IMAGING BIOMARKERS FOR RISK STRATIFICATION IN COLORECTAL AND ANAL CANCER

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Thesis submitted in accordance with the regulations of Imperial College, London, for the PhD degree of Imperial College London and the Diploma of Imperial College

Dedicated to Rob, our daughter Sophia and our son Sam.

ABSTRACT

Introduction: "Imaging biomarkers" (IB) are radiological measurements that predict outcomes. Although IBs have been validated for primary colorectal cancer none are available for predicting disease relapse in colorectal or anal cancer. The aim of this thesis was to develop and implement IBs for relapse in colorectal and anal cancer.

Methods: A systematic review assessed the completeness of reporting of IB publications in colorectal liver metastases. Potential IBs for anal SCC and mrTRG response assessment in rectal cancer were tested retrospectively. Finally IB implementation was tested by retrospectively and then prospectively assessing the potential role of DW-MRI as a screening tool for synchronous liver metastases in colorectal cancer.

Results: Systematic review (n=30) found no IB studies adhered to REMARK but there were areas of good practice. mrT staging and mr-derived depth of extramural spread (DEMS) both predicted for outcome in 131 anal SCC patients, but when combined DEMS >12mm was the only predictor of outcome. mrTRG response in 338 patients predicted for recurrent or metastatic disease (OR 3.6) and described patterns of disease. Retrospectively DW-MRI detected more synchronous liver metastases in high-risk rectal cancer than CT (OR 8.065, P=0.018) with poorer 3 year OS ($p<0.05$). This led to the multicentre SERENADE study which aimed to validate screening DW-MRI in 262 patients; interim analysis showed DW-MRI detected 5% more synchronous liver metastases than CT.

Conclusions and Future Work: REMARK should be required by journals publishing IB work. DEMS is a novel potential IB for anal SCC which could result in a change to TNM staging but first requires external validation against outcomes. Findings related to mrTRG and the timing and site of metastases should be considered when planning follow-up. Risk-adapted screening for liver metastases with DW-MRI has been validated and should be adopted in clinical practice for high-risk patients.

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DECLARATIONS

The work has not already been accepted in substance for any other degree and is not being concurrently submitted in candidature for any other degree.

I declare that this thesis is my work and that everything else is appropriately referenced.

Signed  (candidate).

Date 26/07/2019

ACKNOWLEDGEMENTS

This work was carried out during my appointment as an Academic Clinical Fellow in Clinical Radiology and as a NIHR BRC Clinical Fellow in the Department of Radiology at The Royal Marsden NHS Foundation Trust.

I would like to acknowledge the work and contributions of the following people who have been important in the development and conduct of the work which has contributed to the following chapters:

Chapter 5: A systematic review of published articles in relation to the REMARK guidelines: With my supervisor Prof Brown, I designed the systematic review of published articles in relation to the REMARK guidelines building on preliminary work carried out by Manish Chand and Jenny Mainta. I would like to acknowledge the help and expertise of YiWen Hon, Knowledge Resources Manager (David Adams Library, Royal Marsden Hospital), who helped with the design and conduct of the search strategy and Caroline Martin, who helped with the design of the graphics. Finally I am grateful to my co-authors Katja De Paepe, Christina Messiou and Nina Tunariu who were the additional readers responsible for scoring the papers from which I developed a consensus score and Christos Kontovounisios for his methodological advice.

Chapter 7: Depth of extramural spread for SCC

I designed this study with my supervisor and Diana Tait based on the methodology of previous work I had done prior to undertaking my PhD. I applied for institutional review

board approval for this work and performed the data collection and analysis. I am grateful to L Bernier and SA Khaleq for their preliminary work in assessing the role of IMRT for the treatment of anal SCC within our institution and to Diana Tait who generated the patient cohort and advised on the clinical relevance and application of this study. Finally I would like to acknowledge the role of Sheela Rao in the critical appraisal of this project.

Chapter 8: MRI tumour regression grading for predicting disease relapse according to response to chemoradiotherapy.

The work presented in this chapter was initially developed by previous research fellows of Prof Brown (Stanley Yu and Jemma Bhoday). I then further developed this work in line with biomarker validation, including completion of the dataset and outcomes and data analysis.

Chapter 10: Screening diffusion weighted MRI of the liver results in increased diagnosis of synchronous liver metastases in high risk rectal cancer: A retrospective cohort study.

I generated the idea for this study with my supervisor Prof Brown and co-author Heather Harris. This was based on Heather Harris' experience in performing routine DW-MRI for all patients with rectal cancer in her institution and the previous work of Chris Hunter which showed an increased incidence of metastases in patients with high risk rectal cancer diagnosed by PET-CT and CT*(Hunter, Garant et al. 2012)*. I designed the study, gained Caldicott Approval from Chesterfield Royal Hospital and went to Chesterfield to collect the imaging data for central review. I scored the rectal MRI studies in consensus review with Prof Brown. Heather Harris collected the liver imaging and follow-up data. I then analysed the data. I am grateful to Heather Harris for her enthusiasm and hospitality throughout this study.

This work led to the development of the SERENADE study, the design and set-up of which is presented in Chapter 11 and the interim results are present in Chapter 12.

Chapters 11 and 12: Design, set-up, conduct and preliminary results of the SERENADE Study: Screening for synchronous metastases in colorectal cancer with diffusion-weighted MRI of the liver.

I was responsible for conceiving, designing and drafting the protocol for the SERENADE study and the ongoing running of the study. Along with the chief investigator Professor Gina Brown, I was lead applicant for the grant that the SERENADE Study received from the Pelican Cancer Foundation, I would like to gratefully acknowledge receipt of the £20,000 grant from the Pelican Cancer Foundation. This enabled the trial to be registered on the NIHR portfolio.

With the trials team I was responsible for supporting recruiting sites to the SERENADE study. Clinical trials teams, specialist nurses, surgeons and oncologists in each of the recruiting sites recruited patients to the SERENADE study and I supported these teams by conducting site initiation meetings and answering questions (including those about eligibility and the patient pathway within the study). Supported by Prof Brown I wrote protocol amendments for the SERENADE study. Data analysis and interpretation were performed by me, with the help of my supervisor Professor Gina Brown. I am grateful for the dedication and commitment of the clinical trials team at the Royal Marsden Hospital, including Ceri Evans, Lisa Scerri, Charlene Carvalho, Isabel Carballo-Horton, Faye Holyoake and Xian Van Gelder who managed the data collection and ongoing trial management and support. Statisticians

Karen Thomas, Omar Omar and Ann Petruckevitch provided guidance and statistical support.

I also wish to acknowledge and thank the radiologists, oncologists, surgeons, nurse specialists and research teams from the sites recruiting to the SERENADE study who were responsible for the recruitment of patients and collection of data. A list of the sites open for recruitment to the SERENADE study and the Principle Investigators at each site is provided in Table 11-2.

I would like to acknowledge funding from the Pelican Cancer Foundation (£20,000) and funding and support from the NIHR Biomedical Research Centre at the Royal Marsden and the Institute of Cancer Research. 'This work represents independent research part funded by the National Institute for Health Research (NIHR) Biomedical Research Centre at The Royal Marsden NHS Foundation Trust and the Institute of Cancer Research and The Pelican Cancer Foundation. The views expressed are those of the author and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care.'

Finally, I would like to thank my family and friends for their ongoing belief and support and my supervisors Professor Gina Brown and Dr Christina Messiou, to whom I am greatly indebted for their expert guidance and encouragement over the past years.

PUBLICATIONS RELATED TO THIS THESIS

Background to this thesis has been published as part of a Special Issue of Colorectal Disease (Volume 20, Issue S1): Proceedings of the Future Horizons International Experts Meeting in Colorectal Cancer Held in London on 25th June 2015. Edited by: Dr Anita Wale and Prof Gina Brown.

- **Wale, A.** Wexner, SD. Saur, NM. Massarotti, H. Laurberg, S. Kennedy, E. Rockall, A. Sebag-Montefiore, D. Brown, G. (2018) Session 1: The evolution and development of the multidisciplinary team approach: USA, European and UK experiences–what can we do better? *Colorectal Disease* 20. Page 17-27
- Tudyka, V. Madoff, R. **Wale, A**. Laurberg, S. Yano, H. Brown, G. (2018) Session 1: Colon cancer–10 years behind the rectum. *Colorectal Disease*. 20. Page 28-33
- Balyasnikova, S. Haboubi, N. **Wale, A**. Santiago, I. Morgan, M. Cunningham, D. Mason, M. Berho, M. Brown, G. (2018) Session 2: Extramural vascular invasion and extranodal deposits: should they be treated the same? *Colorectal Disease* 20. Page 43-48
- **Wale, A**. Van Cutsem, E. Rao, S. Cunningham, D. Brown, G. (2018) Session 2: Synchronous metastatic disease-liver first or primary first? The oncologist decides. *Colorectal Disease* 20. Page 52-55
- Patel, A. Holm, T. **Wale, A**. Rutten, H. Nicholls, J. Hawkins, M. Steele, RJC. Marks, J. Brown, G. (2018) Session 3: Beyond the boundaries of Total Mesorectal Excision–where surgeons fear to tread. *Colorectal Disease* 20. Page 61-64

- Patel, A. Chang, G. **Wale, A**. Chong, I. Rutten, H. Nicholls, J. Hawkins, M. Steele, RJC. Marks, J. Brown, G. (2018) Session 3: Intra-operative radiotherapy–creating new surgical boundaries. *Colorectal Disease* 20. Page 65-75
- Balyasnikova, S, Vuong, T, **Wale, A**, Chong, I, Rutten, H, Brown, G. (2018) Session 3: Boosting primary and recurrent rectal cancer: how far can we push the radiotherapy envelope? *Colorectal Disease* 20. Page 88-91

The chapters of this thesis have been prepared as manuscripts ready for submission to peer-reviewed journals.

- **A. Wale**, K De Paepe, C Messiou, N Tunariu, KC. Kontovounisios, G. Brown. "Reporting of prognostic imaging biomarker studies in metastatic colorectal cancer: a systematic review of published articles in relation to the REMARK guidelines".
- **A Wale**, L Bernier, S A Khaleq, S Rao, D, Tait, G, Brown. "MRI predicts for progressive disease and survival in patients with anal cancer treated with chemoradiation. "
- **A Wale**, J Bhoday, S Yu, D Tait, G Brown. "Can magnetic resonance tumour regression grade (mrTRG) predict the timing and patterns of distant metastases in patients with locally advanced rectal cancer post chemoradiotherapy?".
- **A Wale**, H Harris, G Brown. "Screening diffusion weighted MRI of the liver results in increased diagnosis of synchronous liver metastases in high risk rectal cancer: a retrospective cohort study."

Some background and results of this thesis have been presented as a poster:

- **A Wale**, H Harris, G Brown. "Screening diffusion weighted MRI of the liver results in increased diagnosis of synchronous liver metastases in high-risk rectal cancer". Tenth

NIHR Infrastructure Doctoral Research Training Camp, 3rd-5th July 2019. Winner of the Best Poster Award.

CHAPTER 1 – BACKGROUND - GENERAL INTRODUCTION

Colorectal cancer incidence and survival

Approximately 42,000 patients are diagnosed with colorectal cancer annually and colorectal cancer accounts for 10% of all cancer deaths within the UK*(Cancer Research UK 2019b)*. Rectal cancer accounts for 23.1% of new cases of colorectal cancer in females and 31.5% of new cases in males*(Cancer Research UK 2019b)*. The remainder of tumours occur elsewhere within the colon.

Mortality associated with colorectal cancer has reduced by 44% since the 1970s*(Cancer Research UK 2019c)* with survival in the UK more than doubling over the same period*(Cancer Research UK 2019d)*. Overall survival is closely related to the AJCC stage*(American Joint Committee on Cancer (AJCC) 2017)* at diagnosis with 1 year overall survival of 98% for stage I disease, 91-93% for stage II, 85-89% for stage III and 35-44% for stage IV tumours*(Office for National Statistics 2016)*. 5 year overall survival is 95-100% for stage I cancer and drops to 7-8% for stage IV cancer*(Cancer Research UK 2019d)*.

An overview of the clinical presentation of colorectal cancer

Patients with colorectal cancer predominantly present either symptomatically, or through screening, although 20% present as an emergency*(Healthcare Quality Improvement Partnership Ltd. 2018)*; these patients are more likely to present with metastatic disease (26.3% versus 18.6% of GP referrals)*(Healthcare Quality Improvement Partnership Ltd. 2018)*. 76% of all patients are treated with curative intent*(Healthcare Quality Improvement Partnership Ltd. 2018)*.

Colonoscopic evaluation and biopsy is used to obtain a histological diagnosis. Patients are then staged by imaging with contrast enhanced CT of the thorax, abdomen and pelvis (CE-CT) primarily for the identification of metastatic disease*(National Institute for Health and Care Excellence (NICE) 2011a)*, *(The Royal College of Radiologists 2014)* and, for patients with rectal cancer, a high-resolution MRI of the rectum is mandated in the UK*(National Institute for Health and Care Excellence (NICE) 2011a)* and in many other countries*(Australian Cancer Network Colorectal Cancer Guidelines Revision Committee 2005)*, *(Glynne-Jones, Wyrwicz et al. 2017)*, *(Ksar Study Group for Rectal Cancer 2017)* for the identification of validated poor prognostic factors. Local tumour staging can also be derived for colon cancer on CE-CT but anecdotally the provision of CT derived T and N stage for colon cancers is patchy nationally.

Following a histological diagnosis, multi-disciplinary team (MDT) discussion is mandated in the UK to decide upon the treatment pathway. MDT discussion reduces treatment variation nationally*(Healthcare Quality Improvement Partnership Ltd. 2018)* and, specifically for rectal cancer, MDT discussion before surgery has been shown to reduce the rate of pathological circumferential resection margin positivity*(Burton, Brown et al. 2006)*. A similar approach of MDT discussion is therefore being adopted in the USA*(Wale, Wexner et al. 2018)*.

Pathological staging of colorectal cancer

Currently, once a histological diagnosis of malignancy has been obtained, the treatment pathway for colorectal cancer is primarily determined by the presence or absence of poor prognostic features within the primary tumour on imaging and whether the patient presents

with metastatic disease. Further factors, including the patient's performance status, other comorbidities and their wishes also impact upon the management offered. Historically though, staging was performed and prognostic features were only determined following examination of the pathological resection specimen. Irrespective of the modality used, staging aims to determine the how far the tumour has spread and increasingly aims to provide prognostic information.

Dukes' Staging

Pathological staging began in 1926 with a paper from Lockhart-Mummery which assessed the outcomes of 200 patients who'd undergone surgery for rectal cancer. Patients were subcategorised into 3 categories (listed in Table 1-1) which conferred for the chance of disease-free survival (DFS)*(Lockhart-Mummery 1926)*.

Table 1-1: Staging defined by Lockhart-Mummary in 1926(*Lockhart-Mummery 1926*)

Category	Description	5 year DFS
A	"Very favourable cases where the growth was small and had not apparently invaded the muscular coat, and no glands were involved."	73.7%
B	"Medium cases where there was involvement of the muscular coat, but where the growth was not unduly fixed and there was no extensive involvement of glands."	44.1%
C	"Very bad cases, where the growth was large and fixed, or where there was evidence of extensive involvement of glands. These were borderline cases with a bad prognosis."	44.4%

These categories were refined by Cuthbert Dukes in 1932 reporting 3 year survival*(Dukes 1932)*(Table 1-2), and were subsequently refined further in 1958 with a subdivision of stage C according to the presence or absence of disease within the most apical lymph node which was reflected in 5 year overall survival of 40% and 20% respectively*(Dukes and Bussey 1958)*.

Table 1-2: Staging refined by Dukes in 1932*(Dukes 1932)*

Category	Description	3 year OS
A	Growth limited to the wall of the rectum.	80%
B	Extension of growth into the extrarectal tissues, no regional lymph node metastases.	73%
C	Metastases in regional lymph nodes.	7%

Modified Dukes' staging was adapted by other authors in the 1940s and 1950s*(Kirklin, Dockerty et al. 1949)*, *(Astler and Coller 1954)*. In 1967 a final modification to Dukes' staging was made to introduce a D category to include distant spread and/or extensive local disease. This was shown to be prognostic in 1975 with 5 year overall survival of 67% in Dukes C tumours and 14% in Dukes D tumours*(Turnbull, Kyle et al. 1967)*.

TNM Staging and AJCC Stages

Dukes' Staging was augmented and eventually superseded by the 'Tumour, Node, Metastasis' (TNM) method of staging 1980s by a combined effort of the American Joint Committee on Cancer (AJCC) and Union for International Cancer Control (UICC) which resulted in a co-ordinated publication of the AJCC Cancer Staging Manual and the TNM Classification of Malignant Tumours. New editions are published every 6-8 years to reflect advances in cancer care*(American Joint Committee on Cancer (AJCC) 2017)*. Multiple editions of the AJCC staging manual have existed and we are currently on the 8th edition; although each of the editions has not been universally adopted with disagreements from the scientific community regarding their validity.

The T and N components of the TNM Classification and the AJCC stage are primarily derived for and from pathological staging although they have been adopted for imaging-based

staging assessments. In order to distinguish between the modality used to determine the stage the tumour the convention increasingly is to adopt a lowercase letter before the stage to denote the modality. For example, pathological staging is denoted by pTNM and MRI derived staging by mrTNM.

The TNM and AJCC staging systems have traditionally been based on anatomic features only, but since TNM 6 the inclusion of non-anatomic criteria have been included, though only as modifiers of the existing T, N and M groups*(American Joint Committee on Cancer (AJCC) 2017)*. This is increasingly important in colorectal cancer where additional features are being discovered and validated as predictive of prognosis.

Colorectal tumours originate within the mucosal and submucosal layers of the bowel wall and then invade through the muscular layers of the bowel wall into the surrounding fat. The depth of extramural spread therefore determines the T stage, Figure 1-1.

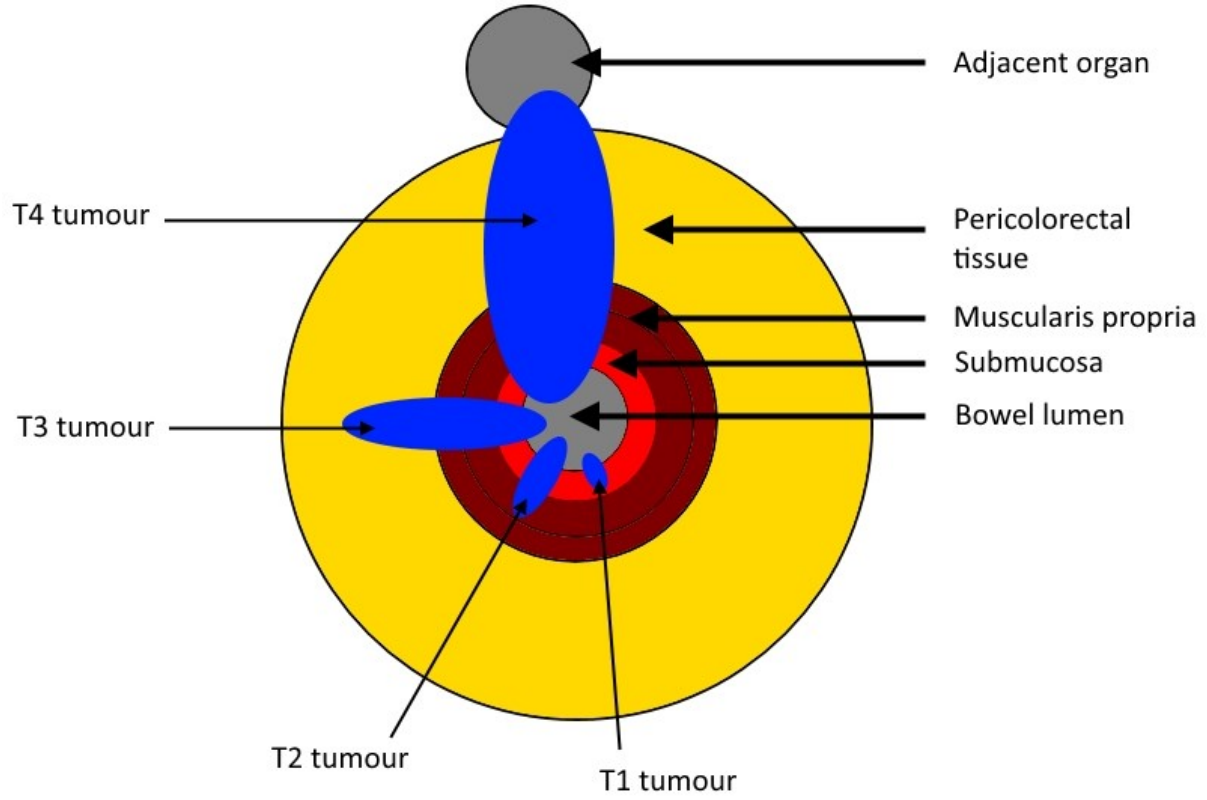


Figure 1-1: T staging for colorectal cancer as determined by the depth of extramural spread

The presence of involved lymph nodes or extranodal tumour deposits (ENTDs) accounts for the N stage, and the presence of distant metastases the M stage. The eighth edition of the TNM Classification for Colorectal Cancer is outlined in Table 1-3 with the corresponding AJCC stage in Table 1-4.

Table 1-3: TNM8 staging for Colorectal Cancer*(American Joint Committee on Cancer (AJCC) 2017)*

Primary tumour (T stage)	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ
T1	Tumour invades submucosa
T2	Tumour invades the muscularis propria
T3	Tumour invades through the muscularis propria into pericolorectal tissues
T4	Tumour invades the visceral peritoneum (T4a) or invades or adheres to adjacent organ or structure(T4b)
Regional lymph nodes (N stage)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	1-3 regional lymph nodes are positive
N1c	No regional lymph nodes are positive but there are tumour deposits in the subserosa, mesentery or non-peritonealised pericolic, perirectal or mesorectal tissues
N2	Four or more regional nodes are positive
Distant metastases (M stage)	
MX	Presence of distant metastases cannot be assessed
M0	No distant metastases by imaging
M1	Metastasis to one or more distant sites or organs or peritoneal metastasis is identified: M1a: Metastasis to one site or organ without peritoneal metastasis M1b: Metastasis to two or more sites or organs without peritoneal metastasis M1c: Metastasis to the peritoneal surface alone or with other site or organ metastases

Table 1-4: AJCC prognostic stage groups for colorectal cancer*(American Joint Committee on Cancer (AJCC) 2017)*

AJCC Stage	T stage	N stage	M stage
Stage 0	Tis	N0	M0
Stage I	T1 or T2	N0	M0
Stage IIA	T3	N0	M0
Stage IIB	T4a	N0	M0
Stage IC	T4b	N0	M0
Stage IIIA	T1 or T2 or T3	N1-3	M0
Stage IIIB	T3-T4a and T2-T3 and T1-T2 and	N1/N1c N2a N2b	M0
Stage IIIC	T4a and T3-T4a and T4b and	N2a N2b N1-N2	M0
Stage IVA-C	Any T	Any N	M1

The AJCC stage is used to report survival figures (see previous) and incidence figures. In England between 2014 and 2016 15% of patients presented with Stage I disease, 23% with stage II, 26% with stage III and 22% with stage IV*(Cancer Research UK 2019b)*, Figure 1-2.

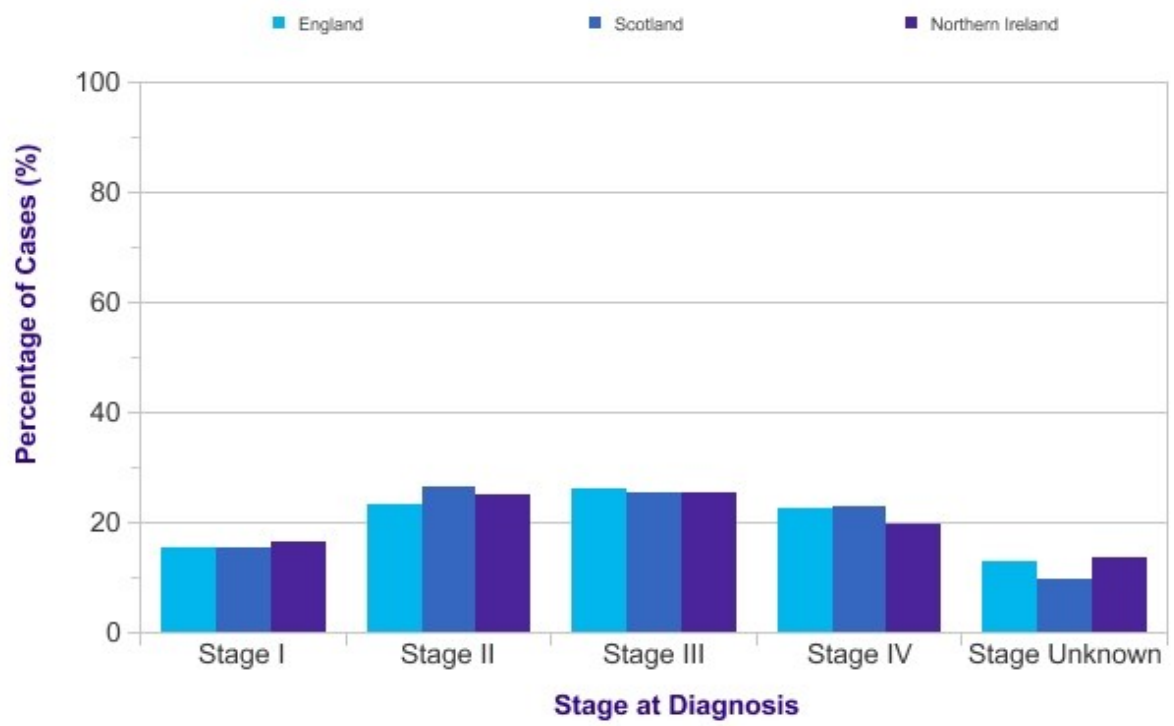


Figure 1-2: Proportion of Colorectal Cancer Cases Diagnosed at Each Stage

Credit: Cancer Research UK*(Cancer Research UK 2019b)*. Specific permission to reproduce this image was not required.

Imaging based staging of colorectal cancer

The adoption of routine imaging-based staging of colorectal cancer led to the identification and validation of imaging staging beyond the AJCC TNM stage to include other poor prognostic factors and imaging biomarkers for disease status. Imaging-based staging is now the mainstream of pre-operative staging and accompanied the transition from purely adjuvant to neoadjuvant chemoradiotherapy which is associated with improved outcomes*(Sauer, Becker et al. 2004)*, *(Ceelen, Fierens et al. 2009)*, *(Roh, Colangelo et al. 2009)*, *(Sebag-Montefiore, Stephens et al. 2009)*.

Patients with rectal cancer should be staged by high-resolution MRI of the rectum for T and N staging and a CE-CT for M staging*(National Institute for Health and Care Excellence (NICE) 2011a)*, *(The Royal College of Radiologists 2014)*. Patients with cancer originating elsewhere within the colon should be staged with CE-CT for T, N and M staging*(National Institute for Health and Care Excellence (NICE) 2011a)*, *(The Royal College of Radiologists 2014)*.

MRI for the staging of primary rectal cancer

Patients with rectal cancer should have their primary tumour (T stage) and associated lymph nodes and/or extranodal tumour deposits (N stage) assessed by high-resolution MRI. The quality of the MRI scans and the experience and degree of specialist training of the reporter are both crucial in ensuring the validated prognostic factors on MRI are accurately identified. This is discussed in detail in Chapter 3 – General Methods.

T staging

T staging is determined by the extension of the tumour through the bowel wall. TNM8 and the AJCC staging system consider any tumour growth beyond the bowel wall to be a poor prognostic factor*(American Joint Committee on Cancer (AJCC) 2017)*, resulting in all patients with T3 tumours treated in the USA receiving neoadjuvant therapy with the aim of downstaging their disease*(National Comprehensive Cancer Network 2018)*.

However seminal work by Hermanek *et al* in 1993*(Hermanek, Henson et al. 1993)* showed T3 tumours are a heterogeneous group on pathological specimens with excellent outcomes for those with ≤1mm extramural spread, good outcomes for those with >1mm - ≤5mm

extramural spread and poorer outcomes for those with >5mm spread. This resulted in the subclassification of T3 tumours into four groups (Table 1-5).

Table 1-5: pT3 subclassification

pT3 subclassification	Definition
T3a	Tumour growth <1 mm beyond the border of the muscularis propria
T3b	Tumour growth 1–5 mm beyond the border of the muscularis propria
T3c	Tumour growth >5–15 mm beyond the border of the muscularis propria
T3d	>15 mm beyond the border of the muscularis propria

These findings were confirmed by Merkel *et al* in 2001 who confirmed that the 5mm cut-off for good and poor prognostic tumours was clinically significant*(Merkel, Mansmann et al. 2001b)*. The authors looked at two groups of patients with pT3 tumours; 514 from the Erlangen Registry for Colo-Rectal Carcinomas and 371 from the Study Group for Colo-Rectal Carcinoma (SGCRC). They showed significant differences in survival and recurrence rates between tumours with ≤5mm invasion and >5mm of invasion beyond the muscularis propria in at least one set of patients*(Merkel, Mansmann et al. 2001b)*. The results are summarised in Table 1-6. Therefore it was concluded again that the pT3 category within the TNM staging should be subdivided*(Merkel, Mansmann et al. 2001a)*, *(Merkel, Mansmann et al. 2001b)* according to the "histological measurement of the maximal tumour invasion beyond the outer border of the muscularis propria"*(Mann 2001)*. The findings have been further confirmed by a recent meta-analysis by Siddiqui *et al(Siddiqui, Simillis et al. 2018)*

Table 1-6: Inhomogeneity of pT3 tumours*(Merkel, Mansmann et al. 2001b)*

	Erlangen Registry for Colo-Rectal Carcinomas n = 514		**Study Group for Colo-Rectal Carcinoma n = 371**	
Outcome	**pT3a (≤5mm)**	**pT3b (>5mm)**	**pT3a (≤5mm)**	**pT3b (>5mm)**
5-year cancer-related survival	85.4% (95% CI 80.5-90.5%)	54.1% (95% CI 48.5 – 60.5%)	71.0% (95% CI 63.9 – 78.8%)	55.0% (95% CI 48.4 – 62.5%)
5-year local recurrence rate	10.4% (95% CI 6.0-14.7%)	26.3% (95% CI 20.6-31.6%)	25.5% (95% CI 18.1-32.2%)	26.3% (95% CI 25.8-39.6%)

Tumours disrupt the normal layers of the rectal wall which is readily appreciated by high resolution MRI, Figure 1-3. The invasion of tumour through and beyond the bowel wall can therefore be seen as intermediate signal intensity which disrupts the normal anatomy of the rectal wall. This is demonstrated in Figure 1-4.

