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Magnetic resonance-based texture parameters as potential imaging biomarkers for predicting long term survival in locally advanced rectal cancer treated by chemoradiotherapy

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Abstract

Aim: The study aimed to investigate whether textural features of rectal cancer on magnetic resonance imaging (MRI) can predict long term survival in patients treated with long-course chemoradiotherapy.

Method: Textural analysis (TA) using a filtration-histogram technique of T2-weighted pre- and six-week post chemoradiotherapy MRI was undertaken using TexRAD, a proprietary software algorithm. Regions of interest enclosing the largest cross-sectional area of the tumour were manually delineated on the axial images and filtration-step extracted features at different anatomical scales (fine, medium, and coarse) followed by quantification of statistical features (mean intensity, standard-deviation, entropy, skewness, kurtosis and mean of

positive pixels [MPP]) using histogram analysis. Cox multiple regression analysis determined which univariate features including textural, radiological and histological, independently predicted overall survival (OS), disease free survival (DFS) and recurrence-free survival (RFS).

Results: MPP (fine-texture, HR: 6.9, 95% CI [2.43–19.55], p= <0.001), mean (medium-texture, HR: 5.6 [1.4-21.7], p=0.007) and extramural venous invasion (EMVI) on MRI (HR: 2.96, [1.04–8.37], p=0.041) independently predicted OS while mean (medium texture, HR: 4.53, [1.58–12.94], p=0.003), MPP (fine texture, HR: 3.36 [1.36–8.31], p=0.008) and threatened circumferential resection margin (CRM) on MRI (HR: 3.1 [1.01–9.46], p=0.046) predicted DFS. For OS; EMVI on MRI (HR: 4.23 [1.41-12.69], p=0.01) and for DFS; kurtosis (medium-texture, HR: 3.97 [1.44–10.94], p=0.007) and CRM involvement on MRI (HR: 3.36 [1.21–9.32], p=0.02) were the independent post-treatment factors. Only TA independently predicted RFS on pre- or post-treatment analyses.

Conclusion: MR based TA of rectal cancers can predict outcome before undergoing surgery and could potentially select patients for individualized therapy.

Key words

Textural analysis, imaging biomarker, rectal cancer, neoadjuvant chemoradiotherapy, MRI

What does this paper add to the literature?

This study shows that MRI-based textural analysis of rectal cancer can act as a prognostic imaging biomarker and is an independent predictor of survival in patients with locally advanced rectal cancer. This finding could contribute to disease risk stratification and allow therapy to be individualised.

Introduction

Currently the standard management of locally advanced rectal cancer is neoadjuvant chemoradiotherapy followed by total mesorectal excision (TME).[1] This strategy combined with high resolution magnetic resonance imaging (MRI) has shown considerable improvement in locoregional control. This is not, however, the case for systemic control and these strategies may not necessarily improve survival.[2] Furthermore, restaging of irradiated rectal cancer is difficult owing to the difficulty MRI has in differentiating fibrosis from viable tumour.[3] In addition, a proportion of such patients will have achieved complete clinical response and could benefit from either a "wait and watch" approach or less invasive local excision.[4] There is, however, a poor correlation of a clinical complete response with a true pathologic complete response.[5] This has resulted in the recent interest on the quantification of imaging biomarkers linked to underlying intra-tumour heterogeneity[6] associated with the adverse outcomes of treatment failure and drug resistance. [7,8] Heterogeneity can be quantified non-invasively by imaging using textural-analysis (TA). TA assesses the distribution of pixel grey-level intensity and the coarseness and regularity of digital images.[9] In the last decade, TA has been employed in oncological studies of lung[10], brain[11], renal[12] and breast[13] cancer as a diagnostic, prognostic and treatment response imaging biomarker. To date there has been no study to assess the potential of MRI-based TA (MRTA) in predicting survival in rectal cancer but a few studies have explored the potential of computerised tomography [CT]-based TA as a prognostic tool to assess survival in patients with colorectal cancer.[14,15] The main object of the present study was to investigate whether MRTA in rectal cancer can predict long term survival.

Method

Patient Selection

Ethical approval was obtained from the institutional ethics committee. A retrospective cohort was included of consecutive patients with stage II and III MRI-defined poor risk, histologically confirmed primary non-metastatic rectal adenocarcinoma originating within 15cm of the anal verge and treated with long course chemoradiotherapy with curative intent from 01/2006 to 06/2011. MRI-defined features of poor risk were a T3 tumour with > 5 mm infiltration into the perirectal fat, T4 tumours, N1 or N2- tumours and tumours with a threatened or involved circumferential resection margin (CRM).[16] Details of long course chemoradiotherapy protocol, technical and operative specifications are described elsewhere.[17]

MR protocols and acquisition parameters

MRI was performed with the same GE Sigma Genesis 1.5-T (software version 9.0) whole-body system using a torso coil (phased array) and a standard imaging protocol without intravenous contrast enhancement (Appendix-1). Restaging CT of the chest, abdomen and a pelvic MRI scan were performed approximately six weeks after the completion of chemoradiotherapy (CRT). Following MDT review, a date for surgery was arranged for patients with no response or with evidence of substantial downstaging,. Those with a partial response were further followed and a second MRI was performed four weeks after the first staging MRI, to optimise the timing of surgery at the point of maximal response.[18]

Image Interpretation

T-staging of tumour at pre-treatment (mrT) and post treatment (ymrT) were standardized (Appendix-2) and based on the interpretation of local extent of persistent tumour signal intensity relative to the layers of bowel wall on T2 weighted images.[19],[20] Nodal stage at baseline (mrN) and after CRT (ymrN) was based on the interpretation of lymph node border characteristics and signal intensity. A node was regarded as positive if either an irregular border or mixed signal intensity was demonstrated.[21] Pre- and post-treatment circumferential resection margin (CRM) involvement (mrCRM and ymrCRM) was predicted to be clear if the

distance of the tumour from the mesorectal fascia was greater than or equal to 1mm. Pre- and post-treatment extramural venous invasion (mrEMVI and ymrEMVI) was visualised as an intermediate signal intensity apparent within vessels with accompanying nodular expansion of the vessel or an irregular vessel contour.[22] The tumour response to CRT was defined by MRI both on the pathological tumour regression grade (TRG) originally described by Dworak (Appendix-3)[19],[23] and a modified Response Evaluation Criteria in Solid Tumours (RECIST) based on the maximum tumour length measured in the sagittal plane.[24],[25] For RECIST, a partial response to treatment was defined as at least a 30% decrease in tumour length in relation to the baseline tumour length. Progression of disease was defined as at least a 20% increase in tumour length and stable disease was defined as neither sufficient shrinkage to qualify for a partial response nor a sufficient increase to qualify for progression of disease. MRI scans were reviewed by two independent radiologists (AA, UP) blinded to the clinical outcome. Any discrepancy in reporting was resolved by discussion and consensus when required.

MR Textural Analysis (MRTA)

T2-weighted pre-treatment and a 6-week post CRT MRI were used for MRTA. Regions of interest (ROIs) enclosing the largest cross-sectional area of tumour area were manually delineated on the axial images under the supervision of a gastrointestinal radiologist (AA) with seven years' experience. The ROIs underwent textural analysis under the supervision of an imaging scientist (BG) with nine years experience in texture analysis using proprietary commercially available TexRAD research software (version 3.3, TexRAD Ltd www.texrad.com, part of Feedback Plc, Cambridge, UK)[26]. MRTA comprised an image filtration-histogram approach where the filtration step employed a Laplacian of Gaussian band-pass spatial scale filter (SSF) to highlight features ranging from SSF=2mm (fine) to SSF=6mm (coarse) in radius with SSF=3mm-5mm in radius corresponding to medium-texture scales (Figure 1). This scale can be considered as the width at which structures in the image will be highlighted and enhanced, while structures less than this width will become

blurred.[8] Histogram analysis comprised quantifying first-order statistics of mean grey level intensity, standard-deviation, entropy, mean of positive pixels (MPP), kurtosis and skewness of the rectal ROI. A recent paper[27]describes the above parameters in detail and what these parameters mean in terms of image features. These parameters have further been shown to be associated with underlying histological features reflecting tumour heterogeneity (solid cancerous tissue, necrosis, angiogenesis, hypoxia and fibrosis[28,29]), predicting the response to neoadjuvant chemoradiotherapy[30]and survival[8], [26]as a potential imaging biomarker.

Histopathological examination of the resected specimen

The histopathological information was retrieved from the institutional pathology database. All the reports contained a standard data-set of histopathology results such as post-treatment pathological T and N stage (ypT and ypN) and included the information regarding the circumferential resection margin (ypCRM) involvement according to rectal carcinoma guidelines of Royal College of Pathologists.[31]

Survival

Overall survival (OS), disease free survival (DFS) and relapse free survival (RFS) were measured. All observations were censored at the date of the last follow-up or at the time lost to follow up. The duration of follow up was calculated from the date of diagnosis to death, last contact or date of conclusion of the study (21.03.2014) whichever came first. Overall survival was defined as the "time from the date of diagnosis to death from any cause". Disease free survival was defined as the "time from the date of diagnosis to any event, irrespective of the cause". Relapse free survival was defined as the "time from the date of diagnosis to any event except for second primary same or other cancers that were ignored"[32]. Local recurrence was defined as evidence of recurrent tumour mass within the pelvis or in the perineum after a surgical resection[33].

