

## Parameters for Predicting Tumour Response Following Neoadjuvant Chemoradiotherapy for Patients with Rectal Cancer

Iustinian Bengulescu, Petru Radu\*, Cristian Iorga, Mircea Bratucu, Costin Pasnicu, Dragos Garofil, Florian Popa, Victor Strambu

Department of Surgery, Carol Davila Clinical Hospital of Nephrology, Bucharest, Romania

\*Corresponding author:  
Petru Radu, MD  
Department of Surgery  
Carol Davila Clinical Hospital of  
Nephrology, Bucharest, Romania  
E-mail: [drradupetru@yahoo.com](mailto:drradupetru@yahoo.com)

### Rezumat

#### *Parametrii de predicție a răspunsului tumoral după radio-chimioterapia neoadjuvantă la pacienții cu cancer rectal*

*Introducere:* Răspunsul patologic tumoral după radio-chimioterapia neoadjuvantă poate varia de la răspuns patologic complet până la „downstaging tumoral” sau lipsa răspunsului. Scopul nostru a fost de a evalua parametrii care ar putea prezice gradul de răspuns tumoral pentru pacienții cu cancer rectal.

*Metodă:* Am realizat un studiu retrospectiv analizând documentația pacienților care au fost tratați în clinica noastră între 2014-2018 după radio-chimioterapie neoadjuvantă.

*Rezultate:* Un număr de 98 de pacienți au fost incluși în studiu, 66 de sex masculin (67,3%) și 32 de sex feminin (32,7%). Vârsta medie a fost de 64,6 ani (39-87). Supraviețuirea la 48 de luni a fost de 81,63% și rata de supraviețuire „cancer free” la 48 de luni a fost de 69,38%. Gradientul de diferențiere tumoral a fost considerat parametru statistic semnificativ în prezicerea răspunsului tumoral. Tumorile cu probabilitatea cea mai mare de a răspunde la radio-chimioterapie au fost din grupa G1 și G2. Tumorile din stadiul T4 în comparație cu stadiile mai mici au prezentat cea mai redusă proporție în obținerea răspunsului patologic complet. Valori crescute ale CEA de asemenea pot anticipa un răspuns tumoral slab.

*Concluzie:* Studiul nostru a evidențiat faptul că parametrii tumorali cum ar fi stadiul T, stadiul N, scorul G dar și markeri biologici precum valorile CEA pot fi folosiți cu valoare predictivă pentru analiza răspunsului patologic tumoral după radio-chimioterapia neoadjuvantă.

*Cuvinte cheie:* cancer rectal, radio-chimioterapie neoadjuvantă, radio-chimioterapie, parametri de predicție

Received: 20.03.2020  
Accepted: 27.04.2020

## Abstract

*Introduction:* Pathologic response following neoadjuvant chemoradiotherapy (nCRT) can vary from pathologic complete response (pCR), to tumour downstaging or minimum to no response. Our goal was to evaluate the parameters that could predict response to neoadjuvant therapy for patients with rectal cancer.

*Method:* We performed a retrospective study and reviewed the medical documentation for patients that received treatment for rectal cancer in our surgical department between 2014-2018 and received nCRT.

*Results:* A total of 98 patients were included in the study. 66 patients were males (67,3%) and 32 were females (32,7%). The mean age was 64,6 (39-87). The 48 months overall survival rate was 81,63% and the 48 months disease-free survival rate was 69,38%. Tumour grading was considered as a statistically significant parameter for evaluating the pathologic response. The tumours most likely to respond to radio-chemotherapy were G1 or G2 grade. T4 tumours compared with lesser T stages were less likely to achieve pathologic complete response. Elevated CEA levels predicted a poor pathologic response to nCRT.

*Conclusion:* Our study concluded that tumour related factors, biologic and imagistic findings such as tumour stage, lymph node, tumour differentiation grade and CEA levels can be used as parameters for predicting the tumour response following neoadjuvant therapy.

