A prospective study of diffusion-weighted magnetic resonance imaging as an early prognostic biomarker in chemoradiotherapy in squamous cell carcinomas of the anus.

Abstract

Purpose

The use of diffusion-weighted magnetic resonance imaging as a prognostic marker of treatment response would enable early individualisation of treatment. We aimed to quantify the changes in mean apparent-diffusion-coefficient (ΔADC_{Mean}) between a DW-MRI at diagnosis and on fraction 8-10 of chemoradiotherapy (CRT) as a biomarker for cellularity, and correlate these with ASCC recurrence.

Methods and materials

This prospective study recruited patients with localised anal cancer between October 2014 and November 2017. DW-MRI was performed at diagnosis and after fraction 8-10 of radical CRT. A region of interest (ROI) was delineated for all primary tumours and any lymph nodes >2 cm on high-resolution T_2 –weighted images and propagated to the ADC map. Routine clinical follow up was collected from NHS electronic systems.

Results

Twenty-three of 29 recruited patients underwent paired DW-MRI scans. 26 ROIs were delineated among the 23 evaluable patients. The median (range) tumour volume was 13.6 cm^3 (2.8 cm^3 to 84.9 cm^3). Ten of 23 patients had lesions with ΔADC_{Mean} ≤20%. With a median follow-up of 41.2 months, 4 patients either failed to have a complete response to CRT, or subsequently relapsed. Three of 4 patients with disease relapse had lesions demonstrating ΔADC_{Mean} ≤20%, the other patient with persistent disease had ΔADC_{Mean} of 20.3%.

Conclusions

We demonstrated a potential correlation between patients with ΔADC_{Mean} ≤20% and disease relapse. Further investigation of the prognostic merit of DW-MRI change is needed in larger, prospective cohorts.

Keywords

Functional imaging; anal cancer; prognostic biomarkers; diffusion weighted MRI; ADC mean

Introduction

Anal squamous cell carcinoma (ASCC) is treated with radical chemoradiotherapy (CRT) with a 3-year disease-free survival (DFS) of 73% (1-3). Although overall outcomes are good, up to 40% of patients with locally advanced disease can have less favourable outcomes (4). Retrospective cohort data and tumour control modelling data support a dose response in anal cancer. Therefore, dose escalation to locally advanced patients is under investigation. The PLATO trial (PersonaLising Anal cancer RadioTherapy dOse, ISRCTN88455282), is an international anal cancer trial funded by Cancer Research UK, randomising patients with locally advanced tumours to standard versus dose-escalated radiotherapy (5). However, approximately 50% of patients will be cured by standard radiotherapy doses. In these patients, unnecessary radiotherapy dose escalation will only serve to increase late toxicities (6-9) which can be lifelong and significantly affect quality of life. These include diarrhoea, faecal incontinence, dyspareunia, and erectile dysfunction (10-12). Early identification of the subgroup of patients who require treatment intensification would allow us to tailor radiotherapy dose to minimise adverse effects.

Diffusion-weighted magnetic resonance imaging (DW-MRI) is an imaging biomarker allowing us to quantify the motion of water in biological tissues (13). A low apparent diffusion coefficient (ADC) suggests reduced water mobility due to increased tumour cellularity and has the potential to identify patients who are unlikely to respond to the standard dose of radiotherapy and require treatment intensification (14).

DW-MRI has a literature base in squamous cell carcinomas of other geographical sites. Its use as a diagnostic imaging tool at outset, to predict outcome, has been investigated with mixed results (15, 16). However, there are a small number of reports evaluating the change in ADC on a pair of DW-MRI images taken at diagnosis and the first 1-3 weeks of radiotherapy in head and neck squamous cell carcinoma (HNSCC) and cervical squamous cell carcinoma. These consistently demonstrate a

correlation between change in ADC and local control (15-19). In anal cancer, initial work has suggested that ADC_{CoV} may be prognostic (20). In addition, its use can improve interobserver agreement in assigning T-stage and aid delineation of gross tumour volume (21). DW-MRI can also assist in assessing response following CRT (22, 23). In terms of predicting outcome using MRI during treatment, there is one study looking at the use of DW-MRI for early therapy response. Jones et al. studied 20 patients with 17.1 month follow up (24) . They had 8 relapses and correlated 7 different MRI parameters taken at 2 different timepoints with local and all recurrence. They concluded there was a number of different parameters from different MRI's from different timepoints that correlated with the two different endpoints. This study was limited by small numbers, short follow up, high relapse rate and a lack of clarity regarding analysis plan at outset; but it does highlight the potential for correlation. We aim to further investigate whether this correlation exists and investigate the validity of a predefined threshold as a prognostic marker for recurrence.

