Original Research

Title: Response prediction of neoadjuvant chemoradiation therapy in locally advanced rectal cancer using CT based fractal dimension analysis.

Abstract

Objectives: There are individual variations in neo-adjuvant chemoradiation therapy (nCRT) in patients with locally advanced rectal cancer (LARC). No reliable modality currently exist that can predict the efficacy of nCRT. The purpose of this study is to assess if CT based fractal dimension and filtration-histogram texture analysis can predict therapeutic response to nCRT in patients with LARC.

Methods: In this retrospective study, 215 patients [average age: 57 years (18-87 years)] who received nCRT for LARC between June 2005 to December 2016 and underwent a staging diagnostic portal venous phase CT were identified. The patients were randomly divided into two datasets: a training set (n = 170), and a validation set (n = 45). Tumor heterogeneity was assessed on the CT images using fractal dimension (FD) and filtration-histogram texture analysis. In the training set, the patients with pCR and non-pCR were compared in univariate analysis. Logistic regression analysis was applied to identify the predictive value of efficacy of nCRT and receiver operating characteristic analysis determined optimal cut-off value. Subsequently, the most significant parameter was assessed in the validation set.

Results: Out of the 215 patients evaluated. pCR was reached in 20.9% (n = 45/215) patients. In the training set, 7 out of 37 texture parameters showed significant difference comparing between the pCR and non-pCR groups and logistic multivariable regression analysis incorporating clinical and 7 texture parameters showed that only FD was associated with pCR (P = 0.001). The area under the curve of FD was 0.76. In the validation set, we applied FD for

predicting pCR and sensitivity, specificity and accuracy was 60%, 89% and 82%, respectively. **Conclusion**: FD on pretreatment CT is a promising parameter for predicting pCR to nCRT in patients with LARC and could be used to help make treatment decisions.

Key words:

Rectal Cancer, neoadjuvant chemoradiation, CT, biomarker

Key points:

・Fractal dimension analysis on pretreatment CT was associated with response to neo-adjuvant chemoradiation in patients with locally advanced rectal cancer.

・Fractal dimension is a promising biomarker for predicting pCR to nCRT and may potentially select patients for individualized therapy.

Abbreviations:

LARC = locally advanced rectal cancer, pCR = pathological complete response, nCRT = neo-adjuvant chemoradiation, FD = fractal dimension, SSF = spatial scaling filter, SD = standard deviation, MPP = mean positive pixel

Introduction

Colorectal cancer is the third leading cause of cancer related deaths in United States [1, 2]. Locally advanced rectal cancer (LARC) is defined as either ≥T3 disease or node positivity [3]. The standard treatment approach for locally advanced rectal cancer (LARC) is neoadjuvant chemoradiation therapy (nCRT) followed by total mesorectal excision (TME) [4]. nCRT prior to surgical resection improves R0-resection in T4 disease or mesorectal fascia invasion and reduces the risk of local relapse. It also allows preservation of sphincter function in low-lying tumors [4], and provides better long-term treatment outcomes [5]. Treatment effects with nCRT can be variable and 15-27% of the patients achieve pathological complete response (pCR) [6]. Patients with pCR have been shown to have improved long-term survival and better quality of life [6]. In patients demonstrating pCR, there is an emerging trend to follow a "wait and watch" approach for management, thereby avoiding the morbidity and mortality associated with surgical resection [7]. Despite the importance of predicting pCR there are no reliable biomarkers currently to predict the efficacy of nCRT. Since pCR is generally determined at pathology using surgical specimens, preoperatively available imaging biomarker for predicting nCRT is required and desirable.