Data analysis

Clinical, MR and histopathological variables were categorized in a binary fashion to enable comparison by multivariate analysis. Clinical variables included age, gender, adjuvant chemotherapy and major post-operative complications based on the Clavien-Dindo classification for surgical complications.[34] Tumours were categorized into “favourable” and “unfavourable” responders to enable binary comparison by multivariate analysis. Based on the known histopathological outcome, “favourable” mrT and ymrT stages were defined as stages T0, T1, T2 and T3a and “unfavourable” were defined as mrT and ymrT stages- T3b, T3c, T3d or T4. Stage T3a and T2 tumours have a similar outcome and therefore were both classified as “favourable”. “Favourable” mrN, ymrN and ypN were defined as N0, while node positivity was unfavourable. “Favourable” mrEMVI, ymrEMVI was defined as having no EMVI, while the presence of EMVI was “unfavourable”. A “favourable” MRI tumour regression grade (mrTRG) was defined as grades 1, 2 & 3 that included tumours with a fibrotic stroma of 50% or more while “unfavourable” was defined as grades 4 and 5 that included tumours in which cancer predominated with minimal or no fibrosis. Similarly a “favourable” histopathological TRG was defined by Dworak stages 2, 3 and 4 while an “unfavourable” TRG was defined as grades 0 and 1. For analysis of the length of the tumour, a partial response was categorised as “favourable”, while stable or progression of disease was “unfavourable”.

Statistical Analysis

Continuous variables were expressed as means and SDs and categorical variables were expressed as percentages and 95% confidence intervals. Univariate Kaplan-Meier survival analysis was employed to identify which texture parameter predicted survival, which further required the identification of the best "optimal" cut-off at which the good and poor survival patient groups were optimally separated (lowest p value from the Log-rank test which assessed the difference between the Kaplan-Meier curves) for each parameter. A p-value of less than 0.05 was considered to be significant. Due to small numbers, significant textural

parameters yielding less than 10 patients per group for comparison were not reported and hence were censored. Multivariate Cox regression analysis (Forward-Wald) was used to determine which of the significant univariate variables were independent predictors of outcome. Analysis was performed separately for pre and post-treatment variables. The hazard ratio (HR) was determined for the variables where HR >1 indicated increased risk of an event associated with the variable and HR <1 indicated a reduced risk related to survival. Statistical analysis was performed using R software (version 2.14.2; R Foundation for Statistical Computing, Vienna, Austria) and SPSS (version 20).

Results

The study population consisted of 56 patients (34 male, 22 female) with meanage of 64 ± 8.8. Complete pathological response (T0N0) was observed in 21% of patients (n-12). Overall recurrence was observed in 23% of patients (n-13). The rate of distant and local recurrence was 20% and 5% (Table 1). Pre- and post-treatment MR TNM staging is shown in Table 2. The average follow up for the entire cohort was 47.2 ± 18.2 months. Thirty six (36/50, 64%) patients were alive and censored when the data were analysed at an average follow up of 56±11.6 months . The mean overall survival was 65.7% (95% CI, 57.9 -73.8 and the five year cumulative survival time was 64%. The mean DFS and five year cumulative DFS were similar i.e. 60 months (95% CI, 51.2-69.2). The mean RFS was 70.8 (95% CI, 62.4 – 79.2) months. All relapses had occurred by 21 months at which time the cumulative survival time was 75%.

Survival

a. Overall Survival

Pre-treatment variables

MRTA was a significant marker of OS on univariate analysis with MPP (fine texture scale) being the best (p=0.008, Table 3). Positive mrEMVI status (p=0.017, Table 4) and threatened mrCRM (p=0.036, Table 4) were also significant MR factors. The clinical variable of a major complication also predicted a worse OS (p=0.002) but as this was a post-operative rather than a pre-treatment or post-treatment factor, it was not included in the multivariate analysis. On multivariate analysis, MPP on fine texture-scale (HR: 6.9, 95% CI: 2.4 – 19.5, p<0.001), MPP on medium texture-scale (HR: 5.7, 95% CI: 1.6 – 20.2, p=0.007) and mrEMVI positive status (HR: 2.9, 95% CI: 1. – 8.3, p=0.041) were the only independent predictors of OS (Table 5, Figures 2-a, 2-b and 2-c).

Post-treatment variables

Texture feature, skewness at fine texture-scale, was the only univariate marker of OS on post-treatment MRTA (p=0.034, Table 3). Positive ymrEMVI status (p=0.002, Table 4), threatened ymrCRM (p=0.027, Table 4) and poorer ymrTRG (p=0.002, Table 4) predicted a worse OS. Among the histological variables, only ypCRM involvement (p=0.007, Table 4) predicted the OS. On multivariate analysis, positive ymrEMVI status (Table 5, Figure 2-d) was the only independent predictor of OS (HR: 4.2, 95% CI: 1.4- 12.6, p=0.01)

b. Disease free survival

Pre-treatment variables

A threatened mrCRM (p=0.006, Table 4) and MRTA (best feature-mean at medium texture, p=0.007, Table 3) were significant markers for DFS on univariate analysis. On multivariate analysis, MPP at fine texture-scale (HR: 3.3 95% CI: 1.3 – 8.3, p=0.008), mean MPP at

medium texture-scale (HR: 4.5, 95% CI: 1.5 – 12.9, p=0.003), and threatened mrCRM (HR: 3.1, 95% CI: 1. – 9.4 p=0.046) were the only independent predictors of DFS (Table 5, Figures 3a, 3b and 3c).

Post-treatment variables

Post-treatment MRTA (best was kurtosis at medium texture-scale, p=0.009, Table 3), positive mrEMVI status (p=0.017, Table 4), threatened mrCRM (p=0.019, Table 4), mrTRG (p=0.02, Table 4) and ypCRM (p=0.035, Table 4) were significant markers of DFS on univariate analysis. On multivariate analysis, kurtosis at medium texture-scale (HR: 3.9, 95% CI: 1.4 – 10.9, p=0.007) and ymrCRM involvement (HR: 3.3 95% CI: 1.2 – 9.3, p=0.02) were the only independent predictors of DFS (Table 5, Figures 3d and 3e).

c. Relapse free survival

Pre-treatment variables

A threatened mrCRM (p=0.016, Table 4) and MRTA were significant markers for RFS on univariate analysis (Table 3).The best textural features were standard deviation and entropy at coarse-textures (p=0.011) and MPP at fine and medium-textures (p=0.011).Using multivariate analysis, texture parameters of MPP at fine texture-scale (HR: 8.9, 95% CI: 2.3 –33.1, p= 0.001) and kurtosis at medium texture-scale (HR: 7.7 95% CI: 2. - 29., p=0.002) were the only independent predictors (Table 5, Figures 4a and 4b).

Post-treatment variables

Post-treatment MRTA (best was entropy at coarse-texture, p=0.002, Table 3), ymrN-stage (p=0.024, Table 4), ypCRM involvement (p=0.009, Table 4) and pCR (p=0.034, Table 4) were significant markers of survival on univariate analysis. On multivariate analysis, texture parameters, entropy at coarse texture-scale (HR: 8.6, 95% CI: 1.8 – 39.8, p=0.005) and kurtosis without filtration (HR: 4.2, 95% CI: 1.4- 13., p=0.01) were the only independent predictors of RFS (Table 5, Figures 4c and 4d).

Discussion

This is the first study to assess the prognostic significance of texture features in addition to morphological MRI and histopathological parameters of rectal cancer undergoing CRT. On pre-treatment MRTA a lower MPP at fine-texture was an independent predictor for all three forms of survival. A lower mean MPP at medium-texture was an independent predictor of OS and DFS and kurtosis at medium-texture was an independent predictor of RFS. Intra-tumour heterogeneity has been attributed to various factors such as hypoxia, necrosis, angiogenesis and genetic variations.[35] [36] Both hypoxia and necrosis reflect increased numbers of dark tumour regions which tend to give a negative mean.[16] MPP considers only pixels greater than zero and reduces the impact of dark areas on the mean histogram value. MPP has been correlated negatively with hypoxia in colorectal cancers exhibiting K-RAS mutations.[37] Lower than threshold MPP values in predicting an inferior outcome are consistent with the possibility of predominance of hypoxic areas in rectal cancer rather than angiogenesis in our study. The finding of lower kurtosis at medium texture predicting poorer DFS and RFS on post-treatment MRTA may suggest more focal radiation induced inactive fibrosis which has previously been associated with an inferior outcome in lung cancer.[38] Post-treatment MR EMVI status was an independent predictor of OS on multivariate analysis. These results are similar to those of Chand et al.[39] In this database patients with ymrEMVI-positivity had a significantly worse DFS at three years (42.7%) compared with ymrEMVI-negative tumours (79.8%). MRI CRM status at pre- and post CRT was noted to be significant on multivariate analysis for DFS, while mrTRG and ymrEMVI were also significant for DFS on univariate analysis. This is similar to previous datasets from Patel et al. [19] (where mrTRG was significant on multivariate analysis for OS and DFS) and Taylor et al. [40] (where involvement of CRM on baseline MRI independently predicted OS, DFS, and LR on multivariate analysis). Significant univariate histopathological parameters such as ypCRM, pCR and ypTRG did not predict survival independently on multivariate analysis.