**Key words:** rectal cancer, neoadjuvant, chemoradiotherapy, predictive parameters, clinical response

## Introduction

The GLOBOCAN study estimates that there will be approximately 18 million new cancer patients in 2018 and one third of them will be consisted of lung, breast and colorectal cancer. Colorectal cancer represents the second cause of death after lung cancer (1). Out of all colorectal cancer one third is represented by rectal cancer. Rectal cancer treatment strategies have changed over the years, from postoperative chemoradiotherapy considered as the principal therapy for stage II-III (2,3), to the introduction of TME (3), and nowadays neoadjuvant chemoradiotherapy being considered as mandatory for stage II-III rectal cancer (4). Furthermore, a more tailored approach to rectal cancer treatment can lead to the development of other strategies such as: transanal endoscopic microsurgery or nCRT followed by close surveillance.

Tumour response to neoadjuvant therapy can be evaluated prior to surgery through the clinical response rate, clinical complete response (cCR) representing no more evidence

of tumour presence, or it can be evaluated through the pathologic response grading system in which a pathologic complete response (pCR) represents tumour absence in the surgical specimen. Obtaining a pCR has been associated with better disease-free survival rates (DFS), better sphincter preservation and improved local control (5-8). Rectal tumours have a heterogenous response to nCRT, pCR rates can vary from 15 to 30 % (9,10). Clinical complete response has been proven with an almost twice rate rate (11,12). Patients can respond to nCRT either with a pathologic complete response, tumour downstaging or with no respond and developing of distant metastasis. Randomized trials proved a 25% rate of distal metastasis for patients treated with nCRT and TME (13-15). There is no consensus of independent parameters that can be used for predicting pCR and current research, although it is performed from a retrospective point of view, is aiming to clarify this topic. The utility of these factors would lead to a more personalized strategy and could help to reduce the number of

unresponsive patients exposed to unnecessary neoadjuvant therapy and its toxicity, could improve surgical morbidity by performing surgical procedures on patients that hadn't been irradiated prior, could help obtain better financial costs, and help improve overall quality of life for rectal cancer patients. On the other hand, the responders would be better classified and thus could benefit from a more tailored approach.

Our goal was to evaluate the parameters that could predict the tumour response to neoadjuvant therapy for patients with rectal cancer.

## Method

We performed a retrospective study and reviewed the medical documentation for patients that received treatment for rectal cancer in our surgical department between 2014-2018 following neoadjuvant chemoradiotherapy. We excluded patients that had other malignant disease, or metastatic dissemination. Data was collected regarding patient demographics, rectal exam, clinical staging, CEA levels, surgical procedure, pathological findings and follow up. Surgical procedure was performed either through open or laparoscopic approach and consisted of anterior resection or abdomino-perineal resection. Patients received long course neoadjuvant chemoradiotherapy with 5 fluorouracil and radiotherapy of 50.4 Gy delivered in 28 fractions. Our goal was to evaluate the possibility of predicting the tumour response using parameters that could be obtained prior to surgery and correlating their value with the pathology report. The premise being that by identifying factors that could predict tumour response it could lead to a better classification and optimisation of the therapeutic strategy. A total of 98 patients were included in this study. Follow up varied from 24 to 60 months. The tumour regression AJCC criteria (16) was used. A complete response is equivalent to a tumour regression score of 0 (TRG0) with no viable cancer cells present, TRG1 represents a near complete response and consists of single cells or rare

small groups of cancer cells remaining, TRG2 represents a partial response and consists of residual cancer with evident tumour regression but more than single cells or rare small groups of cancer cells, TRG3 represents poor or no response and consists of extensive residual cancer with no evident tumour regression. CEA levels were obtained prior to neoadjuvant therapy, prior to surgery and at follow up and were considered either normal  $< 5 \text{ ng/mL}^3$  or elevated  $> 5 \text{ ng/mL}^3$ . Overall survival rate (OS) and disease-free survival rate (DFS) were used throughout the follow up. Clinical T and clinical N stage (cT and cN) were defined as T and N stage prior to treatment. Staging was defined according to the guidelines criteria used at the time of the treatment. Patients were staged using MRI or CT according to the possibilities at the time of the treatment. Endoscopy was used for pre-operative diagnosis or postoperative surveillance and was performed in our clinic by two surgeons. All surgical procedures were performed in our clinic and according to the guidelines at the time of the treatment. The rectum was defined as the surgical rectum and its fractions were defined in cm starting from the external anal orifice as seen on the endoscope as follows : upper rectum 11-15 cm, middle rectum 6-10 cm, low rectum 1-5 cm. All surgical procedures were R0 and the TME resection was complete according to the Quirke protocol (Intact mesorectum with only minor irregularities, no defects deeper than 5 mm, no coning toward the distal margin of the resection, smooth CRM on transverse sections). All patients had imagistic procedures performed both for staging and then preoperative. The Kaplan-Meier curve was used for the OS and DFS, and statistical analysis was performed using GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA, [www.graphpad.com](http://www.graphpad.com).