Intra-tumoral heterogeneity is an integral characteristic of malignant tumors that reflects variations in tumor cellularity, vascular distribution, extracellular matrix, hemorrhage, and necrosis [8]. Tumor heterogeneity is thought to have an influence on sensitivity to anticancer agents or radiation and is hypothesized to be one of the factors contributing to treatment-resistance[9, 10]. Imaging allows non-invasive assessment of intra-tumoral heterogeneity using various modalities and software solutions[8]. Texture analysis is an approach to quantify tissue heterogeneity and CT based texture analysis tools have been investigated to improve

diagnosis as well as provide enhanced assessment of treatment response in oncology as a surrogate biomarker [8]. Statistical-based texture analysis tools describe the distribution and relationship of gray level values in an image and texture parameters obtained by filtration-histogram method have been reported as a useful biomarker to represent tumor heterogeneity[8, 11, 12]. Recent research has shown that tumor heterogeneity assessed by CT texture analysis is an independent predictor of survival in pancreatic ductal adenocarcinoma[13], esophageal cancer [14] and also in non-small cell lung carcinoma [15]. In rectal cancer, texture analysis extracted from MRI have been reported for predicting pathological T and N stages [16] and predicting early progression in patient receiving nCRT [17]. Entropy and energy extracted from T2WI rectal image were valuable for predicting the efficacy of nCRT in rectal cancer [18]. While association of CT texture parameters with nCRT response have been previously described they are limited by small sample size[19, 20]. Fractal dimension (FD) analysis is a mathematical technique that can quantify texture or heterogeneity on digital images[21]. FD analysis is considered as another useful technique for estimating intra-tumoral heterogeneity [22, 23, 24]. These is limited data on the role of FD analysis in evaluating treatment response in patients with rectal cancer. With this background, the purpose of this study was to assess if CT based fractal and filtration-histogram texture analysis can predict therapeutic response to nCRT in patients with LARC.

Methods and Materials

Study Cohort

This retrospective study was HIPAA compliant and approved by our institutional IRB and the need for informed consent was waived. In this study, we included 272 consecutive patients who were diagnosed with LARC and underwent nCRT followed by curative surgery in our

institution between June 2005 to December 2016. All patients had histopathologically proven rectal adenocarcinoma and underwent contrast-enhanced CT prior to initiation of therapy. Inclusion criteria were as follows: (i) an adequate renal function, (ii) no contraindications to chemotherapy and surgery, (iii) no evidence of distant metastases, (iv) all patients successfully underwent nCRT followed by curative surgery. We randomly divided the patients into two datasets: a training set and a validation set. Using the training set, we identified the best parameter for differentiating pCR and non-pCR. We prospectively evaluated the ability of this parameter to predict pCR in the validation set. Clinical TNM stage was determined by colonoscopy, endoscopic ultrasound, computed tomography (CT), magnetic resonance imaging (MRI) and FDG-PET, if necessary.

Presurgical Treatment

All patients included in this study received and completed nCRT. nCRT consisted of delivery of 45-50.4 Gy in 5 fraction per week combined with administration of either capecitabine or infusional 5-fluorouracil. Thirteen patients received total neoadjuvant therapy (TNT), consisting of CRT and neoadjuvant chemotherapy prior to surgical resection. Neoadjuvant chemotherapy consisted of fluorouracil- and oxaliplatin-based chemotherapy. Surgical operation was performed about 6-8weeks after the end of nCRT. Clinical data were retrospectively extracted from the medical records.

Pathological evaluation

All the enrolled patients had histopathological evaluation of the surgical specimens obtained at curative resection. Surgical excision specimens were evaluated by gastrointestinal pathologists and details of histopathology was obtained by review of the pathology records.

Pathological complete response (pCR) was defined in case of ypT0N0 or ypN0/ypNx. Relapse free survival (RFS) was defined as the length of time after completion of primary treatment for rectal cancer that the patient survived without any signs or symptoms of that cancer.

