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Biomarkers in anal cancer

Quantitative imaging outperforms molecular markers when predicting response to chemoradiotherapy for rectal cancer

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ABSTRACT

Background and purpose: To explore the integration of imaging and molecular data for response prediction to chemoradiotherapy (CRT) for rectal cancer.

Material and methods: Eighty-five rectal cancer patients underwent preoperative CRT. ^{18}F-FDG PET/CT and diffusion-weighted imaging (DWI) were acquired before (TP1) and during CRT (TP2) and prior to surgery (TP3). Inflammatory cytokines and gene expression were analysed. Tumour response was defined as ypT0-1N0. Multivariate models were built combining the obtained parameters. Final models were calculated on the data combination with the highest AUC.

Results: Twenty-two patients (26%) achieved ypT0-1N0 response. ^{18}F-FDG PET/CT had worse predictive performance than DWI and T2-volumetry (AUC 0.61 ± 0.04, 0.72 ± 0.03, and 0.72 ± 0.02, respectively). Combining all imaging parameters increased the AUC to 0.81 ± 0.03. Adding cytokines or gene expression did not improve the AUC (AUC of 0.72 ± 0.06 and 0.79 ± 0.04 respectively). Final models combining ^{18}F-FDG PET/CT, DWI, and T2-weighted volumetry at all TPs and using only TP1 and TP3, allowed ypT0-1N0 prediction with a 75% sensitivity, 94% specificity and PPV of 80%.

Conclusions: Combining ^{18}F-FDG PET/CT, DWI, and T2-weighted MRI volumetry obtained before CRT and prior to surgery may help physicians in selecting rectal cancer patients for organ-preservation.

With the implementation of total mesorectal excision (TME), local recurrence rates of rectal cancer decreased from above 20% to about 5% [1]. Local control of locally advanced rectal cancer further improved by the use of preoperative chemoradiotherapy (CRT) [2]. About 15–20% of the patients receiving preoperative CRT achieve a pathological complete response (ypCR) [3]. These patients have an excellent outcome, regardless of their initial T- and N-stages [3,4].

In the era of personalised medicine, the need for extensive surgery in well-responding patients has been questioned, and less invasive alternatives such as local excision and even a "watch-and-wait" policy have been suggested [5–7]. Adopting an organ-preserving strategy for good responders spares patients the morbidity (i.e. postoperative complications, long-term bowel, bladder and sexual dysfunction, or permanent stoma care) and the mortality associated with invasive surgery [8,9].

However, before such less invasive approach can be safely implemented, accurate assessment of response to CRT is of utmost importance. This is considered to be challenging since the concordance between mucosal appearance and pathological response has shown to be poor, and endoscopic biopsies are of limited value in ruling out persisting tumour after neoadjuvant CRT [10,11]. Because conventional morphologic imaging also lacks accuracy for restaging after CRT, alternative ways to assess response to CRT are needed [12,13]. There is growing interest in the use of functional imaging and molecular markers to improve clinical response assessment. Functional imaging techniques depict changes in tumour metabolism and microstructure before morphological changes become apparent. ^{18}F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET) semi-quantitatively assesses tumour glucose metabolic activity through changes in FDG-uptake. A decrease in standardised uptake value (SUV) during treatment has been associated with pathological response in several tumour types, including rectal cancer [14]. Diffusion-weighted

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imaging (DWI) provides information on the microstructure of tissues through the assessment of differences in water diffusion [15]. By quantifying diffusion as the apparent diffusion coefficient (ADC), DWI can be used to monitor and to predict tumour response to CRT. An increase in ADC during and after CRT reflects a decreased cellularity and has been associated with tumour response to therapy [16]. Molecular markers have also been put forward as strategies to predict the response to CRT for rectal cancer. Some promising markers include inflammatory biomarkers and gene expression profiles [17–20].

In general, the predictive performance of ^{18}F-FDG PET, DWI and molecular analysis as single modalities is insufficient to safely guide a patient-tailored treatment. Efforts have been made to investigate whether combining these markers contribute to a more accurate response prediction [21,22].

The purpose of this study was to explore the performance of integrating ^{18}F-FDG PET, DWI, T2-weighted volumetry, inflammatory blood markers and gene expression profiles for prediction of ypT0-1N0 response to CRT.