Limitations of the study

There is a lack of validated published histological correlations of tumour heterogeneity for different MR texture scales in rectal cancer. This is a first exploratory and hypothesis-generating study with regard to MRTA in survival after treatment of rectal cancer. The data are, however, based on small numbers of patients from one centre. Using the same data to identify optimal cut-off values for each marker to divide the population into good and bad prognostic groups could lead to the overstatement of significant results. Acquisition parameters with MRI can introduce higher signal intense variability compared with computerised tomography (CT) or positron emission tomography (PET) which in theory could affect reproducibly of the results.

The study suggests that high resolution pre- and post-treatment MRI-based assessment of CRM and EMVI status and MRTA are superior to Independent imaging markers for predicting survival in locally advanced rectal cancer than the standard TNM-based MR criteria. Treatment for this group could be tailored for example, with more intensive individualized neoadjuvant treatment before undergoing surgery and adjuvant chemotherapy.

Table 1 Baseline characteristics of the patients

TEMS= transanal microsurgery

TME=total mesorectal excision

NA= not available

TRG= tumour regression grade

Male Female	 34 (61%) 22
Age (years median ± SD)	64±8.82
Interval to surgery after completing long course chemo-radiotherapy (weeks median ± SD)	13±3.42
Operation Anterior resection Abdominoperineal resection Hartmann's procedure TEMS inoperable at surgery No surgery (disease progression)	 33 (59%) 16 (28%) 2 (4%) 1 (2%) 3 (5%) 1 (2%)
TME Laparoscopic Open	47 (84%) (4 converted to open) 4 (7%)
Height of tumour from anal verge(cm) >5 <5	 39 (70%) 14 (25%)
ypCRM involvement	6 (11%)
yp T-stage T0 T2 T3 T4	 14 (25%) 14 (25%) 20 (36%) 4 (7%)
yp N-stage N0 N1 N2	 36 (64%) 14 (25%) 2 (4%)
Complete pathological response T0N0	12 (21%)
R0 resection Yes No	 46 (82%) 6 (11%)
yp tumour regression grade(0-4)[a] 0 1 2 3 4 NA Not documented	 3 (5%) 12 (21%) 10 (18%) 1 (2%) 14 (25%) 4 (7%) 12 (21%)
yp tumour regression grade Good responders (TRG 2-4) Bad responders (TRG 0-1)	 25 (47%) 15 (27%)

Adjuvant Chemotherapy Yes No	 11 (20%) 42 (75%)
Major post-operative complication [b] Yes No	 17 (30%) 35 (63%)
Anastomotic leakage Yes No	 6 (18%) 27
Overall Recurrence	13 (23%)
Local Recurrence	3 (5%)
Distant Recurrence	11 (19%)

[a] Dworak 5-stage TRG (tumour regression grade) system[23]

[b] Clavien classification of surgical complications[34]

Table 2 Magnetic resonance imaging (MRI) pre and post chemorqdiotherapy

TRG= tumour regression grade in locally advanced rectal cancer.

	Pre-treatment MRI	Post-treatment MRI
T-stage T0 T1 T2 T3a T3b T3c T3d T4	 4 (7%) 3 (5%) 11 (20%) 12 (21%) 10 (18%) 14 (25%)	 3 (5%) 2 (4%) 7 (13%) 1 (2%) 13 (23%) 14 (25%) 5 (9%) 9 (16%)
N-stage N0 N1 N2	 14 (25%) 24 (43%) 16 (29%)	 40 (71%) 12 (21%) 0
Circumferential resection margin (CRM) threatened	31 (55%)	24 (43%)
Median tumour height from anal verge (cm)	8.4	8.7
Tumour regression grade (mrTRG) (grade 1-5)[a] 1 2 3 4 5		 6 (11%) 17 (30%) 13 (23%) 16 (29%) 2 (4%)
Tumour regression (mrTRG) Good responders (1-3) Bad responders (4-5)		 36 (64%) 18 (32%)
Complete responder T0N0		6 (11%)
Extramural vascular invasion		

(EMVI) Yes No	 14 (25%) 40 (71%)	 8 (14.2%) 44 (79%)

[a]MRI TRG was based on similar principles to the pathological TRG originally described by Dworak (Appendix-3)

Table 3 Magnetic resonance imaging (MRI) Textual analysis.Significant parameters predicting overall survival (OS), disease free survival (DFS) , and recurrence free survival (RFS) on univariate analysis

MPP= mean of positive pixels

EMVI= extramural vascular invasion

Textural parameter	Filter value	Threshold value	Number of patients above and below the threshold value		Mean Survival	95% Confidence interval	p value
Overall Survival: Significant pre-treatment texture parameters							
Mean	3	<-8.2	Poor	27	45.4	38.4-52.3	0.03
			Good	29	72.8	62.5-83.2	
MPP	2	<63.7	Poor	17	40.7	29.2-52.2	0.008
			Good	39	72.2	63.5-80.9	
	3	<75.2	Poor	19	43.5	32.5-54.4	0.029
			Good	37	71.6	62.7-80.5	
	4	<82.3	Poor	22	45.6	35.8-55.3	0.019
			Good	34	74.3	65.6-83	
Overall Survival: Significant post-treatment texture parameters							
Skewness	2	>0.3	Poor	36	38.9	27.3-50.6	.034
			Good	18	65.7	55.-76.3	
DFS: Significant pre-treatment texture parameters							
Mean	2	<-3.5	Poor	25	39.2	30.6-47.7	0.031
			Good	31	68.6	57.3-79.8	
	3	<-8.2	Poor	27	38.2	30.1-46.3	0.007
			Good	29	71.3	60.0-82.5	
	4	<-14.9	Poor	27	39.4	31.2-47.7	0.027
			Good	29	69.2	57.75-80.8	
	6	<-37	Poor	28	40.2	32.11-48.3	0.043
			Good	28	68.6	56.81-80.4	
MPP	2	<64.4	Poor	18	37.1	25.38-48.9	0.022

			Good	38	66.8	56.66-77.	
	3	<75.2	Poor	19	38.7	27.2-50.3	0.045
			Good	37	66.3	55.9-76.7	
	4	<84.7	Poor	24	40.3	30.2-50.4	0.022
			Good	32	69.7	59.1-80.3	
	5	<93.5	Poor	28	42.2	32.7-51.6	0.047
			Good	28	69.6	58.4-80.8	
	6	<102.4	Poor	28	42.2	32.7-51.6	0.047
			Good	28	69.6	58.4-80.8	
Skewness	2	<0.2	Poor	28	42.3	33.2-51.4	0.044
			Good	28	69.2	57.7-80.7	
DFS: Significant post-treatment texture parameters							
MPP	2	>69.5	Poor	37	43	34.9-51.1	0.032
			Good	17	74	61.1-86.8	
Skewness	2	>0.3	Poor	18	38.9	27.3-50.6	0.034
			Good	36	65.7	55-76.3	
Kurtosis	3	<-0.1	Poor	20	36.8	28-45.2	0.042
			Good	34	65.7	54.4-76.9	
	4	<-0.4	Poor	18	34.7	25.9-43.5	0.009
			Good	36	67	56.3-77.7	
RFS: Significant pre-treatment texture parameters							
Mean	3	<-870000	Poor	26	43.4	35.1-51.7	0.0169
			Good	30	80.7	72.3-89.1	
	4	<-14.9	Poor	27	44.1	36-52.1	0.026
			Good	29	80.4	71-89.1	
Standard Deviation	0	<39.7	Poor	21	47.3	35.9-58.7	0.032
			Good	35	77.6	68.8-86.5	
	2	<137.5	Poor	38	64.1	52.9-75.4	0.034
			Good	18	64.5	58.7-70.3	
	4	<151.4	Poor	28	50.5	39.7-61.3	0.018
			Good	28	80.7	72.2-89.1	
	5	<164.8	Poor	32	61.5	48.8-74.2	0.017
			Good	24	63.2	57.5-69	
	6	<162.2	Poor	31	50.8	40.7-61	0.011
			Good	25	82.7	74.9-90.5	
Entropy	0	<5.1	Poor	33	52.6	43.1-62.1	.034
			Good	23	81.7	72.6-90.8	
	4	<6.3	Poor	32	61.5	48.8-74.2	0.016
			Good	24	63.2	57.5-69	
	5	<6.3	Poor	32	61.5	48.8-74.2	0.016
			Good	24	63.2	57.5-69	
	6	<6.3	Poor	31	50.8	40.7-61	0.011
			Good	25	82.7	74.9-90.5	
MPP	2	<63	Poor	16	42.8	29.4-56.2	0.011