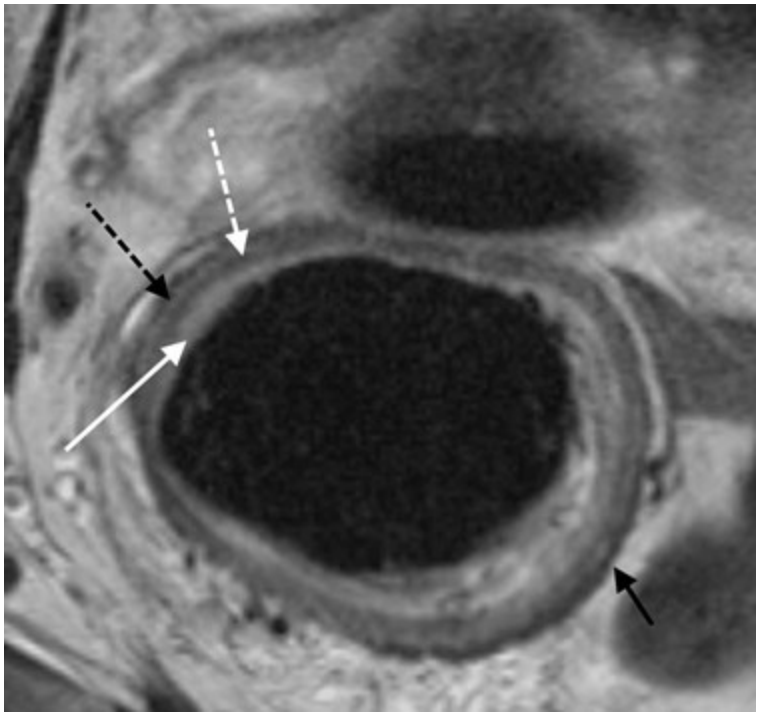
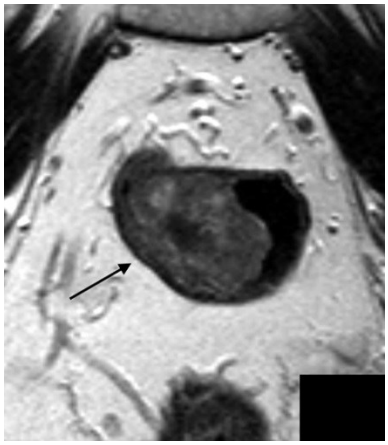


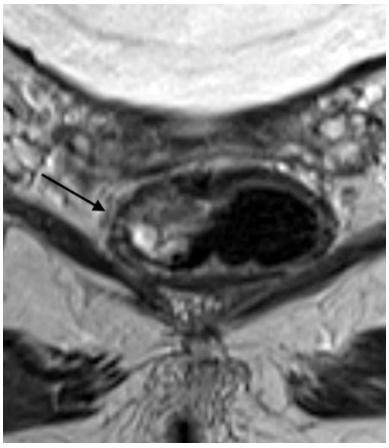
Figure 1-3: High-resolution axial T2-weighted MRI which shows the normal anatomy of the rectal wall.

Annotations: submucosa (solid white arrow), the circular muscle of the muscularis propris (dashed white arrow), the myenteric plexus (dashed black arrow), and the longitudinal muscle of the muscular propria (solid black arrow).

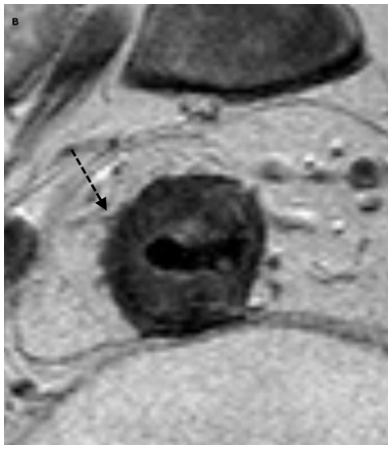
Reproduced with permission from *(Wale and Brown 2014)*



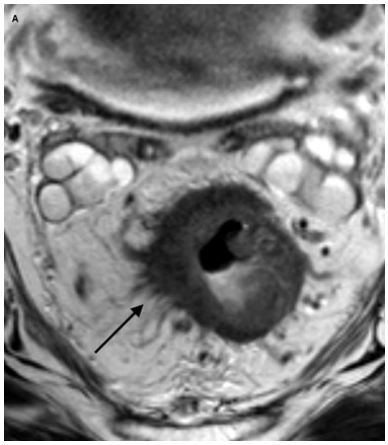
T1 sm2 tumour



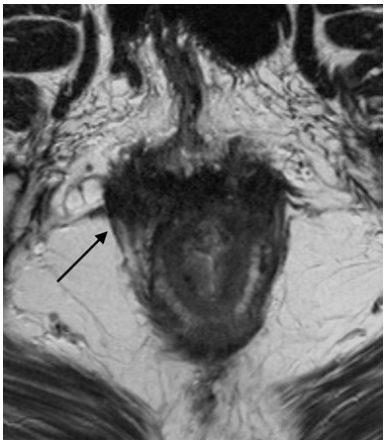
T2 tumour



T3b tumour



T3c tumour



T4b tumour with invasion into the vagina anteriorly

Figure 1-4: High-resolution axial T2-weighted MRI scans of T1, T2, T3b, T3c and T4 tumours. The black arrows show the invasive border of each of the tumours. Reproduced with permission from *(Wale and Brown 2014)*

The assessment of T stage by MRI was advanced in 2003 by Brown *et al* who prospectively assessed the accuracy of preoperative high-resolution MRI for the determination of the amount of tumour spread beyond the bowel wall or "depth of extramural spread"*(Brown, Radcliffe et al. 2003)*. This study of 98 patients showed 94% weighted agreement (weighted κ = 0·67) between the MRI and histopathological assessment of T stage*(Brown, Radcliffe et al. 2003)*.

The Magnetic Resonance Imaging and Rectal Cancer European Equivalence (MERCURY) Study published in 2007 then validated MRI for the assessment of depth of extramural spread (including mrT3 substaging) against the histological gold standard. The MERCURY Study showed MRI and histopathology measurements of depth of extramural spread were considered equivalent to within 0.5mm*(Mercury Study Group 2007)*.

The 5-year follow-up of the MERCURY Study then validated the use of MRI as a prognostic imaging biomarker for differentiating between good and poor prognostic factors (Table 1-7), including T stage, where "good" prognostic tumours were associated with low local recurrence rate and good 5 year overall survival and disease free survival of 3.3%, 68.2% and 84.7% respectively*(Taylor, Quirke et al. 2011a)* .

Table 1-7: MRI features used to determine good prognosis and poor prognosis in the MERCURY Study*(Taylor, Quirke et al. 2011a)*

MRI feature	**Good prognosis**	**Poor prognosis**
T stage	T1, T2, T3 ≤5mm extramural spread (i.e. T3a & T3b)	T3 >5mm extramural spread (i.e. T3c & T3d) and T4
N stage	Any	Any
EMVI	Negative	Positive
CRM	>1mm – clear	<1mm - involved

In addition, the MERCURY Study provided the basis for the methodology for studies validating prognostic features on MRI with a sample size of 679 patients from 11 centres in the UK. Crucially this study used 18 specialist GI radiologists who had undergone workshop training to ensure standardisation of the MRI technique, interpretation and reporting criteria of the features assessed*(Mercury Study Group 2007)*.

Circumferential resection margin

The rectum is housed within its own layer of connective tissue, the mesorectal fascia. Within the mesorectal fascia lies the peri-rectal fat, lymphatics and blood supply to the rectum.

A major step forward was achieved in the treatment of rectal cancer with the development of Total Mesorectal Excision (TME) surgery for rectal cancer in 1982*(Heald, Husband et al. 1982)*. The adoption of TME surgery as standard for rectal cancer reduced local recurrence rates from the then accepted rate of 20-40%*(Dahl, Horn et al. 1990)* to 3%-6% with a complete resection (R0 resection)*(Heald, Moran et al. 1998)*, *(Martling, Holm et al. 2000)* and has been validated by multiple authors*(MacFarlane, Ryall et al. 1993)*, *(Quirke, Steele et al. 2009)*.

The principles of TME surgery are that the surgeon should remove the rectum and the entire mesorectal envelope, defined by the mesorectal fascia, intact. This means that the draining lymphatics and vasculature are removed according to their embryological derivation*(Heald 1988)* which reduces local recurrence. The mesorectal fascia forms the circumferential resection margin (CRM), the plane along which the surgeon must operate during TME surgery. The histopathological assessment of an involved circumferential resection margin, defined as tumour within 1mm of the CRM, is known to be a predictor of local recurrence and poor survival*(Quirke, Durdey et al. 1986)*, *(Cawthorn, Parums et al. 1990)*, *(Adam, Mohamdee et al. 1994)*, *(Birbeck, Macklin et al. 2002)*, *(Wibe, Rendedal et al. 2002)*, *(Nagtegaal and Quirke 2008)*.

The mesorectal fascia and therefore the CRM are well seen on MRI as a thin dark line which surrounds the rectum (Figure 1-5). For this reason, the ability of MRI to identify tumour close to or involving the CRM, and therefore decreasing the risk of an incomplete resection (R1 or R2 resection) was of interest.

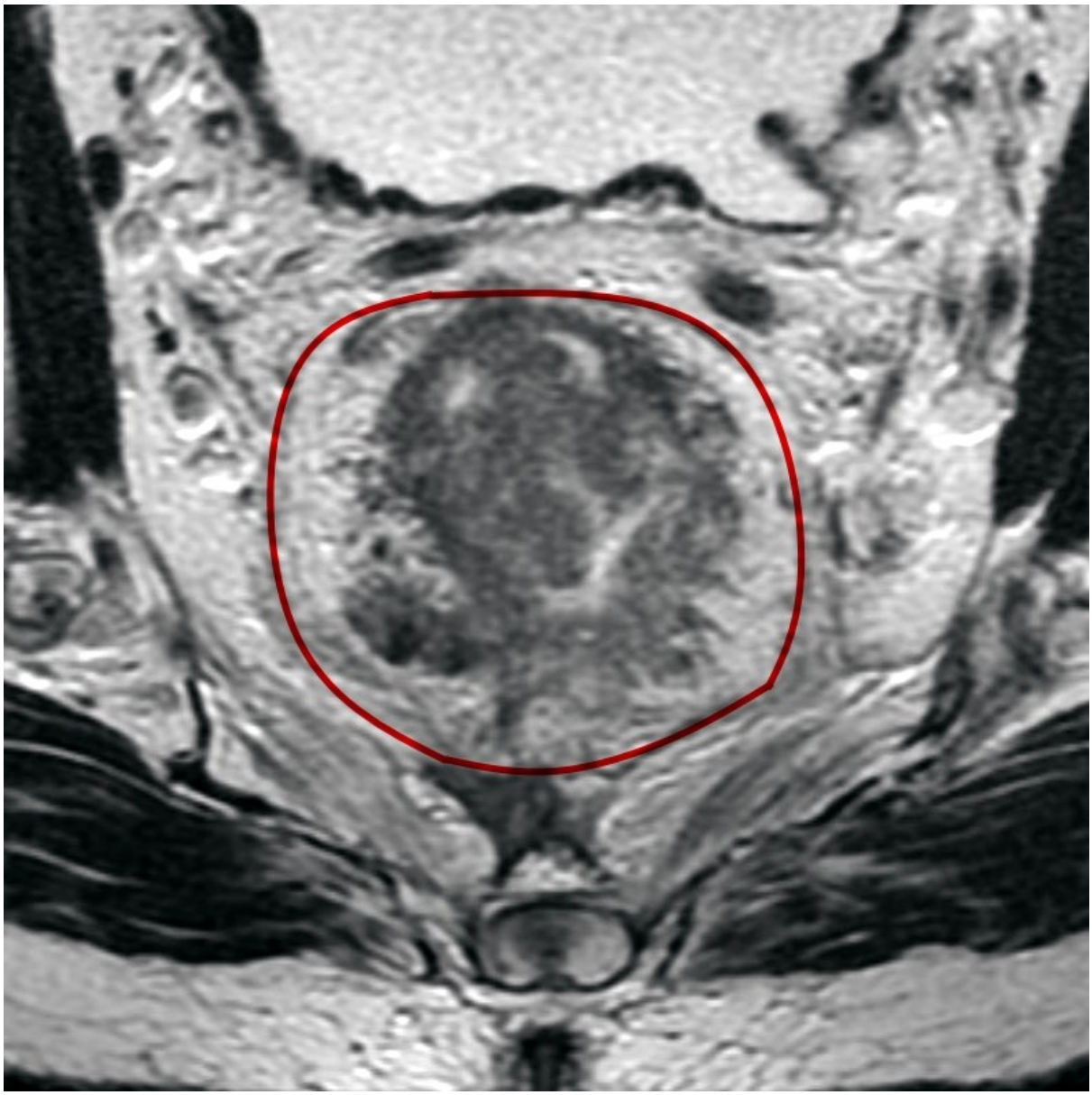


Figure 1-5: Circumferential resection margin as shown on high-resolution T2-weighted MRI

The mesorectal fascia which forms the circumferential resection margin is outlined in red.

Brown *et al* prospectively investigated the correlation between the histological and MRI measurements of the distance between the tumour to the CRM using the 1mm cut-off previously validated by histology*(Brown, Radcliffe et al. 2003)*. This study showed MRI predicted pCRM involvement with 92% agreement (κ = 0·81). Beets-Tan then retrospectively showed the distance between the tumour to the CRM on MRI correlated with histopathological measurements*(Beets-Tan, Beets et al. 2001)*. In 2006, the prospective, multicentre MERCURY Study confirmed the diagnostic accuracy of MRI in measuring the distance from the outer edge of the tumour to the mesorectal fascia and confirmed that MRI could predict for a histologically negative CRM across multiple countries and radiologists*(Mercury Study Group 2006)*.

There was, however, some debate within the literature as to the most appropriate cut-off of mrCRM to predict for local recurrence. Beets-Tan *et al* showed a correlation between a mr-derived 5mm distance between the tumour and the mesorectal fascia and a subsequent negative pathological CRM*(Beets-Tan, Beets et al. 2001)*. Beets-Tan *et al* therefore concluded that neoadjuvant therapy should be offered to all patients with tumour within 5mm of the mrCRM*(Beets-Tan, Beets et al. 2001)*. However the MERCURY Study used the 1mm cut-off previously validated on histopathological specimens*(Mercury Study Group 2006)*.

Analysis of the MERCURY Study data was conducted in 2011 to assess what the best mr-derived cut-off for the tumour distance to the CRM would be*(Taylor, Quirke et al. 2011b)*. 374 patients were included. Multivariate analysis showed that a mr-derived 1mm cut-off of the tumour to the CRM predicted for local recurrence with a HR of 3.72, 95% CI 1.43-9.71,

P=0.007. The 5-year local recurrence rate for patients with tumours ≤1mm from the CRM on MRI (and therefore deemed CRM positive on MRI) was 20% compared to 4-8% with larger margins. This study therefore confirmed a cut-off of 1mm was optimal for the mr-derived definition of an involved CRM*(Taylor, Quirke et al. 2011b)*. The authors then validated this cut-off against outcomes in 2014 and found mrCRM positivity (≤1mm) predicted for 5 year OS, 5 year DFS and local recurrence rates on univariate and multivariate analysis, Table 1-8, and showed mrCRM positivity was significantly associated with the development of distant metastatic disease*(Taylor, Quirke et al. 2014)*

Table 1-8: Univariate and Multivariate Analyses of mrCRM status using Cox Regression Model (N 374)*(Taylor, Quirke et al. 2014)*

	5 year OS			5 year DFS			Time to local recurrence	
mrCRM	**%**	**HR Univariate**	**HR Multivariate**	**%**	**HR Univariate**	**HR Multivariate**	**HR Univariate**	**HR Multivariate**
Clear (>1mm)	62.2%			67.2%				
Involved (≤1mm)	42.2%	**1.99** (95% CI 1.39-2.89) P<0.001	**1.97** (95% CI 1.27-3.04) P<0.05	47.3%	**1.96** (95% CI 1.31-2.94) P<0.01	**1.65** (95% CI 1.01-2.69) P<0.05	**3.9** (95% CI 1.99-7.62) P<0.001	**3.50** (95% CI 1.53-8.00) P<0.05

Extramural vascular invasion

Extramural vascular invasion (EMVI) is defined as "the presence of tumour cells in the vasculature beyond the muscularis propria"*(Smith, Barbachano et al. 2008)* and has been recognised as a poor prognostic factor in rectal cancer since Brown and Warren's 1938 paper*(Brown 1938)*. EMVI is a sign of more advanced disease as, by virtue of its requirement

to be tumour cells with the vasculature beyond the muscularis propria, it is a determinant of T3 and T4 disease*(Smith, Barbachano et al. 2008)*.

Traditionally EMVI has been detected on histopathological specimens but the reported incidence of pEMVI varies between 9-50%*(Brown 1938)*, *(Talbot, Ritchie et al. 1980)*, *(Freedman, Macaskill et al. 1984)*, *(Brown, Radcliffe et al. 2003)*, *(Messenger, Driman et al. 2011)*, *(Messenger, Driman et al. 2012)*. The pathological detection of EMVI is challenging, especially following neoadjuvant treatment with chemoradiotherapy which results in distortion of the normal microarchitecture and false negative reporting of EMVI*(Ouchi, Sugawara et al. 1996)*, *(Liang, Nakada et al. 2007)*, *(Chand, Evans et al. 2015)*.

EMVI is readily appreciated on MRI as serpinginous enlargement of the rectal veins with absence of the normal flow void consistent with the presence of tumour cells within the vessel (Figure 1-6).

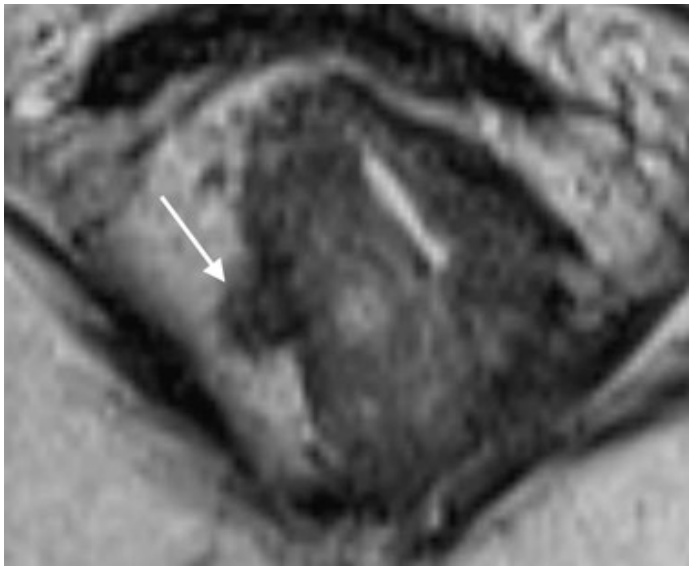


Figure 1-6: EMVI is readily appreciated on high-resolution T2 weighted MRI

White arrow shows the large vessel EMVI which extends into the mesorectal fat. Reproduced with permission from *(Wale and Brown 2014)*.

Whilst the initial studies investigating mrEMVI compared the accuracy of MRI against pathology with good agreement*(Brown, Radcliffe et al. 2003)*, *(Smith, Barbachano et al. 2008)* the mr detection of EMVI is now regarded as the gold-standard method rather than histopathology.

mrEMVI has an average reported prevalence of 34.6% (range 19.8% - 57.4%)*(Siddiqui, Simillis et al. 2017)* but is seen more frequently in advanced disease present in 24.5% of patients with stage II rectal cancer and 44.9% of patents with stage III disease*(Chand, Bhangu et al. 2014)*.

mrEMVI has been validated against outcomes. The presence of mrEMVI is an independent risk factor for 3 year recurrence free survival (74% versus 35% for mrEMVI negative and positive rectal tumours respectively, p<0.001*(Smith, Barbachano et al. 2008)*) and disease free survival (adjusted HR of 2.08, 95% CI 1.66-4.52 for mrEMVI positive tumour*(Chand, Swift et al. 2014a)*. A recent meta-analysis of 6 articles which reported mrEMVI status in 1262 patients showed the presence of mrEMVI conferred a 5-fold increased risk of metastatic disease at presentation (OR 5.68, P<0.001) and a 4-fold increased risk of developing metastases during follow-up (OR 3.91, p<0.001)*(Siddiqui, Simillis et al. 2017)*. mrEMVI status has therefore been incorporated into the risk stratification of patients into those with good versus poor prognostic tumours*(Taylor, Quirke et al. 2011a)* and its

presence is a determinant of the requirement to downstage tumours with neoadjuvant chemoradiotherapy.

Furthermore the presence of persistent mrEMVI after treatment with neoadjuvant chemoradiotherapy (ymrEMVI) has also been shown to be a poor prognostic factor resulting in poorer 3 year disease free survival, although this can be mitigated somewhat by the use of adjuvant chemotherapy*(Chand, Rasheed et al. 2017)*. A study of 631 patients all treated with neoadjuvant chemoradiotherapy and surgery compared the outcomes of the 227 patients in their cohort who had persistent mrEMVI after neoadjuvant chemotherapy according to the adjuvant treatment strategy employed. 158/227 patients were treated with adjuvant chemotherapy and 69 patients were followed up with observation alone. The cohort had a 36% (22/631) incidence of persistent EMVI following CRT. Those treated with adjuvant chemotherapy had a survival benefit on multivariate analysis (HR 0.458, p=0.004) with improved percentage 3 year survival of 74.6% compared to 54.7% in the observation only group*(Chand, Rasheed et al. 2017)*.

Correspondingly the conversion of patients from mrEMVI positive at diagnosis to mrEMVI negative following CRT is associated with improved survival*(Chand, Evans et al. 2015)*.

N staging

The assessment of nodal status has been regarded as important to prognosis since the initial staging systems proposed by Lockhart-Mummery*(Lockhart-Mummery 1926)* and modified by Dukes*(Dukes 1932)* and still remains integral to the TNM 8 and AJCC cancer staging systems*(American Joint Committee on Cancer (AJCC) 2017)*.

However, whilst the presence of involved lymph nodes was a predictor of local recurrence in the pre-TME era, it is now increasingly understood that, with good quality TME which removes the entirety of the mesorectal lymph nodes, lymph node status no longer predicts for local recurrence*(Chand, Moran et al. 2016)*. This is supported by evidence from the CR07 trial which showed that nodal status only predicted for local recurrence when there was poor TME technique*(Quirke, Steele et al. 2009)*. The pelvic recurrence rate was 20% for node positive tumours when poor TME technique was employed compared to 6% and 5% respectively for node-positive and node-negative patients undergoing good quality TME*(Quirke, Steele et al. 2009)*. Similarly an audit of the MERCURY study showed that lymph node status did not predict for local recurrence and local recurrence was instead predicted for by the mrCRM status, mr-derived depth of extramural spread (>5mm) and presence of mr-derived EMVI*(Taylor, Quirke et al. 2014)*.

Initial studies exploring the role of MRI in the staging of rectal cancer investigated the accuracy of MRI staging of nodal disease and showed that size was a poor predictor of lymph node involvement by disease*(Brown, Richards et al. 2003)*, consistent with histopathological studies which showed that there was considerable overlap between the size of normal, inflammatory and metastatic lymph nodes*(Dworak 1991)*. The presence of an irregular nodal border contour and mixed MR signal were more reliable as markers of disease within the lymph nodes on MRI than size*(Brown, Richards et al. 2003)* with 90% of nodes with an irregular contour shown to contain malignancy*(Brown, Richards et al. 2003)*. Using these features a study of 98 patients prospective assessed by MRI showed 85% agreement between the MRI and histopathological assessment of nodal status*(Brown, Radcliffe et al. 2003)*.

The evidence for the lack of importance of nodal status on outcomes have led some centres, including our own, to cease the reporting of nodal status on MRI. However crucially the reporting of the presence of ENTDs is required.

Extranodal tumour deposits (N1c disease)

The definitions of ENTDs have varied since their first description by Gabriel in 1935*(Gabriel 1935)*. This variability continues between individual editions of the TNM staging manual which have varied in their definition of whether ENTDs should be classified by size or by the presence or absence of nodal or vascular tissue*(Lord, D'Souza et al. 2017)*. It is this disagreement which has led to heterogeneous uptake of the various editions of the TNM staging manual by pathologists. This heterogeneity makes evaluation for ENTDs as a prognostic feature within the literature more challenging as, depending on the TNM edition employed within the study, ENTDs may or may not be classified. A current working definition is that a tumour deposits are "separate nodules or deposits of malignant cells in the perirectal or pericolic fact without evidence of residual lymph node tissue"(*Sobin, Gospodarowicz et al.* , *Lord, Knijn et al.*).

It is increasingly believed that the presence of ENTDs and EMVI are a continuity of the same process, consistent with Gabriel's original report*(Gabriel 1935)*, and that the presence of ENTDs represent a more advanced form of EMVI where "nodules are closely related to vessels but are not in continuity with the tumour itself"*(Lord, D'Souza et al. 2017)*. Lord *et al* concluded that "these nodules could be seen as metastases in transit which would make their association with poorer survival and higher rates of recurrence not surprising"*(Lord, D'Souza et al. 2017)*.

This meta-analysis by Lord *et al* of 26 studies showed ENTDs were present in 10.2% to 44.2% with a median prevalence of 21.3%*(Lord, D'Souza et al. 2017)*. ENTDs were associated with adverse overall survival (pooled HR 1.63, 95% CI 1.44-1.61) and disease free survival (pooled HR 1.77, 95% CI 1.37-2.11)*(Lord, D'Souza et al. 2017)*. The association between the presence of ENTDs and poor prognosis was also described by Nagtegaal and Quirke in 2007*(Nagtegaal and Quirke 2007)*.

However many of these studies described the poor prognosis of ENTDs following pathological detection not mr detection of ENTDs. In a similar way to how mr-detection of EMVI is now regarded as the gold standard rather than pathology, it is increasingly believed that the mr-detection of ENTDs may be the gold standard investigation.

Pathologists experience problems in identifying ENTDs which can be summarised as:

- **Issues with sampling technique** – a higher prevalence of histopathologically detected ENTDs are detected in studies which report additional step sectioning rather than standard sampling techniques*(Lord, D'Souza et al. 2017)*
- **Issues with variable TNM classifications***(Lord, D'Souza et al. 2017)*
- **Issues with interobserver variability in distinguishing between ENTDs and lymph nodes***(Rock, Washington et al. 2014)*, *(Lord, D'Souza et al. 2017)*

As ENTDs are hypothesised to represent in-transit metastases from EMVI, MRI has a distinct advantage over pathology in the ability to follow vessels in 3 dimensions and determine the origin of the ENTD*(Lord, D'Souza et al. 2017)*.

As such the mr-detection of ENTDs is regarded as a poor prognostic factor for rectal cancer. Preliminary data from our cancer network has shown MRI diagnoses ENTD in 51% of patients with rectal cancer, which is still present in 37% of patients following neoadjuvant chemoradiotherapy(*Lord, Moran et al. 2018*). This is compared to a prevalence of 13% on histopathological specimens following neoadjuvant chemoradiotherapy in the same cohort(*Lord, Moran et al. 2018*). The comparison of pENTD and mrENTDs is the basis of the ongoing COMET trial(*Lord, Moran et al. 2018*).

Practically for radiologists ENTDs should be actively looked for when reporting imaging examinations of patients with rectal cancer or colon cancer. Radiologists should scrutinise the invasive border of the tumour in multiple planes and look to identify any EMVI. Following the identification of EMVI radiologists should follow the affected veins centrally and scrutinise their path for the presence of any deposits of tumour signal which sit within or adjacent to the vessel which are felt to represent ENTDs. It is important to remember that ENTDs may be seen more centrally than the distal end of the EMVI and therefore there may not be tumour signal within the vessel adjacent to the ENTD. This method is currently being validated as part of the COMET Trial(*Lord, Moran et al. 2018*). Figure 1.7 illustrates how to look for ENTDs.

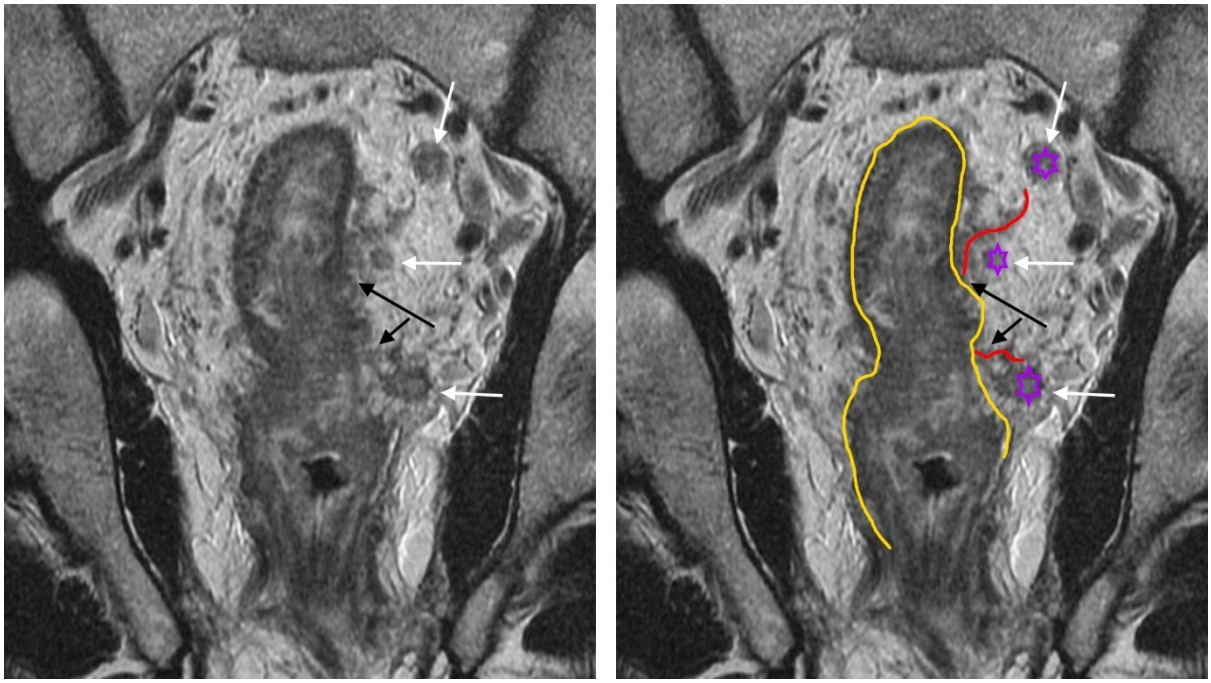


Figure 1-7: High resolution T2-weighted coronal oblique MRI of a locally advanced rectal cancer with large vessel EMVI and ENTDs consistent with N1c disease.

The right hand image has been annotated to demonstrate the key structures. The outline of the rectum is highlighted in yellow. The invasive border is annotated on both images with solid black arrows. Vessels with EMVI are annotated with red lines. As you follow the red lines you will see satellite areas of tumour signal, highlighted with purple stars and solid white arrows, which are ENTDs.

Tumour height

Tumour height is known to be a prognostic factor for rectal cancer. There is variability between studies but the reported histologically determined positive CRM rates for low rectal cancers range from 20-36% *(Marr, Birbeck et al. 2005)*, *(Nagtegaal, van de Velde et al. 2005)*, *(Mercury Study Group 2006)*, *(Sebag-Montefiore, Stephens et al. 2009)*.

The Norwegian Rectal Cancer Project recruited 2136 patients from 47 hospitals between 1993 and 1999. They showed “low rectal” cancers arising between 0 and 5 cm from the anal verge were associated with significantly worse 5 year local recurrence rate 15% (versus 13% for intermediate tumours and 9% for upper rectal tumours, p=0.014), although the local recurrences rates were better than those previously reported. Low rectal cancers were also associated with poorer 5 year overall survival of 59% compared to 62% and 69% for intermediate and upper rectal tumours respectively, p<0.001)*(Wibe, Syse et al. 2004)*. The local recurrence rate was also worse for patients undergoing an anterioperineal resection (APE) compared to anterior resection (AR) (15% versus 10%, p=0.008*(Wibe, Syse et al. 2004)*).

