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*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1635341> since 2017-05-17T11:52:07Z

*Published version:*

DOI:10.23736/S0026-4733.17.07392-8

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Riccardo FALETTI, Marco GATTI, ALBERTO AREZZO, Silvia STOLA, Maria Chiara BENEDETTI, Laura BERGAMASCO, Mario MORINO, Paolo FONIO

*Minerva Chirurgica* 2017 May 11

DOI: 10.23736/S0026-4733.17.07392-8

Article type: Original Article

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## **TITLE**

Preoperative staging of Rectal Cancer using Magnetic Resonance Imaging: comparison with pathological staging

## **AUTHORS**

Riccardo Faletti, MD<sup>1</sup>; Marco Gatti, MD<sup>1</sup>; Alberto Arezzo, Prof.<sup>2</sup>; Silvia Stola, MD<sup>1</sup>; Maria Chiara Benedini, MD<sup>1</sup>; Laura Bergamasco, Prof.<sup>3</sup>; Mario Morino Prof.<sup>2</sup>; Paolo Fonio, Prof.<sup>1</sup>

## **AFFILIATIONS**

1. Department of Surgical Sciences, Radiology Institute, University of Turin, Turin, Italy.
2. Department of Surgical Sciences, University of Turin, Turin, Italy.
3. University of Turin, Turin, Italy.

## **CORRESPONDING AUTHOR**

Marco Gatti

[marcogatti17@gmail.com](mailto:marcogatti17@gmail.com)

Via Genova 3, 10126 Torino

tel: +39 011 6336622

fax: +39 011 6960310

## ABSTRACT

**BACKGROUND:** To evaluate the accuracy of Magnetic Resonance (MR) in loco-regional staging of rectal cancer by comparing the MR results with histologic findings, considered as standard reference.

**METHODS:** Between July 2013 and March 2015, fifty-two patients, 27 (51.9%) males, age  $66.75 \pm 13.77$  years, with rectal cancer, were staged preoperatively with MR and proceeding straight to surgery. Two observers with experience in abdominal MR independently reviewed the images. T stage and N stage were evaluated according to the 7<sup>th</sup> edition of TNM classification. The estimate of Ln probability of malignancy (low, moderate, high) was based on nodal size, border contour and signal intensity and comparison between ADC value of the Ln's belonging to the three different classifications were performed. Statistical testing included Cohen's kappa coefficient, Mann-Whitney's, Kruskal-Wallis, chi-square, Fisher's exact test and Receiving Operating Characteristics curve.

**RESULTS:** MR correctly assessed T stage in 47/52 cases (90.4%;  $k_w=0.89 \pm 0.06$ ), with inter-operator concordance of  $k=0.81 \pm 0.08$ . For Ln staging, concordance between estimate of high probability malignancy and pathology was  $k_w=0.62 \pm 0.11$ . ADC was significantly different for the three grades of estimated malignancy probability ( $p=0.0003$ ), decreasing from  $1.227 \pm 0.298 \times 10^{-3} \text{mm}^2/\text{s}$  (low) to  $1.120 \pm 0.306 \times 10^{-3} \text{mm}^2/\text{s}$  (moderate) and finally to  $0.818 \pm 0.168 \times 10^{-3} \text{mm}^2/\text{s}$  (high). The ROC curve procedure established the good ability of ADC to discriminate high malignancy Ln's (AUC=0.88) with cut-off at  $<1 \times 10^{-3} \text{mm}^2/\text{s}$ . The percentage of high malignancy Ln's in the lateral pelvic space was higher than in other sites (55.6% vs. 17.6%,  $p=0.0003$ ).

**CONCLUSIONS:** MR is an accurate imaging method in T staging and N staging of rectal cancer: prediction of N was improved by considering dimension, morphology and signal characteristic and the ability of ADC to identify high probability malignant nodes underlines its importance in the diagnostic process.

**Key words:** Rectal cancer – Lymph node – Neoplasm staging – Magnetic Resonance Imaging – Diffusion Magnetic Resonance Imaging.