			Good	40	77.3	69-85.6	
	5	<118	Poor	31	50.8	40.7-61	0.011
			Good	25	82.7	74.9-90.5	
	6	<99	Poor	27	47.9	38.1-57.7	0.019
			Good	29	80.5	71.9-89.1	
Skewness	2	<0.4	Poor	38	50.8	42.9-58.8	0.037
			Good	18	84	75.4-92.6	
Kurtosis	4	<0.09	Poor	17	40.1	30.9-49.4	0.047
			Good	39	76.4	67.5-85.3	
RFS: Significant post-treatment texture parameters							
Standard deviation	5	<128.5	Poor	18	56.2	39.4-73	0.018
			Good	36	60.9	54.6-67.2	
	6	<158.1	Poor	30	61.1	48.3-73.9	0.021
			Good	24	63.5	57.3-69.6	
Entropy	3	<6.1	Poor	28	61.6	48.3-74.9	0.042
			Good	26	61.8	55.1-68.5	
	4	<6.1	Poor	26	57.9	44.1-71.7	0.005
			Good	28	64.1	58.6-69.5	
	5	<6.1	Poor	25	56.7	42.6-70.8	0.003
			Good	29	64.2	59.0-69.5	
	6	<6.1	Poor	24	55.4	40.9-69.8	.002
			Good	30	64.4	59.3-69.4	
Kurtosis	0	>0.7	Poor	17	45.5	32.8-58.2	.034
			Good	37	76.1	67.1-85.2	

Table 4 Clinical, MRI and histopathological parameters significantly predicting overall survival (OS), disease free survival (DFS) , and recurrence free survival (RFS) on univariate analysis

Parameters		n=	**Mean OS (95% CI)**	**p-value**	**Mean DFS (95% C1)**	**P-value**	**Mean RFS(95% CI)**	**P-value**
Clinical parameters								
Age	<65 years	29	50.7 (42.8-58.6)	.271	46.3(37-55.5)	.444	51.7(42.5-61)	.150
	≥65 years	27	69.3 (58-80.6)		63.5(51-75.9)		77.1(66.8-87.3)	
Sex	Female	22	52.4 (44.1-60.7)	.633	47.1 (37.2-57)	.711	52 (42.1-61.9)	.360
	Male	34	65.7 (56.5-77.5)		61.7(50.1-73.3)		74.2(64-84.5)	
Adjuvant chemotherapy	Positive	11	55.9(42.7-69.1)	.628	52.4(36.4-68.3)	.948	52.4(36.4-68.3)	.321
	Negative	42	69.1(60.3-77.9)		62.7(52.6-72.8)		72.5(63.2-81.8)	
Major	Positive	17	39.9(30.6-	**.002**	35.7(25.7-	.007	42.7(31.9-	.132

complication			49.2)		45.8)		53.5)	
	Negative	35	77.4(70-84.7)		71.3(61.5-81.1)		74.2(64.7-83.8)	
Anastomotic leak	Positive	6	49.9(34.9-64.8)	.174	47.6(28.8-66.3)	.280	52.7(38.4-66.9)	.795
	Negative	27	74.8(65-84.6)		70.1(58.4-81.9)		72.4(61-83.7)	
Pre-treatment MRI parameters								
mrT Stage	MrT1-T3a	7	62.3(53.1-71.5)	.256	54.1(38.4-69.7)	.493	54.1(38.4-69.7)	.989
	mrT3b-T4	47	65.3(56.5-74.2)		60.4(50.6-70.3)		70.6(61.4-79.8)	
mrN stage	mrN0	14	54.3(43.3-65.4)	.799	47.9(35.5-60.3)	.714	55.6(43.8-67.4)	.895
	mrN1&2	40	66.6(57.3-75.9)		62.1(51.5-72.7)		69.9(59.9-79.9)	
mrEMVI status	Positive	14	46.1(33.6-58.5)	**.017**	42.8(28.9-56.7)	.097	51.5(36.8-66.2)	.221
	Negative	40	72.5(63.9-81.2)		65.8(55.6-76)		73.4(64.1-82.8)	
Height	<5cm	14	46.8(37.9-55.6)	.315	41.5(30.6-52.5)	.226	50.3(39.5-61.1)	.973
	≥5cm	39	66.9(58.7-75.1)		65.1(54.7-75.6)		71.3(61.5-81.1)	
mrCRM status	Clear	23	77.5(67.6-87.4)	**.036**	76.9(66.5-87.3)	**.006**	82.4(74.2-90.5)	**.016**
	Threatened	31	52.1(43.8-60.5)		44(34.4-53.65)		51.41(41.3-60)	
Post-treatment MRI parameters								
ymrT stage	ymrT1-T3a	13	64.3(58.4-70.1)	.056	57.3(47.3-67.4)	.194	60.2(51-69.4)	.306
	ymrT3b-T4	41	62.9(53.4-72.4)		58.1(47.4-68.8)		67.8(57.5-78.2)	
ymrN stage	ymrN0	41	70.7(61.7-79.8)	.171	65(54.9-75.1)	.278	75.4(66.5-84.2)	.024
	ymrN1&2	12	49.9(39-60.8)		42.8(28.5-57.2)		42.8(28.5-57.2)	
ymrEMVI status	Positive	8	38.3(23.8-52.7)	**.002**	33.2(18.2-48.2)	**.017**	46(27.6-64.4)	.236
	Negative	44	72.4(64.1-80.7)		66.1(56.3-75.9)		73.2(64.2-82.1)	
Height	<5cm	13	48.3(39.2-57.4)	.661	42.7(31-54.3)	.445	49.5(38-61)	.895
	≥5cm	38	66.9(57.3-76.6)		62.9(52.2-73.7)		70.8(60.8-80.9)	
ymrCRM status	Clear	29	76(67-85)	**.027**	71.4(60.2-82.5)	**.019**	75.7(65.5-86)	.141
	Threatened	24	49.8(40-59.5)		43.4(32.8-54)		52.7(41.5-63.9)	
mrTRG status	mrTRG1-3(Good responders)	36	63.9(58-69.9)	**.002**	57.8(49.8-65.8)	**.022**	61.5(54-69)	.205
	mrTRG 4-5 (Bad	18	50.3(35.8-64.7)		47(31.5-62.6)		62.2(45.3-79)	

	responders)							
mrRECIST tumour response	Partial response	36	69.4(59.9-78.8)	.319	62.1(51.2-73)	.625	71.9(61.8-82.1)	.417
	Stable disease	16	51.3(38.6-63.9)		47.9(34-61.9)		53.9(40.5-67.4)	
Histopathological parameters								
ypT stage	ypT0-T2	13	64.3(58.4-70.1)	.056	57.3(47.3-67.4)	.194	60.2(51-69.4)	.306
	ypT3-T4	41	62.9(53.4-72.4)		58.1(47.4-68.8)		67.8(57.5-78.2)	
ypN stage	ypN0	36	73(64.2-81.8)	.126	67.6(57.3-78)	.142	74.2(64.7-83.7)	.138
	ypN1-2	16	48.9(38.3-59.4)		42.8(30.6-55)		47.1(34.6-59.5)	
ypCRM involvement	Clear	46	72.3(64.3-80.2)	**.007**	66.1(56.7-75.5)	.058	73.9(65.3-82.5)	**.009**
	Threatened	6	35.1(23.1-47.1)		30.6(16.2-45)		30.6(16.2-45)	
pCR (ypT0N0M0)	Positive	12	82.4(70.9-93.9)	.073	82.4(70.9-93.9)	.035	All cases censored	.034
	Negative	40	56.2(49.4-63)		49.4(41.1-57.6)			
pTRG	Good responder (pTRG 2-4)	25	72.8(61.7-83.9)	.934	71.6(59.7-83.5)	.949	82.2(73.7-90.7)	.159
	Bad responder(p 0-1)	15	61.2(51.2-71.1)		58.3(46.2-70.4)		58.3(46.2-70.4)	
pTRG	Complete response (pTRG 4)	14	77.8(63.9-91.6)	.354	77.8(63.9-91.6)	.301	All cases censored	.072
	Incomplete or no response	26	60.3(52.7-67.9)		57.4(48-66.7)			

Table 5 Parameters significantly predicting overall survival (OS), disease free survival (DFS) ,and recurrence free survival (RFS) on univariate analysis

Pre-treatment multivariate analysis				
Survival endpoints	**parameters**	**p-value**	**Hazard ratio**	**95% confidence interval**
OS	Mean (SSF-3)	0.007	5.7	1.6 – 20.2
	MPP(SSF-2)	<0.001	6.9	2.4 – 19.5
	mrEMVI status	0.041	2.9	1 – 8.37
DFS	Mean(SSF-3)	0.003	4.5	1.5 – 12.9
	MPP(SSF-2)	0.008	3.3	1.3 – 8.3
	mrCRM status	0.046	3.1	1 – 9.4
RFS	MPP(SSF-2)	0.001	8.9	2.3 – 33.1
	Kurtosis(SSF-4)	0.002	7.7	2 - 29
Post-treatment multivariate analysis				
OS	ymrEMVI status	0.01	4.2	1.4-12.6
DFS	Kurtosis(SSF-4)	0.007	3.9	1.4– 10.9
	ymrCRM status	0.02	3.3	1.2 – 9.3
RFS	Entropy(SSF-6)	0.005	8.6	1.8 – 39.8
	Kurtosis(SSF-0)	0.01	4.2	1.4- 13