The poor prognosis for low rectal cancer, despite the adoption of TME surgery, was thought to be secondary to the technical factors of the normal tapering of the mesorectum and the higher perforation risk which makes the surgery more challenging*(Salerno, Daniels et al. 2009)*. The higher risks with APE surgery are thought to be because the mesorectal fascia does not form the surgical margin for an APE procedure*(Salerno, Daniels et al. 2009)*. The potential for MRI assessment of a “safe” low rectal cancer plane (mrLRP) as a roadmap for low rectal cancer operations was initially investigated in 2009 on a subgroup of 101 patients recruited as part of the MERCURY Study, Figure 1-8. This study found that tumours which extended into or beyond the intersphincteric plane, tumours within the anterior quadrant and the MRI assessment of response to neoadjuvant chemoradiotherapy predicted for pathologically assessed positive resection margins*(Salerno, Daniels et al. 2009)*. A further retrospective study of 33 patients supported the potential of an anatomically based MRI

staging system for low rectal cancer to predict the plane of surgery required to achieve a negative resection margin*(Shihab, How et al. 2011)*

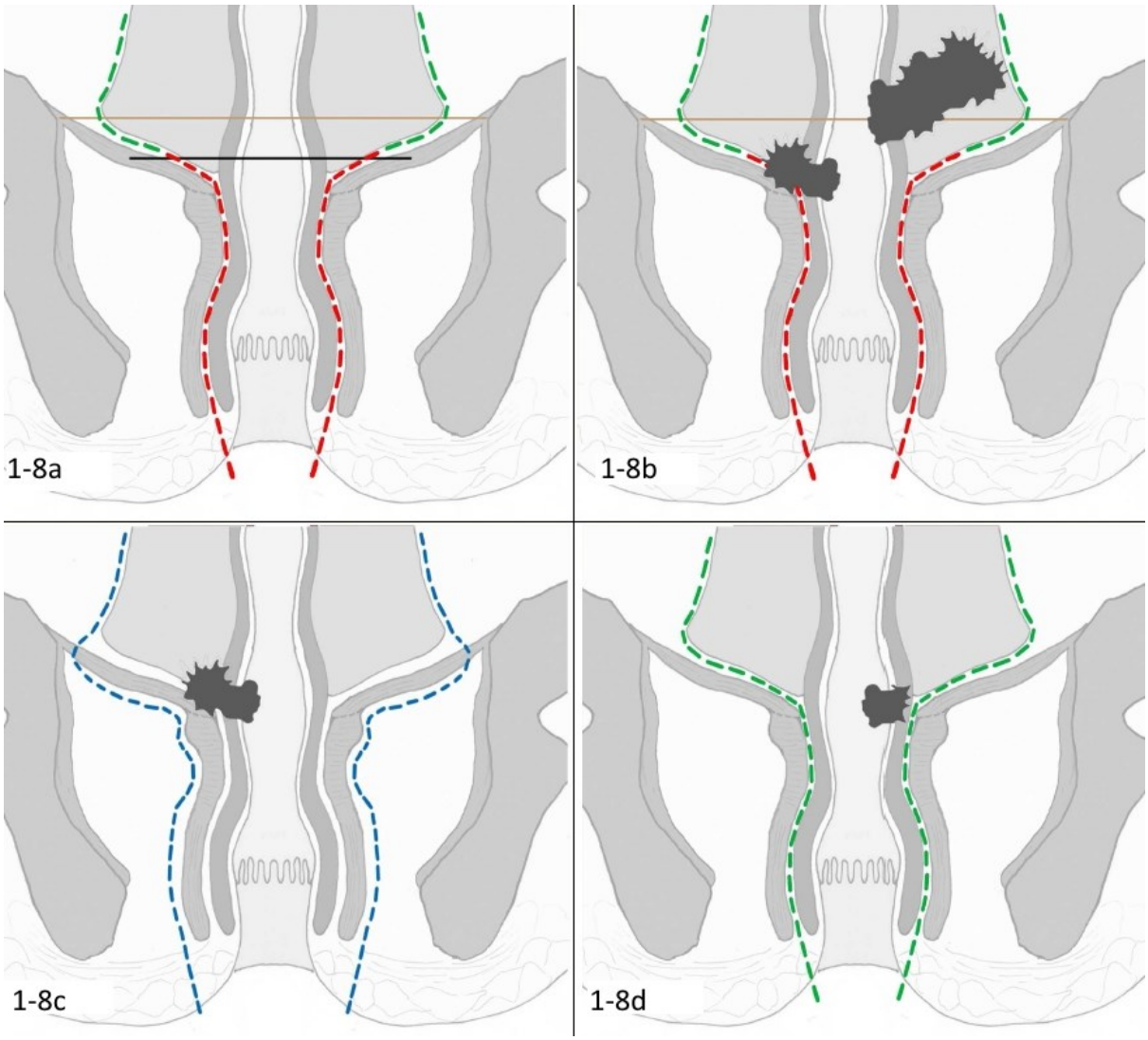


Figure 1-8: Diagrammatic representations of the "safe" and "unsafe" mrLRP

The standard intersphincteric plane is shown as a red line (1-8a and b), tumours which do not come within 1mm of this plane on MRI are deemed mrLRP “safe” (Figure 1-8d). Figure 1-8b shows a tumour which involves the intersphincteric plane and therefore the mrLRP is “unsafe” and the patient requires an ELAPE (the ELAPE excision plane is shown as a blue line in Figure 1-8c).

Reproduced with permission from *(Battersby, How et al. 2015)*.

The five year follow-up data of the 101 low rectal cancer patients from the MERUCRY Study showed advanced low rectal tumour stage (where the tumour extended to or beyond the interspincteric plane) was associated with increased recurrence (P=0.013) and death (P=0.029) and that a good response to pre-operative therapy, as assessed by MRI, was also associated with improved local recurrence rates (P=0.008) and improved survival (P=0.008)*(Shihab, Taylor et al. 2011)*.

The mrLRP was validated in the prospective MERCURY II Study which recruited 279 patients with tumours arising ≤6cm from the anal verge and compared treatment recommendations determined by the mrLRP to the patients' final management and outcomes*(Battersby, How et al. 2015)*. The study showed overall pCRM rates were significantly lower than previously reported studies at 9% and that mrLRP could predict for safe surgery and pCRM positivity rates. In addition EMVI, tumours <4cm from the anal verge and anterior tumours were associated with higher pCRM rates*(Battersby, How et al. 2015)*. Specifically, patients with no MRI adverse features and a "safe" mrLRP could safely undergo sphincter-preserving surgery without the use of preoperative radiotherapy and a resultant 1.6% pCRM rate. The pCRM rate was however increased 5-fold for mrLRP "unsafe" tumours and if the mrLRP remained "unsafe" after CRT this resulted in a 17.5% pCRM rate*(Battersby, How et al. 2015)*.

Imaging staging of colon cancer

As described the validated prognostic features for rectal cancer on MRI are:

- Depth of extramural spread >5mm
- EMVI positivity +/- mr-detected ENTDs
- CRM positivity
- "Unsafe" mrLRP which can also be described as an involved intersphincteric plane

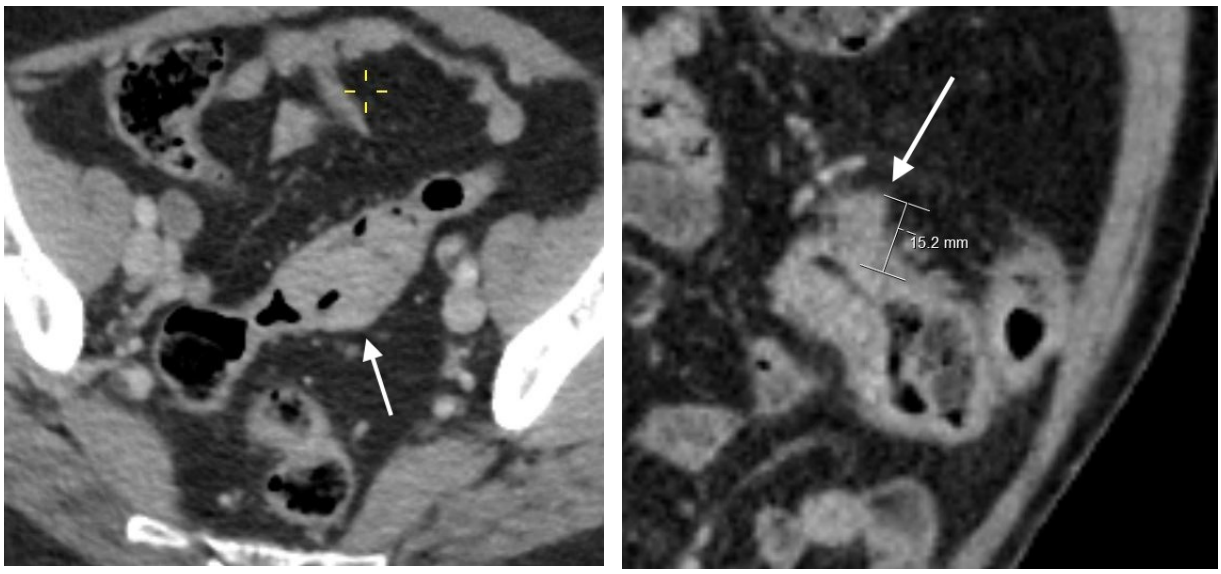
Whilst 5-year overall survival for rectal cancer and colon cancer have both improved since the 1970s the greater improvement has been for rectal cancer secondary to the introduction of TME surgery and the use of preoperative chemoradiotherapy. Survival for colon cancer is now worse than survival for rectal cancer and the treatment options available to patients with colon cancer are limited*(Cancer Research UK 2019d)*.

Whilst MRI of the pelvic sigmoid colon is increasingly employed to identify the poor prognostic factors validated for rectal cancer high quality MRI imaging of the remainder of the colon eludes us. Therefore, there has been interest in seeing whether the factors validated for the MRI assessment of rectal cancer could be applied to the CT assessment of cancer elsewhere within the colon.

CT assessment of depth of extramural spread and resection margin status

A meta-analysis of 8 studies which used CT to identify poor prognostic features in colon cancer found CT has a sensitivity of 92% (95% CI 87%-95%) and specificity of 81% (95% CI 70%-89%) for distinguishing between T3/4 and T1/2 tumours, i.e. for distinguishing depth of

extramural spread beyond the muscularis propria*(Dighe, Purkayastha et al. 2010)*, Figure 1-9. However, the same meta-analysis found that CT was insensitive and non-specific at identifying lymph node involvement with a sensitivity of 70% (95% CI 59%-80%) and a specificity of 78% (95% CI 66%-86%)*(Dighe, Purkayastha et al. 2010)*. It should, however, be noted that none of the studies included looked at the ability of CT to predict prognosis.



(A) – good prognostic tumour (B) – poor prognostic tumour

Figure 1-9: Depth of extramural spread can be assessed by CT for colon cancer

Figure 1-9 (A) shows a contrast enhanced axial CT good prognostic tumour with no extramural spread whereas (B) is a contrast enhanced coronal CT of a poor prognostic tumour with 15mm of extramural spread by virtue of EMVI consistent with a T3c tumour.

In a study of 33 patients with colon cancer assessed by CT, Burton *et al* showed that CT has 70% - 82% accuracy in predicting tumour extension beyond the muscularis propria and 76% - 79% accuracy in the prediction of involvement of the retroperitoneal resection margin*(Burton, Brown et al. 2008)*. Similarly, CT correctly predicted prognosis in 82% - 85%

of cases with moderate agreement with histology(k 0.459, k 0.527, respectively)*(Burton, Brown et al. 2008)*.

CT assessment of EMVI

A further study by Dighe *et al* reviewed the ability of CT to assess for EMVI*(Dighe, Blake et al. 2010)*. Whilst the overall figures for the accuracy and specificity of CT for the detection of EMVI were only 70% and 79% respectively, CT was shown to be able to detect large vessel EMVI*(Dighe, Blake et al. 2010)* which has been shown to be prognostic on MRI, Figure 1-9 (B).

CT categorisation of colon tumours into "good" and "poor" prognosis

In a similar approach to the classification of good and poor prognostic rectal tumours employed by Taylor *et al(Taylor, Quirke et al. 2011a)*, Smith *et al* grouped colon cancers into those with good or poor prognosis according to the CT features present*(Smith, Bees et al. 2007b)*, Table 1-9.

Table 1-9: Prognostic features on CT determining good and poor prognostic colonic tumours on CT

Prognostic feature	**Good prognostic tumour on CT**	**Poor prognostic tumour on CT**
T stage corresponding to depth of extramural spread	T1-T3b (DEMS ≤5mm)	T3c-T4 (DEMS >5mm)
N stage	N0/N1	N2
EMVI	Negative	Positive

It should be noted that the presence of ENTDs was not included in this categorisation of tumours in this study which was performed prior to the work on ENTDs on MRI. But more recent studies which are awaiting publication have included CT detected ENTDs as a poor prognostic factor.

Smith *et al* showed that 56% of the 126 patients included in the study had a poor prognostic tumour and that using the above criteria to categorise colonic tumours had an overall accuracy of detecting poor prognostic tumours of 83.3% and a sensitivity of 92.4%*(Smith, Bees et al. 2007b)*. In addition CT determined DEMS, and so T stage, showed excellent correlation to histology against outcomes; tumours with <5mm invasion beyond the muscularis propria had 87% 3 year recurrence free survival, compared to 53% for tumours with >5mm invasion beyond muscularis propria*(Smith, Bees et al. 2007a)*.

Ongoing work is looking to further validate the CT staging colonic cancer against outcomes in the modern era and the results are expected shortly. However, the evidence to date supports the use of depth of extramural spread and EMVI to prognosticate for colon cancer. There is no reason to suggest that the outcomes determined in rectal cancer cannot be translated to colon cancer but this will be confirmed by the ongoing validation studies.

Summary of the validated imaging biomarkers for colorectal cancer

Table 1-10: Summary of the validated imaging biomarkers for colorectal cancer, including which outcomes they have been validated against

	Validated against which outcomes?				
Imaging Biomarker	**LR**	**DFS**	**OS**	**Metastatic disease**	**Details and reference**
Rectal cancer					
mrT stage (depth of extramural spread)	✓	✓	✓		"Good" prognostic tumours have a low local recurrence rate & good 5 year OS and DFS of 3.3%, 68.2% and 84.7% respectively*(Taylor, Quirke et al. 2011a)*.
mrCRM ≤1mm	✓	✓	✓	✓	mrCRM ≤1mm predicted for local recurrence (HR of 3.72, P=0.007)*(Taylor, Quirke et al. 2011b)*, *(Taylor, Quirke et al. 2014)*, 5 year DFS and OS and the development of metastatic disease*(Taylor, Quirke et al. 2014)*.
mrEMVI positive	✓	✓		✓	Independent risk factor for 3 year recurrence free survival(P<0.001)*(Smith, Barbachano et al. 2008)* and DFS (adjusted HR of 2.08)*(Chand, Swift et al. 2014a)*. Increased risk of metastatic disease at presentation (OR 5.68, P<0.001) or during follow-up (OR 3.91, p<0.001)*(Siddiqui, Simillis et al. 2017)*.
ymrEMVI positive		✓			Persistent EMVI positivity after CRT associated with poorer DFS(*Chand, Evans et al. 2015*, *Chand, Rasheed et al. 2017*)
mrENTD/N1c					pENTDs are associated with poorer DFS and OS(*Lord, D'Souza et al. 2017*). mrENTDs now need validate against outcomes.
mrLRP					mrLRP can predict for safe surgery & pCRM (*Battersby, How et al. 2015*)
Colon cancer					
ctT stage (depth of extramural spread)			✓		<5mm invasion beyond the muscularis propria had 87% 3 year survival, compared to 53% for tumours with >5mm invasion *(Smith, Bees et al. 2007a)*
ctEMVI positive					CT can detect large vessel EMVI(*Dighe, Blake et al. 2010*) which is prognostic when detected on MRI, but ctEMVI has not been validated against outcomes

Treatment options for colorectal cancer

The management of colorectal cancer is multifactorial and depends not only on the tumour site, stage and the presence of validated poor prognostic factors but also the patient's wishes and comorbidities.

Rectal cancer

The management of patients with rectal cancer in our institution depends on the presence or absence of poor prognostic features as described previously. Patients are therefore categorised into those with good or poor prognostic tumours and treated accordingly as summarised in the flowchart (Figure 1-10).

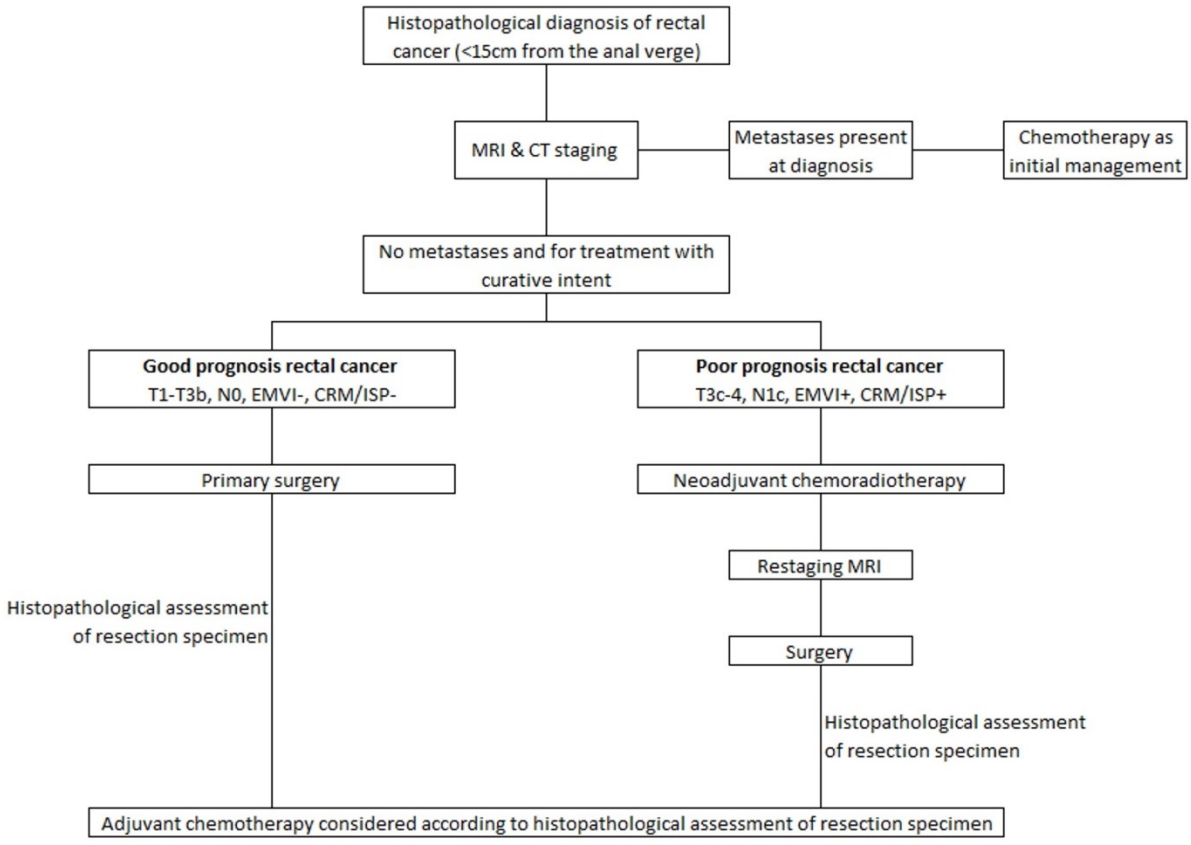


Figure 1-10: Management of rectal cancer according to our local institutional guidelines

However the NICE guidelines*(National Institute for Health and Care Excellence (NICE) 2011a)* and ESMO guidelines*(Glynne-Jones, Wyrwicz et al. 2017)* differentiate tumours into three risk categories according to the risk of local recurrence which determine the need for, and which type of, neoadjuvant therapy is offered to patients. The risk categories and subsequent neoadjuvant treatment options according to the NICE guidelines are described in Table 1-11.

Table 1-11: Use of preoperative therapy according to the risk of local recurrence as recommended by the NICE guidelines*(National Institute for Health and Care Excellence (NICE) 2011a)*.

Risk of local recurrence	**MRI features present**	**Treatment to be offered/not offered if the tumour appears operable at diagnosis**
High	• CRM+ • ISP+ in low rectal tumours	Offer preoperative CRT with an interval before surgery to allow tumour response and shrinkage (rather than SCPRT)
Intermediate	• Any cT3b+ in which the potential surgical margin is not threatened • Any suspicious lymph node not threatening the CRM • EMVI+	• Consider SCPRT then immediate surgery. • Consider preoperative CRT with an interval to allow tumour response and shrinkage before surgery for patients with tumours that are borderline between moderate and high risk.
Low	• cT1-T3a • No lymph node involvement	Do not offer short-course preoperative radiotherapy (SCPRT) or chemoradiotherapy to patients with low-risk operable rectal cancer, unless as part of a clinical trial.

For the remainder of this thesis I will refer to the management offered to patients without metastatic disease at diagnosis and outcomes for patients according to our local institutional management practice outlined in Figure 1-10. This is due to the reasons outlined below:

- The NICE and ESMO guidelines refer to the use of preoperative treatment to reduce the risk of local recurrence but evidence presented previously show that validated poor prognostic factors present on MRI not only confirmed an increased risk of local recurrence but also increased risk of poorer outcomes.
- Not all the validated poor prognostic factors considered in our local management guidelines are used to determine the risk groups in the NICE guidelines, for example the presence of ENTDs as mrN1c disease is not considered as a poor prognostic factor.
- The NICE guidelines recommend short-course preoperative radiotherapy (SCPRT) with immediate surgery for patients with intermediate risk rectal cancer which is not routinely used within our institution.
- The management of patients in the large studies which have validated the poor prognostic factors on MRI, for example MERCURY I and MERCURY II, has been according to our local institutional management guidelines outlined in the flowchart*(Taylor, Quirke et al. 2011a)*, *(Battersby, How et al. 2015)*.
- The management of patients in the studies described within the thesis follows that outlined in the flowchart.

Low risk rectal cancer

Patients with low-risk tumours (depth of extramural spread <5mm, T1-T3b, EMVI negative, CRM/ISP clear) can be safely treated with primary surgery, avoiding the added morbidity and mortality associated with chemoradiotherapy.

The choice of method of primary surgery depends on the location of the primary tumour. Low risk tumours are those which do not involve the intersphincteric plane so by definition the mrLRP is considered "safe"*(Battersby, How et al. 2015)*. The surgery offered will therefore predominantly depend on the height of the tumour in relation to the puborectalis sling.

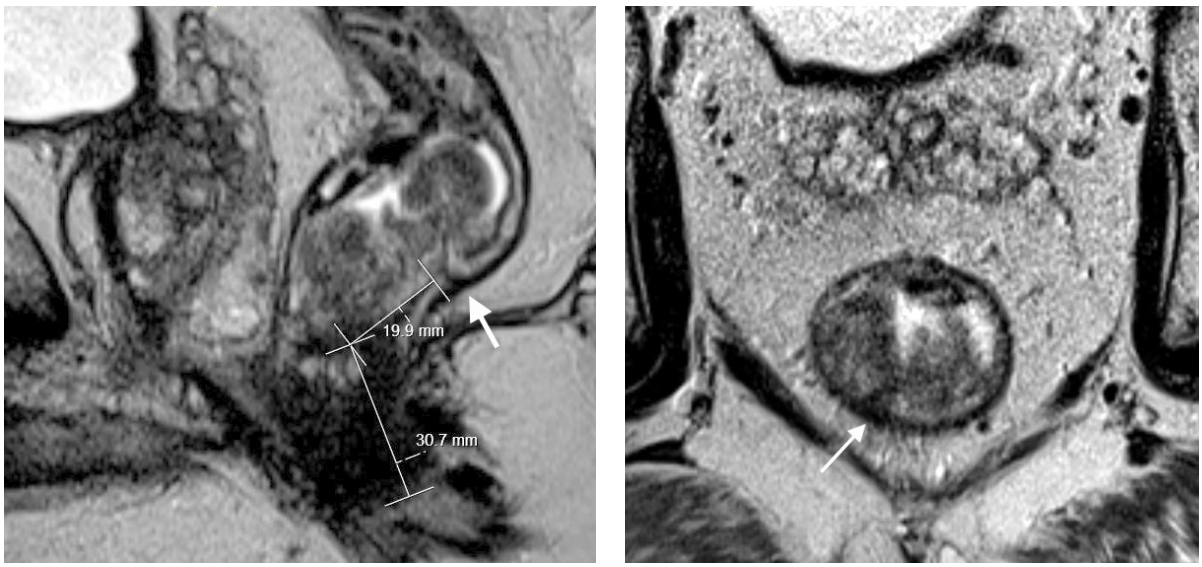
Tumours where the lower border of the tumour lies >1cm above the puborectalis sling should technically be amenable to an anterior resection with the option for primary anastomosis. A temporary defunctioning stoma is normally performed to allow the anastomosis to heal with the option for reversal later. When counselling a patient with low rectal cancer for an anterior resection the surgeon will discuss the functional outcomes including the possibility of low anterior resection syndrome (LARS); a group of symptoms including fecal incontinence, frequency of bowel movements and incomplete bowel emptying. The risk of LARS increases for lower sphincter-preserving procedures*(Chen, Wiltink et al. 2015)*, *(Ekkarat, Boonpipattanapong et al. 2016)*.

For patients with tumours where the lower border lies <1cm from the puborectalis sling, or patients where the morbidity associated with the development of possible LARS is unacceptable, an APE will be offered with a permanent stoma.

However increasingly there are organ preservation options available to patients in the form of local excision and watch and wait or deferral of surgery.

Organ preservation in rectal cancer – local excision

Patients with early rectal cancer are defined as those with "invasive disease which is confined to the submucosa +/- the muscle of the rectal wall without evidence of spread within the mesorectum or beyond"*(PRESERVE Study Group 2019)*, i.e. patients with mrT1 or T2 disease without adverse features, Figure 1-11.



Sagittal T2-weighted MRI

High-resolution axial oblique T2-weighted MRI

Figure 1-11: MRI of an early rectal cancer suitable for local excision

T1 polyp rectal cancer with the polyp stalk located on the posterior wall of the rectum at 7 O'Clock (white arrows). This cancer would be suitable for local excision.

Most of these patients are treated with major surgery (normally TME or APE) but organ preserving local excision surgery is another option for these patients.

There are multiple local excision options available. Transanal excision was superseded by the more stable surgical technique of transanal endoscopic microsurgery (TEM) in the 1980s*(Buess, Theiss et al. 1985)*, *(Williams, Pullan et al. 2013)* and since 2009 the advanced technique of transanal minimally invasive surgery (TAMIS) has been used *(Atallah, Albert et al. 2010)*, *(Albert, Atallah et al. 2013)*, *(Atallah, Albert et al. 2013)*. Each of these excision options have been shown to be safe*(Williams, Pullan et al. 2013)* and are associated with low local recurrence rates, fewer complications, shorter hospital stays, reduced costs when compared to major surgery*(Williams, Pullan et al. 2013)*, *(Juul, Ahlberg et al. 2014)*.

Despite the availability of local excision options the majority of patients with early rectal cancer still undergo major surgical excision*(Association of Coloproctology of Great Britain and Ireland 2014)*, *(Guerrieri, Gesuita et al. 2014)*, *(Greenaway, Hill et al. 2015)*. Data from the National Bowel Cancer Screening Programme and the National Bowel Cancer Audit (NBOCA) reports have shown that rates of early rectal cancer are increasing and this is likely to the be due to the earlier detection of cancers through the screening programme*(Logan, Patnick et al. 2012)*, *(Healthcare Quality Improvement Partnership Ltd. 2017)* – 40% of screen detected cancers are stage 1*(Healthcare Quality Improvement Partnership Ltd. 2017)*. These reports show 10% of patients with rectal cancer are diagnosed with early rectal cancer but only 7-10% of these patients undergo local excision*(Logan, Patnick et al. 2012)*, *(Healthcare Quality Improvement Partnership Ltd. 2017)*. Therefore, there is a concerted effort to increase the availability of local excision using MRI to identify cases which are safe for local excision procedures.

The MRI staging of early rectal cancers follows the principles of MRI staging for all rectal cancers*(Wale and Brown 2014)* but goes further to determine the degree of tumour infiltration into the muscularis propria. In a study from 2017 the authors defined tumours with >1mm preservation of muscularis propria as T2 tumours suitable for local excision*(Balyasnikova, Read et al. 2017b)*. Results from this study showed MRI had 89% accuracy for detecting partial versus full submucosal invasion and 84% specificity for detecting ≤pT2N0 tumours*(Balyasnikova, Read et al. 2017b)*. In addition survival outcomes for patients with early rectal cancer treated with local excision are excellent with single centre results from a study of 34 patients showing 3 year DFS was 85% and overall survival was 100%*(Balyasnikova, Read et al. 2017a)*.

The use of MRI to define safe tumours for local excision will be tested in the upcoming PRESERVE study*(PRESERVE Study Group 2019)*.

Neoadjuvant therapy for poor prognostic rectal tumours

As described previously MRI is able to identify validated poor prognostic features which, when present, are associated with an increased risk of local recurrence(CRM or ISP positivity, “unsafe” mrLRP and persistent “unsafe” mrLRP following neoadjuvant CRT), metastatic disease(mrEMVI positivity and ENTDs) and cancer-specific and overall survival (depth of extramural spread >5mm, mr EMVI positivity and ENTDs and mrCRM/ISP involvement).

Neoadjuvant therapy, initially with radiotherapy alone and then combination chemoradiotherapy (CRT), has been investigated with the aim of downstaging tumours to enable curative resection and improve long-term survival outcomes.

Initial studies were performed in the pre-TME era and offered SCPRT to patients with operable rectal cancer with no consideration of the presence of good or poor prognostic factors. The biggest trials were the Swedish Rectal Cancer Trial (March 1987-Feb 1990)*(Swedish Rectal Cancer, Cedermark et al. 1997)* and the Stockholm II Trial (1987-1993)*(Martling, Holm et al. 2001)* which showed SCPRT reduced pelvic recurrence ($p<0.001$)*(Martling, Holm et al. 2001)* and improved 5 year overall survival($p=0.004$*(Swedish Rectal Cancer, Cedermark et al. 1997)* and $p<0.03$*(Martling, Holm et al. 2001)*). However it is questioned whether these trials are still relevant in the post-TME era.

In the post-TME era the MRC CR07 trial randomised patients between SCPRT and selective postoperative chemotherapy and showed SCPRT lowered local recurrence rates to 4.4% compared to 10.6% with postoperative treatment*(Sebag-Montefiore, Stephens et al. 2009)*. A meta-analysis of trials which compared preoperative CRT with SCPRT showed combination therapy with CRT resulted in improved rates of local recurrence (9.4% if treated with CRT versus 16.5% with SCPRT) but in the pre-TME era studies only*(Ceelen, Fierens et al. 2009)*. A higher rate of pathological completed response (pCR) was also shown in the group treated with CRT prior to TME surgery (11.8%) compared to SCPRT prior to TME surgery (3.5%)*(Ceelen, Fierens et al. 2009)*. These studies therefore suggested that preoperative CRT resulted in improved outcomes over preoperative SCPRT alone.

These findings were confirmed by the National Surgical Adjuvant Breast and Bowel Project R-03 (NSABP R-03) trial which compared outcomes following neoadjuvant versus adjuvant CRT of locally advanced rectal cancer*(Roh, Colangelo et al. 2009)*. This trial showed 5 year DFS was improved for patients treated with preoperative compared to adjuvant CRT (64.7% versus 53.4%, p=0.011) but this did not translate into an improvement of in 5 year OS (P=0.065)*(Roh, Colangelo et al. 2009)*. The German Rectal Cancer Study Group trial similarly showed neoadjuvant CRT lowered local recurrence to 6% versus 13% for postoperative CRT with improved compliance and reduced toxic effects in patients treated preoperatively*(Sauer, Becker et al. 2004)*. These studies therefore confirmed that the use of neoadjuvant CRT downstages disease and reduces the risk of local recurrence, hence it is used as the mainstay of neoadjuvant therapy for patients with poor prognostic rectal tumours in our local treatment pathway.

At The Royal Marsden Hospital neoadjuvant CRT typically consists of treatment with 52.5 Gy in 25 fractions of radiotherapy with capecitabine daily, dose dependent on age and weight.

Restaging of tumours following neoadjuvant therapy

Following neoadjuvant therapy patients are restaged with MRI prior to undergoing surgery with curative intent. The purpose of this restaging examination is to describe whether the tumour has regressed and to ensure the disease has not progressed to an extent where the plane of surgery would need to be changed to ensure a curative resection or to an extent where the tumour is now inoperable.

