



# Predicting response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer with serum biomarkers

TL Clarke<sup>1,2</sup>, DA White<sup>1,2</sup>, ME Osborne<sup>2</sup>, AM Shaw<sup>1</sup>, NJ Smart<sup>2</sup>, IR Daniels<sup>2</sup>

<sup>1</sup>University of Exeter, UK

<sup>2</sup>Royal Devon and Exeter NHS Foundation Trust, UK

## ABSTRACT

**INTRODUCTION** The aim of this study was to identify patient factors including serum biomarkers that may predict response to neoadjuvant chemoradiotherapy (CRT) in patients with locally advanced rectal cancer staged on magnetic resonance imaging. Prediction of response may be helpful when selecting patients for a non-operative programme.

**METHODS** A retrospective review was carried out of patients undergoing neoadjuvant CRT for rectal cancer, conducted at the Royal Devon and Exeter Hospital. All patients were managed through the multidisciplinary team. Receiver operating characteristic (ROC) curve analysis was undertaken to assess the ability of biomarkers to predict response to neoadjuvant CRT. The biomarkers assessed included neutrophils, lymphocytes, monocytes, haemoglobin, platelets, C-reactive protein and carcinoembryonic antigen.

**RESULTS** Seventy-three patients underwent neoadjuvant CRT between January 2006 and December 2011. Nine (12.3%) of these experienced a clinical complete response and were managed with a 'watch and wait' approach. An additional ten patients (13.7%) had a pathological complete response following surgery. Using ROC curve analysis, the biomarkers with the largest area under the curve (AUC) were pre-CRT haemoglobin and post-CRT lymphocyte concentrations, producing AUC values of 0.673 and 0.618 respectively for clinical complete response. Pre-CRT haemoglobin and neutrophil concentrations produced the highest AUC values for pathological complete response at 0.591 and 0.614 respectively.

**CONCLUSIONS** None of the assessed biomarkers offer the ability to predict response to neoadjuvant CRT in patients with rectal cancer. They cannot therefore assist in identifying complete clinical or pathological responders who could be considered for a non-operative, observational approach.

## KEYWORDS

Rectal cancer – Chemoradiotherapy – Surgery – Survival – Recurrence

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## CORRESPONDENCE TO

Ian Daniels, E: i.daniels@nhs.net

Recent advances in magnetic resonance imaging (MRI) staging have led to progress in preoperative prediction of circumferential resection margin involvement.<sup>1–5</sup> The use of preoperative (neoadjuvant) chemoradiotherapy (CRT) is advocated in patients with locally advanced disease to induce clear surgical resection margins,<sup>4</sup> defined as the absence of tumour cells in the margin of healthy tissue.<sup>5</sup>

A patient's response to CRT can be classified in four ways. The first of these is a pathological complete response (pCR), demonstrated by the absence of viable tumour cells in a tissue specimen.<sup>6</sup> Second, there can be a partial tumour response to CRT, assessed histologically by a variety of regression grading scores<sup>7</sup> or radiologically.<sup>8</sup> Third, there may be no response or even disease progression.<sup>9</sup> Finally, some patients have what is known as a clinical complete response (cCR). This category is used if there is absence of clinically and radiologically detectable tumour for a certain

period of time, increasingly defined as one year following completion of therapy.<sup>6</sup>

The 'watch and wait' or 'active surveillance' approach is a method that is being used in some patients who have evidenced a cCR to CRT.<sup>10</sup> As a result of a cCR, patients are offered the option not to undergo surgery, instead being monitored intensively. A retrospective study by our unit in 2012 demonstrated that 12% of patients treated with CRT had a cCR that had been managed without surgery.<sup>11</sup> In addition, an additional 12% of patients who underwent surgery were found to have a pCR, meaning the surgical procedure and subsequent reduction in patient quality of life could have been avoided. This approach was advocated initially by Habr-Gama *et al*, who demonstrated a cCR rate of 26.8% in patients with rectal adenocarcinoma undergoing CRT.<sup>12</sup>

Attitudes to cCR among surgeons are changing rapidly. A 2007 survey of members of the Association of Coloproctology

of Great Britain and Ireland found that 69% of colorectal surgeons were not comfortable offering a non-surgical treatment plan for rectal cancer patients<sup>15</sup> but a repeat survey in 2013 demonstrated wider acceptance of this strategy in selected patients.<sup>14</sup> Prediction of response to CRT would help with preselecting those patients in whom surgery could potentially be avoided. The aim of this study was therefore to identify serum biomarkers that could predict a complete response to neoadjuvant CRT.