We hypothesised that failure to demonstrate an increase of >20% in DW-MRI ADC, following 8-10 fractions of CRT, represents a quantitative imaging biomarker of poor treatment response. The XXX study (ClinicalTrials.gov: XXX) was a single-centre, prospective, observational, imaging study quantifying the ADC change in ASCC.

Materials and methods

Patient population and treatment

All patients had newly diagnosed, histologically confirmed ≥T2N0 ASCC with no prior treatment and were suitable for radical CRT. Exclusion criteria were contraindications to MR imaging and previous pelvic radiotherapy. This study was approved by National Research Ethics Service Committee South Central ~~XXX~~ (14/SC/1130) and all patients provided written informed consent.

CRT was delivered according to UK-based guidance (25). A dose of 50.4Gy or 53.2Gy was prescribed to the primary tumour depending on TNM stage, 50.4Gy to involved nodes and a 40Gy prophylactic

dose to non-involved nodes, all in 28 fractions using a simultaneous integrated boost. Three patients were treated within the PLATO study, two with a dose of 61.6Gy to the primary tumour while maintaining 40Gy to the prophylactic nodes, and the other patient with a reduced dose of 41.4Gy to the primary tumour and 34.5Gy to elective nodes in 23 fractions with simultaneous integrated boost. Treatment was delivered using intensity modulated radiotherapy (IMRT) or volumetric modulated arc therapy (VMAT). Chemotherapy was Mitomycin 12 mg/m^2 Day 1 and Capecitabine 825 mg/m^2 orally twice a day on radiotherapy treatment days.

Data acquisition

Patients underwent MRI prior to and following fraction 8 to 10 of radiotherapy. Patients were scanned on a GE Discovery MR750 3T MR scanner (GE Healthcare Milwaukee, WI, USA) with a flat-topped couch to reproduce the radiotherapy treatment position. Following acquisition of a T_2-weighted image, diffusion-weighted images were acquired with a 2D spin echo EPI sequence employing ASSET to accelerate the acquisition. Four diffusion directions were acquired using the Tetrahedral option that applies four different combinations of x-, y-, and z- gradients simultaneously, using *b*-values of 0 and 1500 s/mm^2. Table 1 details the MRI sequence parameters. Both linear and exponential ADC maps were calculated using the standard GE algorithm provided on the console. The centre slice was determined by the MRI technicians measuring the distance from the patients RT positioning tattoos to the centre of the primary tumour. The landmarking laser on the MRI was then lined up to the tattoos and the centre slice of the MRI sequences were positioned at the given distance from this landmark.

All images and the radiotherapy treatment plans were anonymised and imported into Eclipse software (version 13.0, Varian medical systems, Palo Alto USA). An automatic rigid registration was performed between the radiotherapy planning scan and the two MRIs. Delineation of a region of interest (ROI) around the primary macroscopic tumour and any involved nodes >2 cm in size was

performed by an experienced radiation oncologist (RM) on the multi-slice T_2-weighted images for both data sets for each patient; T_2-weighted volume (cm^3) for the ROI were calculated from these images. Areas of necrosis could not be accurately and consistently identified and removed from the ADC map and were therefore included in all ROIs if present within tumour or nodes. The ROI for each image was propagated to the corresponding ADC map where the ADC_{Mean} was collected.

Statistical analysis

Patients with ADC_{Mean} derived from paired images (PreDW and DuringDW) were evaluable for the primary analysis. The percentage change in ADC_{Mean} (ΔADC_{Mean}) and ~~reduction~~ change in volume from baseline for each ROI between the two scans was calculated.

A "resolving diffusion deficit" on DW-MRI was a change in ADC_{Mean} >20% between baseline and fraction 8-10 CRT scans. A value of 20% was used as a cut off to identify "resolving diffusion deficit" and "non-resolving diffusion deficit" based on previous data from small HNSCC and cervix data [19-23], accepting the limitations of estimating a cut-off in such small studies.