The degree of tumour regression on the histological specimen is known to be a marker of prognosis*(Pahlman, Hohenberger et al. 1998)*, *(Marijnen and Glimelius 2002)*. pCR, where no tumour cells are demonstrated, occurs in between 15-25% of patients following neoadjuvant therapy depending on the series*(O'Neill, Brown et al. 2007)*, *(Roh, Colangelo et al. 2009)*, *(Smith, Waldron et al. 2010)* and is associated with better oncological outcomes. In the NSABP R-03 trial no patient treated with neoadjuvant CRT who had pCR on their resection specimen developed recurrent disease*(Roh, Colangelo et al. 2009)*.

However, whilst the assessment of complete response on pathological specimens is a good prognostic factor, the patient has still undergone major resectional surgery for a tumour which, pathologically, is no longer viable. There is therefore increasing interest in non-operative organ preservation by "watchful waiting"*(Habr-Gama, Perez et al. 2004)*, *(Habr-Gama, Sabbaga et al. 2013)* or "deferral of surgery"*(Battersby, Dattani et al. 2017)* for patients who have had a good response to neoadjuvant CRT.

In order to undergo a non-operative approach to the management of rectal cancer following CRT a reliable technique is required to identify patients who have had a good response.

In the 1980s Angelita Habr-Gama began exploring a non-operative approach for patients who had had a good clinical response to CRT*(Habr-Gama, Perez et al. 2004)*. Between 1991 and 2002 265 patients with low rectal tumours originating at 0-7cm from the anal verge were treated with neoadjuvant CRT. 8 weeks after the completion of CRT patients were assessed clinically by direct visualisation of the tumour to determine their response to CRT.

26.8% of patients who had no abnormality on this assessment were considered to have clinical complete response (cCR)*(Habr-Gama, Perez et al. 2004)*.

Habr-Gama *et al* reported the long-term outcome data from the series of patients with cCR managed with a non-operative approach in 2004*(Habr-Gama, Perez et al. 2004)*. This showed patients with cCR had excellent long-term outcomes irrespective of whether they developed tumour regrowth which was subsequently treated by resection or whether they continued on a surveillance strategy with no tumour regrowth. For the entire group, 10 year OS was 97.7%, with 5 year OS rates of 100% in the observation group and 88% in the resection group. Similarly 10 year DFS was 84% in the whole cohort with 5 year DFS rates of 92% in the observation group and 83% in the resection group*(Habr-Gama, Perez et al. 2004)*. This study showed a non-operative approach could be undertaken for patients who had had a cCR response to CRT.

However, the total number of patients identified as having a cCR response is a relatively small proportion of the total number of patients treated with CRT. In addition there is significant variability between the patients detected as having cCR and pCR with 8-61% of pCR patients missed on clinical examination, even by experienced practitioners*(Hiotis, Weber et al. 2002)*, *(Smith, Waldron et al. 2010)*. This produced interest in identifying a radiological method of identifying good response to CRT and patients who may be suitable for a non-operative approach.

The validated MRI prognostic factors for the reassessment of tumours following CRT are:

- **Regression of EMVI positivity** which is associated with improved 3 year DFS of 79.2% (95% CI 70.0% - 88.4%) compared to 42.7% (95% CI 16.8% - 68.6%) for patients with persistent EMVI positivity*(Chand, Evans et al. 2015)*
- **Regression of CRM/ISP positivity** which, in low rectal cancers, resulted in no patients with a positive pCRM compared to a 17.5% rate of a positive pCRM for "unsafe" mrLRP on the post-treatment MRI*(Battersby, How et al. 2015)*

Regression of the depth of extramural spread has been validated pathologically(*Hermanek, Merkel et al. 2013*) but as good agreement between MRI and histopathological staging of depth of extramural spread has been previously shown(*Brown, Radcliffe et al. 2003*) mr-derived regression of depth of extramural spread has been adopted.

Novel imaging modalities have been investigated for the assessment of response including diffusion-weighted MRI (DW-MRI), mrVolumetric analysis and mrRECIST analysis. However, none of these methods have been validated. For example studies have investigated DW-MRI as a tool which could increase the specificity for detecting complete response but these have failed to validate the use of DW-MRI for this purpose; a study of 50 patients from 2015 showed DW-MRI missed clinical responders and clinical assessment of complete response was more accurate identifying complete response in 15% of patients*(Maas, Lambregts et al. 2015)*.

A mr-method of assessing response to CRT has been developed from the traditional pathological tumour regression grading (pTRG) which assesses the relative proportion of

tumour and fibrosis within the specimen*(Mandard, Dalibard et al. 1994)*, *(Dworak, Keilholz et al. 1997)*. Whilst some studies showed pTRG was related to outcomes*(Rodel, Martus et al. 2005)*, *(Fokas, Liersch et al. 2014)* the literature is inconsistent and heterogeneous*(Fokas, Liersch et al. 2014)* with variability between the scales *(Siddiqui, Bhoday et al. 2016)*, while other studies show no relationship between pTRG and outcomes*(Beddy, Hyland et al. 2008)*, *(Vallbohmer, Bollschweiler et al. 2012)*. Furthermore the interobserver agreement for the various scales is poor*(Chetty, Gill et al. 2012)*, *(Siddiqui, Bhoday et al. 2016)* and thus its reliability in supporting treatment decisions has to be questioned. The pTRG methods of assessing tumour response are also limited by their application only in the post-operative specimen.

The magnetic resonance tumour regression grading (mrTRG) system follows similar principles of an assessment of the relative fibrosis and tumour signal within the treated tumour. Intermediate and poor response to preoperative therapy on the mrTRG system have been shown to have significantly poorer disease free survival (P=.007) and overall survival*(Patel, Taylor et al. 2011)*. Initial studies investigating mrTRG grouped patients with a mrTRG 1, 2 and 3 as those with good response to preoperative therapy and those with an mrTRG 4 and 5 as those with a poor response*(Patel, Taylor et al. 2011)*. However a subsequent study showed those with a mrTRG 1 and 2 response had a better outcome and that the binary categorisation of patients with good (mrTRG 1-2) and poor (mrTRG 3-5) response had a better association with outcomes*(Sclafani, Brown et al. 2017)*.

mrTRG has been validated as a method of assessing response to treatment in terms of identifying patients who could undergo deferral of surgery in a non-operative approach to

the management of rectal cancer following CRT in the Deferral of Surgery Study. Patients who had a mrTRG 1 or 2 response to neoadjuvant CRT were offered follow-up with MRI instead of surgery. Preliminary results suggest this is a safe method of identifying patients eligible for deferral of surgery and that more patients can be identified as eligible for deferral of surgery by mrTRG than by clinical examination alone. mrTRG is now being investigated as a tool to augment treatment offered to patients as part of the TRIGGER trial.

Surgical options for high risk rectal cancer

The surgical options for high risk rectal cancer do not significantly differ for that of low risk cancer. Again, the choice of resectional technique between APE and AR depends predominantly on the height of the tumour from the puborectalis sling.

In addition, further considerations are made for more extensive tumours. For low rectal cancers where the mrLRP is “unsafe” an extra-levator APE is advocated to avoid following the intersphincteric plane and reduce the risk of local recurrence as demonstrated by the MERCURY II study*(Battersby, How et al. 2015)*.

Where tumours extend beyond the TME plane, Beyond TME surgery is required for a curative resection. In order to reduce the high local excision rates associated with ad-hoc beyond TME surgery a Beyond TME Collaborative has defined the variety of procedures which can be performed according to the compartments of the pelvis which are involved by tumour*(Beyond 2013)*.

Compartmental staging by MRI is recommended by the consensus statement to define the compartments involved by tumour*(Beyond 2013)*, Figure 1-12. Despite the expertise of Beyond TME surgeons some disease spread is regarded as inoperable, and this varies between centres. In our centre, disease which involves the sciatic nerve roots at S1 and S2 and some vascular involvement is regarded as inoperable.

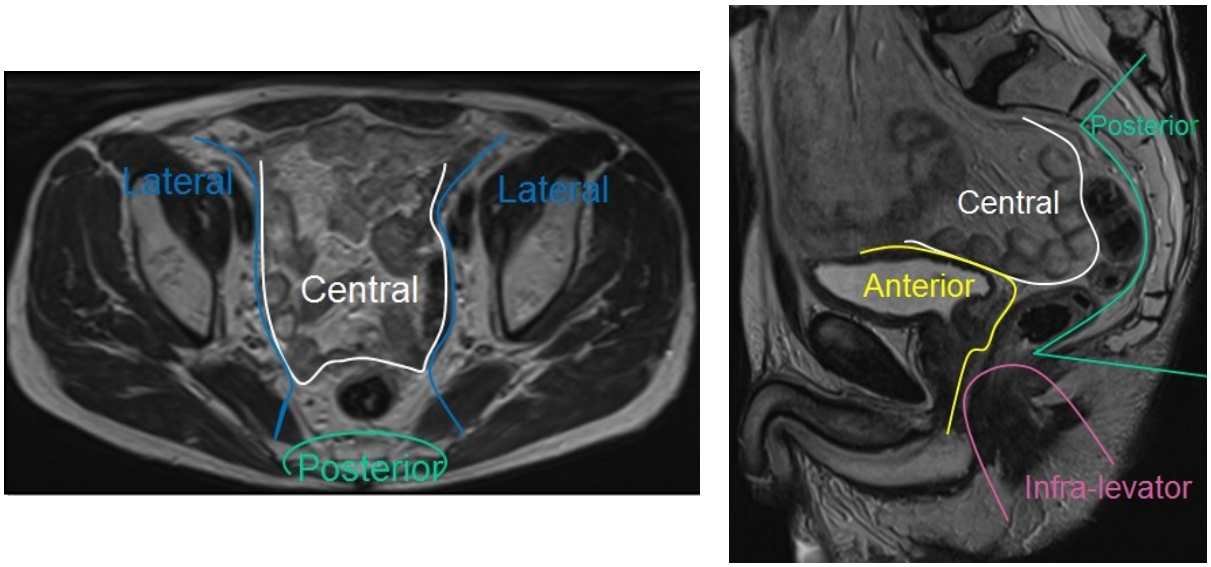


Figure 1-12: Compartmental staging of the pelvis on MRI for tumours which extend beyond the standard TME plane

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Regardless of the surgical procedure performed the aim of the surgery is to achieve a curative resection with an R0 resection. Neoadjuvant CRT is used to increase the chance of achieving a curative resection.

Treatment of colon cancer

Whilst there are multiple treatment options available to patients with rectal cancer, treatment for colon cancer is relatively static without the risk stratification employed for patients with rectal cancer and the option for neoadjuvant therapy. Although, as described previously, the high-risk features validated for rectal cancer on MRI are identifiable on CT for colon cancers.

The mainstay of treatment for colon cancers is primary surgical resection followed by adjuvant chemotherapy if required. The exception is those tumours which originate within the pelvic sigmoid colon, which are increasingly being treated similarly to rectal cancer with assessment by MRI and neoadjuvant CRT for high risk tumours. This is the subject of the ongoing IMPRESS study*(Pelican Cancer Foundation 2019)*.

Colonic tumours are resected according to their location. The most common procedures performed are a right hemicolectomy (for right sided tumours), a left hemicolectomy (for left sided tumours) and/or an anterior resection for tumours of the sigmoid colon. However with outcomes for colon cancer now worse than those for rectal cancer, both in terms of recurrence rates*(O'Connell, Campbell et al. 2008)* and survival*(Cancer Research UK 2019d)*, the surgical committee is turning its attention to colon cancer and considering the importance of embryologically derived surgical strategies*(Tudyka, Madoff et al. 2018)*.

The introduction of embryologically derived surgery in the form of TME for rectal cancer was the turning point in improving outcomes for patients with rectal cancer. Following these

principles two main surgical options have been considered for colon cancer: complete mesocolic excision with central vascular ligation (CME with CVL)*(Bokey, Chapuis et al. 2003)*, *(Hohenberger, Weber et al. 2009)*, *(Bertelsen, Neuenschwander et al. 2015)* and the Japenese D3 surgical procedures*(West, Kobayashi et al. 2012)*. These are both based on the principle of "surgery following the mesocolic plane with transection of the blood vessels at their origin"*(West and Quirke 2018)*. To date studies have shown that surgery using these techniques is associated with greater lymph node yield*(West, Hohenberger et al. 2010)*, can be easily adopted*(West, Kobayashi et al. 2012)* and improves outcomes*(Quirke P* , *(West, Morris et al. 2008)*, *(Tudyka, Madoff et al. 2018)*. But there is no standardisation of the terms or the pathological quality control of the specimens required. This needs to happen before randomised controlled trials can be undertaken to show the possible benefit of embryologically derived surgery for colon cancer*(Tudyka, Madoff et al. 2018)*, *(West and Quirke 2018)*.

Adjuvant treatment following surgery for colorectal cancer

Whilst the mainstay of cancer treatment is a curative resection, adjuvant treatment is used to reduce the risk of local recurrence and metastatic disease. The decision to offer adjuvant chemotherapy is made following the histopathological assessment of the resection specimen, with the exception of patients who have undergone local excision.

The NICE guidelines recommend that adjuvant chemotherapy should be considered for patients with high-risk stage II colorectal cancer and all patients with stage III colorectal cancer. For stage III colorectal cancer the NICE guidelines recommend treatment with

capecitabine monotherapy or oxaliplatin in combination with 5-fluorouracil and folinic acid*(National Institute for Health and Care Excellence (NICE) 2011a)*.

A systematic review of 8507 patients from 22 randomised trials in the pre-TME era showed adjuvant therapy improved overall survival and the risk of local recurrence but it is questionable whether these results are applicable to patients treated with TME surgery. A post-TME era systematic review of 12 randomised controlled trials reviewed the role of adjuvant chemotherapy for stage II colorectal cancer and found adjuvant chemotherapy improved 5 year overall survival (HR 0.81 for colon cancer and 0.72 for rectal cancer) and disease-free survival (HR 0.86 for colon cancer and 0.34 for rectal cancer)*(Wu, Zhang et al. 2012)*. In addition the use of adjuvant chemotherapy reduced the risk of recurrence (risk ratio 0.82)*(Wu, Zhang et al. 2012)*. Similarly the QUASAR trial showed a small but statistically significant benefit for patients with stage II colorectal cancer who were treated with adjuvant chemotherapy versus those who did not receive chemotherapy*(Quasar Collaborative, Gray et al. 2007)*.

The role of chemotherapy to improve survival for patients with poor prognostic factors present at diagnosis has also been investigated. mrEMVI positive rectal tumours are associated with poorer disease free survival*(Chand, Bhangu et al. 2014)*. A study of 227 patients with persistent mrEMVI following CRT explored the potential survival benefit of adjuvant chemotherapy*(Chand, Rasheed et al. 2017)*. It found that whilst the use of adjuvant chemotherapy could not completely reverse the negative impact of mrEMVI on 3 year DFS*(Chand, Bhangu et al. 2014)* it did improve with 3 year DFS of 74.6% in patients

treated with adjuvant chemotherapy versus 53.7% in the observation only group*(Chand, Rasheed et al. 2017)*.

Following local excision many centres offer patients adjuvant chemoradiotherapy according to the presence of poor prognostic factors on the histological specimen which are regarded as conferring a higher risk of residual disease and/or local recurrence*(PRESERVE Study Group 2019)*. Histological features regarded as conferring a higher risk of residual disease include a positive margin or a margin which is not assessable due to a piecemeal excision, a poorly differentiated tumour, or tumours which are staged as T1 SM3 or T2*(PRESERVE Study Group 2019)*.

Local recurrence following surgery for colorectal cancer

The incidence of pelvic recurrence has been reduced by TME surgery*(Heald, Husband et al. 1982)*, the MRI staging of tumours to identify poor prognostic factors*(Brown, Daniels et al. 2006)*, *(Burton, Brown et al. 2006)*, *(Fowler, Beagley et al. 2007)*, *(Taylor, Quirke et al. 2011b)* and neoadjuvant therapy. Pelvic recurrence rates following good quality TME surgery with a negative resection margin are approximately 4-8% in the post-TME era*(Heald, Moran et al. 1998)*, *(Quirke, Steele et al. 2009)*, *(Taylor, Quirke et al. 2011b)*.

Local recurrence rates for colon cancer are higher. The ACCENT trial reported in 2008 included 17,381 patients with stage II and III colon cancer*(O'Connell, Campbell et al. 2008)*. 32.9% of all patients developed recurrence at a median time of 13.3 months after surgery, 84% of which had received chemotherapy after treatment. However, recurrence was more

common in patients with stage III disease, which accounted for 80% of the cases of recurrent disease compared to 20% in patients with stage II disease.

A curative option with chemotherapy and radiotherapy and/or surgery may be available for patients who develop recurrent disease but recurrent disease impacts negatively on survival.

Metastatic disease in colorectal cancer

Two theories exist for the development of metastatic disease; the mechanistical hypothesis*(Ewing 1928)* and the seed-and-soil hypothesis*(Paget 1989)*. Both these hypotheses build on the principle that tumour cells can spread through lymphatic and vascular pathways*(Ewing 1928)*, *(Paget 1989)*. For colorectal cancer, the primary vascular drainage is through the portal vein resulting in liver metastases and the systemic circulation resulting in lung metastases. Lymphatic spread results in lymph node metastases and then systemic metastases*(Ewing 1928)*. The seed-and-soil hypothesis takes this concept one step further suggesting that each cancer type has preferred locations for metastasising and each organ offers an individual microenvironment which determines the distribution of metastatic disease*(Paget 1989)*.

Distribution of metastatic disease

The liver is the first and most common site of metastatic disease, accounting for 60% of metastases*(van Gestel, de Hingh et al. 2014)* in one series. In another series liver only metastatic disease occurred in 76.8% of all patients who developed metastases *(Manfredi,*

Lepage et al. 2006). The incidence of liver metastases has risen in recent years, especially in patients with isolated liver metastases*(van der Geest, Lam-Boer et al. 2015)*.

Following the liver, the lungs and then peritoneum are the next most common sites of disease*(van der Geest, Lam-Boer et al. 2015)*, occurring in 39% and 19% of patients with metastatic disease respectively*(van Gestel, de Hingh et al. 2014)*. Interestingly, extrahepatic disease appears to occur later than liver metastases, with the Dutch Registry database study identifying only 20% of lung metastases within the first year compared to 40% of liver metastases*(van Gestel, de Hingh et al. 2014)*.

Incidence, development and survival for patients with metastatic disease – synchronous versus metachronous

The Dutch Registry study of patients treated with curative intent found 18% of patients who initially presented without metastases developed metastatic disease at least 2 months after diagnosis of the primary tumour *(van Gestel, de Hingh et al. 2014)*. In this series approximately one third of patients with colorectal cancer developed metastases within 1 year*(van Gestel, de Hingh et al. 2014)*.

There is no consensus within the literature as to the definition of synchronous metastases which makes comparison of publications challenging. Synchronous metastases have been defined as those present at diagnosis, within 6 months and within 12 months. Irrespective of the variability in the definition, the incidence of synchronous metastases varies between publications. The incidence of metastases present at diagnosis in all patients with colorectal

cancer ranges from 13% *(Hunter, Garant et al. 2012)* to 18%*(van der Pool, Damhuis et al. 2012)*. Another series found 19% of patients developed metastases within 6 months*(Leporrier, Maurel et al. 2006)* and a registry study found 14.5% of patients were diagnosed with liver metastases before or during treatment *(Manfredi, Lepage et al. 2006)*.

Perhaps unsurprisingly, the rate of curative resection for metastatic disease increases for isolated, single-site, low volume disease*(van der Geest, Lam-Boer et al. 2015)*, suggesting that the earlier diagnosis of metastatic disease when it is still small volume is important. Survival data also supports this concept with the best survival in patients with isolated liver metastases*(Aloia, Vauthey et al. 2006)*, *(Kanas, Taylor et al. 2012)*, followed by those with liver only disease and then those with extrahepatic disease*(Kanas, Taylor et al. 2012)*. Small volume disease is also associated with lower recurrence rates*(Aloia, Vauthey et al. 2006)*. Median survival for patients with synchronous metastases receiving best supportive care is only 5-6 months*(van der Pool, Damhuis et al. 2012)*.

There is also variability in the literature regarding the number of patients with liver metastases who undergo resection with curative intent; this ranges from 6.3%-16.9% for synchronous and metachronous metastases respectively in one French population study*(Manfredi, Lepage et al. 2006)* to 20% in a SEER database registry study*(Kopetz, Chang et al. 2009)*. In the SEER database registry study the 5 year overall survival rate of patients undergoing hepatic resection with curative intent was 50%*(Kopetz, Chang et al. 2009)* but increased to 65.3% if patients are alive at 12 months after diagnosis, compared to 19.5% for patients who did not undergo resection but are alive at 12 months after diagnosis*(Kopetz, Chang et al. 2009)*. 5 year overall survival of 50-60% following hepatic resection is supported

by further studies*(Choti, Sitzmann et al. 2002)*, *(Aloia, Vauthey et al. 2006)* which have shown survival has improved since the 1980s*(Choti, Sitzmann et al. 2002)*. Furthermore most authors agree that the earlier detection of metastatic disease is important as even organ-confined metastatic disease which is initially regarded as irresectable can be downstaged and resected with the advent of new drugs*(Adam, Avisar et al. 2001)*, and whilst the majority of patients are unable to undergo curative metastatectomy, even those patients who cannot receive curative treatment benefit from earlier treatment in terms of survival*(Nordic Gastrointestinal Tumor Adjuvant Therapy 1992)*. This is further supported by the recent results of the SABR-COMET study which reported in 2019 having randomised 99 patients with oligometastatic disease from multiple tumour types, including colorectal cancer, to stereotactic ablative radiotherapy or palliative care. This study found improved overall survival from 28 months with palliative care to 41 months after SABR (p=0.09)*(Palma, Olson et al. 2019)*

Synchronous metastases are also associated with poorer survival. Data from the SEER database showed the best survival was observed in the subset of patients who presented without metastases but developed metastatic disease >6 months after diagnosis*(Cummings, Payes et al. 2007)*, but this study is limited by having excluded patients who developed metastatic disease within 6 months in their primary analysis. A French population based study supports these findings with 1 and 5 year overall survival rates for synchronous metastases of 34.8% and 3.3% respectively compared to 37.6% and 6.1% for metachronous metastases*(Manfredi, Lepage et al. 2006)*. A further study showed that the time to relapse for metachronous metastases did not predict for survival*(Chau, Allen et al. 2004)*.

In summary, the incidence and survival data regarding metastatic disease shows that the liver is the first and most common site of metastases in patients with colorectal cancer. The presence of metastases at diagnosis or the development of synchronous metastases, larger lesions and multiple lesions are all associated with poorer survival. This would therefore support the concept that the earlier diagnosis of metastatic disease when small is crucial to improve survival.

Management of patients who present with metastatic disease

22% of new diagnoses of colorectal cancer are with stage IV (or metastatic) disease*(Cancer Research UK 2019b)*. The NICE guidelines state that the priority in the management of patients should be first to control symptoms arising from the primary tumour*(National Institute for Health and Care Excellence (NICE) 2011a)*, which may include the formation of defunctioning stomas, primary surgery without neoadjuvant therapy, stenting and radiotherapy.

Further management of disease depends on the MDT assessment of whether the patient should be considered for potentially curative treatment (if it is considered that the primary and metastatic disease may be resectable with curative intent at any point in the future) or whether the patient should be treated with palliative intent.

Before treatment is considered it is crucial to ensure the full extent of any metastatic disease is identified by imaging. The NICE guidelines*(National Institute for Health and Care Excellence (NICE) 2011a)* recommend the following investigations for patients with suspected, or confirmed metastatic disease.

1. Contrast enhanced CT of the chest, abdomen and pelvis should be performed as the first line investigation to assess for metastatic disease*(National Institute for Health and Care Excellence (NICE) 2011a)*.
2. "If the CT scan shows metastatic disease only in the liver and the patient has no contraindications to further treatment, a specialist hepatobiliary MDT should decide if further imaging to confirm surgery is suitable for the patient – or potentially suitable after further treatment – is needed"*(National Institute for Health and Care Excellence (NICE) 2011a)*
3. "If the CT scan shows the patient may have extra-hepatic metastases that could be amenable to further radical surgery, an anatomical site-specific MDT should decide whether a positron emission tomography-CT (PET-CT) scan of the whole body is appropriate."*(National Institute for Health and Care Excellence (NICE) 2011a)*
4. "If contrast-enhanced CT suggests disease in the pelvis, offer an MRI of the pelvis and discuss in the colorectal cancer MDT."*(National Institute for Health and Care Excellence (NICE) 2011a)*

In our institution this is interpreted as:

- All patients should undergo CE CT of the thorax, abdomen and pelvis as the first line investigation to assess for metastatic disease
- If liver metastases are suspected on CE CT the extent of the liver disease should be staged by hepatocyte-specific contrast enhanced MRI of the liver with diffusion-weighted MRI and possible extra-hepatic metastatic disease is screened for using PET-CT

- If extra-hepatic metastases only are suspected the patient will be screened for further sites of disease using PET-CT
- If a pelvic site of disease is identified the patient will be scanned with high-resolution MRI of the rectum and pelvis, with alterations to the extent of the body scanned dependent on the CT localisation of suspected disease.

If a patient is to be considered for curative treatment following the imaging staging of the extent of disease, the first line management is systemic chemotherapy which has been shown to improve progression free survival but not overall survival*(National Institute for Health and Care Excellence (NICE) 2011a)*, *(National Institute for Health and Care Excellence (NICE) 2011b)*. Nordlinger *et al* showed the use of chemotherapy prior to primary surgery resulted in improved 3 year PFS with a HR 0.79 (p=0.058), which corresponded to a 7.3% increase in progression-free survival from 28.1% to 35.4% if patients are treated with chemotherapy instead of primary surgery and an increase in median progression free survival from 11.7 months to 18.7 months*(Nordlinger, Sorbye et al. 2008)*.

If the primary and metastatic disease remains operable following chemotherapy, there is little high-quality evidence to dictate whether patients undergo resection of the primary and metastatic disease as a synchronous procedure or, for liver only metastatic disease, resection of the liver disease or primary disease first*(National Institute for Health and Care Excellence (NICE) 2011b)*. Each operative strategy is valid and guidelines exist to guide MDTs according to the individual patient circumstances*(Adam, de Gramont et al. 2015)*, *(Wale, Van Cutsem et al. 2018)*.

Treatment options for patients who develop metastatic disease during follow-up after treatment of the primary tumour do not significantly differ from those described above where chemotherapy should be offered first followed by consideration of a curative procedure.

Screening for local recurrence and metastatic disease

There is heterogeneity in the literature regarding active surveillance strategies for patients with primary colorectal cancer treated with surgical resection. Despite this heterogeneity, all surveillance strategies aim to identify not only locally recurrent and/or metastatic disease but also metachronous secondary colorectal primaries*(Chau, Allen et al. 2004)*.

Surveillance strategies vary in their composition. The NICE guidance recommends regular surveillance including at least two CTs of the thorax, abdomen and pelvis in the first 3 years and CEA tests at least biannually for 3 years and a surveillance colonoscopy at 1 year*(National Institute for Health and Care Excellence (NICE) 2011a)*.

Studies of surveillance strategies place the overall risk of recurrent disease in patients treated for stage II and III colorectal cancer in the order of 15-30%*(Chau, Allen et al. 2004)*, *(Primrose, Perera et al. 2014)*. Two meta-analyses of surveillance strategies found intensive follow-up results in improved overall survival*(Jeffery, Hickey et al. 2002)*, *(Renehan, Egger et al. 2002)* but these and other studies did not utilise contrast enhanced CT of the thorax, abdomen and pelvis*(Minton, Hoehn et al. 1985)*, *(Moertel, Fleming et al. 1993)*, *(Arnaud, Cervi et al. 1997)*, *(Jeffery, Hickey et al. 2002)*, *(Renehan, Egger et al. 2002)*, *(Northover*

2003), *(Rodriguez-Moranta, Salo et al. 2006)* which is now regarded as a standard technique and thus the results may not be applicable today.

A more recent prospective study of 530 patients, published in 2003, investigated the modality by which recurrence was detected for 530 patients with stage II and III colorectal cancer. This study found that recurrence was identified earlier (and in more patients) by CT and then CEA than awaiting symptomatic presentation*(Chau, Allen et al. 2004)*. Patients diagnosed by CT more frequently underwent curative resection for their recurrent disease with improved survival compared to those with a symptomatic presentation or recurrence diagnosed by a CEA rise*(Chau, Allen et al. 2004)*.

However, a recent randomised study of surveillance strategies again found that more intensive surveillance resulted in a greater number of resections of recurrent disease with curative intent but this did not translate into a survival difference*(Primrose, Perera et al. 2014)*. Similarly, a further randomised controlled trial found no difference in survival irrespective of the intensity of surveillance*(Primrose, Perera et al. 2014)*, *(Wille-Jorgensen, Syk et al. 2018)*. To the best of my knowledge no studies of surveillance strategies have stratified patients according to the presence of mr-derived poor prognostic factors and there have been no studies which have investigated risk stratified surveillance.

Active surveillance appears to have the following benefits:

- Earlier diagnosis of metastatic disease by CT and CEA than by awaiting symptomatic presentation*(Chau, Allen et al. 2004)*
- Increasing numbers of patients identified at a stage in which their disease is resectable (23.8% of patients identified by CT +/- CEA versus 3.1% identified by

symptomatic presentation*(Chau, Allen et al. 2004)* which then translates into more patients undergoing resection with curative intent*(Chau, Allen et al. 2004)*, *(Primrose, Perera et al. 2014)*.

- In some studies, better survival if recurrence is identified by CT than by symptomatic presentation, hypothesised to be secondary to the earlier diagnosis of small volume disease enabling more patients to have curative resection*(Chau, Allen et al. 2004)*

Squamous cell cancer of the anus

Squamous cell cancer of the anus accounts for less than 1% of all cancers diagnosed within the UK annually (1438 new cases were diagnosed annually between 2014 and 2016)*(Cancer Research UK 2019a)*. The incidence of anal SCC is however rising; incidence has increased by 70% since the early 1990s and 40% in the last decade*(Cancer Research UK 2019a)*. This may be secondary to an increase in HPV infection which has been identified as the most important risk factor for the development of anal SCC*(World Health Organization 2000)*, *(Machalek, Poynten et al. 2012)*.

Clinical presentation and staging

Patients normally present with symptoms and as a result the majority present with relatively early stage tumours*(Gervaz, Allal et al. 2003)*. TNM staging(Table 1-12) and the assignment of AJCC Stage(Table 1-13) was developed on the clinical assessment of the maximal size of the tumour (T stage) and the presence of enlarged, abnormal-feeling lymph nodes (N stage). MRI is now recommended for the local staging of anal SCC*(The Royal College of Radiologists 2014)* but there has been no validation of the staging systems using

MRI. The presence of metastatic disease (M stage) continues to be assessed by CT*(American Joint Committee on Cancer (AJCC) 2017)*.

Table 1-12: TNM staging of anal SCC*(American Joint Committee on Cancer (AJCC) 2017)*

Primary tumour (T stage)	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ
T1	Tumour ≤2cm in greatest dimension
T2	Tumour >2cm - ≤5cm in greatest dimension
T3	Tumour >5cm in greatest dimension
T4	Tumour of any size which invades adjacent organs (vagina, urethra, bladder). NB: direct tumour invasion of the rectal wall, perirectal skin, subcutaneous tissue or sphincter muscles is not classified as T4
Regional lymph nodes (N stage)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Metastases in perirectal lymph nodes
N2	Metastases in unilateral internal iliac and/or inguinal lymph node(s)
N3	Metastases in perirectal and inguinal lymph nodes and/or bilateral internal iliac and/or inguinal lymph nodes
Distant metastases (M stage)	
MX	Presence of distant metastases cannot be assessed
M0	No distant metastases
M1	Distant metastases

Table 1-13: AJCC prognostic stage groups for anal SCC*(American Joint Committee on Cancer (AJCC) 2017)*

AJCC Stage	**T stage**	**N stage**	**M stage**
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2 or T3	N0	M0
Stage III	T1 or T2 or T3	N1-3	M0
	or T4	N0	M0
Stage IV	Any T	Any N	M1

50-60% of patients present with T1 and T2 lesions*(Salmon, Zafrani et al. 1986)*. It is estimated that 10% present with nodal metastases (increasing to 20-40% for T4 lesions)*(Salmon, Zafrani et al. 1986)* and a further 10% of patients present with metastatic disease*(Sebag-Montefiore 2017)*.