**Methods**

A retrospective review of data from our local cancer database was carried out. Tumour and patient characteristics were retrieved from a database of patients undergoing neoadjuvant CRT for rectal cancer with curative intent between January 2006 and December 2011. The protocol for investigation and treatment of rectal cancer in our unit has been described in detail previously.<sup>11</sup> Following multidisciplinary team discussion, patients are considered for neoadjuvant CRT if the circumferential resection margin is threatened ( $\leq 1\text{mm}$ ) or involved by tumour, or if there is an involved lymph node. All patients received 45Gy in 25 fractions over 5 weeks with concurrent capecitabine ( $825\text{mg}/\text{m}^2$ ). Outcomes (recurrence, local or distant metastasis or death from another cause) were determined from patient records. Full haematological data were available for 58 patients from hospital computer records.

**Statistical analysis**

Pretreatment patient demographics and clinical factors were assessed in the context of cCR and pCR to neoadjuvant CRT. In order to determine the predictive qualities of biomarkers, receiver operator characteristic curve analysis was undertaken to generate an area under the curve (AUC) value. An AUC of 0.75 is considered a ‘good’ predictor of outcome.<sup>15</sup> In addition, so as to ascertain correlations between biomarkers,  $R^2$  values were generated using linear correlation analysis. Adjusted  $R^2$  values can range from -1 to +1, with +1 indicating a 100% correlation. Likelihood ratios were calculated from these  $R^2$  values.<sup>16</sup>

The Mann–Whitney U test was employed to establish the extent to which biomarkers could predict response to neoadjuvant CRT. In order to determine the effects of age and sex on response to CRT, Fisher’s exact test was used. All statistical analysis was performed using SPSS<sup>®</sup> (IBM, New York, US), StatsDirect (StatsDirect, Altrincham, UK), MATLAB<sup>®</sup> (MathWorks, Natick, MA, US) and Excel<sup>®</sup> (Microsoft, Redmond, WA, US). A  $p$ -value of  $<0.05$  was considered statistically significant.

**Results**

Between January 2006 and December 2011, 73 patients with a rectal adenocarcinoma underwent neoadjuvant CRT at the Royal Devon and Exeter Hospital. Of these, 56 (77%) proceeded to surgery. Eight patients (11%) did not undergo