## Results

A total of 98 patients were included in the study. 66 patients were males (67.3%) and 32

were females (32.7%). The mean age was 64.6 (39-87). The majority of patients were from an urban area (69%) compared to 31% from a rural area. The 48 months overall survival rate was 81.63% and the 48 months disease-free survival rate was 69.38%. Clinical T staging: cT0 2 patients (2.04%), cT1 6 patients (6.12%), cT2 10 patients (10.2%) and cT3 69 patients (70.4%) and cT4 11 patients (11.22%). Clinical N staging was represented as follows: 21 patients cN0 (21.42%), 35 patients cN1(35.71%) and 42 patients cN2 (42.85%). 87 patients were graded stage II and III (88.77%). G grading: G1 12 patients (12.24%), G2 60 patients (61.22%), G3 18 patients (18.36%) and G4 8 patients (8.16%). The patient's characteristics are shown in *Table 1*. According

**Table 1.** Patients general characteristics

Patients general characteristics	No of patients N=98
Sex	
Men	66 (67.3%)
Women	32 (32.7%)
Age	64.6 (29-87)
Clinical T stage	
cT0	2 (2.04%)
cT1	6 (6.12%)
cT2	10 (10.2%)
cT3	69 (70.4%)
cT4	11 (11.22%)
Clinical N stage	
cN0	21 (21.42%)
cN1	35 (35.71%)
cN2	42 (42.85%)
Clinical Stage	
Stage 0-I	11 (11.22%)
Stage II-III	87 (88.77%)
G stage	
G1	12 (12.24%)
G2	60 (61.22%)
G3	18 (18.36%)
G4	8 (8.16%)
TRG grading	
TRG 0	25 (25.51%)
TRG 1	36 (36.73%)
TRG 2	24 (24.48%)
TRG 3	13 (12.74%)
Tumour location	
Upper rectum	11 (11.22%)
Middle rectum	33 (33.67%)
Lower rectum	54 (55.10%)

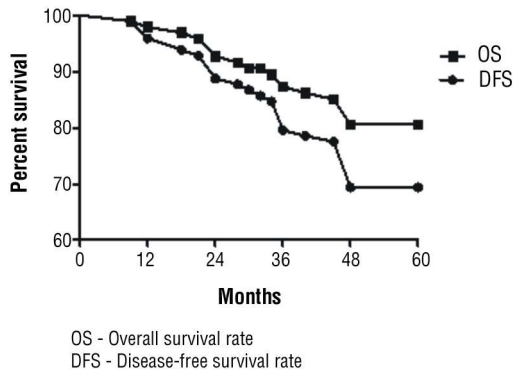
to the TRG score the patients were divided into 4 groups: TRG0 25 patients (25.51%), TRG 1 36 patients (36.73%), TRG 2 24 patients (24.48%) and TRG 3 13 patients (12.74%). There were no significant differences regarding age, sex, or comorbidities between the TRG groups of patients.

Tumour grading was considered statistically significant for both TRG0 and TRG3 groups. The tumours most likely to respond to neoadjuvant therapy were G1 or G2 with a 24%, and 64% for TRG0 and 7.69%, 30.7% for TRG3,  $p=0.0243$  and  $0.0030$ . And similarly, tumours that did not respond well to neoadjuvant therapy were G3 and G4, 12% of them for TRG0 and 61.53% for TRG3. T4 tumours were more likely not to achieve pCR (4%) compared to lesser T stages, for the TRG3 group T4 tumours being the most represented category (46.15%). Clinical N stage reported a better TRG score for N0,N1 patients compared to N 2 patients (40%, 52%, vs 8 %)  $p=0.0074$  and  $p=0.0004$  and amongst TRG 3 group the most likely not to respond to nCRT were the N2 patients (53%) compared to N0 and N1 (15.38% and 30.76%)  $p=0.0405$  and  $p=0.0250$ . Elevated CEA levels were a negative predictor for a good response to nCRT, with elevated levels ratio for TRG0 being lesser then for TRG 3, 28% vs 69.23%.