Treatment and outcomes for SCC anus

Anal SCC is an exquisitely radiosensitive tumour which is treated with combined-modality therapy following data from three international randomised studies in the late 1980s and 1990s. This data showed that incorporating radiotherapy with systemic chemotherapy (5-FU and mitomycin) improved outcomes compared to treatment with radiotherapy alone*(Flam, John et al. 1996)*, *(UKCCCR Anal Cancer Working Party 1996)*, *(Bartelink, Roelofsen et al. 1997)*. The UK ACT I study showed this combination treatment resulted in a 46% reduction in the risk of local failure*(UKCCCR Anal Cancer Working Party 1996)* and reduced the risk of relapse and death up to 13 years later*(Northover, Glynne-Jones et al. 2010)*. Similar results were found in the European EORTC study and USA RTOG study which found improved complete recurrence rates, locoregional control and survival with combination therapy*(Flam, John et al. 1996)*, *(Bartelink, Roelofsen et al. 1997)*. As a result, practice changed to offer combination therapy routinely*(Downing, Morris et al. 2015)*.

80% of patients in the EORTC trial achieved complete response to combination therapy*(Bartelink, Roelofsen et al. 1997)* however 25%*(Ajani, Winter et al. 2008)* to 35.3%*(Renehan, Saunders et al. 2005)* of patients will develop locoregional recurrence (at a median time of 20.4 months*(Renehan, Saunders et al. 2005)*) and 15% of patients will develop metastases within 5 years*(Ajani, Winter et al. 2008)*. Unfortunately the outcomes

for patients who do not achieve complete response to, or develop locoregional recurrence following, combination therapy are poor and the only curative option that remains is radical salvage surgery*(Kochhar, Mullan et al. 2017)*. A study from 2017 found an R0 resection is required to offer a chance of cure but R1/R2 rates are between 16-32%*(Mullen, Rodriguez-Bigas et al. 2007)*, *(Schiller, Cummings et al. 2007)*, *(Sunesen, Buntzen et al. 2009)*, *(Eeson, Foo et al. 2011)*, *(Lefevre, Corte et al. 2012)*, *(Kochhar, Mullan et al. 2017)* with typically no surviving patients at 3-5 years*(Renehan, Saunders et al. 2005)*, *(Sunesen, Buntzen et al. 2009)*, *(Eeson, Foo et al. 2011)*, *(Lefevre, Corte et al. 2012)*, equivalent to that if the patient had not undergone surgery*(Renehan, Saunders et al. 2005)*.

Predictors of response to treatment and outcomes

A number of factors have been identified as predictors of response to treatment and outcomes for anal SCC. However, it should be noted that each of these factors has been investigated in patients who have undergone clinical rather than MRI based staging which is the norm today.

Tumour size (defined by T stage) is related to outcomes. A binary categorisation of patients into good and poor prognosis by T stage is made between T1-2 (≤5cm maximal diameter) and T3-4 tumours (>5cm maximal diameter) and nodal disease by N stage N0 versus N1-3. The UK ACT II study showed clinically derived, size based T staging predicted for 3 year PFS (80% for T1-2 versus 65% for T3-4) but clinically derived N staging did not predict for 3 year PFS (76% for cN0 versus 68% of cN1-3 tumours)*(James, Glynne-Jones et al. 2013)*. Secondary analysis of the USA RTOG study showed tumours >5cm and N positive disease was associated with poorer 5-year DFS (P=0.0003 and P=0.001 respectively) and poorer 5-year

OS (P0 .0031 and p<0.0001 respectively). Interesting the EORTC study did not identify tumour size as a prognostic factor*(Bartelink, Roelofsen et al. 1997)*.

More recently these findings have been confirmed by further studies, for example a retrospective study of patients treated with definitive combination therapy showed, on multivariate analysis, that higher T stage (P = .023) and higher N stage (P = .030) independently predicted for a higher rate of locoregional failure*(Das, Bhatia et al. 2007)*. This study does not explicitly state how patients were assigned to T and N stage, however no patients underwent an MRI*(Das, Bhatia et al. 2007)* so it is likely that this was clinical T and N staging.

TNM staging combined as AJCC stage is related to 5 year survival as shown by the SEER database registry study with 5 year survival of 76.9% for stage I tumours, 66.7% for stage II, 50.7-57.7% for stage III and 15.3% for stage IV tumours*(National Cancer Institute 2015)*, *(American Joint Committee on Cancer (AJCC) 2017)*.

Tumour location defines the sites of nodal metastases*(American Joint Committee on Cancer (AJCC) 2017)* and whilst anal SCC is more common in females*(Cancer Research UK 2019a)* prognosis is worse for males*(Bartelink, Roelofsen et al. 1997)*, *(Ajani, Winter et al. 2008)*, *(Gunderson, Winter et al. 2012)*, *(Glynne-Jones, Sebag-Montefiore et al. 2013)*, *(American Joint Committee on Cancer (AJCC) 2017)*. Grade of differentiation has also been shown to be a predictor of survival*(American Joint Committee on Cancer (AJCC) 2017)*.

In summary, anal SCC is a highly treatable, radiosensitive tumour but up to one third of patients will develop locoregional recurrence and a further 15% will develop metastatic disease. The majority of studies have shown that size and nodal status predict for survival but these studies have been performed on patients undergoing clinical not MRI based staging and therefore may not be applicable to patients treated today with MRI staging. Further work is needed to define whether the current TNM staging system applies to MRI staging and to identify potential imaging biomarkers for progressive disease.

CHAPTER 2 - AIMS AND HYPOTHESES

The overarching aim of this thesis is to investigate the use of imaging biomarkers for risk stratification for disease relapse in patients with colorectal and anal cancer.

This thesis is formed of three parts which tell the story of imaging biomarker development, testing and application into clinical practice in the context of colorectal cancer and anal squamous cell carcinoma. Specifically:

- Part 1 reports a systematic review investigating the adherence of imaging biomarker studies to the REMARK guidelines.
- Part 2 describes two studies which have investigated the use of novel imaging biomarkers for colorectal cancer and anal squamous cell carcinoma.
- Part 3 describes the application of imaging biomarkers for the prediction of disease relapse in colorectal cancer.

Hypotheses

- Peer-reviewed publications of prognostic studies of imaging biomarkers for liver metastases in patients with colorectal cancer do not adhere to the "REporting recommendations for tumour MARKer prognostic studies" (REMARK) guidelines.
- The MRI assessment of tumour length and depth of extramural spread for anal squamous cell carcinoma can be used for risk stratification for disease relapse.
- MRI tumour regression grading following chemoradiotherapy in rectal cancer can be used to risk stratify patients for disease relapse in terms of the timing and site of metastatic disease.

- There is increased prevalence of synchronous liver metastases diagnosed by diffusion-weighted MRI in patients with MRI-defined high-risk versus low-risk rectal cancer.
- Diffusion-weighted MRI of the liver can be used as a screening tool for the diagnosis of synchronous liver metastases in patients with imaging-defined high-risk colorectal cancer.

CHAPTER 3 - GENERAL METHODS

Imaging methods

High-Resolution MRI of the Rectum

For chapters in this thesis which refer to colorectal cancer high-resolution MRI of the rectum has been used for the identification of validation of poor prognostic factors.

The poor prognostic factors which can be identified on MRI have been validated using a high-resolution technique which has been shown to be reproducible between centres and countries*(Mercury Study Group 2006)*, *(Mercury Study Group 2007)*, *(Group, Shihab et al. 2011)*, *(Taylor, Quirke et al. 2011b)*, *(Taylor, Quirke et al. 2011c)*.

Technical factors to ensure adequate imaging acquisition

MRI of the rectum is performed in our institution following the MRI protocol validated as part of the MERCURY study*(Brown, Daniels et al. 2006)*. In summary the sequences performed are:

- 1.5T Sagittal T2 fast (turbo) spin-echo
- Axial T2 FSE
- Oblique-axial and oblique-coronal T2 FSE small (16 cm) field of view (voxel size 1.1mm^3) as validated by the MERCURY Study.

The detailed scanner parameters are provided in Table 3-1.

The examination should be performed with an anti-spasmodic (hyoscine butylbromide) which is normally delivered via intramuscular injection*(Wale and Brown 2014)* and an

anterior saturation band should be employed to reduce bowel movement artefact. Every endeavour should be made to make the patient as comfortable as possible to reduce movement artefact, as a standard patients should be advised to empty their bladder and any pain should be controlled*(Taylor, Swift et al. 2008)*.

The scanner parameters as validated by the MERCURY Study group as shown in Table 3-1. Since the MERCURY Study some institutions, including our own, have elected to enlarge the field of view to 20 x 20cm but this should be off set by changes to the matrix size and slice thickness to maintain a voxel size of 1.1mm^3.

Table 3-1: High resolution pelvic MRI parameters as validated by the MERCURY Study Group

	Fast (Turbo) Spin Echo, T2 Weighted		
Parameter	**Sagittal (LFOV)**	**Axial (LFOV)**	**Obl-Axial and Obl-Coronal High Resolution**
Repetition time (TR), ms	3961	4018	5362
Echo time (TE), ms	125	80	100
TSE factor	23	20	16
Field of view / rectangular field of view	250/100%	300/100%	160/90%
Thickness/gap, mm	3/0.4	5/1	3/0.3
No. slices	24	32	24
No. acquisitions (NSA)	4	2	6
Matrix	512 x 320	512 x 256	256 x 256
Saturation bands	Anterior & superior	No	No
Acquisition time, min	6.0	3.28	7.35
Purpose of the scan	Localize tumour Scans enable height of tumour above anal verge and length of tumour to be assessed	Scans enable pelvic disease outside the mesorectum to be assessed.	High-resolution scans should be undertaken to assess the primary tumour and tumour spread within mesorectum i.e. high-resolution coverage to the L5/S1 level. Scans perpendicular to the long axis to assess the intersphincteric and levator planes.

Reproduced by permission from Wolters Kluwer Health, Inc. from Wale A, Brown G. A Practical Review of the Performance and Interpretation of Staging Magnetic Resonance

Imaging for Rectal Cancer. Topics in Magnetic Resonance Imaging (2014)*(Wale and Brown 2014)*.

Figure 3-1 shows an example of the same patient imaged at a voxel size of 1.6mm^3 (A) and then again at 1.1mm^3 (B). (A) T2-weighted axial image of the rectum that has been obtained with a low-resolution technique (voxel size of 1.6 mm^3); the early-stage rectal cancer is not clearly identified (solid arrow). (B), The same patient who has been rescanned with a high-resolution technique (voxel size of 1.1 mm^3) where the early T2 tumour is clearly demonstrated (white arrow).

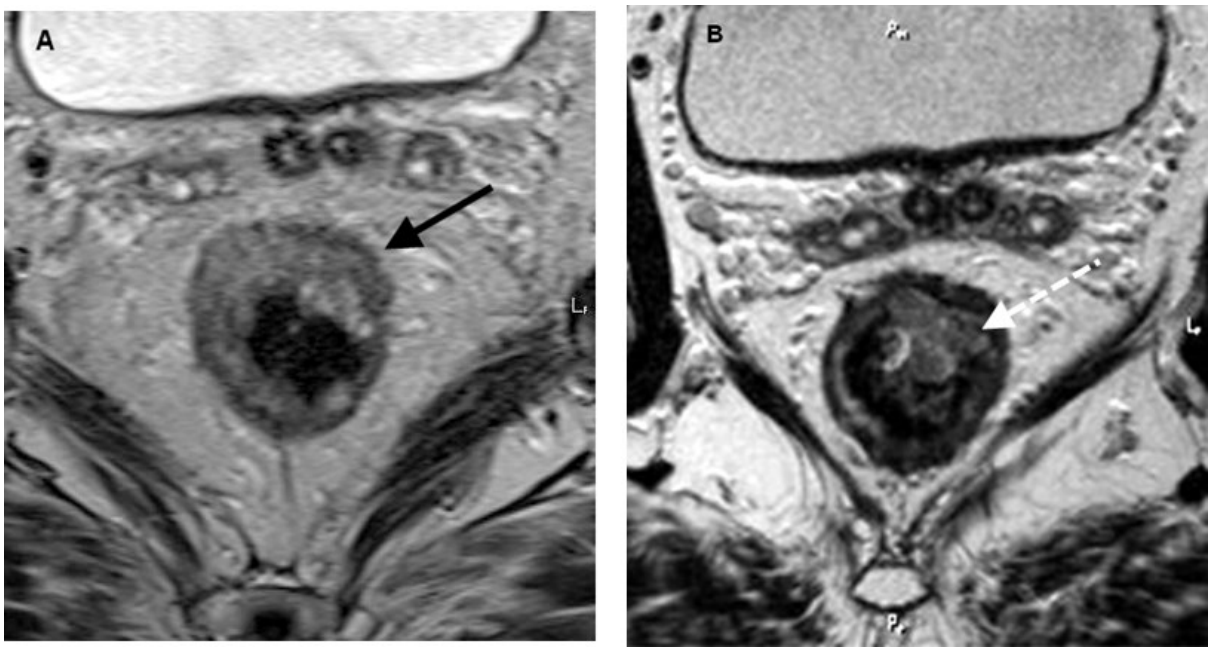


Figure 3-1: MRI technique - the importance of voxel size

Reprinted with permission from *(Wale and Brown 2014)*.

Image interpretation

Numerous publications have discussed the technique for the interpretation of MRI rectal examinations*(Taylor, Swift et al. 2008)*, *(Wale and Brown 2014)*. In addition, further publications have also described the nuances of the interpretation of examinations for the identification of individual validated poor prognostic factors*(Smith, Shihab et al. 2008)*, *(Patel, Blomqvist et al. 2012)*. Furthermore, the importance of workshop training and specialist, structured reporting have been demonstrated in the accurate identification of validated poor prognostic features on MRI*(Mercury Study Group 2006)*, *(Mercury Study Group 2007)*, *(Taylor, Mangat et al. 2010)*, *(Group, Shihab et al. 2011)*, *(Taylor, Quirke et al. 2011b)*, *(Taylor, Quirke et al. 2014)*, *(Siddiqui, Bhoday et al. 2016)*, *(Siddiqui, Gormly et al. 2016)*, *(Patel, Rockall et al. 2018)*. As a result good interobserver variability has been demonstrated for the identification and reporting of validated poor prognostic features on MRI*(Mercury Study Group 2007)*, *(Taylor, Quirke et al. 2011b)*, *(Taylor, Quirke et al. 2011c)*, *(Battersby, How et al. 2015)*, *(Siddiqui, Gormly et al. 2016)*.

High-resolution pelvic MRI for the staging of anal SCC

In recent years there has been a move to using high-resolution pelvic MRI for the staging of anal SCC. The Royal College of Radiologist's guidelines state that:

"MRI is the modality of choice to assess the extent of local invasion to sphincter pelvic floor and adjacent structures. Clear pre-treatment delineation of pelvic disease by MRI enables optimal planning of radiotherapy to the target volume"(The Royal College of Radiologists 2014).

The technical parameters for image acquisition are the same for high-resolution rectal MRI examinations with a couple of specific requirements for the imaging of anal SCC:

- Small field of view images of the inguinal lymph nodes should be obtained for lymph node staging
- The anal canal should be imaged with small field of view axial images which are perpendicular to the canal to assess for infiltration into adjacent organs
- As with rectal MRI, the most superior small field of view images should encompass the presacral space up to L5-S1 to assess for high nodal spread

Contrast-enhanced CT of the thorax, abdomen and pelvis

Contrast enhanced CT of the thorax, abdomen and pelvis is the routine examination for the M staging of colorectal and anal SCC and is recommended by the NICE guidelines*(National Institute for Health and Care Excellence (NICE) 2011a)*. The individual scanner parameters and protocols vary between hospitals but with little effect on the sensitivity and specificity of the examination provided the following principles are followed:

- The patient is comfortable to minimise the negative effect of movement artefact
- Contrast enhancement of the abdomen and pelvis is undertaken so that the images are acquired in the portal venous phase to ensure to ensure adequate soft tissue contrast between the abdominal organs. An example imaging protocol would be the use of 100ml of Omnipque 300© or Visipaque 270© with a 70 second delay for portal venous phase imaging.
- Contrast enhancement of the thorax may be obtained in the arterial or portal venous phase as the phase of contrast does not impact upon the identification of pulmonary nodules which may represent pulmonary metastatic disease. More

recently the use of maximum intensity projections has become standard to increase the accuracy of readers in identifying small pulmonary nodules*(Valencia, Denecke et al. 2006)*

For patients with colon cancer T and N staging can and should be performed on the portal venous phase imaging. The identification of validated poor prognostic factors is readily appreciated on the CT images, although often require the use of multiplanar reformats (sagittal and coronal) which are provided as standard.

The tumour should be identified following the principles of rectal cancer imaging*(Wale, Pawlyn et al. 2016)* and the depth of extramural spread, invasive border (mesenteric or peritoneal) and the presence or absence of extramural venous invasion should be reported.

Statistical methodology

Statistical analysis was performed by SPSS 25.0.0 (SPSS, Chicago, IL.) and Medcalc Software 2019. For all analyses, a P-value of <0.05 was considered significant.

Survival analysis

Studies which have reported survival outcomes used the Kaplan-Meier product limit method and Mantel-Cox log-rank tests of significance according to standard methodology*(Bland and Altman 1998)*.

PART 1: THE DEVELOPMENT AND VALIDATION OF IMAGING BIOMARKERS

CHAPTER 4 - INTRODUCTION TO PART 1

Making assessments and taking measurements is routine within medicine, from simple bedside measurements such as blood pressure, height and blood glucose, to more sophisticated but still routine laboratory examinations such as the full blood count. In the interpretation of an imaging examination, irrespective of the modality, assessments are made and measurements are taken, for example the morphology of a rectal tumour*(Wale and Brown 2014)* or the width of the common bile duct on an ultrasound when looking for a cause of upper abdominal pain.

However, imaging differs from laboratory examinations as in imaging the overall interpretation of the findings comes not only from the measurements but from the skill of the interpreter in making a visual assessment and deciding on whether the appearances of this particular scan are normal or abnormal, and if abnormal whether they are significant. This perhaps subjective interpretation can result in interobserver variability where two interpreters may review the same imaging but come to different conclusions.

As such there has been a desire to quantify some imaging characteristics with the hope that this may reduce interobserver variability and enable mathematical correlations between imaging findings and outcomes. In this scenario the measurements taken on imaging would be regarded as biomarkers, although not all biomarkers need to be quantified.

A background to biomarkers

There have been a number of definitions of the term "biomarker" since its inception in a paper from 1980 which looked at the amount of a membrane-bound enzyme (UDP-galactose: N-acetylglucosamine galactosyltransferase (GT)) present and its relationship to the presence or absence of, and the stage of, breast cancer*(Paone, Waalkes et al. 1980)*, *(Aronson 2005)*. The authors concluded that "Serum GT may be potentially useful in the detection of recurrent breast carcinoma and as a marker of tumor response to therapy for advanced disease"*(Paone, Waalkes et al. 1980)* and entitled the paper "Serum UDP-galactosyl transferase as a potential biomarker for breast carcinoma"*(Paone, Waalkes et al. 1980)*.

Moving forward a couple of decades, the term "biomarker" was defined by the National Institutes of Health Biomarker Definitions Working Group in 2001 as:

"Characteristics that are objectively measured and evaluated as indicators of normal biological processes, pathological processes, or pharmaceutical responses to a therapeutic intervention"(Biomarkers Definitions Working Group 2001).

Also in 2001, the World Health Organisation in collaboration with the United Nations and the International Labour Organization*(Strimbu and Tavel 2010)* defined biomarkers as:

"…any substance, structure or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease." And said that "Biomarkers can be classified into markers of exposure, effect and susceptibility."

More recently in 2016 the FDA-NIHR Biomarker Working Group defined biomarkers as:

> *"A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions.(FDA-NIH Biomarker Working Group 2016)"*

Essentially each of the definitions of the term "biomarker" agree that biomarkers are **objectively measured** and can be used as "**indicators of processes within the body**"*(Biomarkers Definitions Working Group 2001)*. This makes a biomarker distinct from a clinical endpoint which measures disease from the subject's perspective as biomarkers are biological characteristics*(Strimbu and Tavel 2010)*.

Many biomarkers are also used as standard clinical measurements. Blood pressure, for example, is considered a biomarker for the risk of stroke*(Desai, Stockbridge et al. 2006)*. Other biomarkers are more specialised and have been developed and validated specifically to be biomarkers, for example the ACR BI-RADS breast morphology score*(American College of Radiology 2013)* which is used worldwide as a diagnostic decision making tool in mammography*(O'Connor, Aboagye et al. 2017)*.

Measurement and use of biomarkers

Biomarkers as objectively measured indicators of processes within the body*(Biomarkers Definitions Working Group 2001)* can been categorised according to the endpoint for which they can be used to predict*(Waterton and Pylkkanen 2012)*, *(FDA-NIH Biomarker Working Group 2016)* or the method used to obtain them*(Waterton and Pylkkanen 2012)*.

Broadly speaking, biomarkers can be used for prognostication, prediction, for monitoring or for response, and some can be used as surrogate markers for survival*(Waterton and Pylkkanen 2012)*. Table 4-1 defines these terms with examples from Oncology.

Table 4-1: Definitions of the endpoint classifications of biomarkers with Oncology examples.

Term	FDA-NIH Definition	Examples from Oncology
Predictive Biomarker	"A biomarker used to identify individuals who are more likely than similar individuals without the biomarker to experience a favorable or unfavorable effect from exposure to a medical product or an environmental agent."*(FDA-NIH Biomarker Working Group 2016)*	• Squamous differentiation in non-small cell lung cancer to predict which patients should not be treated with pemetrexed as this is associated with poorer survival*(Scagliotti, Hanna et al. 2009)*, *(FDA-NIH Biomarker Working Group 2016)* • TNM stage*(Waterton and Pylkkanen 2012)*
Prognostic Biomarker	"A biomarker used to identify likelihood of a clinical event, disease recurrence or progression in patients who have the disease or medical condition of interest."*(FDA-NIH Biomarker Working Group 2016)*	• Elevation of prostate specific antigen (PSA) to predict for the likelihood of disease progression in patients with prostate cancer*(Roberts, Blute et al. 2001)*, *(FDA-NIH Biomarker Working Group 2016)* • TNM stage*(Waterton and Pylkkanen 2012)*
Monitoring Biomarker	"A biomarker measured serially for assessing status of a disease or medical condition or for evidence of exposure to (or effect of) a medical product or an environmental agent."*(FDA-NIH Biomarker Working Group 2016)*	• Cancer antigen 125 (CA 125) for assessing disease burden in ovarian cancer during and after treatment*(Rustin, Marples et al. 2001)*, *(Gundogdu, Soylu et al. 2011)*, *(FDA-NIH Biomarker Working Group 2016)* • Recurrence with FDG PET-CT*(Waterton and Pylkkanen 2012)*
Validated Surrogate Endpoint in the context of biomarkers	"An endpoint supported by a clear mechanistic rationale and clinical data providing strong evidence that an effect on the surrogate endpoint predicts a specific clinical benefit."*(FDA-NIH Biomarker Working Group 2016)*	• Splenic volume in assessment of response in patients with myelofibrosis*(O'Connor, Aboagye et al. 2017)*

In terms of the method of obtaining the biomarkers they can be grouped into those which are “bio-specimen” biomarkers i.e. biochemical*(McShane, Altman et al. 2005)*, molecular or genetic and “bio-signal” biomarkers i.e. imaging biomarkers*(Waterton and Pylkkanen 2012)*.

“Bio-specimen” biomarkers and the development of the REMARK criteria

Initial biomarker studies concentrated on “bio-specimen” biomarkers as in the first biomarker paper from 1980 which looked at serum GT as a biomarker for breast cancer*(Paone, Waalkes et al. 1980)*.

By 2000, with two decades of investment and resources into biochemical biomarkers in oncology, a tiny number of biochemical biomarkers had been translated into clinical practice which was a concern for the biomarker community*(Hayes, Bast et al. 1996)*, *(Bast, Ravdin et al. 2001)*, *(Schilsky and Taube 2002)*, *(McShane, Altman et al. 2005)*. It was proposed that a major reason for this was the inconsistencies between initial studies which showed promise and subsequent studies which show “inconsistent and/or contradictory” results*(McShane, Altman et al. 2005)*. The possible causes for this which were proposed can be divided into three broad categories:

1. **Methodological problems**: poor design, lack of standardisation and reproducibility, sample sizes which are too small*(McGuire 1991)*, *(Fielding, Fenoglio-Preiser et al. 1992)*, *(Burke and Henson 1993)*, *(Concato, Feinstein et al. 1993)*, *(Gasparini, Pozza et al. 1993)*, *(Simon and Altman 1994)*, *(Gasparini 1998)*, *(Hall and Going 1999)*

2. **Statistical problems**: inadequately powered studies, data mining, "subset analysis"*(McShane, Altman et al. 2005)* and "cutpoint optimisation"*(Altman, De Stavola et al. 1995)*, *(McShane, Altman et al. 2005)*.
3. **Reporting problems**: incomplete reporting with insufficient information to allow for an assessment of the possible methodological and statistical problems*(McShane, Altman et al. 2005)*. These problems were highlighted in systematic reviews of imaging biomarkers for non-small cell lung cancer*(Brundage, Davies et al. 2002)*, breast cancer*(Mirza, Mirza et al. 2002)*, *(Burton and Altman 2004)*, neuroblastoma*(Riley, Abrams et al. 2003)*, *(Riley, Heney et al. 2004)*, the Ewing's sarcoma family of tumours*(Riley, Burchill et al. 2003)* and colorectal cancer*(Burton and Altman 2004)*, *(Popat, Matakidou et al. 2004)*. These systematic reviews all found similar problems with deficiencies of reporting throughout the publications.

As a result the first meeting of the European Organisation for Research and Treatment of Cancer (NCI-EORTC) First International Meeting on Cancer Diagnostics (From Discovery to Clinical Practice: Diagnostic Innovation, Implementation, and Evaluation) held in Nyborg, Denmark in 2000 set out to discuss the successes and problems in the field of cancer diagnostics*(McShane, Altman et al. 2005)*. A major recommendation of this meeting was to develop reporting guidelines for prognostic biochemical biomarker studies*(McShane, Altman et al. 2005)* similar to the CONSORT guidelines for the reporting of randomised controlled trials*(Moher, Schulz et al. 2001)* and the STARD guidelines for the reporting of diagnostic accuracy studies*(Bossuyt, Reitsma et al. 2003)*. The thinking behind this was it is not possible to improve upon the methodological and statistical problems with biomarker

studies if the quality of publications is insufficient so that these problems cannot be assessed for.

The resulting "REporting recommendations for tumour MARKer prognostic studies (REMARK)" Guidelines were developed*(McShane, Altman et al. 2005)*, *(Altman, McShane et al. 2012)* based on previous publications*(Altman and Lyman 1998)*, *(Gion, Boracchi et al. 1999)*, *(Altman 2001)*, *(Altman DG 2001)*, *(McShane LM 2001)*, *(R 2001)*, *(Biganzoli, Boracchi et al. 2003)*, *(Riley, Abrams et al. 2003)*, *(Schumacher M 2005)*.

The REMARK Guidelines

The REporting recommendations for tumour MARKer prognostic studies (REMARK) guidelines were developed for studies evaluating a single biochemical prognostic tumour marker and published in 2005*(McShane, Altman et al. 2005)*. Whilst they are largely relevant for studies which look at more than one biomarker, they do not specifically address the statistical considerations which need to be made when prognostic models are developed from a very large numbers of candidate biomarkers.

The REMARK guidelines are a 20-point checklist of reporting recommendations which should be adhered to when publishing the results of biochemical biomarker studies. The checklist is reproduced in Table 4-2.

Table 4-2: REporting recommendations for tumour MARKer prognostic studies

(REMARK)*(McShane, Altman et al. 2005)*

Introduction

1. State the marker examined, study objectives, and any pre-specified hypotheses.

Materials and methods

Patients

2. Describe the characteristics (e.g. disease stage or comorbidities) of the study patients, including their source and inclusion and exclusion criteria.
3. Describe the treatments receives and how chosen (e.g. randomised or rule-based).

Specimen characteristics

4. Describe type of biological material used (including control samples) and methods of preservation and storage.

Assay methods

5. Specify the assay method used and provide (or reference) a detailed protocol, including specific reagents or kits used, quality control procedures, reproducibility assessments, quantification methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to study endpoint.

Study design

6. State the method of case selection including whether prospective or retrospective and whether stratification or matching (e.g. by stage of disease or age) was used. Specify the time period from which cases were taken, the end of the follow-up period, and the median follow-up time.
7. Precisely define all clinical end points examined.
8. List all candidate variable initially examined or considered for inclusion in the models.
9. Give rationale for sample size; if the study was designed to detect a specified effect size, give the target power and affect size.

Statistical analysis methods

10. Specify all statistical methods, including details of any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data values were handled.
11. Clarify how marker values were handled in the analyses; if relevant describe methods used for cut point determination.

Results

Data

12. Describe the flow of patients through the study, including the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the number of patients and the number of events.
13. Report distributions of basic demographic characteristics (at least age and sex), standard (disease-specific) prognostic variables, and tumour marker, including number of missing values.

Analysis and presentation

14. Show the relation of the marker to standard prognostic variables.
15. Present univariate analyses showing the relation between the marker and outcome, with the estimated effect (e.g. hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analysed. For the effect of a tumour marker on a time-to-event outcome, a Kaplan-Meier plot is recommended.
16. For key multivariable analyses, report estimated effects (e.g. hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model.
17. Among reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their statistical significance.
18. If done, report results of further investigations, such as checking assumptions, sensitivity analyses and internal validation.

Discussion

19. Interpret the results in the context of the pre-specified hypotheses and other relevant studies; include a discussion of limitations of the study.
20. Discuss implications for future research and clinical value.

Since the introduction of the REMARK guidelines many journals state adherence to the REMARK guidelines is a requirement for publication of biochemical tumour biomarker studies*(Mallett, Timmer et al. 2010)*. Prior to the introduction of the REMARK guidelines a systematic review found the completeness of reporting of biochemical biomarker studies was 53.4% (range: 10%-90%)*(Mallett, Timmer et al. 2010)*. Following implementation of the REMARK criteria the completeness of reporting was 58.1% (range: 30%-100%)*(Sekula, Mallett et al. 2017)*. Overall there was no significant difference in the completeness of reporting following implementation of the REMARK guidelines*(Sekula, Mallett et al. 2017)*. The authors therefore concluded that a further combined effort is needed from all involved in the reporting of these clinical studies before a difference in the quality of biochemical biomarker study reporting will be seen*(Sekula, Mallett et al. 2017)*. This conclusion is supported by other authors; for example a Nature editorial in 2011 concluded that biomarkers are developed without consideration of the methodology required to correlate

the biomarker with clinically relevant endpoints, that validation studies of an adequate size are challenging logistically and that general methodological and statistical challenges have been identified*(Poste 2011)*.

Similar methodological and statistical inadequacies and inadequate reporting are likely to be encountered with imaging biomarker studies. A large number of studies of imaging biomarkers have been identified of which very few have been translated into clinical practice*(Poste 2011)*, *(Sullivan, Obuchowski et al. 2015)*, *(O'Connor, Aboagye et al. 2017)*; O'Connor found 10'000 studies reported on new or established imaging biomarkers between 2004 and 2014*(O'Connor, Aboagye et al. 2017)* and Sullivan found 43'000 studies in a search for publications which report on quantitative imaging biomarkers*(Sullivan, Obuchowski et al. 2015)*. As with biochemical biomarkers it is hypothesised that poor reporting and contradictory results*(Mallett, Timmer et al. 2010)* may also be hampering the implementation of imaging biomarkers into clinical practice.