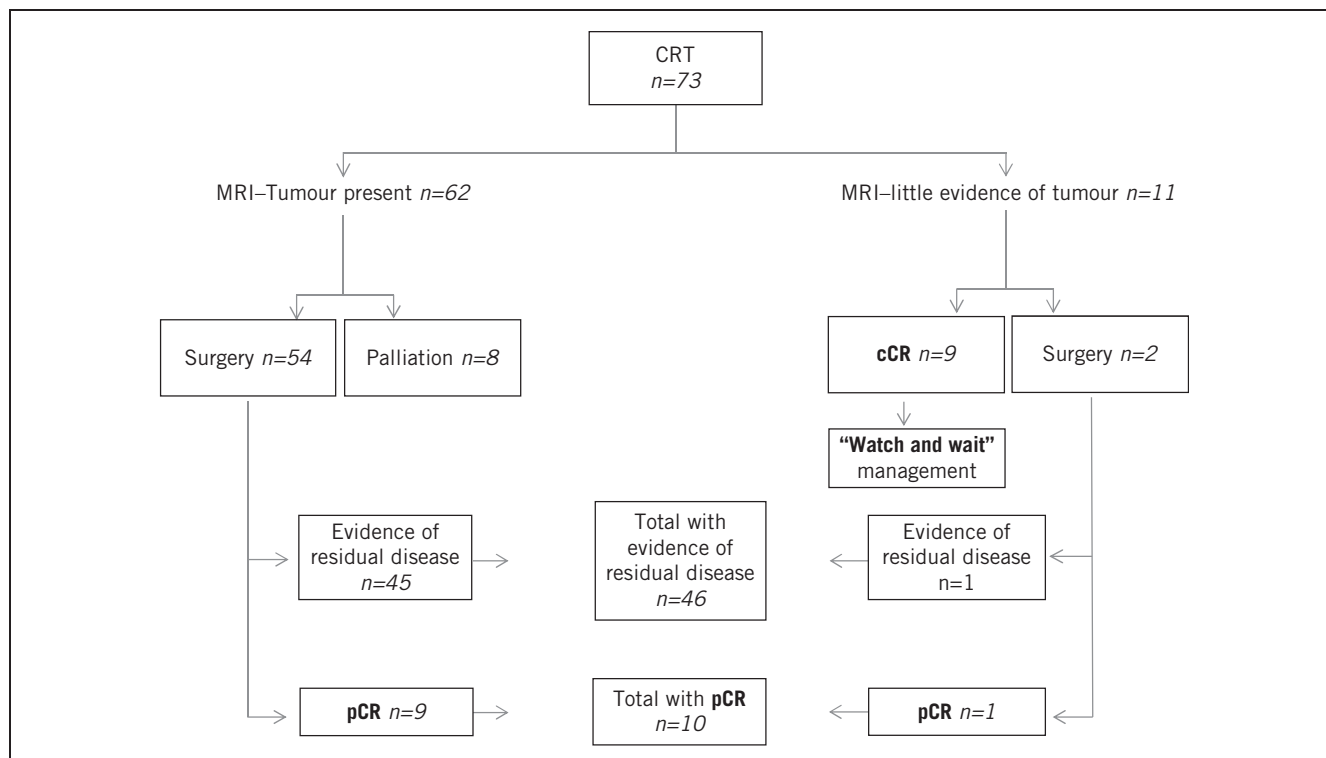


Figure 1 Response to chemoradiotherapy in patients with rectal adenocarcinoma

surgery as a result of disease progression despite therapy. A further nine patients (12%) avoided surgery, having experienced a cCR to CRT, and were managed with a ‘watch and wait’ approach. Of the 56 patients who underwent surgery, 10 (18%) had a pCR. Overall, therefore, a quarter of the patients who had neoadjuvant CRT (19/73, 26%) experienced a complete response, following either surgical or non-surgical management (Fig 1). After treatment with neoadjuvant CRT, there was a downstaging of MRI T stage and the number of potentially node positive tumours (Table 1).

From the initial AUC analysis, two biomarkers, post-CRT lymphocytes and pre-CRT haemoglobin (Hb), were identified as potential predictors of cCR, albeit both with an AUC of <0.75 (Table 2). Post-CRT lymphocyte concentration produced an AUC of 0.618. Nevertheless, this biomarker had a positive likelihood ratio (LR) of 1 and a negative LR of 1, indicating very little diagnostic or predictive use.

Hb was analysed separately to understand its effectiveness in predicting a cCR following CRT. Pre-CRT Hb concentration produced an AUC of 0.673, suggesting that higher Hb offers a greater chance of achieving a cCR to CRT. A positive LR of 1.258 and a negative LR of 0.578 further support the idea that Hb may have some predictive value, albeit not at the level that would be considered clinically relevant.

AUC analysis was also undertaken to elucidate whether any biomarkers would predict a pCR to CRT. A number were identified as potentially offering a predictive insight with an AUC of >0.5 (Table 2). These included pre-CRT neutrophil, lymphocyte and Hb concentration, and post-CRT neutrophil-to-lymphocyte ratio (NLR). Pre-CRT neutrophil and Hb concentration yielded the strongest AUC values. For that reason, these biomarkers were analysed individually.

Pre-CRT neutrophil concentration produced an AUC of 0.591. However, a positive LR of 0.152 and a negative LR of 0.934 indicates that it has no predictive value.

**Table 1** Comparison of T stage and node positivity among patients who underwent neoadjuvant chemoradiotherapy, prior to and following treatment

MRI T stage	n	Node positive	Histological T stage	n	Node positive
T1	0	0	T1	2	0
T2	5	3	T2	11	4
T3	48	38	T3	27	15
T4	14	10	T4	6	2
			pCR	10	–
			cCR**	9	–
<b>Total</b>	<b>67*</b>	<b>51</b>	<b>Total</b>	<b>65</b>	<b>21</b>

cCR = clinical complete response; MRI = magnetic resonance imaging; pCR = pathological complete response  
 \*MRI T stage data were unavailable for 6 patients, who were excluded from this analysis.  
 \*\*Based on MRI following chemoradiotherapy