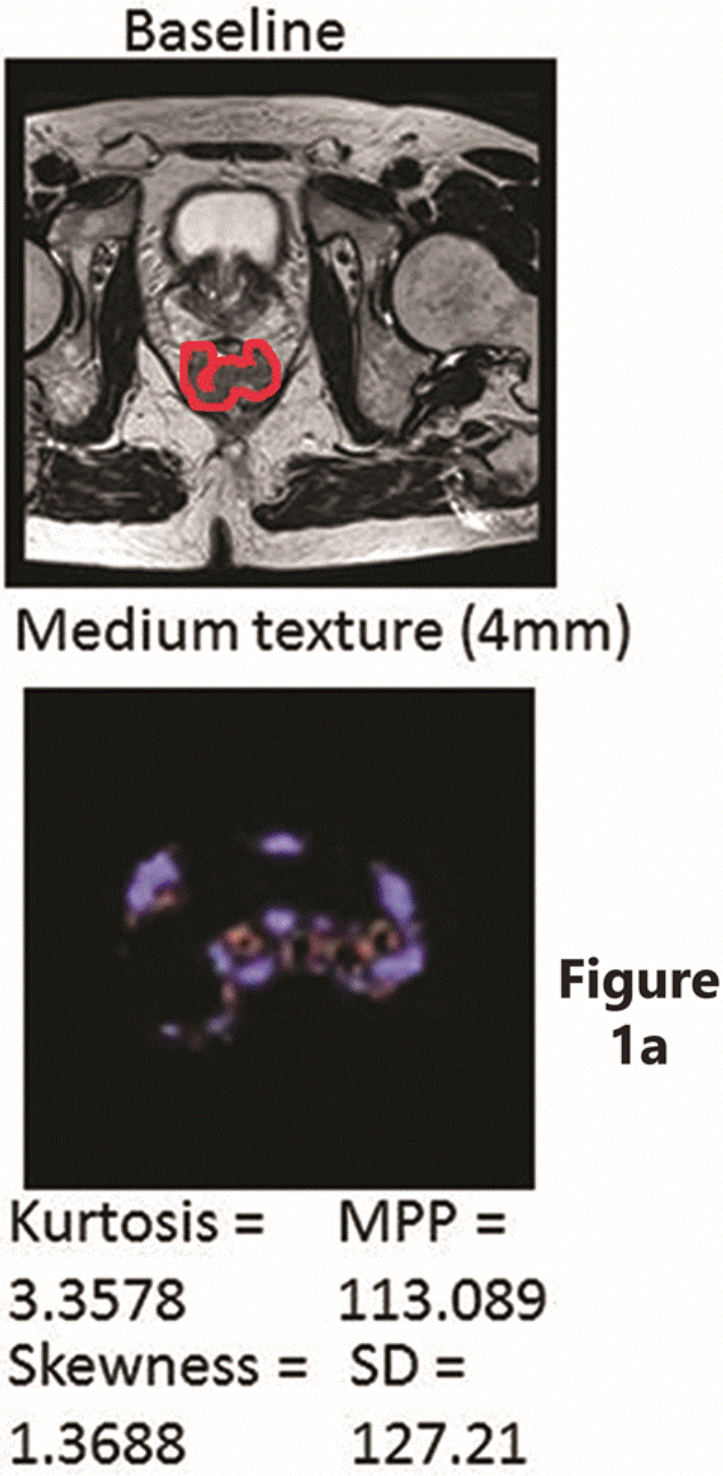

Figure 1a

Figure 1a- Textural analysis of rectal cancer at medium-texture scale for baseline magnetic resonance imaging (MRI)

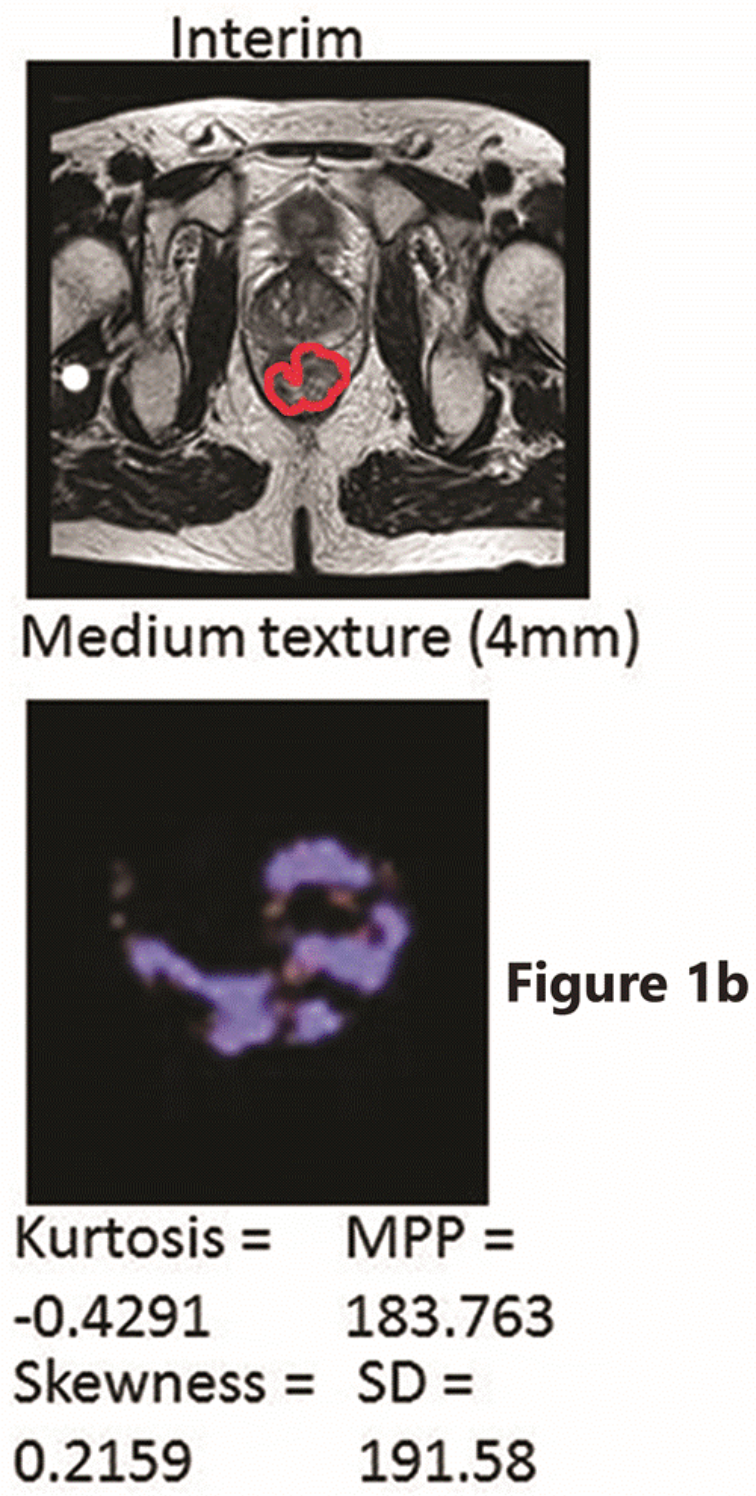

Figure 1b

Figure 1b- Textural analysis (MRTA) of rectal cancer at medium-texture scale for interim magnetic resonance imaging (MRI)

