volume change. ymrT was based on the interpretation of local extent of persistent tumor signal intensity relative to the layers of bowel wall on T2-weighted images. Comparison was made with the pre-treatment images. Tumour response manifested as either replacement of tumor signal by low signal intensity fibrosis (dark stroma) or the development of high signal intensity mucin pools; such areas were not considered to be tumour, as they did not contribute to T staging. ymrT staging is was conducted as described by Sobin and Brierley (23,28). T3 sub-staging was conducted as described by Patel and colleagues (25).

Based on known histopathological outcomes according to ypT stage, the patient’s ymrT was divided into favourable and unfavourable response to enable binary comparison. Favourable was defined as stages ymrT0, 1, 2, and 3a, while unfavourable was defined as ymrT3b, c, d and ymrT4 (29).
MRI TRG is based on principles similar to the pathological ypTRG system originally described by Dworak. Scans were reviewed to determine the degree of tumor replacement by fibrotic stroma, as previously described (17,18,26). Favourable MRI tumor regression grade was defined as grades 1, 2, and 3, and unfavourable regression as grades 4 and 5, as in previous studies (17,25).

The MRI scans were also assessed for percentage volume change (25). Tumor volume was obtained by multiplying tumor length, width, and height. Percentage volume reduction was defined as $100 \times \left( \frac{\text{Volume at baseline} - \text{Volume post-CRT}}{\text{Volume at baseline}} \right)$, and categorized into two groups (unfavourable <80%, and favourable ≥80 %).

**Surgical Resection**

All patients underwent total mesorectal excision (TME) (30) with or without abdominoperineal excision, performed 15 weeks (+/- 4 days) after the completion of NACRT.

**Histopathology Assessment**

After surgical resection, the specimen was fixed in formalin for 48 h, cross-sectioned into 3–5 mm slices, and histologically sampled. A predefined protocol assessed pathological complete response, with a minimum of 5 blocks of tumour taken. If no tumour was found on the first set of haematoxylin and eosin sections the rest of the tumor area was embedded, and if no tumour was seen then a final three levels were taken through each block to look for tumor to confirm a complete response. Each specimen was graded by degree of tumour regression, according to the Dworak system and also by ypT stage. As well as grading and staging by the five-point ypTRG and TNM version 5 systems, a simplified pathological grading of favourable and unfavourable pathology was also undertaken. Favourable pathology was
defined as ypT stages 0, 1, 2, and 3a or ypTRG stages 3 and 4. Unfavourable pathology was defined as ypT stages 3b, c, d, and 4 or ypTRG stages 0, 1, and 2. ypT3a was included in the favourable group as these tumours have been shown to have a similar prognostic outcome as ypT2 tumours (18,29).

Statistical Analysis

Central reviewer (Royal Marsden; GB) data was used for analysis; agreement between the two observers grading categorical variables (ymrT, ymrTRG and volume change) was determined by kappa statistic ($\kappa = 0$, poor agreement; $\kappa = 0–0.20$, slight agreement; $\kappa = 0.21–0.40$, fair agreement; $\kappa = 0.41–0.60$, moderate agreement; $\kappa = 0.61–0.80$, substantial agreement; and $\kappa = 0.81–1.00$, almost perfect agreement).

Data were described as frequency (percentage) and mean (SD), with 95% confidence intervals (95%CIs), as appropriate. To analyse the association between demographic variables (age and sex), MRI parameters (ymrT, ymrTRG, volume change) and pathologic tumor response (ypT and ypTRG), univariate logistic regression analysis was used. This enabled calculation of odds ratio (OR) of a favourable category outcome (low grades from 1 to 3) from the pathological examination, along with 95%CIs. Receiver operating characteristic (ROC) curve analysis was also performed, with calculation of the area under the curve (AUC) as an indicator of overall accuracy, together with sensitivity, specificity, positive and negative likelihood ratios. Significant univariate relationships with the outcome were adjusted by multifactor logistic regression analysis for baseline values of the predictor variables if this was possible. Two-tailed $p<0.05$ was considered statistically significant unless specified otherwise. Calculations were performed using Statistical Package for Social Sciences program, version 22.0 (SPSS, IBM, USA).
RESULTS

Table 1 shows the baseline demographic characteristics of the patients who were eligible for this study. All patients completed the standardised course of NACRT. One patient needed capecitabine dose reduction, while 4 patients sustained perineal radiation skin changes (maximum score 2 out of 4).