Materials and methods

Patients

Eighty-five rectal cancer patients were prospectively included between January 2012 and February 2015. Inclusion criteria were (1) primary histologically proven adenocarcinoma of the rectum, clinical stage T3-4N0 or T1-4N1-2, (2) WHO performance scale $\leq$2, and (3) adequate bone marrow, hepatic, and renal function assessed by biochemical examination. Exclusion criteria were (1) distant metastases (n = 4), (2) prior chemotherapy or radiotherapy for rectal cancer (n = 0), (3) previous or concurrent malignancies at other sites (n = 0), and (4) known allergies to intravenous contrast agents or other contraindications for ^{18}F-FDG PET/CT and MRI acquisition (n = 0). Patients received CRT (45 Gy delivered in 1.8 Gy fractions, with a continuous infusion of 5-fluorouracil (225 mg/m^2/d)). Six patients received capecitabine (825 mg/m^2 twice daily). TME was performed after an interval of eight weeks from completion of CRT (Fig. 1). Patients underwent ^{18}F-FDG PET/CT and DWI scans at regular time intervals. Blood and tissue samples were obtained. Endoscopy and digital rectal examination (DRE) were not consistently performed and were therefore not included as potential explanatory variables. This trial (NCT01171300) was approved by the institutional ethical committee and all patients gave written informed consent prior to study entry.

PET acquisition and evaluation

^{18}F-FDG PET/CT scans were performed prior to CRT (TP1), after 10–15 fractions of CRT (TP2), and prior to surgery (TP3). Analyses were performed by a staff member of Nuclear Medicine (CD) who was unaware of the pathological and DWI results. Following PET parameters were extracted for each time point: SUV_{max}, SUV_{mean}, SUV_{min}, SUV_{median}, SUV_{peak}, metabolic tumour volume (MTV), metabolic diameter, and total lesion glycolysis (TLG = SUV_{mean} * MTV). Absolute and relative changes in PET parameters between different time points were calculated, leading to a total of 72 PET variables. Details on PET acquisition can be found in Supplementary Material.

MRI acquisition and evaluation

MRI studies were acquired at the same time points as the ^{18}F-FDG PET/CT scans. MRI analyses were performed by a staff member of Radiology (VV) who was blinded for pathological and ^{18}F-FDG PET/CT results. Tumour volumetry was assessed by manually delineating tumour boundaries on the axial T2-weighted images. Besides the tumour volume in cm^3, a diameter of the equivalent sphere was calculated. DWI images were acquired using six different b-values (b = 0, 50, 100, 300, 600, 1000 s/mm^2). Following DWI parameters were extracted for each time point: ADC_{low} (b0-b300), ADC_{avg} (b0-b1000), ADC_{high} (b600-b1000). Additionally, absolute and relative changes in T2-volumetry and relative changes in ADC between different time points were calculated, generating 18 T2-volumetry and 18 DWI variables. Details on MRI acquisition are described in Supplementary Material.

ELISA assays

Blood samples were collected prior to CRT (TP1), two weeks into radiotherapy (TP2), and at the end of CRT (TP3′). Blood markers (interferon-gamma (IFN-γ), interleukin (IL) 10, IL12p70, IL13, IL1β, IL2, IL4, IL6, IL8, and tumour necrosis factor-alpha (TNF-α) were investigated by using a multiplex ELISA platform (Proinflammatory Human MSD assay, Mesoscale Diagnostics). The absolute and relative changes in cytokines between the time points were also calculated, leading to a total of 90 variables.

Microarray and gene expression profiling

Microarray analysis was performed on tumour biopsies obtained by endoscopy prior to CRT (details described in Supplementary Material). We used the following existing gene expression signatures for predicting rectal cancer outcomes: the Ghadimi et al. 54-gene signature, the Watanabe et al. 33 gene signature and the Kim et al. 95 gene signature [18–20]. Next, we added an Epithelial-to-Mesenchymal (EMT) signature and a hypoxia signature for colorectal cancer [23,24]. Finally, a five-gene signature for predicting metastasis of colorectal cancer was applied [25].

Pathology

Pathological TNM staging served as the gold standard. Histological evaluation of the resection specimen was independently performed by an expert pathologist (XS) according to the method described by Quirke et al. [26]. The primary outcome measure for our study was tumour response defined as ypT0-1N0. Two patients (2%) did not undergo surgery due to strong clinical evidence of a complete response (repeated digital rectal examination, endoscopic evaluation, and DWI). These patients were strictly followed and were disease-free 42 and 38 months after the end of CRT, which we considered a surrogate endpoint for ypCR.

Statistical analysis

The primary outcome measure was ypT0-1N0 response. Patients who had more than 30% missing variables in a particular data set (i.e. PET/CT, DWI, T2-weighted MR, ELISA and gene expression-specific features) were excluded from the analysis. Remaining missing data were estimated using a 15-Nearest Neighbour algorithm [27]. All variables were standardised to a zero mean and unit standard deviation. In a first step, models were built on each data set separately to identify the baseline performance of each modality. Secondly, we built models using different combinations of PET, T2-volumetry, DWI, ELISA, and microarray data applying logistic regression with lasso regularisation. We used a ten-fold stratified cross validation strategy to assess the models' performance on unseen data. We repeated this process ten times to randomise the process to split the data in folds. Performances were expressed as the area under the curve (AUC) of receiver operating