Imaging ("Bio-signal") Biomarkers

Increasingly imaging biomarkers are favoured over biochemical biomarkers as they allow non-invasive, serial measurements with the option to image the entire patient*(Mankoff, Pryma et al. 2014)*.

Guidelines have been published with recommendations for image acquisition and analysis*(Food and Drug Administration 2015)*, standardisations of acquisition, analysis and terminology*(National Cancer Institute* , *(Tofts, Brix et al. 1999)*, *(Leach, Brindle et al. 2005)*,

(Hunter 2008), *(Woodcock and Woosley 2008)*, *(Shankar 2012)*, *(Waterton and Pylkkanen 2012)*, *(European Society of Radiology 2013)*, *(Clarke, Nordstrom et al. 2014)*, *(Boellaard, Delgado-Bolton et al. 2015)*, *(Huang, Wang et al. 2015)*, *(Sullivan, Obuchowski et al. 2015)*, *(FDA-NIH Biomarker Working Group 2016)* and the validation and qualification of imaging biomarkers*(O'Connor, Aboagye et al. 2017)*. Table 4-3 describes the guidelines published to date with their scope.

Table 4-3: Summary of the imaging biomarker guidelines and their scope

Guideline & issuing body	Date	Main aim	Key recommendations
Image acquisition and analysis			
Clinical Trial Imaging Endpoint Process Standards Guidance for Industry *(Food and Drug Administration 2015)*	2015	"To assist sponsors in optimizing the quality of imaging data obtained in clinical trials intended to support approval of drugs and biological products" Appears to be written for sponsors of phase III trials.	Existing standards, for example the use of PACS systems and DICOM images, could be augmented to create "trial-specific imaging process standards".
Standardisation of acquisition, analysis and terminology			
BEST (Biomarkers, EndpointS, and other Tools) Resource *(FDA-NIH Biomarker Working Group 2016)*	2016	"To harmonize and clarify terms used in translational science and medical product development and to provide a common language used for communication by those agencies"*(Cagney, Sul et al. 2018)*	Provided definitions of key biomarkers terms, for example diagnostic biomarker, predictive biomarker, prognostic biomarker and validated surrogate endpoint.
Cancer Imaging Programme *(National Cancer Institute*	Last updated 2019	"Fosters advances in in vivo medical imaging sciences through support of basic and applied research in cancer imaging as well as promotion of imaging in clinical trials in order to gain greater understanding of the pathways of cancer biology for the benefit of cancer patients and people at cancer risk."	• Set of focussed imaging guidelines e.g. for DCE-MRI, MRS but no specific guidelines related to colorectal cancer • General publications e.g. quality control of PET-CT • No publications regarding the requirements for publication.
Estimating kinetic parameters from dynamic contrast-enhanced T(1)-weighted MRI of a diffusable tracer: standardized quantities and symbols. Review article in JMRI *(Tofts, Brix et al. 1999)*	1999	To "issue standardised terms for the estimation of kinetic parameters from DCE-MRI".	Standard set of quantity names and symbols for the "estimation of kinetic parameters from DCE T1-weighted MRI data, including: 1. Volume transfer constant Ktrans 2. Volume of extravascular extracellular space (EES) per unit volume of tissue 3. Flux rate constant

Guideline & issuing body	Date	Main aim	Key recommendations
The assessment of antiangiogenic and antivascular therapies in early-stage clinical trials using magnetic resonance imaging: issues and recommendations. Pharmacodynamic/Pharmacokinetic Technologies Advisory Committee (PTAC)*(Leach, Brindle et al. 2005)*	2005	"Reports the outcome of a workshop that considered the methodology and design of magnetic resonance studies (DCE-MRI), recommending how this new tool might best be used."	• "Recommendations for MR measurement methods and end points for use in Phase I/II trials of anticancer therapeutics" • "Recommendations for analysis of DCE-MRI data in ROI or VOI" • "Standardisation, validation and reproducibility guidelines" but no specific guidelines regarding the publication of imaging biomarker studies.
The FDA critical path initiative and its influence on new drug development. *(Woodcock and Woosley 2008)*	2008	"Issued with the intent of modernizing drug development by incorporating recent scientific advances, such as genomics and advanced imaging technologies, into the process."	Identified development gaps, critical path processes and project & the deliverables.
The clinical evaluation of novel imaging methods for cancer management. National Cancer Institute*(Shankar 2012)*	2012	Description of the different NCI funding streams for clinical trials in imaging.	Description of the different NCI funding streams for clinical trials in imaging.
Qualification of imaging biomarkers for oncology drug development. QuIC-ConCePT (Quantitative Imaging in Oncology: Connecting Cellular Processes to Therapy) *(Waterton and Pylkkanen 2012)*	2012	Describes the challenges of imaging biomarker development, a roadmap for imaging biomarker development & the QuIC-ConCePT initiative.	• Makes a distinction between qualification and technical validation, but did not described validation against clinical outcomes. • Developed a roadmap for qualifying imaging biomarkers with "robust and standardised procedures", "correlation with pathology", "effect size, reproducibility and timing", "cross-sectional correlations" and then "correlation with outcomes".

Guideline & issuing body	Date	Main aim	Key recommendations
ESR statement on the stepwise development of imaging biomarkers. *(European Society of Radiology 2013)*	2012	Describes the unique challenges posed in the qualification and technical validation of imaging biomarkers.	• Distinguishes between technical validation and qualification • Describes the process of the development of imaging biomarkers • States "Imaging biomarker(s) should bring new information on top of existing diagnostic tools or existing risk factors and have the potential to modify the patient management"*(Wang 2011)*
The Quantitative Imaging Network: NCI's Historical Perspective and Planned Goals *(Clarke, Nordstrom et al. 2014)*	2014	Editorial provides a brief history of National Institutes of Health National Cancer Institute workshops related to quantitative imaging within the oncology setting and recently supported NCI initiatives, including the Quantitative Imaging Network (QIN) initiative.	• No specific recommendations made
FDG PET/CT: EANM procedure guidelines for tumour imaging: version 2.0. European Association of Nuclear Medicine*(Boellaard, Delgado-Bolton et al. 2015)*	2015	"To assist physicians in recommending, performing, interpreting and reporting the results of FDG PET/CT for oncological imaging of adult patients."	• Harmonisation/standardisation of diagnostic quality and quantitative information in oncology imaging of adult patients. • Presents a standardised imaging procedure for static FDG PET/CT data acquisition, QC and QA.
Meta-analysis of the technical performance of an imaging procedure: guidelines and statistical methodology. *(Huang, Wang et al. 2015)*	2015	Statistical guidelines for how meta-analyses of imaging biomarkers should be performed.	• Application of statistical methodology • Describes how meta-analyses should be reported in a "complete and transparent fashion in order to ensure proper interpretation and dissemination of the results".

Guideline & issuing body	Date	Main aim	Key recommendations
Metrology Standards for Quantitative Imaging Biomarkers RSNA-QIBA Metrology Working Group*(Sullivan, Obuchowski et al. 2015)*	2015	"To review some of the important statistical concepts relevant to technical performance, describe methods that can be used for evaluating and comparing quantitative imaging biomarkers, and discuss some of the technical performance issues related to imaging biomarkers"	• Distinguishes between technical performance and clinical validation • Requires the framework in which quantitative imaging biomarkers are acquired is "described rigorously, including context of use, acquisition parameters, and measurement methods." • Provides recommended terminology • Describes six steps for designing quantitative imaging biomarker technical performance studies
Validation and qualification of imaging biomarkers			
Imaging biomarker roadmap for cancer studies. Cancer Research UK and EORTC*(O'Connor, Aboagye et al. 2017)*	2017	Describes the developed, detailed roadmap for the validation and qualification of imaging biomarkers to improve translation.	• Describes how all biomarkers must cross two "translational gaps" before they can be used to guide clinical decisions. • Describes 14 key recommendations for accelerating the clinical translation of imaging biomarkers including, but not limited to, parallel tracks of technical assay validation, assessment of cost effectiveness, the need for standardisation and accreditation systems • Recommends that the "REMARK guidelines provide a framework for the assessment of clinical utility and validation"*(McShane, Altman et al. 2005)*

Some of these guidelines have described recommendations regarding the publication of imaging biomarker studies but no dedicated guidelines regarding the reporting of imaging biomarker studies exist. Guidelines from the Pharmacodynamic/Pharmacokinetic Technologies Advisory Committee *(Leach, Brindle et al. 2005)* described requirements for "standardisation, validation and reproducibility"*(Leach, Brindle et al. 2005)*, guidelines about how meta-analyses of imaging biomarker studies should be performed and described how the results should be reported in a "complete and transparent fashion"*(Huang, Wang et al. 2015)*. The Cancer Research UK and EORTC Imaging Biomarker Roadmap recommended use of the REMARK guidelines*(O'Connor, Carano et al. 2009)* for the reporting of imaging biomarker studies.

Since imaging biomarkers have been proposed for use in clinical practice in the same way as the biochemical prognostic markers, it is logical that they follow the principles of the REMARK guidelines*(McShane, Altman et al. 2005)*. But to date it is unknown whether the REMARK guidelines could be applied to imaging biomarker studies successfully and if so whether imaging biomarker studies would adhere to them. I therefore performed a systematic review of imaging biomarker studies and their adherence to the REMARK guidelines. The results of this work are presented in Chapter 5.

CHAPTER 5 - REPORTING OF PROGNOSTIC IMAGING BIOMARKER STUDIES IN METASTATIC COLORECTAL CANCER: A SYSTEMATIC REVIEW OF PUBLISHED ARTICLES IN RELATION TO THE REMARK GUIDELINES

This chapter is based on the manuscript **A. Wale**, K De Paepe, C Messiou, N Tunariu, KC. Kontovounisios, G. Brown. "Reporting of prognostic imaging biomarker studies in metastatic colorectal cancer: a systematic review of published articles in relation to the REMARK guidelines". *Manuscript ready for submission.*

Introduction

Whilst there has been extensive time and monetary investment in biomarker research few biomarkers have been translated into routine clinical practice*(Kern 2012)*. Many biomarkers (both biochemical and imaging) initially show promise but their implementation into clinical practice is limited by inconsistencies in the results of subsequent studies, reporting bias and poor or incomplete reporting of studies in the published literature. As a result many biomarker studies are ineligible for inclusion in systematic reviews and meta-analyses*(Mallett, Timmer et al. 2010)*.

For biochemical biomarkers it was hypothesised that the incomplete reporting of biochemical biomarker studies may contribute to the lack of implementation*(McShane, Altman et al. 2005)*. This led to the REporting recommendations for tumour MARKer prognostic studies (REMARK) guidelines*(McShane, Altman et al. 2005)*, adherence to which is a requirement for publication of biochemical tumour biomarker studies*(Mallett, Timmer et al. 2010)*. Various guidelines have been published for imaging biomarker studies

(National Cancer Institute , (Tofts, Brix et al. 1999), *(Leach, Brindle et al. 2005)*, *(Hunter 2008)*, *(Woodcock and Woosley 2008)*, *(Shankar 2012)*, *(Waterton and Pylkkanen 2012)*, *(European Society of Radiology 2013)*, *(Clarke, Nordstrom et al. 2014)*, *(Boellaard, Delgado-Bolton et al. 2015)*, *(Food and Drug Administration 2015)*, *(Huang, Wang et al. 2015)*, *(Sullivan, Obuchowski et al. 2015)*, *(FDA-NIH Biomarker Working Group 2016)*, *(O'Connor, Aboagye et al. 2017)* but there are no guidelines for reporting of these studies.

As with biochemical biomarkers it is hypothesised that poor reporting and contradictory results*(Mallett, Timmer et al. 2010)* may also be hampering the implementation of imaging biomarkers into clinical practice. Since imaging biomarkers have been proposed for use in clinical practice in the same way as the biochemical prognostic markers, it is logical that they follow the similar principles as the REMARK guidelines*(McShane, Altman et al. 2005)*.

I undertook a systematic review which aimed to determine the extent of the reporting of items from the REMARK guidelines in prognostic studies of imaging biomarkers for liver metastases in patients with colorectal cancer.

The hypothesis was that peer-reviewed publications of prognostic studies of imaging biomarkers for liver metastases in patients with colorectal cancer do not adhere to the "REporting recommendations for tumour MARKer prognostic studies" (REMARK) guidelines.

Methods

Ethics committee approval was not required as only published data from studies in humans were included.

Literature search

I conducted the systematic review in keeping with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines*(Liberati, Altman et al. 2009)*. Records were identified through MEDLINE database searching performed on 24th August 2018.

The search strategy was designed to identify studies which had included patients with liver metastases from colorectal cancer, prior to undergoing an intervention (i.e. prognostic biomarker studies), with any comparator and survival outcomes. The terms used were “colorectal neoplasm”, bowel* OR colon* OR colorectal OR rectal OR rectum) ADJ3 (cancer* OR tumor* OR tumour* OR malignan* OR neoplas*, liver OR hepatic) ADJ3 metastas*,”TOMOGRAPHY, X-RAY COMPUTED”, "MAGNETIC RESONANCE IMAGING", MRI, recist, k trans, SUV, percist, predict* OR stag* OR prognos* OR evaluat* OR assess* OR indicat*. The complete search strategy is available in Appendix 3. The search terms were kept intentionally broad to avoid missing relevant studies.

Inclusion criteria

Articles were included if they examined the impact of a prognostic imaging biomarker on cancer outcomes (one or more of overall survival, disease free survival, progression free

survival or recurrence) in patients with liver metastases from colorectal cancer. Prognostic imaging biomarkers were chosen as the focus of this review as the few prognostic imaging biomarkers which have been validated against outcomes form part of routine clinical practice*(Brown, Radcliffe et al. 2003)*, *(National Institute for Health and Care Excellence (NICE) 2011a)*, *(Taylor, Quirke et al. 2011b)*, *(Chand, Bhangu et al. 2014)* and so there is increasing interest in finding other prognostic biomarkers.

Prognostic imaging biomarkers derived from any imaging modality were eligible for inclusion including, but not limited to, CT, MRI and PET-CT.

As per prior studies which investigated the completeness of the REMARK reporting criteria for biological biomarkers*(Mallett, Timmer et al. 2010)*, prognostic studies evaluating an imaging biomarker were included irrespective of whether the patient data originated from prospective or retrospective data collection. There were no limitations on the sample size of the included studies or the language of the included studies.

Validity assessment, data abstraction and consensus review

I performed an initial hand review of the abstracts to exclude the majority of ineligible studies which could be excluded by hand review.

I then assessed the full-text manuscripts initially for eligibility and then against the REMARK guidelines in a random order using a pre-piloted data extraction form of 59 items based on the REMARK guidelines*(McShane, Altman et al. 2005)*{Appendix 4). Only studies which assessed a prognostic imaging biomarker for patients with colorectal liver metastases were

included. Studies were excluded if they did not report original work or if they assessed predictive imaging biomarkers or prognostic imaging biomarkers in response to locoregional therapy, if a time to event outcome was not assessed, or if the results were duplicated.

In order to generate a consensus score for each of the 59 items the studies were then reviewed by other academic radiologists (KDP, CM and NT) who each reviewed and scored 10 of the manuscripts blinded to my score.

I compared these scores to my own and then determined the consensus score with a further review of the original manuscript. Agreement between the readers was not planned or performed as due to the large number of items to be scored reader fatigue was likely to be a cause of disagreement and reader agreement was not the aim of this study.

Results synthesis

The reporting of each of the 59 items within the data extraction form was assigned a score of 1 if clearly reported, and of zero if an inadequate amount of detail was provided, if it was unclear or not reported as per the methodology of other systematic reviews which have assessed the completeness of reporting against the REMARK criteria*(Mallett, Timmer et al. 2010)*. A score for each of the twenty items of the REMARK criteria was not planned as not all information included within the 59 items of the data extraction form has equal importance and an overall score would not allow the detail to be determined. For each of the items the completeness of reporting was presented as the number and percentage of the 30 articles which clearly reported the item and in terms of the total number and

percentage of patients who were involved in those studies. Summary measures, further results synthesis or a meta-analysis were not planned or performed.

Bias and the assessment of study quality

The risk of bias for individual studies was minimised by having multiple readers and consensus decision making and including papers of any language published in any journal. Selective reporting bias has been identified in biomarker studies within other cancer groups*(Altman, McShane et al. 2012)* and is presumed to exist within colorectal cancer biomarker studies too but we are unable to make a further assessment of this within this review.

Study quality assessment was not planned or undertaken beyond adherence to the REMARK criteria which was the objective of this systematic review.

Development of the 59 item data extraction form

The REMARK criteria (Table 4-2) was developed as reporting guidelines for prognostic biochemical tumour marker studies evaluating a single tumour marker of interest, often including adjustment for standard clinical prognostic variables. They are largely relevant for studies exploring more than one marker, but they are not intended to specifically address statistical considerations in development of prognostic models from very large numbers of candidate markers. The same considerations have been made in selecting studies for this systematic review where studies which predominantly evaluate one prognostic imaging biomarker have been assessed. If more than two imaging biomarkers were assessed within

the same study, the study was included in this systematic review only if the results from each biomarker were reported so it could be assessed independently.

The principles of the REMARK guidelines are wholly applicable to imaging biomarker studies but with some adaptations to the explanatory notes. I made these adaptions prior to undertaking the systematic review in discussion with my supervisor and in consultation with the explanation and elaboration documents for the REMARK criteria*(Altman, McShane et al. 2012)*. The explanatory notes for data extraction are presented in Appendix 4 and the case report form is available in Appendix 5.

Item 1 and 4 of the REMARK guidelines have adapted the information required for biochemical biomarkers to those required to perform an imaging biomarker study (imaging modality, protocol including contrast agent or tracer use and sequences and timing of the imaging) with enough detail to allow reproducibility without further contact to the article authors. Item 5 was reworded for imaging biomarker studies but with the same intent as the original REMARK guidelines requiring details of quality control procedures, reporting criteria and whether the reporters were blinded to the clinical outcome. No significant changes were made to the remaining items of the REMARK criteria.

Each of the 59 items were not regarded as equally important. Prof Brown and I decided upon the most important items based on the REMARK criteria, other guidelines and personal experience. Whilst all 59 items are important, the following items were regarded to be crucial in the reporting of imaging biomarker studies:

- The study objective

- Pre-defined hypothesis
- The protocol for imaging
- The reporting criteria for the biomarker
- Whether reporters were blinded to the patient outcome
- A precisely defined endpoint
- Details of which variables were initially included in the model
- A definition of all the variables and how they were measured
- What the detectable effect would be given the sample size
- Whether and how univariate and multivariate analysis was performed to assess the variables against outcomes
- Any association between the biomarker, patient outcome and the gold standard (as defined by the study)
- Whether any further investigations were performed to check the findings
- In the discussion to state the purpose of the study and any pre-specified hypothesis
- To distinguish between pre-specified hypotheses and post-hoc conclusions
- A critical evaluation
- How the results relate to the body of evidence

Analysis methods

Descriptive statistics were used to describe the results. ≥80% completeness of reporting was regarded as the minimum acceptable standard. Any item which was reported in <80% of studies would be regarded as inadequately reported.

Results

363 articles were screened, 111 full text articles were assessed for eligibility and 30 studies were included in the review. Figure 5-1 shows the PRISMA flowchart*(Liberati, Altman et al. 2009)* of the included articles and reasons for exclusions.

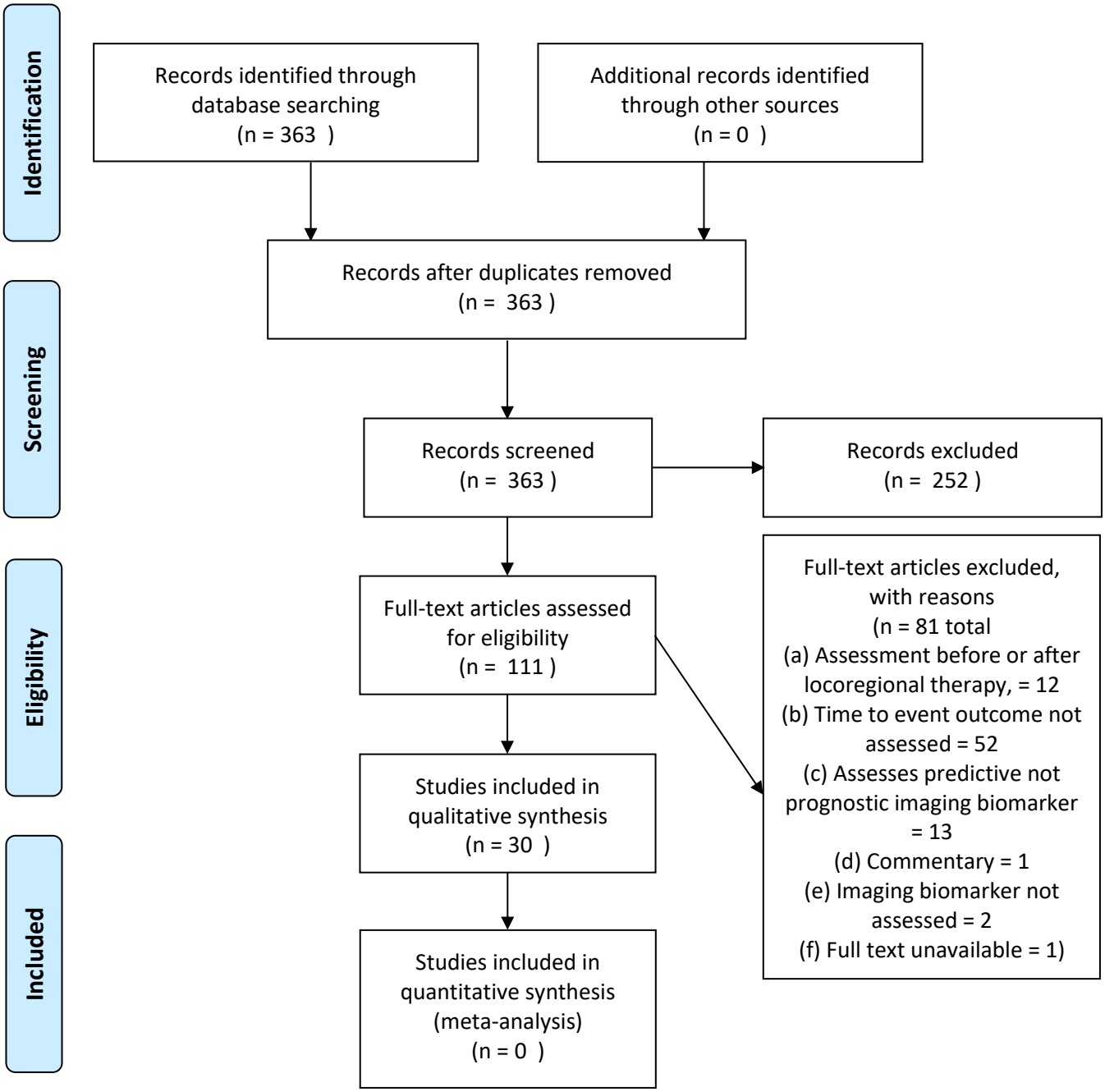


Figure 5-1: PRISMA flow chart of study selection

Of the 30 studies included in the review the imaging modalities investigated were CT (13 articles), MRI (7 articles) and PET-CT (13 articles). Three articles examined potential imaging biomarkers from two imaging modalities in the same cohort of patients (PET-CT and MRI, PET-CT and CT, and CT and MRI). A total of 40 different potential prognostic imaging biomarkers were assessed (Table 5-1) with only one biomarker assessed in 13 studies, two biomarkers assessed in 11 studies, three biomarkers assessed in 4 studies and four biomarkers assessed in 2 studies.

In total 3286 patients were included in the 30 articles, with a median sample size of 65 patients (range 18 – 418 patients). One third of articles included prospectively collected patient data (10/30, 33%) but in only two studies (2/30, 7%) was a description of stratification or matching provided.

Table 5-1: Prognostic imaging biomarkers assessed in the studies included in this systematic review according to modality

Modality	Details of the prognostic imaging biomarker examined	No. studies
CT	Attenuation	1
CT	Changes in tumour morphology	1
CT	Deepness of response (DpR)	1
CT	Early tumour shrinkage (ETS)	1
CT	Initial distribution of metastatic disease	1
CT	Morphological criteria	1
CT	Number of liver metastases	1
CT	Radiological heterogeneity	1
CT	Response Evaluation Criteria in Solid Tumors (RECIST)	6
CT	Texture features	2
CT	Tumour morphology	1
CT	Tumour shrinkage ratio	1
MRI	DCE-MRI - AUC of liver metastases	1
MRI	DCE-MRI - Hepatic Perfusion Index	1
MRI	DCE-MRI - IAUC60	1
MRI	DCE-MRI – Ktrans	1
MRI	DW-MRI - Mean apparent diffusion coefficient (ADC)	1
MRI	DW-MRI - Mean apparent diffusion coefficient high (ADC high)	1
MRI	DW-MRI - Apparent diffusion coefficient (ADC)	1
MRI	Area under the receiver operating characteristic curve (AUROC) for ECA-MRI	1
MRI	Area under the receiver operating characteristic curve (AUROC) for Gd-EOB-MRI	1
MRI	Number of liver metastases	1
MRI	T2* value	1
MRI	Target tumour enhancement (TuEn)	1
PET-CT	% change in standardised uptake value (SUV)	1
PET-CT	Complete metabolic response	1
PET-CT	Maximum standardized uptake variable (SUV max)	6
PET-CT	Metabolic response	1
PET-CT	Metabolic tumor diameter	1
PET-CT	Metabolic tumor volume [MTV]	1
PET-CT	Metabolic tumour volume (MTV)	1
PET-CT	Peak standardized uptake value [SUVpeak]	1
PET-CT	Positron Emission Tomography Response Criteria in Solid Tumors (PERCIST)	1
PET-CT	Ratio of tumour SUVmax to normal liver SUVmean	1
PET-CT	Reconstructed tumour volume (RTV)	1
PET-CT	Staging	1
PET-CT	Standardized added metabolic activity (SAM)	1
PET-CT	Total glycolytic volume (TGV))	1
PET-CT	Total lesion glycolysis [TLG]	4
PET-CT	Mean standardized uptake variable (SUVmean)	1
Total		**54**

Each of the 20 individual elements of the REMARK guidelines signpost a number of smaller recommendations which were each assessed individually (see Appendix 4). In total 59 recommendations were assessed for each of the 30 prognostic imaging biomarker studies included.

Overall there was 51% adherence to the REMARK guidelines. Only 19/59 items had ≥80% completeness of reporting. The full numerical results of completeness of reporting for each of the 59 guideline recommendations are tabulated in Appendix 7.

Figure 5-2 graphically represents the completeness of reporting of the 59 items extrapolated from the REMARK criteria. Each of the 59 items is represented as a column as either positive or negative percentage point deviation from the required 80% compliance. Those items previously identified as being crucially important are highlighted in blue and labelled.

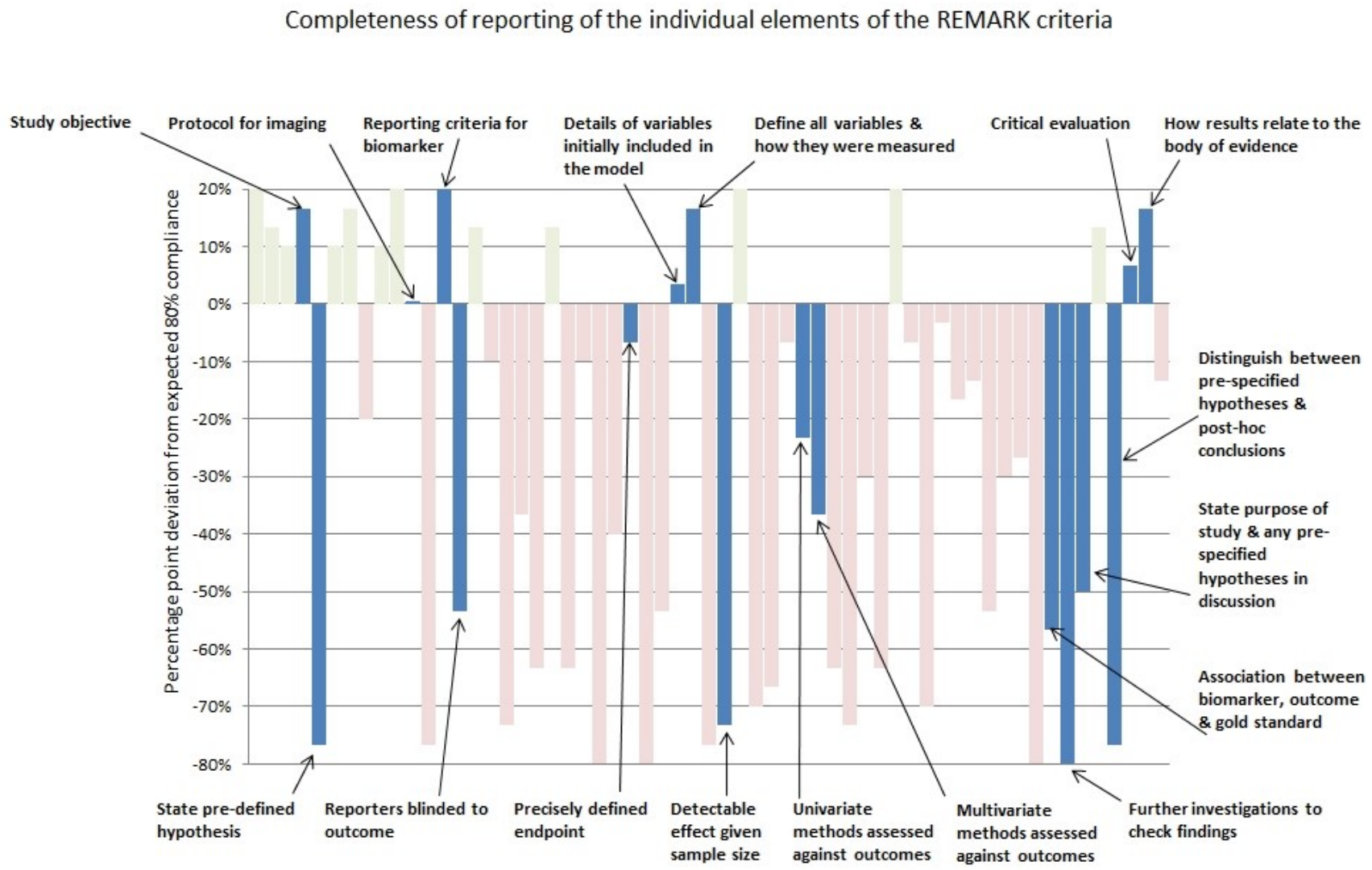


Figure 5-2: Graphical representation of the results of the systematic review of the completeness of reporting against the REMARK criteria

Key items which achieved ≥80% compliance

There was ≥80% completeness of reporting regarding the study objective, protocol for the imaging undertaken and the method of case selection, defining the variables, listing the statistical methods used and summarizing the main findings with critical evaluation and comparison to existing literature.

Whereas key areas of incomplete reporting were the lack of a pre-defined hypothesis in 97% of publications, blinding of reporters to the clinical outcome in only 27% of publications, sample size calculations with detectable effect size reported in only 7%, a distinction between pre-specified and post-hoc conclusions made in only 3% of publications and an investigation of an association between the imaging biomarker, outcome and the gold standard was only reported in 23% of publications.

Item-by-item analysis

Reporting of the study objectives and any pre-specified hypotheses

Item 1 of the REMARK guidelines requires the study objective and any pre-specified hypotheses to be reported*(McShane, Altman et al. 2005)* in the introduction of a biomarker publication. 29/30 (97%) of articles stated the study objective whereas only 1 article (3%), involving only 2% (65/3286) of the patients, stated a pre-specified hypothesis for testing.

<u>Methods</u>

Items 2-11 of the REMARK criteria describe the requirements for reporting the methods and material of biomarker study publications.

Reporting of the imaging protocol

Item 4 of the REMARK guidelines requires a description of the type of biological material used. For imaging biomarker studies details of the imaging protocol is required in enough detail to allow reproduction without further information from the study authors. This requires details of the imaging modality, contrast or tracer use, timing, scanner type, sequences used and sequence parameters. 80% (24/30) of the studies reported the imaging protocol adequately.