Table 2 shows MRI T-stage, TRG, volume change at Week 9 and Week 14 and pathological T-stage and TRG. The mean baseline distance from anal verge was 54 mm, standard deviation (SD) 28 mm. The mean baseline tumour length was 50 mm (SD 18 mm). The mean baseline tumor volume was 47773 mm\(^3\) (SD 72005 mm\(^3\)). The mean post-treatment tumor distance from anal verge at Week 9 was 56 mm (SD 27mm), and at Week 14 was 66 mm (SD 28 mm). The mean Week 9 tumour volume was 16277 mm\(^3\) (SD 29386 mm\(^3\)) and at Week 14 was 8831 mm\(^3\) (SD 18060 mm\(^3\)). The mean tumor volume reduction at Week 9 was 61% (SD 39%) and at Week 14 was 80% (SD 22%). At histopathological examination the mean number of blocks taken was 7 (SD 4) and the mean number of sections taken per block was 7 (SD 4). The mean nodal harvest was 12 (SD 8).

Interobserver Agreement

Appendix 1 shows the raw data for the interobserver agreements between central and local MR reviewers for ymrT, ymrTRG and volume change. Agreements ranged between moderate (κ = 0.44) to almost perfect (κ = 0.92) for continuous and categorical variables at all 3 time points (baseline, Week 9 and Week 14).

T stage, tumour regression grading and volume change at Week 9 and Week 14 on MRI images obtained after NACRT
Table 3 shows a univariate logistical regression model of age, gender, ymrT, ymrTRG and volume change at Week 9 and Week 14 compared to ypT histopathology grade. Tumour grading of ymrT stage T0-T3a was significantly associated with favourable pathology at Week 14, compared with ymrT stage T3b-4 (p=0.006). Week 14 showed a stronger association of ymrT and favourable pathology than Week 9 (p=0.005). Furthermore, tumours graded as ymrTRG stage 1-3 were significantly associated with favourable pathology at Week 14, compared with ymrTRG stage 4-5 (p= 0.009). Similarly, Week 14 showed a stronger association of ymrTRG and favourable pathology than Week 9 (p=0.056). These models were not amenable to adjustment for baseline values.

Tumours graded as favourable volume change (≥80%) were significantly associated with favourable pathology at Week 14 (p= 0.011). Week 14 showed a stronger association of favourable volume change when compared to favourable ypT stage than volume change at Week 9 (p=N/a). Moreover, when adjusting for baseline values, volume change at Week 14 was significantly associated with favourable ypT stage (p=0.025). The sensitivity, specificity, positive and negative likelihood ratios for all of the variables described above are plotted in Figure 1 and tabulated in Appendix 2. Higher sensitivity, specificity and respective area under the receiver operating characteristic (AUC) curve measurements were received for Week 14 measurements.

Table 4 shows a similar univariate logistical regression model of age, gender, ymrT and ymrTRG at Week 9 and Week 14 compared to ypTRG derived at histopathology. Again, ymrT stage T0-T3a is significantly associated with favourable pathology at Week 14, compared with ymrT stage T3b-4 (p=0.017). Week 14 showed a stronger association of ymrT and favourable pathology than Week 9 (p=0.013). After adjusting for baseline values, ymrT at Week 14 was still significantly associated with favourable ypTRG stage (p=0.035).
Tumours graded as ymrTRG stage 1-3 were significantly associated with favourable pathology at Week 14, compared with ymrTRG stage 4-5 (p= 0.022). This showed a stronger association with favourable pathological outcome than ymrTRG at Week 9 (p=0.031). These models were not amenable to adjustment for baseline values.

Similarly, tumours graded as favourable volume change (≥80%) were significantly associated with favourable pathology at Week 14 (p= 0.009). Week 14 showed a stronger association of favourable volume change when compared to favourable ypTRG stage than volume change at Week 9 (p=0.047). When adjusting for baseline values, volume change at Week 14 was still significantly associated with favourable ypTRG stage (p=0.015). The sensitivity, specificity, positive and negative likelihood ratios for all of the variables described above are plotted in Figure 1 and tabulated in Appendix 2. Higher sensitivity, specificity and respective area under the receiver operating characteristic (AUC) curve measurements were received for Week 14 measurements.