CT Technique

CT scans were performed over a period of eleven years and different generations of CT scanners from multiple manufacturers were used. A complete description of the scanner models and manufacturers is provided in the online supplement (***Table E1, Supplement***). All patients underwent a contrast-enhanced CT of the abdomen and pelvis in the portal venous phase after injection of 80–120 ml of iodinated contrast media (Isovue 370 mg/ml, Bracco Diagnostics Inc.), at a flow rate of 3-3.5 ml/second followed by a 40 ml saline chaser at the same rate. Image acquisition was triggered with bolus-tracking when an attenuation of 150HU was attained in the aorta at the mid-liver level. Images were reconstructed in the axial plane at 5 mm thickness. Coronal and sagittal reformatted images were obtained at 3 mm thickness.

Image Processing and Data Analysis

Tumor texture analysis was performed on axial contrast-enhanced CT images by an independent observer (T.T, with 12 years of experience in CT interpretation). A single axial CT image representative of the tumor depicting the largest cross-sectional area of the tumor was selected. Data analysis was performed by manually drawing a region-of-interest (ROI) to delineate the tumor area. Heterogeneity was determined quantitatively using fractal analysis and filtration-histogram based texture analysis. The same ROI of CT image was used for both analyses. The same process was followed for determination of texture features on both training set and validation set.

I. Fractal analysis

Fractal analysis[21] was obtained using ImageJ (ver. 1.39s; National Institutes of Health; available at http://rsb.info.nih.gov/ij) with a plugin (FracLac version 2.5; available at http://rsb.info.nih.gov/ij/plugins/fraclac/FLHelp/Introduction.htm). The plugin was specifically designed for analyzing difficult-to-describe morphological features and successfully used to analyze the FD of various medical digital images[23, 25]. Contrast-enhanced CT images were retrieved from the institutional archive and loaded to ImageJ (***Figure 1)***. CT images which were adjusted at a window width of 400 HU and a window level of 40 HU and converted to gray-scale 8-bit images on ImageJ. In FracLac, differential box-counting method was applied to calculate the FD as a parameter for tumor heterogeneity. FD is defined as $N_L = KL^{-FD}$, where L is the box size, N_L is the number of boxes at size L needed to cover the object being studies, and log K can be obtained from the y-intercept obtained by linear regression on a log–log plot of N_L versus L. The box sizes were gradually increased during the sampling period until the maximum size of 45 % of the total area selected was reached.

II. Filtration-Histogram Analysis

Filtration-histogram based texture analysis was performed using a commercially available research software TexRAD software (version 3.3, Feedback Medical Ltd.) (***Figure 2***). This research software has been studied extensively for different lesions[11, 12, 26]. In initial filtration step, derived image features from CT image are highlighted by different spatial scaling factor (SSF), which ranges from SSF2 (fine, object radius of 2 mm) to SSF6 (coarse, object radius of 6 mm) with SSF3-5 (medium, object radius of 3–5 mm) by using a Laplacian

of Gaussian spatial band pass filter. And then, for each filtration, six statistical and histogram based measures are used to quantify tumor heterogeneity by summarizing the distribution of pixel intensity: Mean (mean gray level intensity), standard deviation (SD), Entropy (irregularity of pixel intensities in space), mean positive pixel (MPP; average value of all the pixels with positive values), Skewness (measure of asymmetry of the histogram) and Kurtosis (a measure of peakedness or pointedness or sharpness of the histogram). In addition, these statistical and histogram parameters were also quantified from the conventional CT image without filtration (i.e. SSF0).

Statistical analysis

Patient characteristics were compared using chi-square test (categorical variables) and Mann–Whitney's *U* or Kruskal–Wallis rank tests (continuous variables). FD and histogram parameters were compared between patients who achieved pCR and non-pCR and all parameters were assessed as predictive value for efficacy of nCRT using Mann–Whitney's *U* test. Then, logistic multivariate regression analysis was performed for variables that were found to be statistically significant in univariate analysis. The best cutoff values and area under the curve (AUC) were determined by receiver operating characteristic (ROC) analysis for the variable which was significant on multivariate analysis. Sensitivity, specificity and accuracy were calculated at the best cut-off point. In the training set, we identified the best parameter in this way. And then we applied cut-off value to the best parameter (as determined in the training set.) in the validation set for assessing the predictive value (diagnostic criteria). Kaplan Meier survival analysis assessed the ability of the best parameter(s) to predict relapse free survival (RFS), and the difference in the survival curves were analyzed using log-rank test. All statistical analyses were carried out using Statview 5.0 (SAS Institute, Inc.) and SPSS version

24 (IBM Corp), and p values < 0.05 were considered to be statistically significant.