Reporting of quality control procedures, reporting criteria and the blinding of the reporters to the clinical outcomes

Item 5 of the REMARK guidelines requires specification of the quality control procedures and the scoring and reporting protocols, and whether assessment is performed blinded to the study endpoint. One study (1/30, 3%) described quality control procedures for the imaging biomarkers. All the studies specified the reporting criteria for the imaging biomarker but only 27% (8/30) studies stated the reporters were blinded to the clinical outcome.

Reporting of the clinical endpoints examined

Item 7 of the REMARK guidelines requires precise definition of all the clinical endpoints examined. 73% (22/30) of the studies precisely defined the endpoint but no studies reported the cause of death. The REMARK guidelines recommend reporting of the cause of death for cancer studies and the importance of indicating how the cause of death was classified. Only 8/30 studies (27%) stated that the endpoint assessments were made blinded to the marker measures.

Reporting of the variables with complete definitions and details of how they were measured

Item 8 of the REMARK guidelines requires complete definitions of the variables so that the study can be reproduced without further information*(McShane, Altman et al. 2005)*, this information could be included in the body of the text, the appendices or in a reference if the referenced paper provides adequate detail. 25/30 (83%) of studies gave details of the variables initially considered for inclusion in the model and 29/30 (97%) of studies defined all the variables and how they were measured.

Reporting of sample size calculations and statistical analysis plan

Item 9 of the REMARK guidelines requires a rationale for the sample size and item 10 requires specification of all the statistical methods. Within the eligible studies the median sample size was 65 patients (range 18 – 418 patients). 1/30 (3%) of studies involving 79 patients gave a rationale for the sample size and 2/30 (7%) of studies described the detectable effect size with a power calculation provided. All studies listed the statistical methods used.

67% (20/30) of studies reported univariate analysis, 17/20 of these studies described the method of univariate analysis assessed against clinical outcomes (including confidence intervals). 16/30 studies (53%) of studies reported multivariate analysis and only 15/16 of these adequately described how the model was made.

Results

Items 12-18 of the REMARK criteria describe the requirements for reporting the results of biomarker study publications.

Reporting of the flow of patients through the study

Item 12 of the REMARK guidelines requires a description of the flow of patients through the study including the number of patients included at each stage of the analysis. A participant diagram is helpful to describe the flow of patients through the study but only 5/30 studies (17%) included a participant flow diagram.

Reporting of the distributions of patient demographics, standard prognostic variables and markers, including the missing values

Item 13 of the REMARK guidelines requires reporting of the patient demographics and prognostic variables. All studies described the patient demographics. For a total of 22/30 studies (73%), the number of missing patients for each variable was reported or could be derived from the text and tables. 77% (23/30) studies described the distribution of the biomarker e.g. in a frequency table, bar chart or by mean, median or percentiles, with a range and standard deviation.

Reporting of the relationship of the imaging biomarker to standard prognostic variables

Item 14 of the REMARK guidelines require a description of the association of the biomarker with the standard prognostic variables. 19/30 studies (63%) described the relationship of the imaging biomarker to standard prognostic biomarkers.

Reporting of univariate and multivariate analysis

Item 15 of the REMARK guidelines requires presentation of univariate analyses showing the relationship between the marker and outcome with estimated effect. If the effect of a marker on a time-to-event outcome is assessed, a Kaplan-Meier plot with the number of patients at selected time points is recommended. 67% (20/30) of articles reported univariate analysis between the marker and outcome with confidence intervals and P values. All articles reported time-to-outcome but only 24/30 articles presented a Kaplan-Meier plot and of these only 8 articles (33%) had a Kaplan-Meier plot with the number of patients at selected time points as required by the REMARK guidelines.

Item 16 of the REMARK guidelines also require estimated effects and confidence intervals. 53% (16/30) of articles reported multivariate analysis either in the results or the discussion and 15/16 of the articles described how the multivariate model was built.

Reporting of the association between the biomarker and gold standard prognostic variables

Item 17 of the REMARK guidelines requires estimated effects (with confidence intervals) from an analysis which includes the marker and gold standard prognostic variables (as identified by the authors), regardless of their clinical significance. 7/30 (23%) of studies reported the possible association between the biomarker and the gold-standard prognostic variables. No studies reported further investigations to check their findings within the same publication.

Discussion

Items 19-20 of the REMARK criteria describe the requirements for the discussion of biomarker study publications.

Reporting of the discussion of results

Item 19 of the REMARK guidelines recommends interpretation of the results in the context of pre-specified hypothesis and other relevant studies. 30% (9/30) of studies state the purpose of the study and any pre-specified hypotheses in the discussion and 93% (28/30) of studies summarised the main findings.

Within the discussion one study (3%) distinguished between the results of pre-specified hypothesis and post hoc conclusions.

26/30 (87%) of studies reported critical evaluation regarded as consideration of the limitations of the study and 29/30 (97%) of the studies described how the results relate to the body of evidence.

Discussion

With this systematic review I aimed to assess the completeness of reporting of prognostic imaging biomarker studies in patients with colorectal cancer liver metastases against the REMARK guidelines*(McShane, Altman et al. 2005)*; criteria which are not currently applied to imaging biomarker studies. This study systematically evaluated the quality of reporting of prognostic imaging biomarker studies in colorectal cancer. Analysis of 30 eligible reports showed no study achieved complete reporting of all elements of the REMARK guidelines. Only 19/59 (32%) items had ≥80% completeness of reporting. Individual elements of the REMARK guidelines do not have equal importance and the range of completeness of reporting was 0 to 100%.

Limited numbers of imaging biomarkers have been translated into clinical practice and there are major concerns that poor quality studies and poor quality reporting leads to inaccurate utilisation of biomarkers*(Sekula, Mallett et al. 2017)*. The REMARK guidelines were developed to improve the quality of reports for prognostic biochemical biomarker studies*(McShane, Altman et al. 2005)* and, with limited amendments, are relevant to prognostic imaging biomarker studies.

A precisely defined endpoint is crucial to ensure accurate reporting of the remainder of the study. 73% (22/30) of studies within this cohort precisely defined the endpoint. For time-to-event outcomes, as in prognostic biomarker studies, the endpoint definition should include the time origin, e.g. overall survival from the data of colonoscopy to date of death from a cancer cause*(Altman, McShane et al. 2012)*.

Multiple guidelines have been published with recommendations for image acquisition and analysis*(Food and Drug Administration 2015)*, standardisations of acquisition, analysis and terminology*(National Cancer Institute , (Tofts, Brix et al. 1999)*, *(Leach, Brindle et al. 2005)*, *(Hunter 2008)*, *(Woodcock and Woosley 2008)*, *(Shankar 2012)*, *(Waterton and Pylkkanen 2012)*, *(European Society of Radiology 2013)*, *(Clarke, Nordstrom et al. 2014)*, *(Boellaard, Delgado-Bolton et al. 2015)*, *(Huang, Wang et al. 2015)*, *(Sullivan, Obuchowski et al. 2015)*, *(FDA-NIH Biomarker Working Group 2016)* and the validation and qualification of imaging biomarkers but each of these are different to the REMARK criteria which provide a framework for the adequate reporting of biomarker studies. Without adequate reporting of imaging biomarker studies it is not possible to assess for and improve the methodological and statistical quality which is hypothesised to be part of the reason for the incomplete translation of initially promising biomarkers into clinical practice.

For example the Imaging Biomarker Roadmap for Cancer Studies requires hypothesis-driven research with validation against gold-standard prognostic variables and patient outcomes in an independent dataset *(O'Connor, Aboagye et al. 2017)*. Only one article stated a pre-specified hypothesis for testing. The REMARK guidelines require clear delineation of what analysis was pre-specified and what was performed post-hoc*(Altman, McShane et al. 2012)*. Again, only one of the studies in this review achieved this. Furthermore only 53% (16/30) of studies explored the relationship of the proposed imaging biomarker to standard prognostic markers in multivariate analysis and only 23% (7/30) of all studies attempted to validate the proposed imaging biomarker against the author-identified gold standard prognostic variables.

Imaging biomarkers for metastatic colorectal cancer

This is the first study to investigate the quality of imaging biomarker studies and also the first systematic review of prognostic imaging biomarkers for colorectal liver metastases. Other systematic reviews have reported the prognostic significance of biological and molecular biomarkers for colorectal liver metastases*(Yamashita, Chun et al. 2018) (Das, Kalita et al. 2017)* and the economic impact of biomarkers for targeted therapies*(Seo and Cairns 2018)* but these did not include imaging biomarkers.

Impact of the REMARK guidelines

Prior to the introduction of the REMARK guidelines a systematic review found the completeness of reporting of biochemical biomarker studies was 53.4% (range: 10%-90%)*(Mallett, Timmer et al. 2010)*. Following implementation of the REMARK criteria the completeness of reporting was 58.1% (range: 30%-100%)*(Sekula, Mallett et al. 2017)*. Overall there was no significant difference in the completeness of reporting following implementation of the REMARK guidelines*(Sekula, Mallett et al. 2017)*. The authors showed that a further combined effort is needed from all involved in the reporting of these clinical studies before a difference in the quality of biochemical biomarker study reporting will be seen. It is therefore likely that a similar approach is needed for imaging biomarker studies.

Limitations of the systematic review

Only 30 eligible studies were identified between 2013 and 2018. The small sample size may reflect reporting bias of negative studies as has been identified as a problem in the field of biochemical biomarker studies.

This is the first study to investigate the quality of reporting of prognostic imaging biomarker studies. Previous systematic reviews in other disease processes have shown clinical applicability is limited by poor quality studies in a number of cancer sites (including breast cancer*(Altman 2009)*, neuroblastoma*(Riley, Abrams et al. 2003)*, prostate cancer*(Sutcliffe, Hummel et al. 2009)* and bladder cancer*(Malats, Bustos et al. 2005)*), and Kyzas *et al* reported evidence of selective reporting bias in head and neck squamous cell cancers*(Kyzas, Cunha et al. 2005)*. It is therefore hypothesised that the limited sample size may be secondary to selective reporting of prognostic imaging biomarker studies of colorectal cancer liver metastases.

A further limitation is the restriction of the systematic review to studies of patients only with colorectal cancer liver metastases. This was a conscious decision during the design of the systematic review to choose a study population which is research active. By restricting the study to one disease process, we are able to give a snapshot of the problem. However, imaging biomarkers may be used in all disease processes and populations, not just those in cancer and it is recommended that further systematic reviews are conducted of studies focussed on other disease processes to corroborate the findings here.

Conclusion

Prior to conducting this systematic review it was known that the translation of imaging biomarkers into clinical practice has been slow despite many so-called “promising results”. I conducted this systematic review which aimed to determine the extent of the reporting of items from the REMARK guidelines in prognostic studies of imaging biomarkers for liver metastases in patients with colorectal cancer. This systematic review has shown that deficiencies in study design are widespread in imaging biomarker research and that there is a need to apply better standards in this area. The REMARK guidelines should be made mandatory for the publication of imaging biomarker studies which may highlight the need for adherence to authors, although it is noted that following the implementation of the REMARK guidelines for biochemical biomarker studies there was no significant difference in the completeness of reporting of biochemical biomarker studies*(Sekula, Mallett et al. 2017)*.

The choice of imaging biomarkers to be investigated in this thesis

As illustrated by Table 5-1 a total of 40 different imaging biomarkers were assessed in the 30 studies included in the systematic review. However for only 4 imaging biomarkers was there more than one investigating study and none of these imaging biomarkers had gone through a process of validation to become appropriate for clinical use.

When deciding upon the imaging biomarkers I would investigate in Parts 2 and 3 of this thesis I chose not to further investigate any of the imaging biomarkers identified by this review as, as demonstrated by this systematic review, they showed flawed investigative methodology and a lack of validation. Instead I chose the imaging biomarkers investigated in

the remainder of this thesis by review of the literature and identification of imaging biomarkers which had been validated in other cancer sites or in the same cancer site but for a different clinical situation. Therefore:

- mrT and mrN stage were investigated as novel imaging biomarkers for MRI staging of SCC in Chapter 7 as clinical T and clinical N stage had been previously validated. MRI assessment of depth of extramural spread was chosen as a further novel imaging biomarker for investigation as its use has been validated in rectal cancer.
- mrTRG was chosen for Chapter 8 as the imaging biomarker used to predict for disease relapse in patients with rectal cancer as mrTRG has previously been validated as a method of assessing response to neoadjuvant chemoradiotherapy in rectal cancer.
- The presence of any previously validated poor prognostic imaging biomarkers was chosen as the definition of high-risk colorectal cancer for the work looking to apply imaging biomarkers for the prediction of disease relapse in colorectal cancer in Chapters 10, 11 and 12 as these imaging biomarkers had previously been validated for the use in primary colorectal cancer.

PART 2: INVESTIGATION OF NOVEL IMAGING BIOMARKERS FOR DISEASE RELAPSE IN ANAL AND COLORECTAL CANCER

CHAPTER 6 - INTRODUCTION TO PART 2

Whilst imaging biomarkers have been proposed for many cancer types, very few have made it into routine clinical practice. One of the explanations for this may be the lack of validation of imaging biomarker studies and lack of adherence to the REMARK guidelines as explored in Chapter 4 – Introduction to Part 1.

The ultimate aim of an imaging biomarker is one that can predict and prognosticate without the need for an invasive procedure. Whilst there are multiple biomarkers, both biochemical and imaging, which have been investigated as potential prognostic and/or predictive biomarkers for colorectal cancer, very few of these have been translated into clinical practice. However within rectal cancer the depth of extramural spread, the presence of mr-detected EMVI and involvement of the circumferential resection margin/intersphincteric plane as determined by MRI have been validated following the principles set out in the REMARK criteria.

The example of the validation of the use of the mrCRM is given in to show how this has been achieved, Figure 6-1.

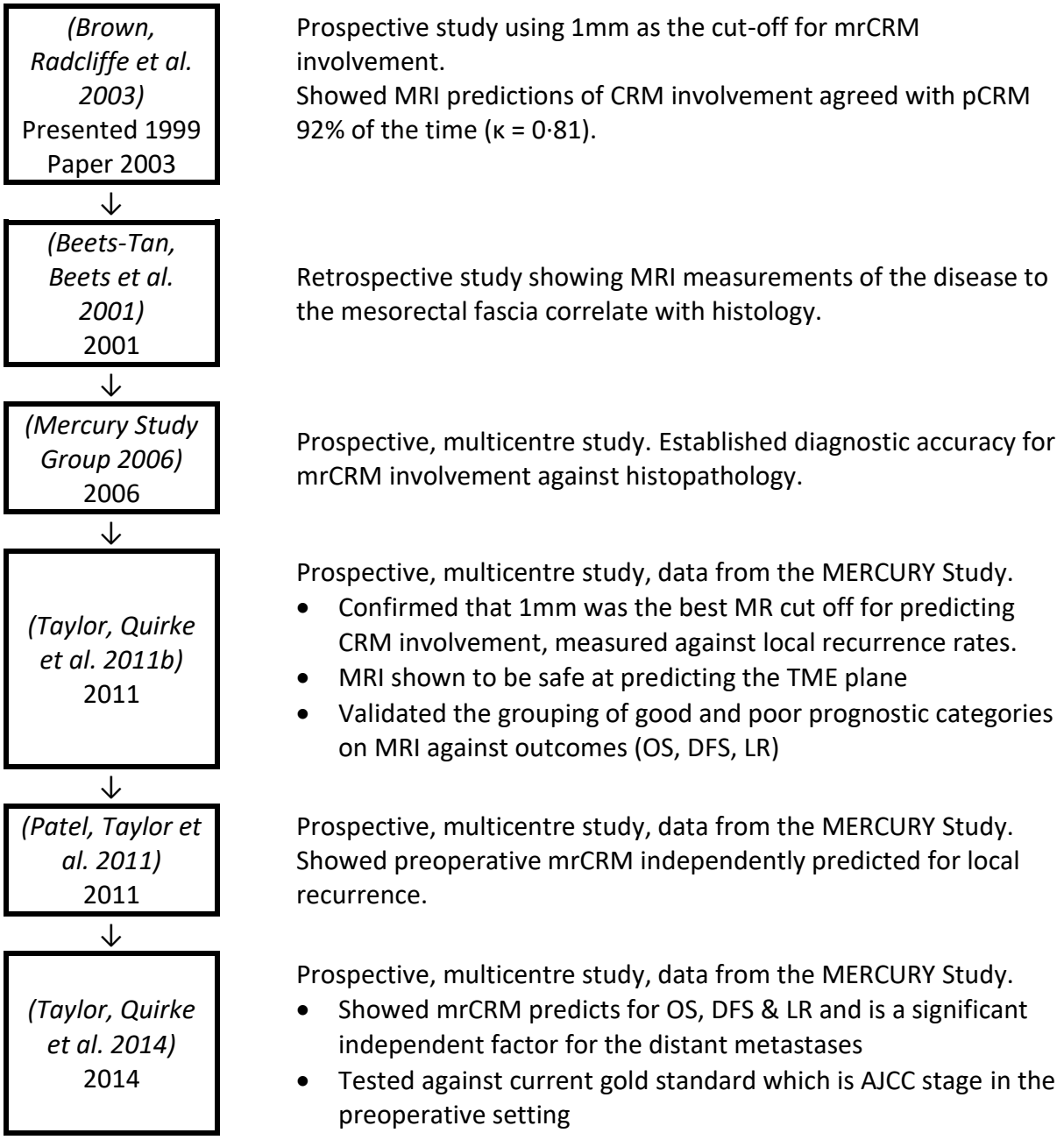


Figure 6-1: Process of mrCRM imaging biomarker validation in rectal cancer

Imaging biomarkers for anal SCC

Traditionally anal SCC has been staged clinically, i.e. assessing length of disease on clinical examination. Although MR staging is now standard practice in many pelvic malignancies, the available guidelines for its use in anal cancer are less prescriptive and vary between countries and issuing bodies as shown in Table 6-1.

Table 6-1: Guidelines for staging anal SCC

Authors	Guideline	Date	Recommendation
(Goh, Gollub et al. 2010), *(Glynne-Jones, Nilsson et al. 2014)*	European Society of Medical Oncology	2014	MRI is an option for the staging of anal SCC
(Benson, Arnoletti et al. 2012)	National Comprehensive Cancer Network (USA)	2012	MRI is not indicated for the staging of anal SCC
(Muirhead, Adams et al. 2016)	UK National Practice Guidelines for IMRT	2016	MRI is not prescribed
(The Royal College of Radiologists 2014)	Recommendations for cross-sectional imaging in cancer management. Second edition.	2014	MRI is recommended as the modality of choice for both staging and treatment planning

Within the UK most centres use MRI to locally stage anal SCC and for treatment planning. However, the use of MR staging and the application of TNM staging on MRI has not been validated and it is unknown whether MRI can provide predictive or prognostic imaging biomarkers for anal cancer in the same way as there are validated imaging biomarkers for rectal cancer.

Validation of MRI for Anal Cancer

Limited literature is available regarding the use of MRI for staging and response assessment of anal cancer. An initial study from our group showed that in 15 patients there was good

agreement in T staging between clinical examination and MR imaging (kappa = 0.68), and tumour shrinkage and stabilisation of the T2 signal at 12 months was associated with a good outcome*(Koh, Dzik-Jurasz et al. 2008)*. A small study of 35 patients by Goh *et al* looked to identify MRI features which were predictive of response at 6-8 weeks following treatment*(Goh, Gollub et al. 2010)*. In this small series no features were predictive of response*(Goh, Gollub et al. 2010)*.

Kochhar *et al* demonstrated that following CRT tumour size on MRI reduces incrementally at 3 months and then 6 months*(Kochhar, Mullan et al. 2017)*. The authors developed and investigated the potential role of their mr tumour regression regarding system, which differs from those validated for rectal cancer. Kochhar *et al* found that their poor TRG scores were predictive of early local recurrence but not late recurrence*(Kochhar, Mullan et al. 2017)*. There are no papers which have validated the use of mrTRG in SCC however mrTRG assessment of tumours forms part of the PLATO study (National Anal Cancer Treatment Study) but there is no secondary endpoint to validate mrTRG as part of this study.

Predictive and prognostic biomarkers for anal cancer

A retrospective study of 167 patients with nonmetastatic SCC of the anus treated with definite CRT by Das *et al(Das, Bhatia et al. 2007)* showed, on multivariate analysis, that higher T stage (P = .023) and higher N stage (P = .030) independently predicted for a higher rate of locoregional failure. The 3-year rate of locoregional control was 90% for Tx/T1, 86% for T2, 77% for T3, and 63% for T4 tumors*(Das, Bhatia et al. 2007)*. The 3-year rate of locoregional control was 84% to 88% for N0–2 and 39% for N3 tumors*(Das, Bhatia et al.*

2007). This study does not explicitly state how patients were assigned to T and N stages, though as no patients underwent an MRI it is likely that this was clinical T and N staging. Another retrospective study of 106 patients by Myerson *et al (Myerson, Kong et al. 2001)* showed, on multivariate analysis, extent of disease (T1–2N0 vs. T3N0 vs. T4 or N+) was the only factor that independently predicted for ultimate freedom from disease, local control, and freedom from relapse*(Das, Crane et al. 2008)*.

Tumour size has been reported as an independent predictor of poor prognosis in anal SCC. Firstly, a secondary analysis of the RTOG 98-11 trial *(Ajani, Winter et al. 2010)* showed tumours >5cm were associated with poorer 5-year DFS (P0 .0003) and poorer 5-year OS (P0 .0031). Secondly, N+ was associated with poorer 5-year DFS (P=.0001) and poorer 5-year OS (P <0.0001) in the multivariate analysis. Staging was derived by a combination of proctoscopy or sigmoidoscopy, chest radiography and computed tomography or magnetic resonance imaging of the abdomen/pelvis. These results were not shown in a previous EORTC study which did not identify tumour size as a prognostic factor*(Bartelink, Roelofsen et al. 1997)*.

A further database study by Goffredo *et al (Goffredo, Garancini et al. 2018)* confirmed the findings of Ajani *et al(Ajani, Winter et al. 2010)*. A total of 9230 stage IIA (2–5 cm) and 2418 stage IIB (>5 cm) patients were identified. 5-year OS was 72% and 69% for stage IIA versus 57% and 50% for stage IIB in the NCDB and SEER databases, respectively (p=0.001). After adjustment for available demographic and clinical confounders, stage IIB was significantly associated with worse survival in both cohorts (HR 1.58 and 2.01, both p=0.001).

In rectal cancer, the depth of extramural spread is a validated imaging biomarker as extramural spread >5mm is associated with poor prognosis*(Taylor, Quirke et al. 2011c)*. No literature has been identified which has assessed the impact of mr-assessed depth of extramural spread in anal SCC; this will therefore be assessed as a possible imaging biomarker.

I therefore performed a retrospective, hypothesis-generating study which aimed to validate mr-derived T staging against prognosis and investigate whether mr-derived depth of extramural spread was related to outcomes in patients with anal SCC. This work is reported in Chapter 7.

Assessment of response to chemoradiotherapy in rectal cancer and the potential of mrTRG as an imaging biomarker

24-61% of patients with rectal cancer present with locally advanced tumours and so are treated with preoperative radiotherapy in the form of chemoradiotherapy or short course radiotherapy*(Healthcare Quality Improvement Partnership Ltd. (HQIP) 2018)*. The use of preoperative therapy for patients with "locally advanced" rectal cancer is known to improve survival outcomes*(Roh, Colangelo et al. 2009)*, *(Sebag-Montefiore, Stephens et al. 2009)*, downstage disease*(Sauer, Becker et al. 2004)* and reduce the risk of local recurrence*(Sauer, Becker et al. 2004)*, *(Sebag-Montefiore, Stephens et al. 2009)*.

Traditionally, response to preoperative therapy has been assessed on the histological resection specimen with pathological tumour regression grading (pTRG) which assesses the

relative proportion of tumour and fibrosis within the specimen*(Mandard, Dalibard et al. 1994)*, *(Dworak, Keilholz et al. 1997)*. Whilst some studies showed pTRG is related to outcomes*(Rodel, Martus et al. 2005)*, *(Fokas, Liersch et al. 2014)* there is inconsistency and heterogeneity in the literature*(Fokas, Liersch et al. 2014)* and between the scales*(Siddiqui, Bhoday et al. 2016)* with other studies showing no relationship between pTRG and outcomes*(Beddy, Hyland et al. 2008)*, *(Vallbohmer, Bollschweiler et al. 2012)*. Furthermore the interobserver agreement for the various scales is poor*(Chetty, Gill et al. 2012)* and its reliability in supporting treatment decisions can therefore be questioned.

The magnetic resonance tumour regression grading system (mrTRG) follows similar principles of an assessment of the relative fibrosis and tumour signal within the treated tumour. Intermediate and poor response to preoperative therapy on the mrTRG system have been shown to have significantly poorer disease free survival and overall survival*(Patel, Taylor et al. 2011)*. However, the sites of recurrence and timing of recurrent disease following treatment with preoperative therapy is currently unknown. As a result, limited information is available to patients and clinicians to understand the ongoing risk of metastatic or recurrent disease and to determine the optimum surveillance strategy.

I therefore undertook a retrospective study which aimed to determine the timing and pattern of metastatic or recurrent disease following preoperative therapy for locally advanced rectal cancer and whether this can be predicted by the mrTRG response to preoperative therapy. This work is presented in Chapter 8.

CHAPTER 7 - DEPTH OF EXTRAMURAL SPREAD FOR SCC

This chapter is based on the manuscript **A Wale**, L Bernier, S A Khaleq, S Rao, D, Tait, G, Brown. "" MRI predicts for progressive disease and survival in patients with anal cancer treated with chemoradiation."

Introduction

TNM staging for anal SCC was developed for clinical rather than MRI staging but has been adopted for MRI staging without any validation. T staging is based on the maximal size of the tumour rather than the depth of extramural spread which has been validated and used for the staging of rectal cancer*(Mercury Study Group 2007)*. Size greater than 5cm*(van der Wal, Cleffken et al. 2001)*, defined as T3 or T4 tumours*(Das, Bhatia et al. 2007)*, *(James, Glynne-Jones et al. 2013)* and a circumference of greater than one third*(Allal, Mermillod et al. 1997)* have previously been proposed as poor prognostic features, however these studies used clinical not MRI staging. A retrospective study of 22 patients who had surgical salvage for anal cancer found that "invasion through the bowel wall"*(Smith, Whelan et al. 2001)* and "advanced T stage"*(Smith, Whelan et al. 2001)*, as assessed pathologically on the surgical resection specimen, were both associated with poor prognosis, but other studies have questioned whether these factors are independent of each other*(Gervaz, Allal et al. 2003)*. Experience in colorectal cancer would suggest that these factors are indeed independent of each other, and that depth of extramural spread (mr-DEMS) may be an independent prognostic factor for progression free survival in SCC.

I therefore undertook a hypothesis-generating, retrospective study which aimed to begin the validation process of mr-derived T staging against prognosis and investigate whether mr-DEMS was related to outcomes in patients with anal SCC.

The hypothesis was that the MRI assessment of tumour length and depth of extramural spread for anal squamous cell carcinoma can be used for risk stratification for progression-free survival.

Methods

Patient population

I undertook a retrospective service evaluation of patients with anal cancer who were treated with IMRT. Patients were identified from a prospectively maintained database of all patients treated with radiotherapy between January 2006 and December 2017. The search was performed on 29th June 2018 and the study took place in 2019. The follow-up of all participants ended on 31st May 2019 or at 36 months from the date of their baseline MRI, whichever was sooner.

Inclusion and exclusion criteria

Patients who met the following inclusion criteria were eligible for the study:

- Aged over 18 years
- Biopsy proven squamous cell carcinoma of the anus
- Primary tumour treated with radical intent
- Baseline and post-treatment high-resolution rectal MRI available for review

Patients who did not meet the inclusion criteria or those with additional malignancies or suboptimal quality imaging were excluded.

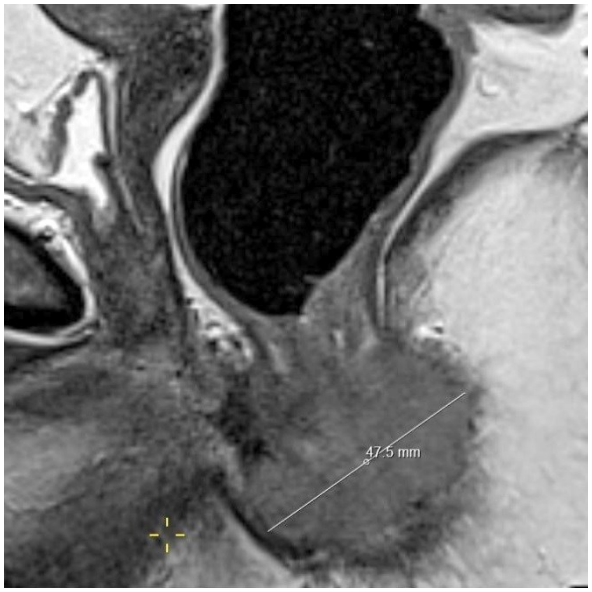
Image interpretation

Patients were staged with high-resolution MRI for T and N staging and CT TAP for M staging according to national guidelines*(The Royal College of Radiologists 2014)*. Details regarding

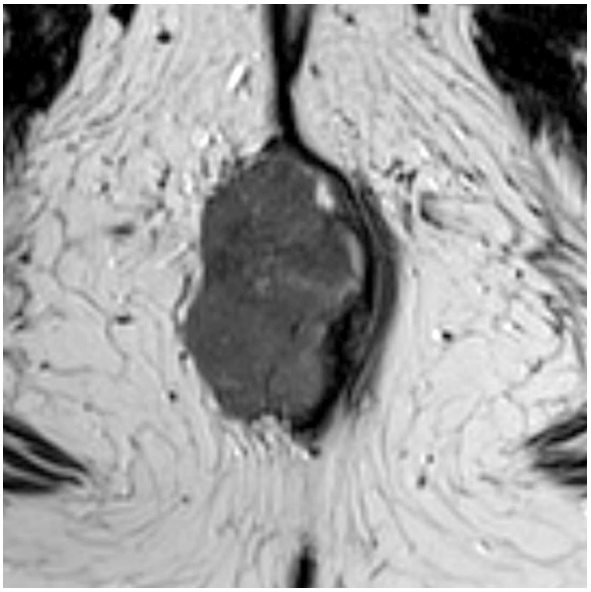
the technical performance of the MRI and CT scans are given in Chapter 3 – General Methods.

MRI images and reports were reviewed by me with support from Prof Brown. Where the MRI report had not been reported by Prof Brown I determined the T and N staging and the depth of extramural spread.

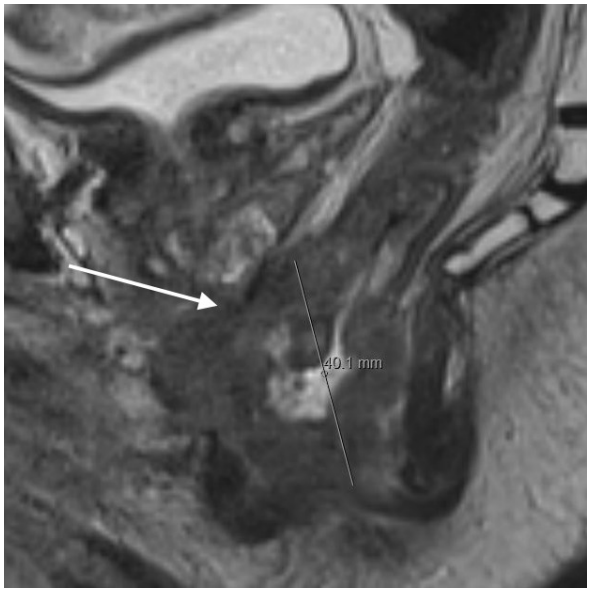
For all cases, whether reported previously by Prof Brown or by me for this study, the imaging was reported blinded to the patient outcome. Using multi-planar sequences the T stage was determined according to the longest diameter of the tumour on small field of view axial or coronal oblique sequences in combination with the sagittal sequence, except for T4 tumours which were any tumours which infiltrated an adjacent organ as per the TNM staging system*(American Joint Committee on Cancer (AJCC) 2017)*, Table 1-11; N staging was determined according to the presence of abnormal lymph nodes on MRI. Examples are given in Figure 7-1 and Figure 7-2. The MRI criteria for an involved node was adopted from the criteria used to validate nodal staging for rectal cancer where an involved node needed to have an irregular border contour and/or mixed MRI signal*(Brown, Richards et al. 2003)*. Size was not used as a criteria for disease within the nodes according to the evidence from rectal cancer which showed size did not predict for involvement of the nodes*(Brown, Richards et al. 2003)*.