**DISCUSSION**

In this study we have compared MRI evaluation of ymrT-staging, ypTRG and tumour volume assessments at two time-points (Week 9 and Week 14) post-NACRT with the pathology gold standards of ypT and ypTRG. This is the first attempt at understanding the relationship between MRI derived predictors and histopathological outcomes at two time points post-NACRT prior to surgery, in an attempt to inform clinical decision making about the optimal time interval between the end of NACRT and surgery. This is the first prospective study of tumour changes on MR at two pre-operative restaging time points, and the first report of substantial agreement for ymrT, mrTRG and tumour volume at baseline, Week 9 and Week 14 between two blinded reviewers at two different tertiary colorectal cancer referral centres.
Considering ypT as an outcome variable, there was a stronger association of favourable ymrT, ymrTRG and volume changes at Week 14 compared to Week 9. Similarly, considering ypTRG as an outcome variable, there was a stronger association of favourable ymrT, ymrTRG and volume changes at Week 14 compared to Week 9. Thus, all predictor variables at Week 14 show a strong relationship with both histopathological parameters. Clearly tumour regression is still ongoing until Week 14 post-NACRT, the mean tumor volume reduction being larger at Week 14 (80% (SD 22%)) than ato Week 9 (61 % (SD 39%)) . Interestingly, after correcting the volume change regression models for baseline values, the Week 14 models were still significantly related to both histopathological outcome measures, unlike the Week 9 models. The Week 14 predictive models corrected for baseline still retain their significant association with outcome variables, and are indicative of optimal variables that can be used in clinical practice; however larger validation studies are necessary to confirm this finding.

The pre-operative MRI staging of locally advanced, circumferential margin-threatened rectal cancers is closely associated with survival outcomes. MRI assessment post-NACRT has implications for surgical planning, timing of surgery, sphincter preservation and (for favourable responders) perhaps the deferral of surgery. Thus the ability to use re-staging MRI variables like ypT, ypTRG and volume change to predict favourable and unfavourable pathological outcomes in a clinical setting is crucial, as the subgroup of patients with MR-predicted unfavourable outcomes are at a higher risk of local or systemic failure following oncological resection. In these cases pre-operative MR may not only direct surgical dissection, but also alert the MDT to the need for further upfront systemic chemotherapy, contact radiotherapy or extended surgical resection. In this cohort of patients, the identification of an optimal time for surgery post-NACRT which coincides with maximal oncological down-staging is an urgent question (19,31). This pilot study suggests that further
volume reduction and down-staging occurs between Week 9 and Week 14 post-NACRT, with more favourable ymrT, ymrTRG and volume changes found at Week 14. Moreover, the longer time to surgery post-NACRT was associated with a 23% pathological complete response rate (pCR), a high rate comparable to literature rates of 17-27% (32). These results lend support to previous work where a greater delay to surgery following completion of NACRT is associated with better pathological outcome (20,25). The link between greater down-staging, completed pathological responses and long-term impact on disease-free survival are however yet to be established. This highlights the need for a randomised controlled trial.

The main limitation of this study was the limited number of patients recruited in what was a nested study within a larger published trial. We suggest that this potential weakness is offset by the novelty of the study design, the serial MRI assessment, the strength of the association between MRI predictor variables and histopathological outcomes and the magnitude of change between weeks 9 and 14. Other limitations include: the lack of tumour outlining on a workstation to calculate volume; the lack of MRI nodal status reporting (we felt that by limiting our predictor variables to ymrT, ymrTRG and volume change, type-1 errors due to multiple testing would be reduced) and recruitment of patients from a single centre, with uncertain generalizability.

Strengths of our study include: (i) the prospective study design; (ii) the homogenous study population (only operable MRI defined locally advanced rectal cancer patients); (iii) the blinded two-centre reporting of predictor variables (blind to patient demographics, clinical status and timeline); (iv) the standardized NACRT regime; (v) the targeted MRI and pathological analyses, conducted by strictly following a specific protocol; and (vi) rigorous statistical modelling showing significant ORs (even after adjustment for baseline values) with
calculation of predictive performance descriptors including accuracy, sensitivity, specificity, positive and negative likelihood ratios values.

Future studies of more patients may be able to refine our current findings in order to better inform decisions about optimal timing for surgical intervention following neoadjuvant cancer therapies in this cohort of patients. This will enable optimal timing of oncological resection based on objective, validated MRI defined tumour assessments. We suggest that clinical MRI directed re-staging based on ymrT, ymrTRG and percentage volume change is essential in order to fully inform multi-disciplinary patient tailored decision-making.

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Declaration of interest

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References