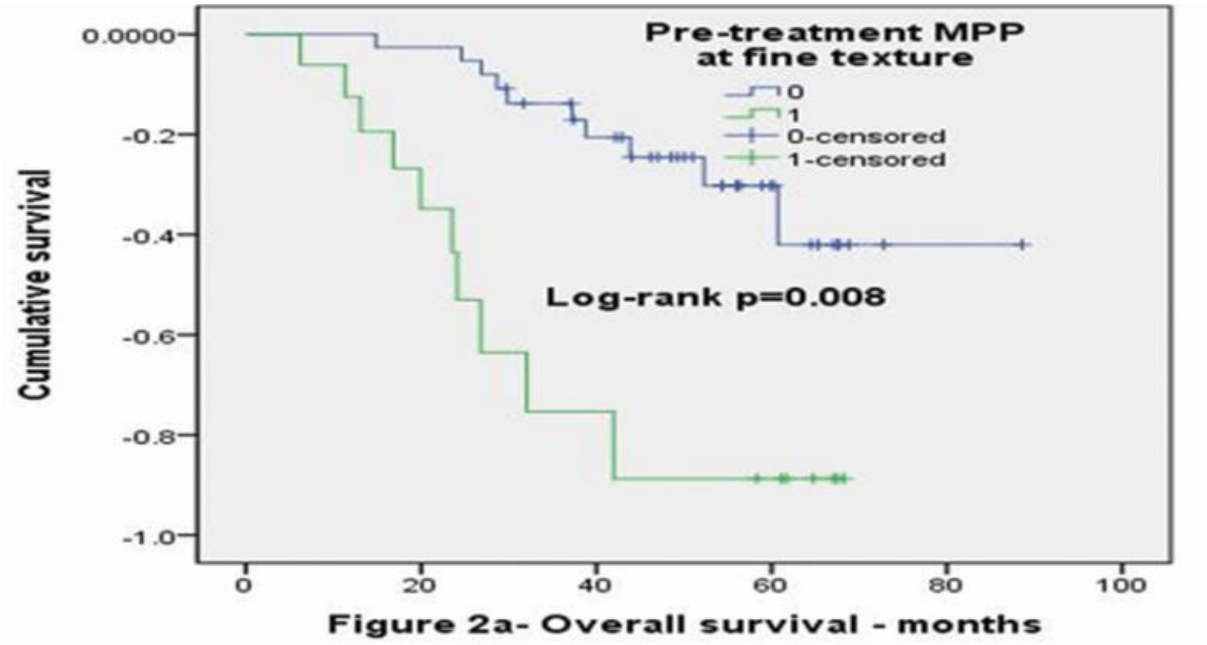

Figure 2a- Overall survival - months

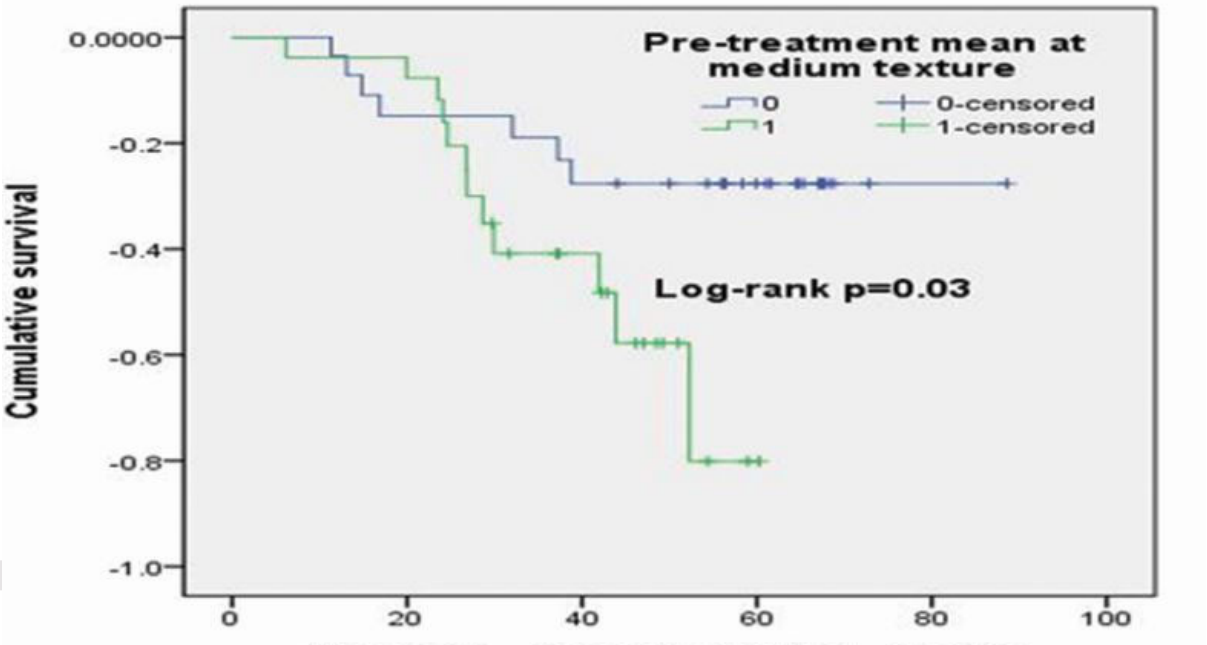

Figure 2b - Overall survival - months

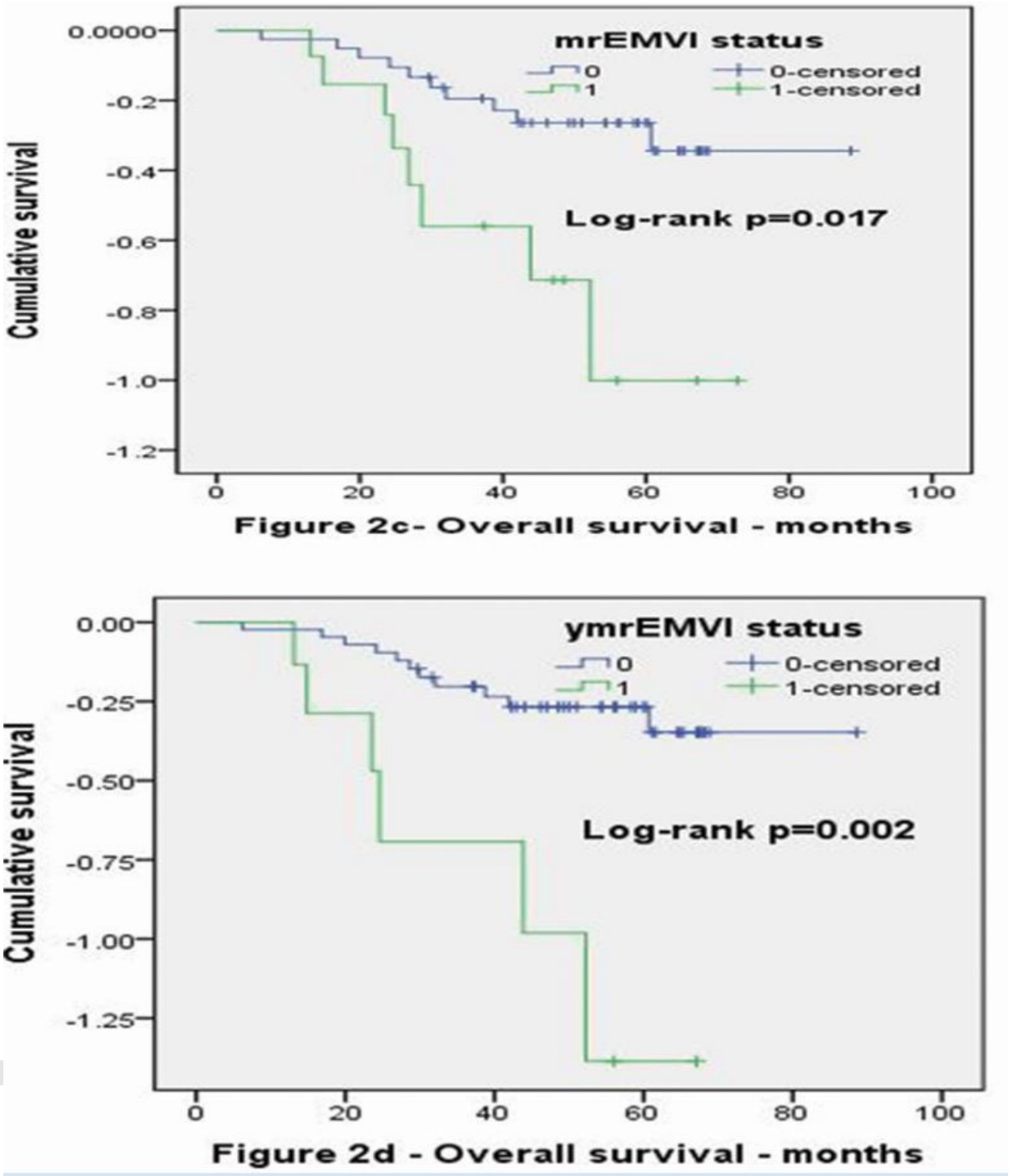

Figure 2- Kaplan-Meier curves show a significant difference in overall survival for (a) pre-treatment mean positive pixel (MPP) at fine texture (b) pre-treatment mean at medium texture (c) pre-treatment extramural venous invasion (mrEMVI) and (d) post-treatment extramural venous invasion (ymrEMVI) with log-rank p values of 0.008, 0.03, 0.017 and 0.002 respectively