Median (lower quartile (LQ), upper quartile (UQ) / range) and numbers (with percentages) were used to summarise continuous and categorical variables, respectively. Clopper–Pearson 95% confidence intervals (CI) were estimated for proportions, and 95% CIs for medians, including the median percentage change in ADC_{Mean} were derived using the binomial method. Statistical analyses were performed using STATA 15.1.

The sample size was calculated using the A'Hern single-stage design [23]. We expected at least 20% of patients to show limited improvement in water mobility ("non-resolving diffusion deficit"), based on the hypothesis that patients with no improvement correlate with those with local relapse (40%), and half of those patients could be identified by this imaging technique with a ~~≤~~≤20% change in

ADC_{Mean}. With the hypothesis (H_1) that the proportion with non-resolving diffusion deficit is at least 20% and a null hypothesis (H_0) that the proportion with non-resolving diffusion deficit is 5% maximum, 70% power and 3% significance level, at least four of 23 evaluable patients need to demonstrate non-resolving diffusion deficit ($ADC_{Mean} \leq 20\%$) to consider taking the concept of individualised treatment intensification forward.

Clinical Follow up

Patients consented to progress reports on routine follow up within standard routine clinical follow up. In routine follow up patients are seen for a clinical exam 3 monthly for the first 2 years, 6 monthly in year 3 and yearly ~~for~~ in years 4 and 5. A CT scan is performed annually. On 20th November 2019, electronic systems in the XXX were reviewed to collect the date of last follow up or death; and whether or not the patient was disease-~~-~~free based on routine follow up. Relapsed disease was a failure to demonstrate a complete response 6 months after CRT, or evidence of local, regional or distant disease after CR had been achieved.

Sensitivity and specificity were used to assess the performance of the 20% cut-off in percentage change in ADC between baseline DW-MRI and DW-MRI at fraction 8-10 of radiotherapy.

Results

Patients and Treatment

Twenty-nine patients were registered to the trial between October 2014 and November 2017 from the XXX. Patient flow is illustrated in Figure 1 using ~~the~~ a CONSORT diagram. Six patients were excluded for the primary endpoint analysis, as they did not have paired DW-MRI scans. This resulted in 23 evaluable patients for analysis. Among the 23 patients, the median (LQ, UQ) time from the first scan to commencing CRT was 13 (12, 19) days. All 23 patients completed the radiotherapy as planned. Planned Mitomycin dose was delivered to all 23 patients, with one patient prescribed a

planned dose reduction of 9 mg/m^2. Twenty-one patients completed planned Capecitabine, two underwent dose reduction during treatment due to neutropenia and admission for sepsis.

Patient demographics for the 23 patients for whom paired imaging was achieved is described in Table 2. Patient demographics for all 29 registered patients is available as supplementary material.

Imaging results.

26 ROIs were delineated among the 23 evaluable patients: 23 primary tumours with median (LQ, UQ) primary gross tumour volume (GTV) 13.8 (9.7, 21.6) cm^3 and three nodes >2cm on clinical exam with volumes 16.3 cm^3, 6.5 cm^3 and 3.4cm^3 on MRI. The median (LQ, UQ) ADC_{Mean} for all 26 ROI's was 9.416 x 10^{-3} mm2/s (8.262 x 10^{-3}, 10.092 x 10^{-3}) before and 12.086 x $10^{-3}$$mm^2$/s (10.332 x 10^{-3}, 12.626 x 10^{-3}) post 8-10 fractions. Table 3 lists ADC and volumes of all 26 ROI's.

Figure 2 illustrates T_2-weighted and ADC parametric maps with ROI for both PreDW and DuringDW MRI in patient 4 who demonstrated excellent resolution of ADC. Supplementary material Figure A, demonstrates patient 15 who failed to demonstrate resolution of ADC. Among all regions, the median (95% CI) percentage change in ADC_{Mean} between scans was 20.7% (12.7%, 34.1%). Eleven of 26 lesions, had "non-resolving diffusion deficit" ROIs (Figure 3), as they showed $-\leq\leq$20% change in ADC_{Mean}. These 11 lesions were in ten patients, hence 10 of 23 patients (43% [95% CI: 23%, 66%] of patients) demonstrated "non-resolving diffusion deficit". Table 3 illustrates the ΔADC_{mean} for all patients. In the supplementary material, Figure B, demonstrates the lack of correlation between the percentage change in volume and the percentage change in ADC_{Mean}.