## INTRODUCTION

Colorectal cancer is a common neoplastic disease, representing the third leading cause of cancer death [1]. Unfortunately, only 40% of patients with colorectal cancer get diagnosed when the disease is still at a local stage [2]. In fact, about half of them are affected by rectal cancer, their survival and risk for local recurrence being highly influenced by the presence of lymph nodes (Ln) metastasis (MTS) [3], as observed in up to 2/3 of patients [4,5].

In case of suspected rectal cancer international guidelines recommend Magnetic Resonance (MR) as fundamental for primary staging, because of its good accuracy for tumour (T) staging, being EUS preferred only in early stages suitable for local excision [6,7] and CT to identify possible distant MTSs [8]. Studies which investigated the ability of MR to predict also Ln involvement in rectal cancer [6,9-13], did not converge on uniformly accepted criteria on how to define Ln positivity [12], and how to assess it on the basis of morphologic criteria as dimension, spiculated or indistinct border and mottled heterogeneous appearance [4,5]. Diffusion Weighted Imaging has been considered effective in the detection of Ln [9], but failing in its characterization [11]. A few authors investigated Ln specific contrast agents [13], without however reaching a general consensus on their usefulness [14].

The aim of our study was to evaluate the accuracy of MR in the loco-regional staging of rectal cancer by comparing its results to pathology findings, considered as standard reference, avoiding the confounding factor of neoadjuvant therapy between initial MR staging and pathology examination of the surgical specimen.

## MATERIALS AND METHODS

Between July 2013 and March 2015, a total of fifty-two consecutive patients, 27 (51.9%) males, mean age  $66.7 \pm 13.7$  years, with histologically proven rectal cancer, underwent a multi-parametric rectal MR as part of an institutional standard work up before undergoing to surgery without neo-adjuvant therapy. Reasons for not performing chemo-radiotherapy before surgery in pT3 and pT4 Ln positive patients were advanced age, location of tumour between 12 and 16 cm from the anal verge, or the patient's preference.

At the moment of the MR examination, all patients were informed about the possible use of their data for study purposes, and signed an informed consent form. Patients' information was anonymized prior to the analysis. The study is a retrospective trial without any study-related clinical intervention and conforms to the Helsinki Declaration of 1975 and subsequent modifications.

## **MR protocol**

The MR examination was performed by means of a 1.5 T (Achieva, version 2.6, Philips Medical Systems, Eindhoven, The Netherlands) with body coil phased array (16-channel Sense XL Torso). MR protocols consisted of a T2-weighted fast spin-echo sequence. Sagittal sequences were used to determine the longitudinal tumour axis in order to angle the axial and coronal planes as much as possible perpendicular and parallel to the tumour axis. Axial DWI was performed with a free-breathing single-shot echo planar imaging sequence immediately after the routine sequences. b 0, 300 and 600 s/mm<sup>2</sup> were used.

## **Imaging analysis**

MR Extended Work Space 2.6.3.2 2009 software (Philips Medical Systems) was used for imaging interpretation.

Two observers (M.G. and S.S.) with experience in abdominal MR independently reviewed all images in a randomized and blinded way and classified the primary tumour according to the 7<sup>th</sup> edition of TNM classification [15]. Discordant readings were examined and discussed with a senior radiologist (R.F.) to reach a general consensus.

For nodal staging, the Ln estimate probability of malignancy was based on: 1) dimension ( $\geq 5$  mm), 2) irregular border contour and 3) inhomogeneous signal intensity. The absence of all previous characteristics was considered at low probability of malignancy (P1); the presence of only one of these features was considered at moderate probability of malignancy (P2); the presence of two or more features was considered at high probability of malignancy (P3). For each Ln the Apparent Diffusion Coefficient (ADC) value was measured by drawing manually regions of interest (ROI) on the ADC map; the ROIs were placed on the center of each Ln to reduce the possibility of signal contamination caused by the partial volume effect of the surrounding structures.

Ln's were classified also according to their location in the three main different lymphatic districts: mesorectal, presacral and lateral pelvic.