Results

Patient characteristics

Of the 272 consecutive patients who were eligible in this study, 57 patients were excluded because of following reasons: (i) contrast-enhanced CT was not performed at our institute (n = 47), (ii) distant metastases (n = 7), and (iii) lack of R0 surgery (n = 3). Therefore, a total of 215 patients were finally included in this study **(*Figure 3*).** Patients were divided into (1) a training set (n = 170), and (2) a validation set (n = 45) in a random order. Clinicopathological features in each group are shown in ***Table1*** and each dataset have same proportion of disease / patient categories. For the entire cohort, the median age was 57 years (range, 18–87 years) and 134/215 were male (62.3%). The tumor location from anal verge was 6 cm (range, 0–15 cm) and the median size of maximum tumor diameter was 4.8 cm (range, 1.3–12 cm). All patients underwent R0 surgery. Low anterior resection (LAR) was performed in 153/215 patients (71.2%), abdominal perineal resection (APR) was done in 53/215 patients (24.7%) and other kinds of surgeries, such as total proctocolectomy, total pelvic exenteration (TPE) and posterior pelvic exenteration (PPE), were done in 9/215 patients (4.2%). The median value of carcinoembryonic antigen (CEA) was 2.9 ng/mL (range, 0.4–137.4). pCR was achieved in 45/215 patients (20.9%) and the rest 170/215 patients (79.1%) did not represent pCR on pathological examination (non-pCR). In Kaplan-Meier analysis, patients with pCR showed better RFS than those with non-pCR for the entire cohort (***Figure 4***, P = 0.042).

Tumor heterogeneity in Training set

In training set, pCR was achieved in 35/170 patients (20.6 %). Pretreatment clinical features

were compared between pCR and non-pCR ***(Table2)***. There was no difference in age, sex, cT, cN, cStage, tumor location, and surgical resection method. Maximum tumor diameter in non-pCR was significantly larger than that in pCR (non-pCR median 4.9 cm, range 1.3–12 cm vs pCR median 3.7 cm, range 1.6–9 cm, $P = 0.012$). CEA of the patients with non-pCR was higher than those with pCR (non-pCR median 3.95 ng/mL, range 0.4–137.4 ng/mL vs pCR median 1.95 ng/mL, range 0.5–14.5 ng/mL, $P = 0.003$).

In the textural features, 7 parameters out of 37 parameters showed significant difference comparing between the pCR and non-pCR groups ***(Table3)***. FD (non-pCR median 1.157, range 0.86–1.367 vs pCR median 1.058, range 0.863–1.275, $P < 0.0001$), skewness at SSF0 (non-pCR median -0.53, range -1.55–0.19 vs pCR median -0.57, range -2.09–0.27, $P = 0.030$), SD at SSF2 (non-pCR median 63.15, range 40.29–205.09 vs pCR median 72.72, range 45.46–114.79, $P = 0.014$), SD at SSF3 (non-pCR median 61.57, range 35.49–232.35 vs pCR median 76.78, range 44.86–114.14, $P = 0.001$), MPP at SSF3 (non-pCR median 60.67, range 30.24–139.62 vs pCR median 68.48, range 46.95–106.16, $P = 0.017$), SD at SSF4 (non-pCR median 64.06, range 37.02–271.05 vs pCR median 72.12, range 43.73–122.27, $P = 0.012$), and MPP at SSF4 (non-pCR median 68.72, range 32.11–130.43 vs pCR median 76.91, range -37.18–120.27, $P = 0.034$) demonstrated significant difference. And then, logistic multivariable regression analysis for predicting pCR was performed using the pretreatment clinical and textural parameters which showed significant difference in univariate analysis. In logistic regression analysis, only FD showed statistically significant difference (Odds ratio 6.21×10^{-6}, Confidence interval 3.162×10^{-9}–0.012, $P = 0.001$) ***(Table4)*** and FD emerged as a statistically significant predictor of pCR. ROC analysis to assess the discriminatory power of FD for distinguishing pCR from non-pCR revealed an AUC of 0.76. In the training set, the best cut-