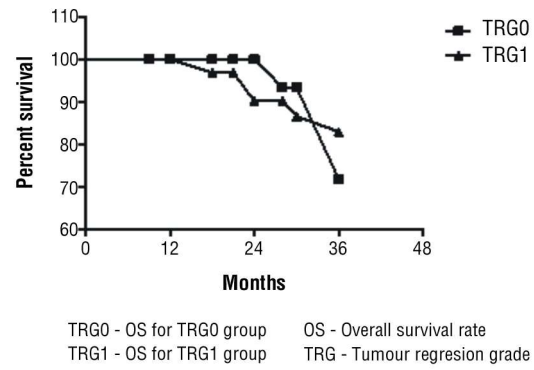
## Discussions

Our study aimed to find parameters that could be used to predict tumour response to neoadjuvant therapy. Our results associated tumour related factors and biological findings with a degree of tumour regression, such as clinical tumour stage, clinical lymph node, tumour differentiation grade and CEA levels.

TRG0 is associated with a better OS and DFS, compared to TRG 3, for a mean follow up of 36 months. The DFS was significantly higher for TRG 0 and decreasing for every other group of TRG (88.8% TRG0, 86.10% TRG 1, 79.10% TRG2 and 69.10% TRG3). The Kaplan-Meier OS and DFS rates are shown in *Fig. 1, 2, 3*. A meta-analysis of 16 studies with a total patient number of over 3000 estimated



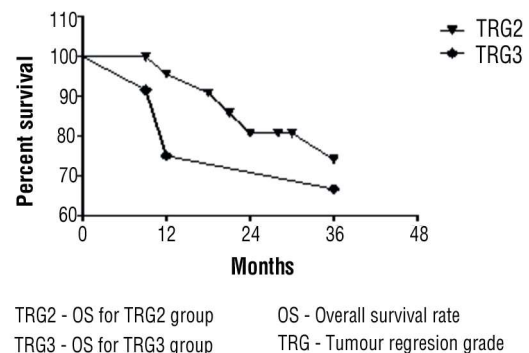
**Figure 1.** Kaplan-Meier OS vs DFS curve



**Figure 2.** Kaplan-Meier OS curve for TRG0 and TRG 1 group

a better OS rate and lower local recurrence rates associated with pCR (OR 0.23  $p < 0.001$  and OR=0.25  $p = 0.002$ ) (17). Other studies reported better DFS and OS rates for patients developing pCR or even intermediate levels of pathological response (18,19). Although numerous studies focus on establishing the pCR rate, a consensus is yet to be obtained regarding the factors that could predict it. Even the TRG score has several proposed gradings systems such as the Mandard score, the Dworak scale, the Rodel percentage model, the Aberdeen/Murray residual percentage model, the AJCC score (16,20-23). Tumour size has been considered as a pCR predictive factor in other studies, Garland et al (24) analysed a group of 297 patients and reported that tumour size is an independent predictor for pCR. Another study published in 2016 analysed clinical predictive factors for locally advanced rectal cancer and concluded that tumour size  $< 5$  cm is a predictive factor for positive pCR and also showed a positive trend for nodal status and pCR (25). A large study,  $n > 13.000$  patients focussed on evaluating factors associated with tumour response, concluded that lower tumour stage and grade, negative lymph nodes, CEA normal levels, association of chemotherapy and increased doses of radiotherapy can induce a greater regression (26). nCRT, especially for locally advanced rectal cancer has improved local control of the disease, and managed to reduce LR rates, but the distant metastasis rate

remains higher for this group of patients. Survival rates could be improved for locally advanced rectal cancer patients by a better understanding and treatment of the disseminated disease. Adjuvant therapy is directly involved in this matter, however by failing to identify the patients at risk of developing distal metastasis following nCRT +TME and treating them all with adjuvant chemotherapy would lead to unnecessary overexposure for the majority of 75% patients that will not develop distant metastasis. Basically, the patients with a higher risk for disease recurrence represent the candidates for adjuvant chemotherapy. TRG score is not always associated with lymph node regression, and studies reported a 7% lymph node invasion remnant even when there is a TRG0 for the primary tumour (27).