## **Histological analysis**

Pathology evaluation of the surgical specimens was the standard reference for the histological parameters. Pathology reports were reviewed to determine the tumour T stage and N stage according to the 7<sup>th</sup> edition of TNM classification [15].

## **Statistical analysis**

Concordance between different qualitative (categorical) items classified in two different

ways was measured by Cohen's kappa coefficient with linear weighting  $k_w$  ( $0 \leq k_w \leq 1$ ). The performance of MR in correctly staging tumours and Ln was assessed by cross correlating its outcome with the histological findings and computing, beside the kappa coefficient, also the five diagnostic parameters, i.e. sensitivity, specificity, diagnostic accuracy, positive predictive value and negative predictive value computed with the relative 95% Confidence Interval (CI).

Categorical variables were compared with non chi-square or Fisher's exact test. The ADC distributions met the normality conditions according to the Kolmogorov-Smirnov/Lillienfors test and the Shapiro-Wilks W test: for greater confidence, their comparison was carried out besides ANOVA also with the non-parametric Mann-Whitney's and Kruskal-Wallis tests, respectively for 2 and 3 distributions. Statistical significance was set at two-tails  $p < 0.05$ .

The discriminatory ability of ADC as indicator of high probability of malignancy (P3) was assessed by the Receiving Operating Characteristics (ROC) curve, a plot of True Positive Rate (Sensitivity) versus False Positive Rate (1-Specificity). The Area Under the Curve (AUC) measures the quality of discrimination: conventionally 0.6 corresponds to poor and 1 to excellent. The threshold for P3 was set at the ADC value for which both the harmonic mean (HM) of specificity and sensitivity and Cohen's coefficient  $k$  reached their maximum.

Open source software OpenEpi ([www.openepi.com](http://www.openepi.com)) and VassarStats ([www.vassarstats.net](http://www.vassarstats.net)) were used along with StatPlus:mac Pro (Analyst Soft Inc. Walnut, CA, US). All tests were run on at least two different packages.

## RESULTS

### T-stage

The inter-observer concordance on MR T-stage (cT) as measured by Cohen's coefficient was  $k_w = 0.81 \pm 0.08$ . The end result was the cross-correlation table between MR (cT) and pathology (pT) reported as Table 1. The relative Cohen's concordance coefficient was  $k_w = 0.89 \pm 0.06$ .

The comparison of the identification of mesorectal fat invasion by MR and histology yielded 24 true positive, 25 true negative, 3 false positive and 0 false negative interpretations. The diagnostic parameters were sensitivity 100 % (95%CI 86-100), specificity 89.3% (95%CI 73-96), diagnostic accuracy 94.2% (95%CI 84-98), positive predictive value 88.9% (95%CI 72-96) and negative predictive value 100% (95%CI 87-100).

### N-stage

MR qualitative analysis recognized 265 Ln's. Our estimate of the probability of malignancy classified the Ln as follows: 198 P1, 17 P2 and 50 P3. Figure 1 shows as an example T2WI, DWI

(b600) and ADC map for a P1, P2 and P3 Ln.

Table 2 reports the cross-correlation table of the MR node stage (cN) for Ln's classified with score P3, i.e. with high probability of malignancy, versus pathology node staging (pN) for the 52 patients. Cohen's concordance coefficient was  $k_w=0.62\pm 0.11$ .

The comparison of the ability of MR to classify the nodal staging (cN) with histology (pN) yielded 14 true positive, 26 true negative, 11 false positive and 1 false negative. The diagnostic parameters were sensitivity 93.3% (95%CI 70-99), specificity 70.2% (95%CI 54-83), diagnostic accuracy 76.9% (95%CI 64-86), positive predictive value 56% (95%CI 37-73) and negative predictive value 96.3% (95%CI 82-99).