off value of FD was 1.072 and sensitivity, specificity and accuracy were 63%, 84% and 79%, respectively ***(Figure 5)***.

Validation Set

In validation set, pCR was achieved in 10/45 patients (22.2 %). In the validation set, we evaluated the ability of FD to predict patients with pCR from patients with non-pCR. Using the optimal threshold FD value of 1.072, sensitivity, specificity and accuracy of 60% 88.6% and 82.2% was achieved in the prediction of pCR in the validation set (***Table5***).

Discussion

The standard treatment for locally advanced rectal cancer (LARC) is neoadjuvant chemoradiation (nCRT) followed by total mesorectal excision (TME) [4]. Pathological complete response (pCR) at the time of surgery is an important prognostic biomarker and patients with pCR after nCRT demonstrate less propensity for local or distant recurrence and show improved survival [6]. Prediction of pCR prior to treatment initiation has important implications for patient selection and determining therapeutic strategies. In this study we investigated and demonstrated that CT based fractal dimension and filtration-histogram texture analysis could help predict treatment response to nCRT. In the initial training set we found that several texture parameters could distinguish between pCR and non-pCR. Fractal Dimension (FD) emerged as the most significant and validated predictor of pCR with a sensitivity, specificity and accuracy was 60%, 89% and 82%, respectively. To the best of our knowledge, this is the first study evaluating the usefulness of FD analysis on CT in patients with LARC.

Intra-tumoral heterogeneity as depicted on texture analysis is multifactorial and is related to

several factors such as hypoxia, necrosis, angiogenesis and genetic variations[27, 28]. Hayano et al. reported that structural heterogeneity leads to a heterogeneous blood supply in the tumor, which may result in a hypoxic tumor environment [12]. In general, significant resistance to radiation and chemotherapy have been shown in hypoxic regions within solid tumors [29]. Hypoxia and necrosis lead to increase in the regions of low density within the tumor contributing to tumor heterogeneity. We hypothesized that the texture parameters could potentially predict therapeutic resistance through depiction of intra-tumoral structural heterogeneity. Single slice CT-based texture analysis is a simple tool that can be used in routine practice. Therefore, it is very meaningful to demonstrate the usefulness of texture analysis on pretreatment contrast-enhanced CT. In a study of 95 patients with LARC treated with nCRT, Chee CG et al [19] reported that homogeneous texture features on pretreatment CT images were associated with better nCRT response and higher disease free survival. In their study cohort, only 14.7% of patients (14/95) had pCR. Benjamin et a. also reported that texture analysis using histogram is useful to assess the response to nCRT in patients with LARC [20]. In their study of 121 patients, they used a combination of 6 features derived from 36 texture features with and without filtration using ELASTIC-NET method. They presented a prognostic model for down staging and AUC was estimated 0.70. Our study has a large sample size compared to prior studies and has a higher proportion of patients with pCR. Prior investigators have not compared texture features between patients with and without pCR and there is no reported study comparing FD and histogram analysis for quantification of tumor heterogeneity in LARC.