Clinical Follow Up

All patients remain in routine clinical follow up or have died. Twenty-two patients are alive with median FU of 41.2 months (range 22.1 to 56.3 months). Of those that failed to achieve CR or

relapsed: patient 3 in Table 3, failed to have a CR and underwent an APR for recurrent disease 9 months after completing CRT and was found to have ypT1ypN0 tumour with tumour regression grade of 2. ~~She~~ The patient~~then~~ subsequently went on to develop distant disease and is currently on systemic therapy for this; patient 13 in Table 3, had a T2N0 ASCC which went into CR at 6 months but developed metastatic disease 2 months after completing CRT, ~~he~~ and remains on systemic therapy; patient 15 in Table 3, had a T2N1 tumour developed a local relapse, identified on imaging, 11.1 months following completion of CRT and died of ASCC 12 months later; finally, patient 23 in Table 3 had a T3 N0 tumour, developed a local relapse 13.3 months after completion and underwent an APR and groin node dissection which demonstrated ypT2 ypN1a with tumour regression grade of 3.

Three of the four patients with recurrence had a ΔADC_{mean} ≤~~<~~ 20%, the other had ΔADC_{mean} 20.3%. Sensitivity and specificity of the 20% cut-off for percentage change in ADC was 75% and 63% respectively.

Discussion

Our primary endpoint was to confirm a subset of patients demonstrated a “non-resolving diffusion deficit” on DW-MRI and we identified 10 such patients, well above the 4 patients required to consider taking the concept of individualised treatment-intensification forward. In addition, we have demonstrated feasibility of acquiring, analysing and quantifying ADC changes during a course of CRT in ASCC. A secondary endpoint of correlating a predefined cut off of non-resolving diffusion deficit with relapse demonstrated that all 4 patients that relapsed were on or below the cut-off. This finding highlights that DW-MRI might have potential to select patients, who have poor outcomes on routine CRT, at an early time point to enable treatment intensification.

The studies to date of DW-MRI during CRT as an early prognostic factor in squamous cell carcinoma have repeated the DW-MRI at different timepoints during CRT and used different measures of outcome (15, 17, 19, 26, 27). There are varying results on the validity of using absolute ADC levels at outset or during CRT to predict response however the change in ΔADC between baseline and scans from the 2nd week onwards, consistently correlates with outcome. In terms of potential thresholds for prediction, Makino et al. arbitrarily selected a cut-off of 50% and reported a strong correlation with patients demonstrating a percentage change in ADC >50% and complete tumour response (28). Vandecaveye et al, King et al, and Kuang et al suggested thresholds, above which patients may be considered to have a "resolving diffusion deficit", of 14%, 15.5% and 18% respectively (16, 18, 29). With the baseline variability of ADC, a chosen threshold must incorporate some baseline variability. These previous studies were the basis of our primary endpoint of >20% increase in ADC representing "resolving diffusion deficit". However, each of these studies has a small sample size. Larger studies are needed to reliably estimate the performance of these thresholds; those reported by these small studies vary widely, making it difficult to draw firm conclusions.

DW-MRI is an attractive translational modality, MRI is non-invasive and does not use ionizing radiation. However there remain multiple challenges in DW-MRI data acquisition and interpretation including claustrophobia, motion, and image registration. As both normal and pathologic tissues can exhibit high signal intensity on high-b value DW-MRI, unambiguous definition of necrotic tissue is impossible by this method. Consequently, necrosis is dealt with differently in different publications; due to this ambiguity we chose to leave areas of possible necrosis within the volume. A further limitation is that it is generally recommended only to use the mean as an average value when data are normally distributed which is not the case in ADC analysis. There may be improved interpretation with analysis using the shape of the distribution (30), however in this analysis we are investigating the efficacy of the standard, published approach used in similar publications.