**Table 2** Ability of biomarkers to predict cCR and pCR

Biomarker	cCR AUC	pCR AUC
Pre-CRT neutrophils	0.421	0.591
Pre-CRT lymphocytes	0.432	0.588
Pre-CRT NLR	0.521	0.503
Post-CRT neutrophils	0.536	0.506
Post-CRT lymphocytes	0.618	0.506
Post-CRT NLR	0.436	0.567
Pre-CRT monocytes	0.439	0.506
Pre-CRT haemoglobin	0.673	0.614
Pre-CRT PLR	0.436	0.387
Pre-CRT CEA	0.304	–

AUC = area under the curve; cCR = clinical complete response; CEA = carcinoembryonic antigen; CRT = chemoradiotherapy; NLR = neutrophil-to-lymphocyte ratio; pCR = pathological complete response; PLR = platelet-to-lymphocyte ratio

Pre-CRT Hb concentration was again suggestive of a positive response to CRT. Specifically, in the context of pCR, pre-Hb levels produced an AUC of 0.614 and this was therefore the biomarker offering the best predictive value for a pCR.

The ability of the change in NLR to predict response to CRT was evaluated by comparing pre and post-CRT values. For cCR, the change in NLR produced an AUC of 0.390 while for pCR, the AUC was 0.560. Consequently, there appears to be little predictive value with regard to NLR change during treatment with CRT determining tumour response.

The medians of the different biomarkers were compared for complete and incomplete responders to CRT (Table 3). These data yielded no statistically significant results. Nevertheless, the difference between pre-CRT Hb concentration in complete responders and that in incomplete responders had a *p*-value of 0.064, suggesting that a larger sample size could potentially demonstrate a significant difference. Pre-CRT carcinoembryonic antigen (CEA) and C-reactive protein yielded *p*-values of 0.401 and 0.970 respectively. Our data also showed that neither age nor sex were associated with a significant difference in response to CRT (Table 4) and the same was true for pretreatment clinical T stage (Table 5).

**Discussion**

The ability to accurately predict how individual patients would respond to neoadjuvant CRT would enable the tailoring of treatments to specific subsets of patients. Although non-operative management following cCR remains controversial,<sup>17</sup> the possibility of poor functional outcome due to low anterior resection syndrome and the associated reduced quality of life<sup>18,19</sup> or a permanent end colostomy means that it remains an attractive proposition for patients who have a

cCR. Risk stratification, particularly for those patients unlikely to respond to neoadjuvant CRT, would allow appropriate patient counselling and consideration of alternative treatment strategies. In our series, 11% of patients developed disease progression despite CRT. This aspect of response is not often reported in trials and demonstrates the diversity of response to CRT.

Numerous attempts have been made to identify factors that predict the degree of response to neoadjuvant CRT. Most have focused on genetic, epigenetic or molecular factors on tissue analysis from resected specimens, with a few using pretreatment biopsy samples. Several investigative techniques have been employed, including whole genome, single and multimarker analyses. Translation into routine clinical practice has not yet occurred owing to a combination of conflicting results and the retrospective nature of the studies, meaning there is a lack of independent prospective validation.<sup>20</sup> Furthermore, some authors have argued that the whole concept of personalising treatment is deeply flawed because of intratumour heterogeneity and plasticity.<sup>21</sup>

Serum biomarkers may offer a cheaper and more pragmatic approach to response prediction. Our data suggest that higher levels of Hb prior to the initiation of CRT are associated with cCR (AUC 0.675) or pCR (AUC 0.614) in rectal cancer patients. However, no significant relationships between clinical T stage and response to neoadjuvant CRT were found (Table 2). Previous studies have reported that pretreatment Hb concentration is important in terms of tumour downstaging.<sup>22,25</sup> In addition, it has recently been reported that patients with anaemia prior to neoadjuvant CRT are less likely to experience a pCR.<sup>24,25</sup> Only one study has previously examined Hb levels in the context of cCR and it failed to demonstrate any relationship.<sup>26</sup>

Our results have shown that there is a limited role for NLR in predicting response to CRT. Pre and post-CRT NLR did not offer any predictive insight into response to CRT (Tables 2 and 3). Furthermore, the change in NLR from before CRT to after CRT also appears to be limited as an indicative predictor of pCR to CRT as this only produced an AUC of 0.56. In addition, in terms of predicting cCR, the AUC was 0.59, suggesting no predictive value.