In patients with LARC demonstrating clinical complete response to nCRT, “watch-and-wait” approach allows potential avoidance of major surgery with subsequent organ preservation[7,

[30, [31]. In this study, we found that patients with pCR showed significantly better relapse free survival than those without pCR. Recent studies have identified quality of life as an important factor for patients with rectal cancer [31]. It is therefore critical to identify reliable biomarkers for predicting the efficacy of nCRT in patients with LARC and be able to predict pCR preoperatively. This has been previously studied using a genomic approach [32], but it is also critical to determine whether non-invasive radiologic parameters can serve as reliable predictive biomarkers for response to nCRT. As shown in our study, FD is a potential biomarker for identifying patients who will likely experience pCR and might be suitable for a "watch-and-wait" approach. Fractal analysis is an appropriate technique for quantifying heterogeneity of the tumor that are hard to describe quantitatively. Medical images of colorectal cancer contain information that reflects underlying tumor biology and have correlation with genetic, histologic, clinical, and prognostic and/or predictive data [33].

Our study has a few limitations. First, this study was performed using single-center retrospective data. Therefore, it should be confirmed in multicenter prospective investigations. Second, the definition of the tumor ROIs was subjective and performed by one reader. ROIs were manually delineated, and automated tumor segmentation methods can be evaluated in the future. Thirdly, CT scans were acquired over an eleven-year period on different scanners that can potentially influence noise and heterogeneity analysis. However, CT protocols for staging of rectal cancer are standardized for injection protocols and slice thickness at our institution and across most cancer centers. Moreover, small variations in CT protocols have been found to have no significant effect on heterogeneity analysis for portovenous phase CT imaging [34]. Previous studies have demonstrated good reproducibility for filtration-histogram based texture

analysis using multi-center clinical validation[35, 36], robustness to variation in image acquisition parameters[34, 37] and good inter- and intra-operator repeatability (good intra class correlation from test-retest technique) [38]. The contour of the tissue was drawn on a single axial slice. Although the multi-slice or volume delineation would be a better representation of the whole tumor, it is not practical in clinical settings due to a time-consuming process and increased variability in the ROI segmentation process arising from multiple delineations. Furthermore, Ng et al [39] reported comparable results in heterogeneity assessment between cross-sectional area versus whole volume analysis in primary colorectal cancer patients on CT.

Conclusions

This study suggests that textual parameters derived from routine CT using fractal dimension and filtration-histogram based analysis are significant predictors of response to neo-adjuvant chemoradiation therapy in patients with locally advanced rectal cancer. Particularly, FD analysis on initial CT demonstrates to be a robust and validated imaging biomarker and can potentially contribute towards personalized treatment of rectal cancer.

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Figure Legends

Figure1. Fractal Dimension (FD) analysis using FracLac plugin on ImageJ. Contrast-enhanced CT image in the axial section at the largest area of the tumor was 8-bit gray-scaled, and the region of interest (ROI) was drawn to include entire tumor (A). FD was measured using a differential box-counting method at 12 different grid positions, and then the FD was calculated as a parameter of heterogeneity (B, C, and D).

Figure 2. Histogram analysis was performed using TexRAD software. Contrast-enhanced CT image of the tumor in the axial section with region of interest (ROI) was delineated around the tumor (A). Corresponding images in the same patient with fine, medium, and coarse filter values by using spatial scaling factor (SSF) 2 (fine; B), SSF3 (medium, C), SSF4 (medium; D), SSF5 (medium; E), and SSF6 (coarse; F), respectively. Texture parameters were obtained at each filtration.

Figure 3. Flowchart of the study sample

Figure 4. Kaplan-Meier analysis in patients with complete pathological response (pCR) and non-complete pathological response (non-pCR) for relapse free survival (RFS). Patients with pCR significantly showed better survival in entire cohort ($P = 0.042$).

Figure 5. (A) This boxplot displays the distribution of fractal dimension (FD) in patients with complete pathological response (pCR) and non-complete pathological response (non-pCR) in test dataset. The FD of pCR group is significantly lower than non-pCR group ($p<0.001$). (B)Receiver operating characteristic (ROC) curve for FD. The area under the curve (AUC) is 0.76. The sensitivity was 63%, specificity was 84% and accuracy was 79% at a cut-off value of 1.072 in the training set.