One of the limitations of this study was the small number of relapses in ASCC. This reflects the excellent outcomes with the UK IMRT guidance (31). We chose to scan only once during CRT as we envisage DW-MRI as an early prognostic factor to enable change in management plan in a subset of patients. The timeframe at which the decision to modify treatment is made, must be at a point where the patient has received a sufficiently small radiation dose that changing management is possible should dose escalation or introduction of biological agents be required, or after which the late toxicity of CRT will be negligible should the patient proceed to early surgical abdominoperineal resection (APR). Finally, we selected ≥T2 lesions, and only analysed nodes >2cm as a result of head and neck data suggesting the use of ADC analysis on lesions less than 2cm is limited and the larger the lesion the more robust the analysis. However it must be acknowledged that seven lesions were small, <10 cm^3. The potential errors in delineation, registration and calculation of ADC_{mean} are greater in small lesions. We therefore acknowledge that the potential for errors in this group will be larger.

Further work is required to better explore the potential role of DW-MRI in ASCC treatment. In a larger cohort, investigation of the correlation between ΔADC and 3-year relapse should offer further confirmation of the prognostic potential of DW-MRI, as well as provide the statistical power to confirm a threshold above which risk of relapse is sufficiently high to consider treatment intensification. Investigation into the pulse sequences and method of data analysis is required to optimise clinical relevance of DW-MRI, particularly in view of the variation in the literature. In addition, investigation is ongoing into the role of other imaging biomarkers such as FDG-PET/CT, DCE MRI, and perfusion CT in ASCC and comparisons between these modalities and with DW-MRI will ascertain which are worthy of further development. The evolving field of biological translational markers such as expression of p16/Human Papilloma Virus (HPV), Epidermal Growth Factor Receptor (EGFR), p53, presence or absence of tumour infiltrating lymphocytes and immune response (32-34) would be an excellent addition to imaging biomarkers. Whether treatment individualisation should be dose escalation, incorporation of a biological agent specifically targeted to the detected biological

markers, or abandonment of CRT for early surgery, is a question for the future. However, a decision tree, using multiple translational factors to tailor treatment to biology, is a desirable and necessary long-term goal.

Conclusions

Our observational study, with a predefined cut off, ~~confirmed~~ identified that in ~~all~~ 3 of the 4 patients with ASCC recurrence the change in ADC was below or on the predefined cut off. Further investigation in a larger cohort could optimise the technique and potentially lead the way for more radiological biomarker-led individualised treatment.

Figure 1. CONSORT diagram demonstrating the flow of patients screened, registered and eligible for analysis.

Figure 2. T_2-weighted (a) and ADC parametric maps (b) with ROI for both PreDW and DuringDW MRI in Patient 4 who demonstrated excellent resolution of ADC.

Figure 3. The percentage change in ADC_{Mean} and the disease free status of all patients to date.

Funding and Support

The authors would like to thank the OUH NHS Foundation Trust Radiology department, particularly Dr Andrew Slater for facilitating the trial; as well as the patients who took part in the ART study. The Oxford C REC ethically approved the trial. The trial was sponsored by the University of Oxford, funded by the CRUK & EPSRC Cancer Imaging Centre Oxford and managed by the Oncology Clinical Trials Office. Statistical support was provided by the Centre for Statistics in Medicine, Oxford, supported by Cancer Research UK (grant C5529/A16895). Independent oversight was provided by the Radiotherapy and Imaging Oversight Committee. M Hawkins is supported by Medical Research Council grant MC_UU_00001/2.

1. James RD, Glynne-Jones R, Meadows HM, Cunningham D, Myint AS, Saunders MP, et al. Mitomycin or cisplatin chemoradiation with or without maintenance chemotherapy for treatment of squamous-cell carcinoma of the anus (ACT II): a randomised, phase 3, open-label, 2×2 factorial trial. The Lancet Oncology. 2013;14(6):516-24.

2. Glynne-Jones R, Nilsson PJ, Aschele C, Goh V, Peiffert D, Cervantes A, et al. Anal cancer: ESMO-ESSO-ESTRO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2014;25 Suppl 3:iii10-20.

3. Gunderson LL, Winter KA, Ajani JA, Pedersen JE, Moughan J, Benson AB, 3rd, et al. Long-term update of US GI intergroup RTOG 98-11 phase III trial for anal carcinoma: survival, relapse, and colostomy failure with concurrent chemoradiation involving fluorouracil/mitomycin versus fluorouracil/cisplatin. J Clin Oncol. 2012;30(35):4344-51.