The ADC value of the Ln's belonging to the three different classifications of probability of malignancy was  $1.23\pm 0.30\times 10^{-3}$  mm<sup>2</sup>/s for P1,  $1.12\pm 0.31\times 10^{-3}$  mm<sup>2</sup>/s for P2 and  $0.82\pm 0.17\times 10^{-3}$  mm<sup>2</sup>/s for P3 ( $p=0.0003$ ). Since the ADC values for P1 and P2 were not significantly different ( $p=0.15$ ), the two sets were pooled to form the low probability MTS Ln sample. The pooled ADC was  $1.22\pm 0.30\times 10^{-3}$  mm<sup>2</sup>/s significantly higher ( $p<0.0001$ ) than the value relative to the high probability MTS Ln sample P3  $0.82\pm 0.17\times 10^{-3}$  mm<sup>2</sup>/s. The performance of ADC as possible discriminator was tested by the ROC curve procedure, obtaining an appreciably high AUC = 0.88 indicating a good-very good discrimination (Figure 2). High probability MTS Ln's were then found to correspond to  $ADC \leq 1.0\times 10^{-3}$  mm<sup>2</sup>/s (Figure 3), with sensitivity 90% and specificity 77.5%.  $ADC \leq 1\times 10^{-3}$  mm<sup>2</sup>/s held for 90% (45/50) of high probability malignant Ln's and for 22% (48/215) of low-moderate probability MTS Ln's (OR = 31, 95%CI 12-82.8;  $p<0.0001$ ).

Of the 265 Ln identified, 153 were identified in the mesorectal district, 103 in the presacral and 9 in the lateral pelvic; the incidence of P3 in this last district was 5/9 (55.6%), significantly higher than in the mesorectum (27/153, 17.6%) and in the presacral (18/103, 17.5%) districts ( $p=0.02$ ).

## DISCUSSION

The results of the study herein presented allowed us to confirm the accuracy of MR for tumour (T) staging in patients with rectal cancer. We also obtained encouraging results on MR capability to correctly characterize Ln's and on the ability of ADC to discriminate between metastatic and non-metastatic Ln's.

Accurate determination of the T stage is important in rectal cancer because certain T3 and T4 tumours are treated when not contraindicated and accepted with neoadjuvant chemoradiation prior to resection. Our study demonstrates that the ability of MR to identify tumour mesorectal fat invasion, when compared to histology, is characterized by good diagnostic accuracy: 100%



sensitivity 100%, 89.3% specificity and overall 94.2% diagnostic accuracy, are in agreement with the literature showing 75-91%, 76-90%, and 77-86% data respectively [16-18].

Ln involvement is another indication for preoperative neoadjuvant chemoradiation, and this fact makes a reliable characterization of N stage a highly desirable requisite. To explore this feature, the MR outcome was expressed as P1, P2 or P3 according to the increasing probability of Ln metastases computed on the basis of dimension, irregular morphology and signal characteristics [4,5]. Considering only the high probability malignant Ln's (P3) our results indicate a particularly high sensitivity and specificity, with a considerable negative predictive value close to 100%. This is slightly better than literature were sensitivity ranges between 45 and 85%, but with high specificity, although accuracy, positive predictive value and negative predictive value waver around 70% [3,4,19]. Our very high negative predictive value is an important result, being crucial, if confirmed, for the choice of the most appropriate treatment, with impact on the potential risk for local tumour recurrence.

We also established the important role of the ADC. We found that the Ln's with high MTS probability (P3) had  $ADC = 0.82 \pm 0.17 \times 10^{-3} \text{ mm}^2/\text{s}$ , significantly lower ( $p < 0.0001$ ) than the value  $1.22 \pm 0.30 \times 10^{-3} \text{ mm}^2/\text{s}$  for low and moderate MTS probability Ln's. The ROC curve procedure assessed the very good performance of ADC as discriminator between the two ( $AUC = 0.88$ ), determining  $ADC \leq 1 \times 10^{-3} \text{ mm}^2/\text{s}$  as the values associated with high probability of MTS Ln (P3), with 90% sensibility and 78% specificity, and an  $OR = 31$ .