**Figure 3.** Kaplan-Meier OS curve for TRG2 and TRG 3 group

Which raises the questions: Is the degree of tumour response more significant than the ypTNM staging? Could the tumour response reflect the overall therapeutic response better than the yp staging? The NAR score which is calculated using cT and pathological T and N degrees standardises the nCRT response into two categories. Recent studies validated the predictive value of the NAR score regarding DFS, (28) and concluded that it can even have better value than the pCR in predicting OS (29). Another NAR study that was published this year addressed the relation between the NAR score and the indication for adjuvant therapy and concluded that patients in the low NAR group (< 16) that received neoadjuvant therapy had a better prognostic, suggesting that adjuvant chemotherapy could improve survival for patients with a better therapeutic effect of the neoadjuvant therapy (30).

Studies in the field of molecular profiling, immunotherapy, biomarkers or tumour pattern of response represent alternative strategies for establishing parameters that could predict tumour response, however they depend on the surgical specimen. Patients that are MSI+ (test positive for microsatellite instability) are prone to a poorer prognosis (31), and it is considered that this prognosis may be associated to a reduced response to the neoadjuvant therapy (32). Immunotherapy could prove to be an alternative solution for patients MSI+ that respond poorly to nCRT. A prospective trial studying the effect of the pembrolizumab PD-1 (programmed death) blocker, revealed a 40% response rate for colorectal cancers (33). DNA mutations – KRAS and p53 mutations are considered to confer some degree of tumour resistance to radiotherapy (34,35). Further DNA mutations studied like the apoptotic LUM genes and the DNA repair gene XRCC3 and SMC1 have been reported to decrease the response to nCRT (36,37). The tumour patterns of response is of recent interest, the idea that following nCRT the tumour shrinks lumen centred for tumours that have yet to breach the muscularis propria would represent the ideal response, and could easily be evaluated

through endoscopy or imagistic strategies. However, it has been postulated that rectal cancer can respond via two ways to nCRT: shrinkage and fragmentation (38). Fragmentation can be present in up to 40% locally advanced rectal cancer patients (39). The presence of these small groups of tumour cells, can be responsible of less actual downstaging, the presence of positive resection margins, residual positive lymph nodes and overall a poorer outcome.

Finally, the duration between the completion of the neoadjuvant therapy and surgery could have an impact on the tumour regression. All of our procedures were performed between 6 to 8 weeks after neoadjuvant therapy. A recent study published improved pCR results if the interval between neoadjuvant therapy and surgery is greater than 8 weeks (40), other studies proved that this interval represents an independent predictor for pCR rates (41,42).

## Conclusions

Rectal cancer requires a more patient-centred approach performed in experienced centres. The key to lowering the distant metastasis rate remains in understanding the response to neoadjuvant therapy. We know that the pathologic complete response is a great predictor for good outcomes, however the heterogeneity of tumour response remains challenging. In overcoming this task efforts must be focused on identifying factors capable of predicting the response to nCRT. Response recognition has yet to meet a consensus regarding evaluating methods. Patients could be better stratified if an optimal prediction strategy would be developed. Clinical assessments, imagistic findings and biomarkers have been studied. At the moment, the best method to predict pCR eludes us. A better understanding of the response pattern, the discovery and utilisation of clinically proven biomarkers are essential and could lead to the development of individualised treatment.

However, clinical practice in concordance with national and international guidelines has

been well established. Sphincter preserving surgery is dictated by the axial tumour spread, the “watch and wait” strategy is suited for incipient stage tumours but could become dangerous without complete surveillance and patient compliance. A patient centred approach would be ideal but usually multidisciplinary teams, when making decisions in the tumour board, rely on established guidelines. Their decision has a tendency to insert the patients into the guidelines group and continue in a nonpatient centred manner. Standard therapy for rectal cancer, in the locally advanced stages remains the same, neoadjuvant chemoradiotherapy, standardised surgical technique (TME either open or laparoscopic/robotic, regardless of the intent to preserve the sphincter) and adjuvant therapy. Of course a better understanding of the heterogeneity of the tumour response to neoadjuvant therapy is essential and would help in optimising the treatment for rectal cancer, but are we ready and willing to adhere to these future principles just yet? Future prospective randomized trials will probably bring more clarification to this subject.

### Conflict of Interest

The authors declare no conflicts of interests.

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