4. Gunderson LL, Moughan J, Ajani JA, Pedersen JE, Winter KA, Benson AB, 3rd, et al. Anal Carcinoma: Impact of TN Category of Disease on Survival, Disease Relapse, and Colostomy Failure in US Gastrointestinal Intergroup RTOG 98-11 Phase 3 Trial. International journal of radiation oncology, biology, physics. 2013.

5. Sebag-Montefiore D, Adams R, Bell S, Berkman L, Gilbert DC, Glynne-Jones R, et al. The development of an umbrella trial (PLATO) to address radiation therapy dose questions in the locoregional management of squamous cell carcinoma of the anus. International Journal Radiation Oncology Biology Physics. 2016;96 (2S):E164-E5.

6. Ng M, Ho H, Skelton J, Guerrieri M, Guiney M, Chao M, et al. Intensity-modulated Radiotherapy for Anal Cancer: Dose-Volume Relationship of Acute Gastrointestinal Toxicity and Disease Outcomes. Clinical oncology. 2018;30(10):634-41.

7. Kavanagh BD, Pan CC, Dawson LA, Das SK, Li XA, Ten Haken RK, et al. Radiation dose-volume effects in the stomach and small bowel. International journal of radiation oncology, biology, physics. 2010;76(3 Suppl):S101-7.

8. Michalski JM, Gay H, Jackson A, Tucker SL, Deasy JO. Radiation dose-volume effects in radiation-induced rectal injury. International journal of radiation oncology, biology, physics. 2010;76(3 Suppl):S123-9.

9. Roach M, 3rd, Nam J, Gagliardi G, El Naqa I, Deasy JO, Marks LB. Radiation dose-volume effects and the penile bulb. International journal of radiation oncology, biology, physics. 2010;76(3 Suppl):S130-4.

10. Joseph K, Vos LJ, Warkentin H, Paulson K, Polkosnik LA, Usmani N, et al. Patient reported quality of life after helical IMRT based concurrent chemoradiation of locally advanced anal cancer. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology. 2016;120(2):228-33.

11. Han K, Cummings BJ, Lindsay P, Skliarenko J, Craig T, Le LW, et al. Prospective evaluation of acute toxicity and quality of life after IMRT and concurrent chemotherapy for anal canal and perianal cancer. International journal of radiation oncology, biology, physics. 2014;90(3):587-94.

12. De Francesco I, Thomas K, Wedlake L, Tait D. Intensity-modulated Radiotherapy and Anal Cancer: Clinical Outcome and Late Toxicity Assessment. Clinical oncology. 2016;28(9):604-10.

13. Padhani AR, Liu G, Mu-Koh D, Chenevert TL, Thoeny HC, Takahara T, et al. Diffusion-Weighted Magnetic Resonance Imaging as a Cancer Biomarker: Consensus and Recommendations. Neoplasia. 2009;11(2):102-25.

14. Bollineni VR, Kramer G, Liu Y, Melidis C, deSouza NM. A literature review of the association between diffusion-weighted MRI derived apparent diffusion coefficient and tumour aggressiveness in pelvic cancer. Cancer treatment reviews. 2015;41(6):496-502.

15. Kim S, Loevner L, Quon H, Sherman E, Weinstein G, Kilger A, et al. Diffusion-weighted magnetic resonance imaging for predicting and detecting early response to chemoradiation therapy of squamous cell carcinomas of the head and neck. Clinical cancer research : an official journal of the American Association for Cancer Research. 2009;15(3):986-94.

16. King AD, Mo FK, Yu KH, Yeung DK, Zhou H, Bhatia KS, et al. Squamous cell carcinoma of the head and neck: diffusion-weighted MR imaging for prediction and monitoring of treatment response. European radiology. 2010;20(9):2213-20.

17. Harry VN, Semple SI, Gilbert FJ, Parkin DE. Diffusion-weighted magnetic resonance imaging in the early detection of response to chemoradiation in cervical cancer. Gynecologic oncology. 2008;111(2):213-20.

18. Vandecaveye V, Dirix P, De Keyzer F, de Beeck KO, Vander Poorten V, Roebben I, et al. Predictive value of diffusion-weighted magnetic resonance imaging during chemoradiotherapy for head and neck squamous cell carcinoma. European radiology. 2010;20(7):1703-14.