Our results on the association of  $ADC \leq 1 \times 10^{-3} \text{ mm}^2/\text{s}$  with possible metastatic Lns and their classification confirm with a more powerful statistics the conclusions suggested by two previous studies [11,12]. Yasui et al. [11] observed that the mean ADC value was significantly lower for metastatic Ln's ( $1.36 \times 10^{-3} \text{ mm}^2/\text{s}$ ) than for non-metastatic Ln's ( $1.85 \times 10^{-3} \text{ mm}^2/\text{s}$ ). Cho et al. [12] found the same trend, with an ADC value of  $0.9 \pm 0.15 \times 10^{-3} \text{ mm}^2/\text{s}$  vs.  $1.1 \pm 0.22 \times 10^{-3} \text{ mm}^2/\text{s}$  for high risk and low risk groups respectively ( $p < 0.0001$ ). When they followed the ROC curve procedure ( $AUC = 0.73$ ) they identified the value of  $1.0 \times 10^{-3} \text{ mm}^2/\text{s}$  as cut off for ADC, with moderate sensitivity and specificity.

The low ADC values of malignant tissues are probably related to a combination of higher cellularity, tissue disorganization, and increased extracellular space tortuosity, all contributing to reduce the mobility of water. Low ADC values were associated also with a more aggressive rectal tumour profile [20] and with a better response to neoadjuvant chemoradiation [21].

The last result concerned the lateral pelvic Lns. It is well known that the lymphatic drainage of the upper rectum involves predominantly mesorectal lymphatics, with upward drainage along the inferior mesenteric artery. The lymphatic system of the lower rectum, in contrast to the upper

rectum, drains instead via lateral lymphatic vessels and this way it likely involves lateral pelvic Lns. In our series, although only 3.4% were located in the lateral pelvic district, they had very high risk to be metastatic (55.6%); those data are important because suspicious metastatic Ln in the lateral pelvic area are a risk factor for loco-regional recurrence in patients who have undergone curative resection with preoperative concurrent chemo-radiotherapy [22].

Our study has some limitations. First it was a retrospective review of a limited number of examinations on a patient-per-patient basis. Furthermore, sometimes it is difficult to indicate which of the Ln was truly positive in patients with nodal involvement, but the good correlation with pathology achieved makes us confident of right matching.

## **CONCLUSION**

In conclusion, our study shows that MR is certainly an accurate imaging method in T staging and N staging of rectal cancer cases, allowing reliable prediction of nodal involvement by considering dimension, morphology and signal characteristic. The association with low ADC value and high probability malignant nodes suggests the importance of the routine use of MR with DWI in the diagnostic process.

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## FIGURES CAPTION

Figure 1. T2WI, DWI (b600) and ADC map for a P1 (first line), P2 (second line) and P3 (third line) Ln.

Figure 2. ROC curve procedure to test the discriminating ability of ADC between high probability and low probability of Ln MTS; Area Under the Curve (AUC) = 0.88.

Figure 3. Plot of harmonic mean (HM) of sensitivity and specificity and k (Cohen's coefficient) as a function of ADC: their maximum indicates the ADC threshold. The ADC values are  $1 \times 10^{-3} \text{mm}^2/\text{s}$ .

Table 1. *Cross-correlation table between MR (cT) and pathology (pT)*

<b>MR</b>	<b>cT1</b>	<b>cT2</b>	<b>cT3</b>	<b>cT4</b>
<b>AP</b>				
<b>pT1</b>	<b>6</b>	<b>1</b>		
<b>pT2</b>	<b>1</b>	<b>17</b>	<b>3</b>	
<b>pT3</b>			<b>20</b>	
<b>pT4</b>				<b>4</b>

Table 2. Cross-correlation table of MR(cN) versus pathology (pN) for the 52 patients.

<b>AP</b>	<b>RM</b>	<b>cN0</b>	<b>cN1</b>	<b>cN2</b>
<b>pN0</b>		<b>26</b>	<b>11</b>	
<b>pN1</b>		<b>1</b>	<b>12</b>	
<b>pN2</b>			<b>2</b>	