This is in contrast to the results of two previous retrospective studies that implied a relationship between lymphocyte ratios and response to neoadjuvant CRT.<sup>26,27</sup> The underlying hypothesis postulated by these studies is that the proportion of lymphocytes is an indicator of the adaptive immune response against the cancer while the neutrophil count reflects the innate inflammatory response that may cause the suppression of lymphocyte mediated immunity via the production of reactive oxygen species, nitric oxide and arginase.<sup>28</sup> Despite being a plausible hypothesis, our data suggest alternative factors are likely to be as important in determining cCR.

In our study, male patients had a greater response to CRT although this failed to reach statistical significance with the limited number in the series. There was no difference in

**Table 3** Comparison of median biomarker levels for patients with complete and incomplete responses to CRT

Biomarker	Complete response	Incomplete response	p-value
Pre-CRT neutrophils	5.09 × 10 <sup>9</sup> /l	4.87 × 10 <sup>9</sup> /l	0.585
Pre-CRT lymphocytes	1.91 × 10 <sup>9</sup> /l	1.57 × 10 <sup>9</sup> /l	0.290
Pre-CRT NLR	3.00	3.32	0.774
Post-CRT neutrophils	3.54 × 10 <sup>9</sup> /l	3.98 × 10 <sup>9</sup> /l	0.423
Post-CRT lymphocytes	0.83 × 10 <sup>9</sup> /l	0.68 × 10 <sup>9</sup> /l	0.408
Post-CRT NLR	4.37	4.81	0.542
Pre-CRT monocytes	0.58 × 10 <sup>9</sup> /l	0.61 × 10 <sup>9</sup> /l	0.713
Pre-CRT haemoglobin	13.8g/dl	13.2g/dl	0.064
Pre-CRT CRP	9.5mg/l	7.5mg/l	0.970
Pre-CRT CEA	3.1ng/ml	4.3ng/ml	0.401

CEA = carcinoembryonic antigen; CRP = C-reactive protein; CRT = chemoradiotherapy; NLR = neutrophil-to-lymphocyte ratio

**Table 4** Influence of age and sex on pCR and/or cCR to chemoradiotherapy

Variable	All patients	cCR	p-value	pCR	p-value	cCR/pCR	p-value
Male	45	8	0.218	9	0.290	17	0.063
Female	20	1		1		2	
Age ≤65	33	7	0.159	4	0.313	11	0.733
Age >65	32	2		6		8	

cCR = clinical complete response; pCR = pathological complete response

response between those aged ≤65 years and those aged >65 years (Table 4).

There was no evidence of CEA providing any insight into predicting patient response to CRT. In all of the AUC analyses undertaken, CEA failed to produce any values of clinical relevance. This is surprising given the findings from other studies, which have indicated that circulating CEA levels could offer some prognostic value in determining response to neoadjuvant CRT.<sup>29,50</sup> However, one possibility for the difference in results could be related to the accepted notion that CEA is increased in smokers, with suggestions that smokers commonly exhibit CEA levels that are almost double those of non-smokers.<sup>51</sup> Unfortunately, in this study, there was limited information on patient smoking status. It is therefore difficult to interpret the results presented here because it is not possible to accurately attribute CEA concentration as the sole consequence of presence of the rectal neoplasm.

**Table 5** Numbers of patients with cCR/pCR and incomplete response to chemoradiotherapy by pretreatment T stage

MRI T stage	Incomplete response	cCR	p-value
T1/T2	5	0	0.910
T3/T4	44	9	
<b>pCR</b>			
T1/T2	5	0	0.575
T3/T4	36	8	

cCR = clinical complete response; MRI = magnetic resonance imaging; pCR = pathological complete response

## Conclusions

None of the serum biomarkers tested in this study reliably predicted either cCR or pCR to neoadjuvant CRT for rectal cancer. Determinants of response to CRT are likely to be multifactorial and it is therefore unlikely that response can be predicted using clinical parameters alone. The impact of genetic, epigenetic and molecular factors on response to CRT needs further investigation, and selection of patients for a 'watch and wait' approach cannot therefore be recommended on currently available pretreatment factors in patients with locally advanced rectal cancer staged by MRI.

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