19. Galbán CJ, Mukherji SK, Chenevert TL, Meyer CR, Hamstra DA, Bland PH, et al. A Feasibility Study of Parametric Response Map Analysis of Diffusion-Weighted Magnetic Resonance Imaging Scans of Head and Neck Cancer Patients for Providing Early Detection of Therapeutic Efficacy. Translational Oncology. 2009;2(3):184-90.

20. Owczarczyk K, Prezzi D, Cascino M, Kozarski R, Gaya A, Siddique M, et al. MRI heterogeneity analysis for prediction of recurrence and disease free survival in anal cancer. Radiotherapy and Oncology. 2019;134:119-26.

21. Prezzi D, Mandegaran R, Gourtsoyianni S, Owczarczyk K, Gaya A, Glynne-Jones R, et al. The impact of MRI sequence on tumour staging and gross tumour volume delineation in squamous cell carcinoma of the anal canal. Eur Radiol. 2018;28(4):1512-9.

22. Liu Y, Bai R, Sun H, Liu H, Zhao X, Li Y. Diffusion-weighted imaging in predicting and monitoring the response of uterine cervical cancer to combined chemoradiation. Clinical radiology. 2009;64(11):1067-74.

23. Vandecaveye V, De Keyzer F, Nuyts S, Deraedt K, Dirix P, Hamaekers P, et al. Detection of head and neck squamous cell carcinoma with diffusion weighted MRI after (chemo)radiotherapy: correlation between radiologic and histopathologic findings. International journal of radiation oncology, biology, physics. 2007;67(4):960-71.

24. Jones M, Hruby G, Coolens C, Driscoll B, Stanwell P, Kumar M, et al. A prospective, multi-centre trial of multi-parametric MRI as a biomarker in anal carcinoma. Radiotherapy and Oncology. 2020;144:7-12.

25. Muirhead R, Adams RA, Gilbert DC, Glynne-Jones R, Harrison M, Sebag-Montefiore D, et al. Anal cancer: developing an intensity-modulated radiotherapy solution for ACT2 fractionation. Clin Oncol (R Coll Radiol). 2014;26(11):720-1.

26. Ju F-J. Evaluation of the efficacy of chemoradiotherapy in cervical cancer using diffusion-weighted imaging and apparent diffusion coefficient. OncoTargets and Therapy 2016;9:7555-61.

27. Zhang Y, Chen J-Y, Xie C-M, Mo Y-X, Liu X-W, Liu Y, et al. Diffusion-weighted magnetic resonance imaging for prediction of response of advanced cervical cancer to chemoradiation. J Comput Assist Tomogr. 2011;35:102-7.

28. Makino H, Kato H, Furui T, Morishige K, Kanematsu M. Predictive value of diffusion-weighted magnetic resonance imaging during chemoradiotherapy for uterine cervical cancer. J Obstet Gynaecol Res 2014;40:1098-104.

29. Kuang F, Yan Z, Wang J, Rao Z. The value of diffusion-weighted MRI to evaluate the response to radiochemotherapy for cervical cancer. Magnetic resonance imaging. 2014;32:342-9.

30. Just N. Improving tumour heterogeneity MRI assessment with histograms. British journal of cancer. 2014;111:2205-13.

31. Shakir R, Adams R, Cooper R, Downing A, Geh I, Gilbert D, et al. Patterns and predictors of relapse following radical chemoradiotherapy delivered using intensity modulated radiotherapy with a simultaneous integrated boost in anal squamous cell carcinoma. Int J Radiat Oncol Biol Phys. 2019;In press pre-proof. .

32. Gilbert DC, Serup-Hansen E, Linnemann D, Hogdall E, Bailey C, Summers J, et al. Tumour-infiltrating lymphocyte scores effectively stratify outcomes over and above p16 post chemo-radiotherapy in anal cancer. British journal of cancer. 2016;114(2):134-7.

33. Gilbert DC, Williams A, Allan K, Stokoe J, Jackson T, Linsdall S, et al. p16, p53, EGFR expression and KRAS mutation status in squamous cell cancers of the anus: Correlation with outcomes following chemo-radiotherapy. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology. 2013.

34. Morris VK, Salem ME, Nimeiri H, Iqbal S, Singh P, Ciombor K, et al. Nivolumab for previously treated unresectable metastatic anal cancer (NCI9673): a multicentre, single-arm, phase 2 study. The Lancet Oncology. 2017;18(4):446-53.