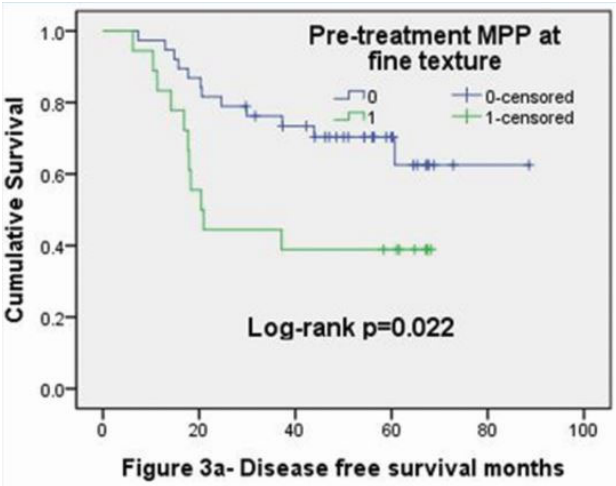

Figure 3a- Disease free survival months

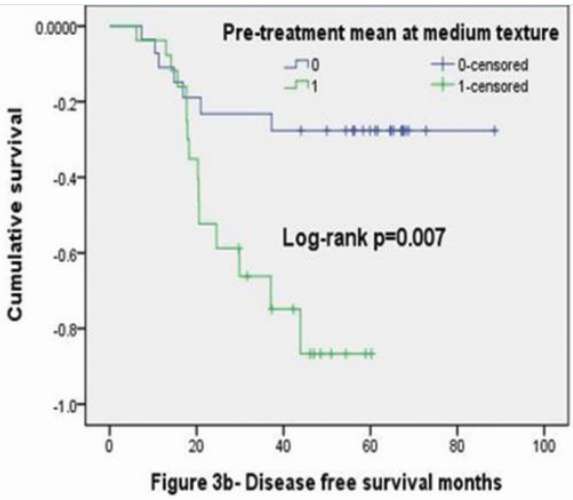

Figure 3b- Disease free survival months

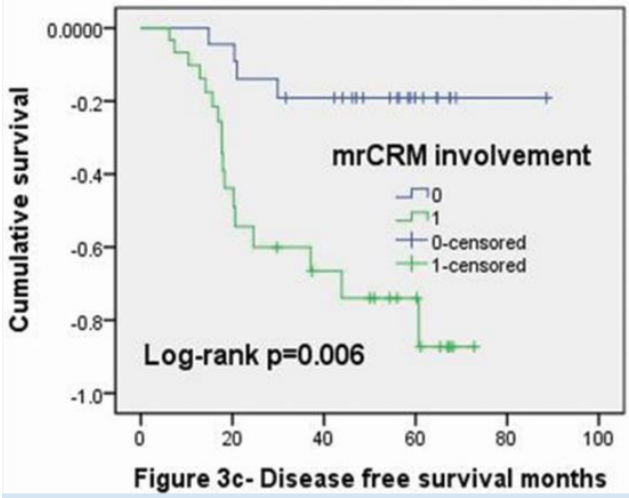

Figure 3c- Disease free survival months

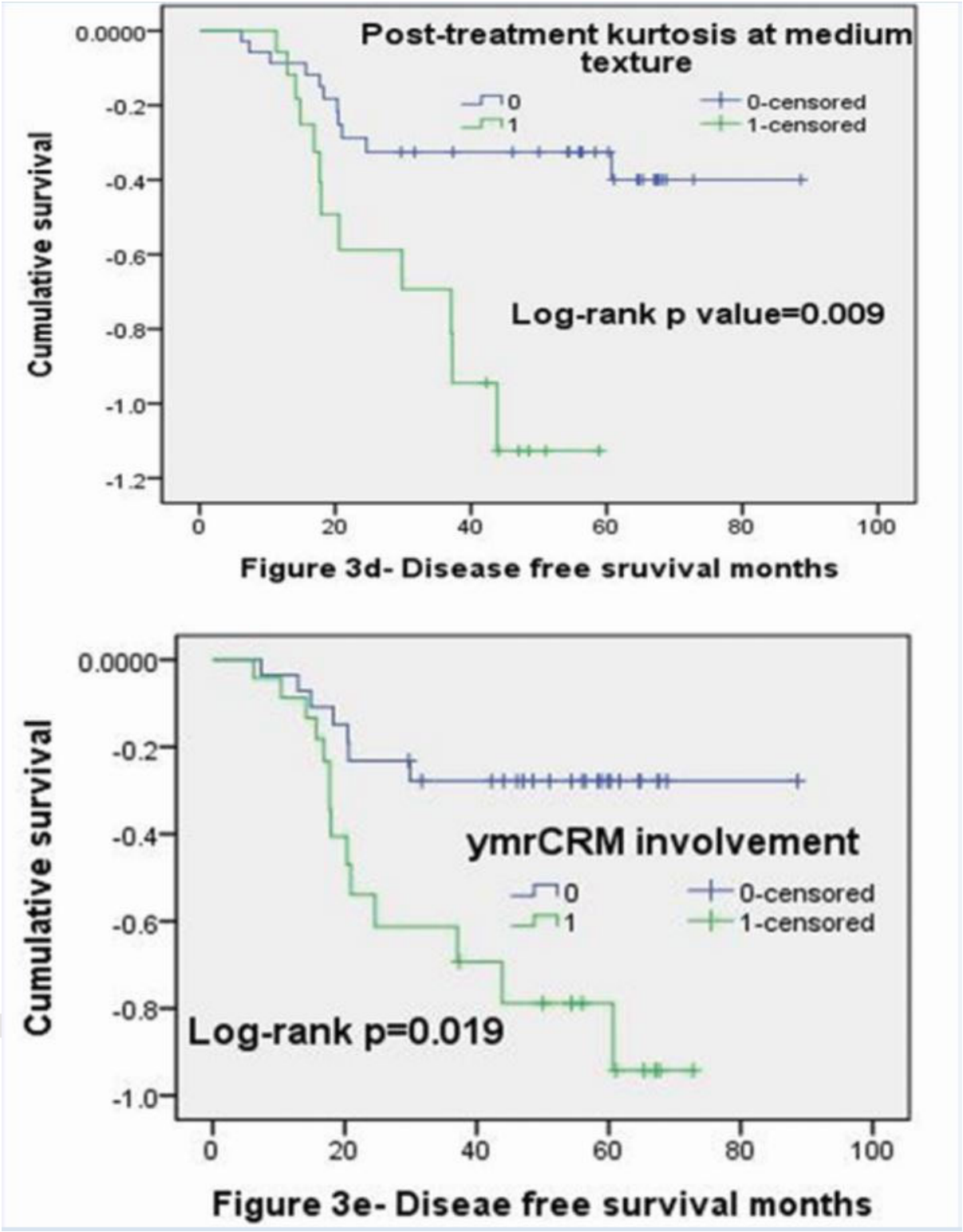

Figure 3-Kaplan-Meier curves show a significance difference in disease free survival for (a) pre-treatment mean positive pixel (MPP) at fine texture (b) pre-treatment mean at medium texture (c) pre-treatment circumferential resection margin involvement (mrCRM) (d) post-treatment kurtosis at medium texture (e) post-treatment circumferential resection margin involvement (ymrCRM) with log-rank p values of 0.022, 0.007, 0.006, 0.009 and 0.019 respectively.

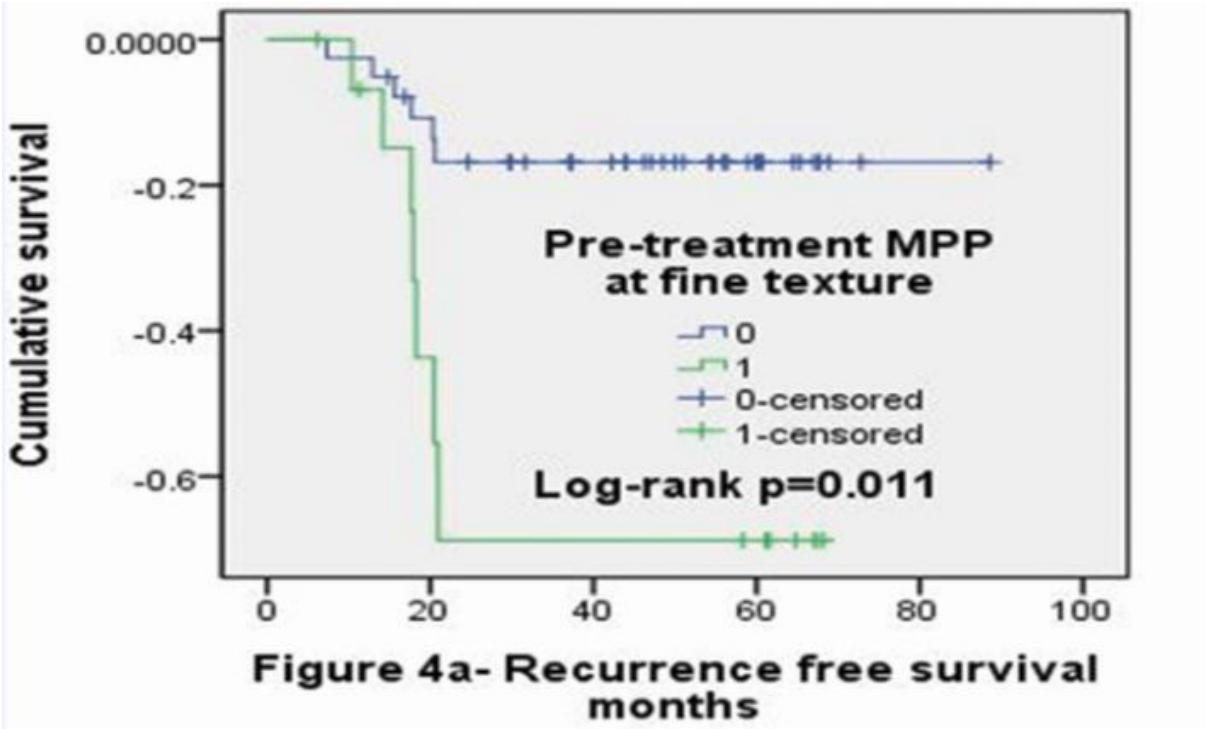

Figure 4a- Recurrence free survival months

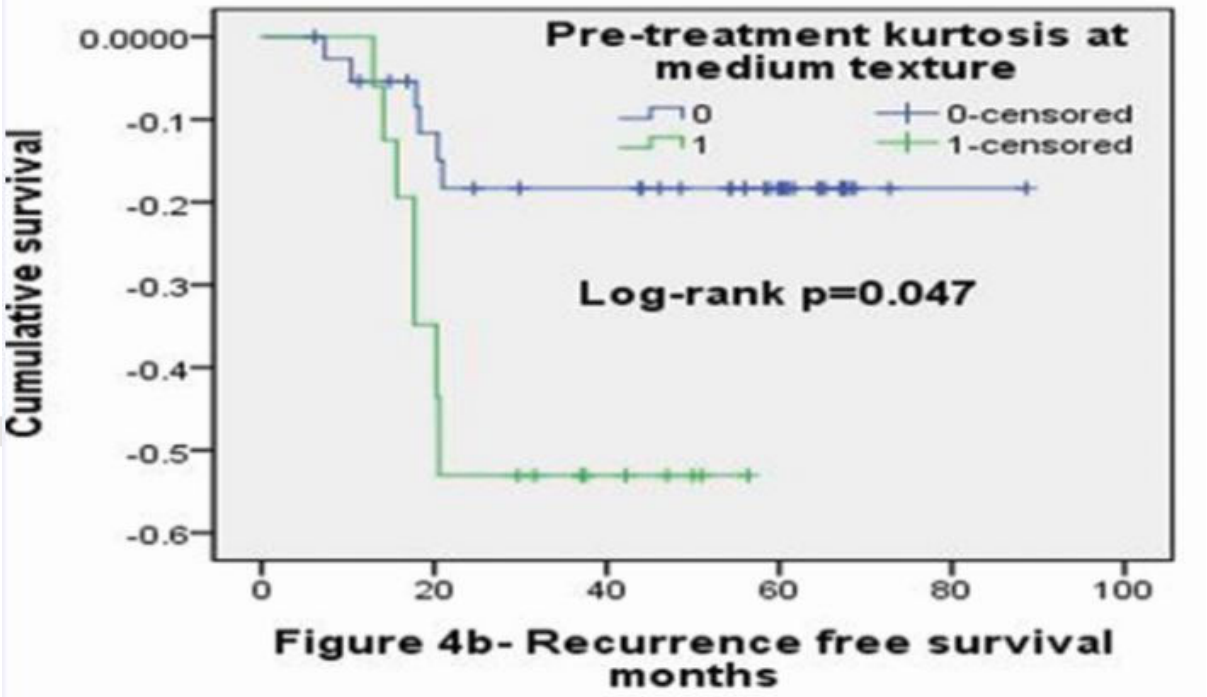

Figure 4b- Recurrence free survival months

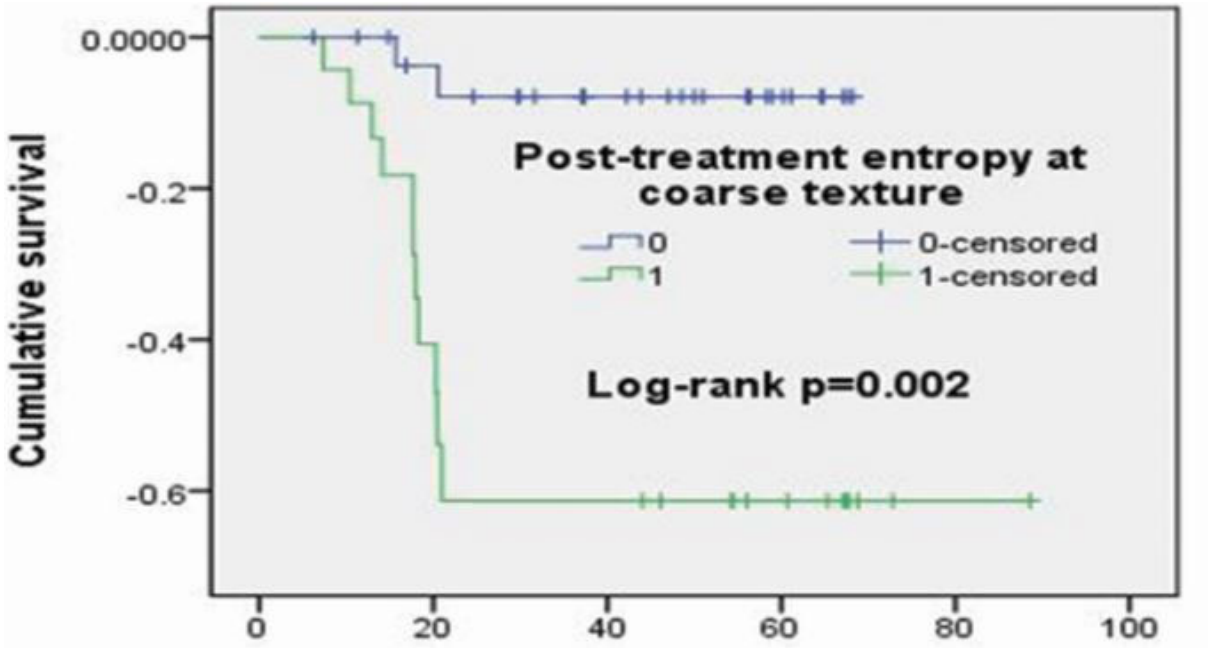

Figure 4c- Recurrence free survival months

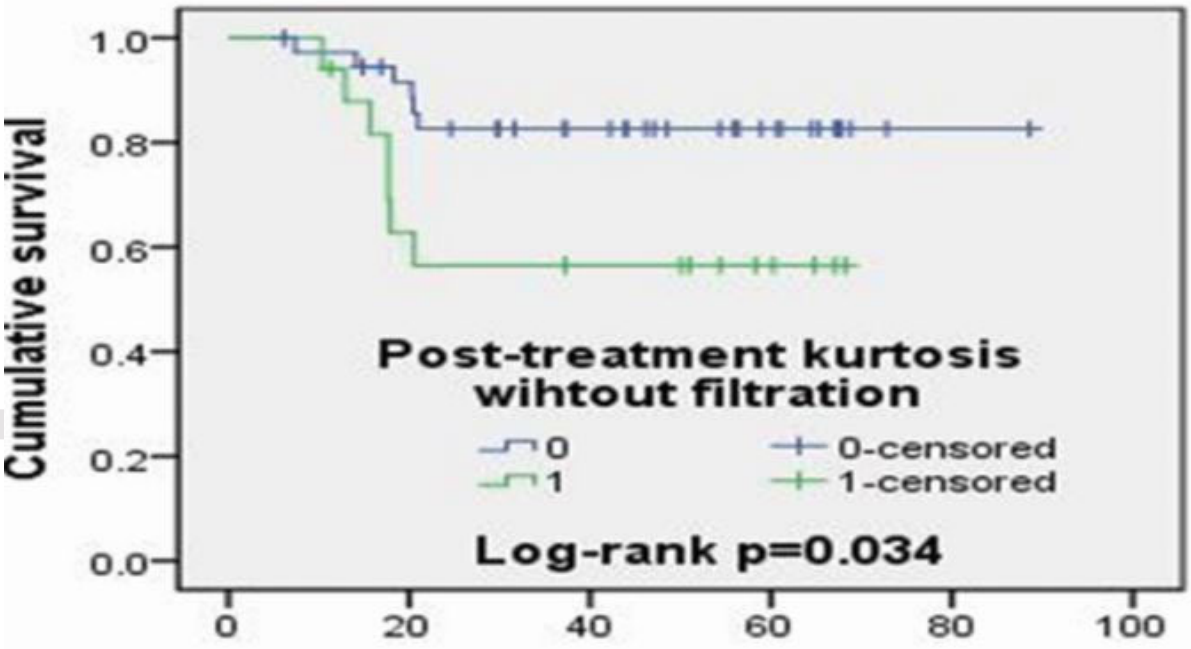

Figure 4d- Recurrence free survival months

Figure 4-Kaplan-Meier curves show a significance difference in recurrence free survival for (a) pre-treatment mean positive pixel (MPP) at fine texture (b) pre-treatment kurtosis at medium texture (c) port-treatment entropy at coarse texture and (d) post-treatment kurtosis without filtration with log-rank p values of 0.011, 0.047, 0.002 and 0.034 respectively

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