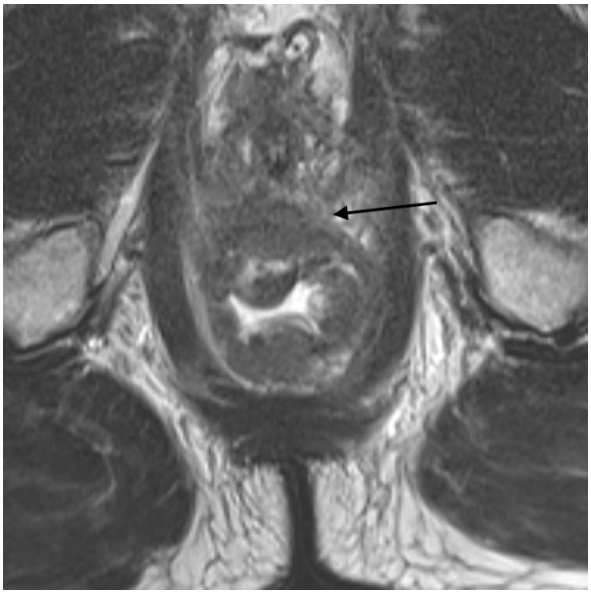
T2-weighted sagittal image of an anal SCC measuring 47.5mm in maximal diameter consistent with a T2 tumour.



T2 weighted high-resolution axial image of the T2 anal SCC.

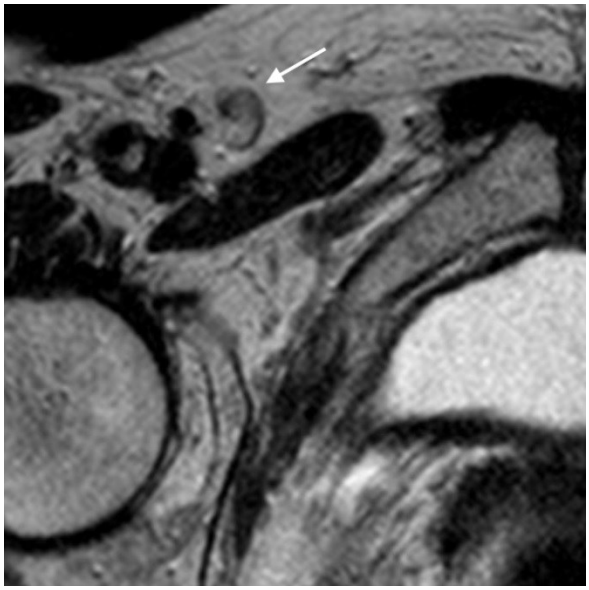


T2-weighted sagittal image of an anal SCC measuring 40mm in maximal diameter but with local infiltration of the prostate gland consistent with a T4 tumour (white arrow).

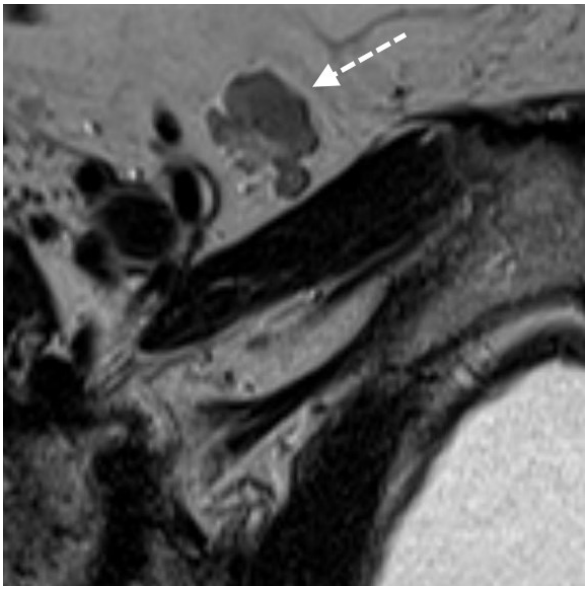


T2-weighted high-resolution axial imaging of the T4 anal SCC, the arrow shows the direct infiltration of the posterior prostate gland.

Figure 7-1: Examples of a T2 and T4 anal SCC lesion as demonstrated on MRI



T2-weighted MRI of the inguinal region showing a normal appearing lymph node with the central bright signal consistent with fat.



T2-weighted MRI of the inguinal region showing a malignant appearing lymph node with an irregular margin and intermediate signal.

Figure 7-2: Examples of normal appearing and malignant lymph node in patients with anal SCC.

The mr-DEMS was assessed on the small field of view axial MRI images at the point where the tumour was thickest, Figure 7-3. Measurements were taken with the images zoomed in using the calibrated callipers integral to the PACS system on the DICOM images.

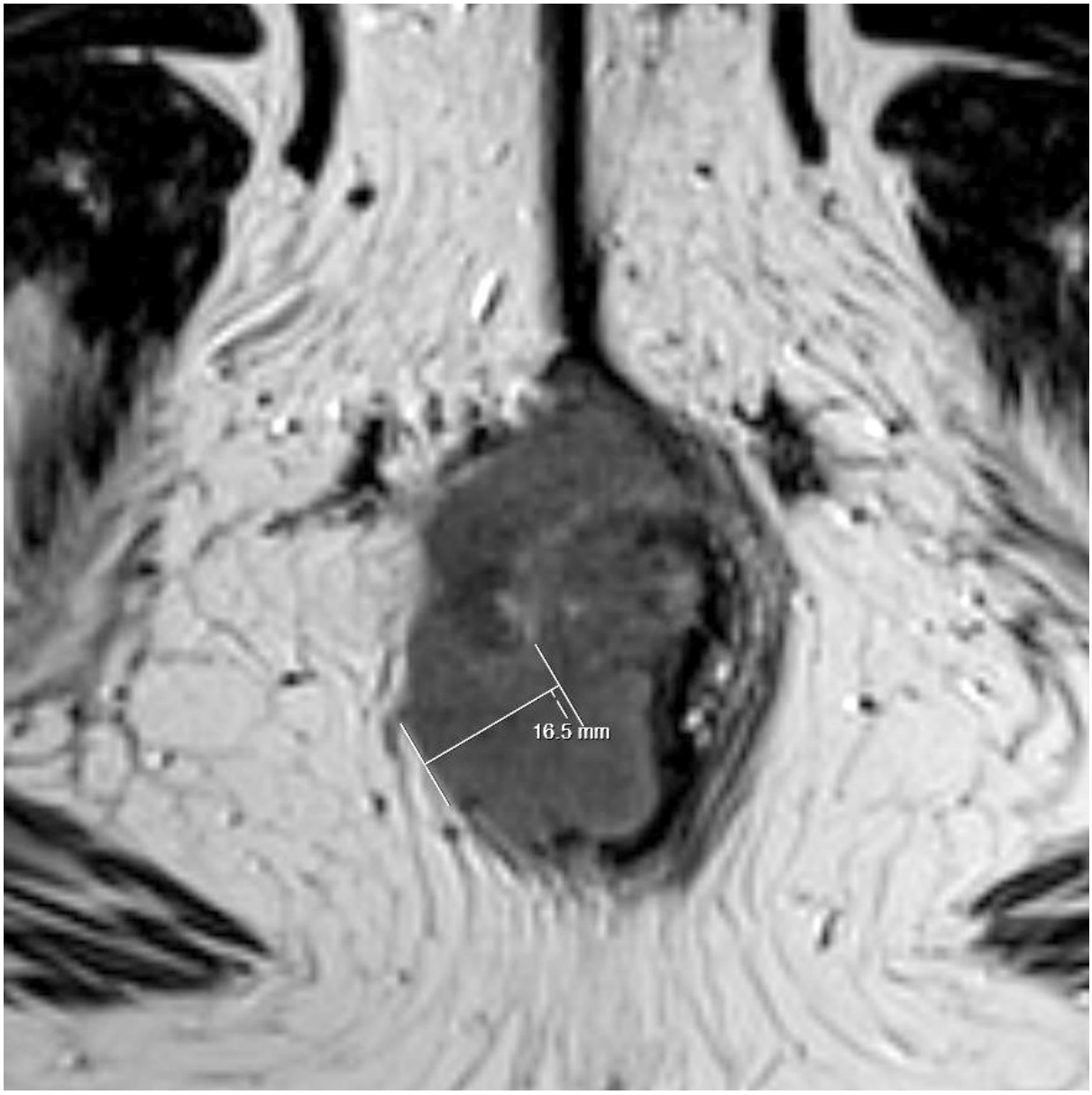
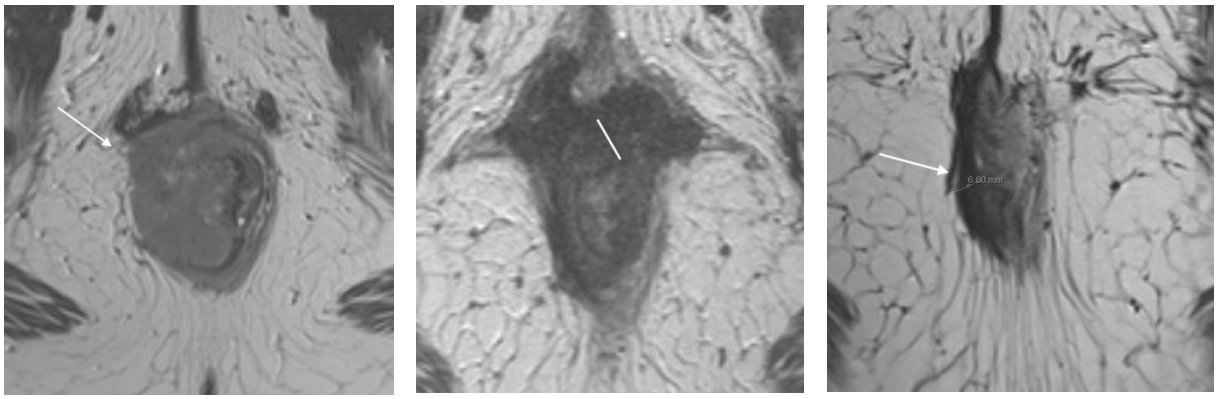


Figure 7-3: Example of how depth of extramural spread was measured on MRI (mrDEMS)

Follow-up MRI scans were performed at 12 and 24 weeks following the completion of treatment as per the standard treatment protocol in our hospital. Further imaging was performed as required by the clinical team. The MRI scans were assessed for the response to treatment according to the following criteria, Table 7-1, Figure 7-4.

Table 7-1: MRI assessment of response in anal SCC

Radiological response	MR characteristics
Complete radiological response	Complete resolution of the intermediate tumour signal, residual low signal scar or no abnormality may be demonstrated.
Partial radiological response	Partial regression of the intermediate tumour signal with either a reduction in the size of the tumour or some, but not complete, low signal change indicative of fibrosis
Stable disease	Tumour is unchanged from the baseline imaging
Progressive disease	Intermediate tumour signal has increased in size or there are new sites of disease



Baseline anal SCC tumour (white arrow)

Partial response 12 weeks following treatment for anal SCC with residual tumour signal seen at 12 O'Clock (white line)

Complete response 24 weeks following treatment for anal SCC with a low signal fibrotic scar seen and no residual tumour signal (white arrow)

Figure 7-4: Example of different radiological response categories for anal SCC

CT scans at baseline and follow-up were reviewed for the presence of metastatic disease.

The response to treatment on the 12 and 24 week MRI scans was recorded with the date of the scans. In addition the following information was recorded with the relevant dates:

- Whether the patient ever achieved complete response and if so if they developed recurrent or metastatic disease.

- If the patient did not achieve complete response the date progressive disease or metastatic disease was evident on imaging

Treatment received

All patients were treated with radical intent according to the standard protocol within our institution with IMRT and mitomycin C according to national and local guidelines.

Outcomes and Statistical Analysis

Patient outcomes were derived from the electronic patient record. Progressive disease was defined as any of the following outcomes occurring within 36 months (3 year PFS) from the date of the baseline staging MRI:

- Patient had MRI defined complete response and then developed recurrent disease.
- Patient did not achieve MRI defined complete response and then showed worsening of disease on MRI
- Patient developed metastatic disease
- Patient died

Patients who did not have a PFS event within 36 months were censored at 36 months.

The primary outcome was two-fold:

1. To determine appropriate cut-offs for depth of extramural spread and assess these against time to disease progression.
2. To assess mrT staging against time to disease progression.

Secondary outcomes were to assess time to disease progression against mrN staging.

Categorical analysis was planned and performed based on the binary categorisation shown to be significant for survival in previous trials*(James, Glynne-Jones et al. 2013)*:

- mrT1-2 versus mrT3-4
- mrN0 versus mrN1-3

Receiver operating curve (ROC) with calculation of the area under the curve was used to derive the optimal cut-off for depth of extramural spread. mrDEMS was dichotomised following determination of the appropriate cut-off from the ROC curve as described in the results. Progression-free survival (PFS) and overall survival (OS) was calculated using the Kaplan–Meier product limit method and Mantel-Cox log-rank tests.

Univariate and then multivariate cox regression analysis was performed against 3 year PFS and 3 year OS. Factors initially chosen for inclusion in the univariate model were those which are currently used for clinical decision making; mrT staging and mrN staging, and mrDEMS (the investigative factor). Those factors which were significant, or near significant, for 3 year PFS were included in the multivariate cox regression analysis.

Results

131 eligible patients were included; median follow-up was 50 months. All patients were followed up for a minimum of 15 months unless they had a PFS event before this point.

Table 7-2 details the characteristics of the patients according to their final outcome.

Table 7-2: Patient characteristics according to their final outcome at 3 years

	Disease status at 3 years					
	Developed progressive disease (n = 38/131)			**Died (with or without progressive disease) (n = 20/131)**		
Characteristic	N	(%)	RR	n	(%)	RR
Gender						
Female (n = 84)	20	(24)		9	(11)	
Male (n = 47)	18	(38)	**1.63 95% CI 0.99 - 2.90 P = 0.0545**	11	(23)	2.19 95% CI 0.98 – 4.89 P = 0.0572
mrT stage (at baseline)						
T1-2 (n = 73)	12	(16)		5	(7)	
T3-4 (n = 58)	26	(45)	**2.73 95% CI 1.51 – 4.92 P = 0.0009**	15	(26)	**3.78 95% CI 1.46 – 9.78 P = 0.0062**
mrN stage (at baseline)						
N0 (n = 63)	13	(21)		8	(13)	
N1-3 (n = 68)	25	(37)	**1.78 95% CI 1.00 – 3.17 P = 0.05**	12	(18)	1.39 95% CI 0.61 – 3.18 P = 0.44
ctM stage (at baseline)						
M0 (n = 129)	36	(28)		19	(15)	
M1 (n = 3)	2	(67)	**2.37 95% CI 1.02 – 5.53 P = 0.05**	1	(33)	1.68 95% CI 0.29 – 9.67 P = 0.56
TOTAL	38	(29)		20	(15)	

Statistically significant relative risk is shown in bold.

Relative risk of a PFS event at 3 years was predicted for by male gender, mrT stage and mrN stage, ctM stage at diagnosis, p=0.05, p=0.0009, p=0.05 and p=0.05 respectively. Relative risk of death at 3 years was predicted for by mrT stage, p= 0.0062.

Patients undergoing surgical salvage for local disease

A total of 38 patients (29%) developed a PFS event within 36 months from the date of their first treatment. 23/38 (61%) of first PFS events were local recurrence or locally progressive disease, in 3/23 patients the local disease was accompanied by with nodal recurrence.

Of those 23 patients only 5 patients were scheduled for surgery, and for one of these 5 the patient was lost to follow-up before the surgery happened. Therefore 4/23 patients (17%) with local recurrence or locally progressive disease underwent surgical resection, in all cases the surgery was APER or ELAPE, with one patient also having a vaginectomy.

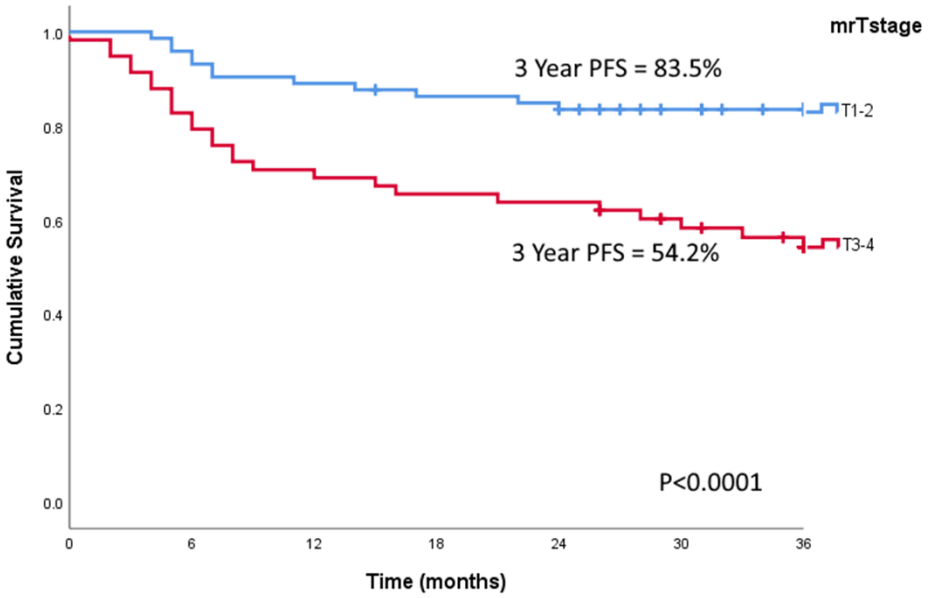
A comparison of the patient characteristics of those 5/23 patients considered for surgical resection of progressive disease (surgical salvage) compared to the remainder who were not considered for surgical salvage is given in Table 7-3. The distribution of baseline characteristics is similar between those patients who were planned for surgical salvage and those who weren't. The histopathological factors were available for 2/4 patients who underwent surgery.

Table 7-3: Patient characteristics of those with local recurrence or progressive local disease, comparison of those planned for surgical salvage and those who weren't.

	Planned for surgical salvage of local disease (n=5/23)		**Local disease – not planned for surgical salvage (n = 18/23)**	
Characteristic	n	(%)	n	(%)
Gender				
Female	2	(40)	6	(33)
Male	3	(60)	12	(66)
mrT stage (at baseline)				
T1-2	2	(40)	6	(33)
T3-4	3	(60)	12	(66)
mrN stage (at baseline)				
N0	2	(40)	7	(39)
N1-3	3	(60)	11	(61)
ctM stage (at baseline)				
M0	5	(28)	17	(95)
M1	0	(67)	1	(5)
pTN stage				
T4N0	2	(40)	N/A	
Missing	3	(60)	N/A	
Pathological R0 or R1				
R0	1	(20)	N/A	
R1	1	(20)	N/A	
Missing	3	(60)	N/A	

T staging as a determinant of progression free survival at 3 years

A binary categorisation of mrT staging (mrT1/2 versus mrT3/4) was performed against PFS at 3 years. 3 year PFS of patients with mrT1 or mrT2 disease was 83.5% (standard error 0.044) compared to 54.2% (standard error 0.067) for patients with mrT3 or mrT4 disease (P<0.0001), Figure 7-5.

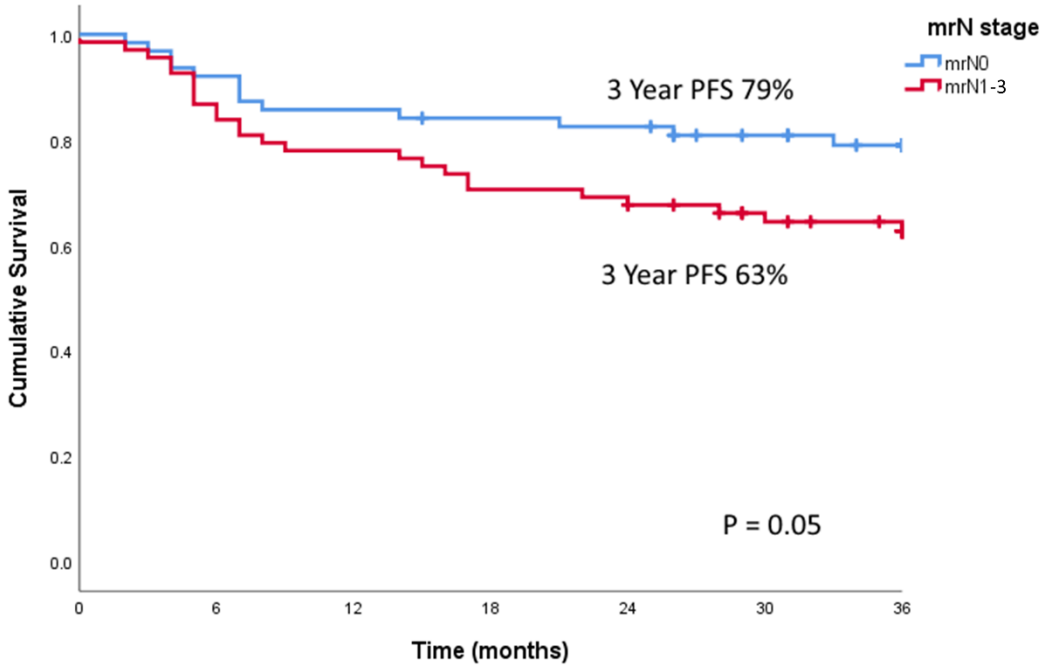


mrT1-2							
No. at risk	73	68	65	62	59	54	49
No. of events		5	8	10	12	12	12
mrT3-4							
No. at risk	58	46	40	38	37	30	26
No. of events		12	18	20	21	24	26

Figure 7-5: 3 year progression free survival according to T stage

N staging as a determinant of progression free survival at 3 years

A binary categorisation of mrN staging (mrN0 versus mrN1-3) was performed against PFS at 3 years. 3 year progression-free survival of patients with N0 disease was 79% (standard error 0.052) and 63% (standard error 0.059), p = 0.05, Figure 7-6.



mrN0							
No. at risk	63	58	54	52	51	45	40
No. of events		5	9	10	11	12	13
mrN1-3							
No. at risk	68	57	53	48	45	39	35
No. of events		11	15	20	22	24	25

Figure 7-6: 3 year progression free survival according to N stage

Depth of extramural spread as a determinant of progression free survival at 3 years

To determine the mr cut-off for depth of extramural spread in relation to progressive disease at 36 months I generated a ROC curve for depth of extramural spread against progressive disease (binary yes/no), Figure 7-7.

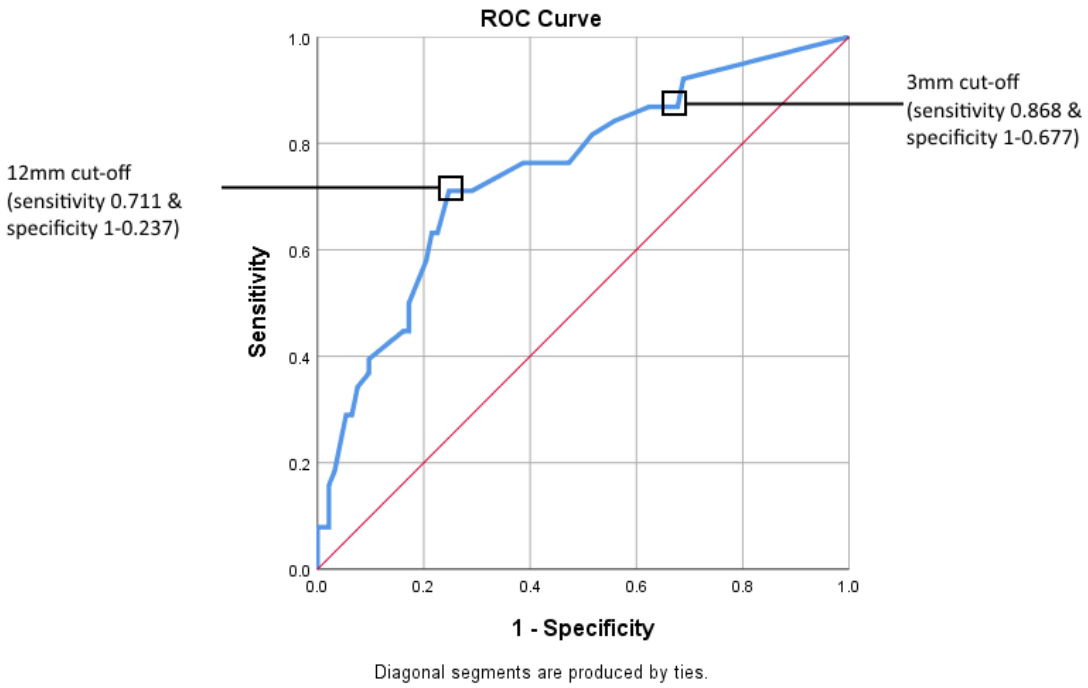
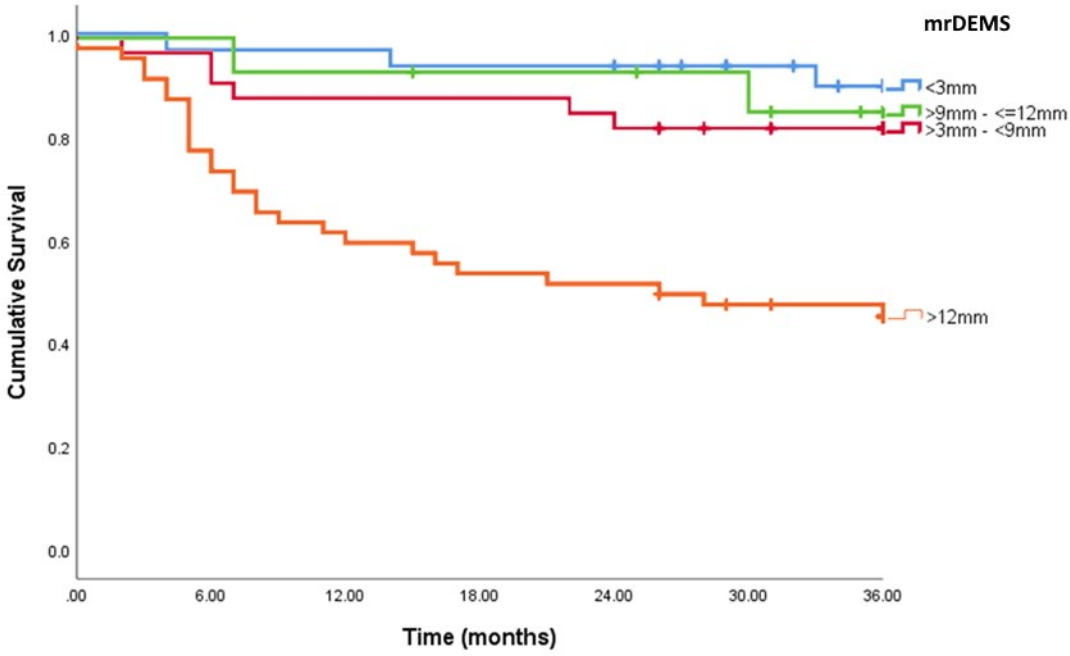


Figure 7-7: ROC curve of depth of extramural spread against progressive disease at 3 years

Cut-off values were determined from the ROC curve according to standard methodology. A cut-off of 12 mm depth of extramural spread was chosen as the pair of values as closest to 0.8 for sensitivity and 1-specificity (12.5mm had values of 0.711 and 1-0.237 respectively) and a cut-off of 3mm was chosen as the value with improved sensitivity with sacrificed specificity (3.5mm had values of 0.868 and 1- 0.677 respectively) . An interim cut off of 9mm was chosen and a final cut-off of less than 3mm was chosen. I then generated Kaplan-Meier

curves for the respective cut offs to determine the 3 year progression free survival (Figure 7-8).



mrDEMS <3mm							
No. at risk	32	31	31	30	29	25	21
No. of events		1	1	2	2	2	3
mrDEMS ≥3mm-≤9mm							
No. at risk	34	31	30	30	28	26	24
No. of events		3	4	4	6	6	6
mrDEMS >9mm - ≤12mm							
No. at risk	15	15	14	13	13	11	8
No. of events		0	1	1	1	2	2
mrDEMS >12mm							
No. at risk	50	37	30	27	26	22	20
No. of events		13	20	23	24	26	27

Figure 7-8: Time to progressive disease in relation to mr-derived depth of extramural spread (mrDEMS)

The impact of depth of extramural spread on 3 year PFS appears to split into 3 subcategories with depth of extramural spread of <3mm with good prognosis, extramural spread of >12mm with poor prognosis and extramural spread of 3-12mm with intermediate prognosis.

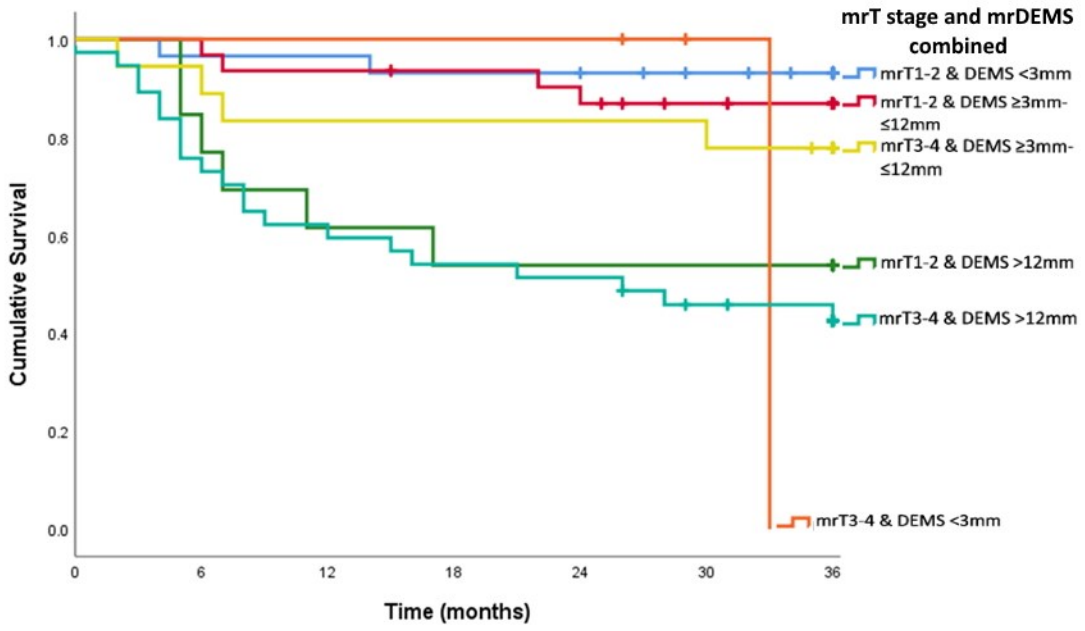
Additional of depth of extramural spread to T staging

mr-derived T1-2 and T3-4 binary categorisation has a statistically significant impact on 3 year PFS and depth of extramural spread appears to split into 3 subcategories as described above. It was important to determine whether depth of extramural spread adds to the prognostic information gained from T staging, Table 7-4.

Table 7-4: Subcategorisation of T stage and depth of extramural spread with number alive without progressive disease at 3 years and mean survival.

mrT stage	**mrDEMS**	**No. patients in each subcategory** N	*(%)*	**Alive without progressive disease at 3 years** n	*(%)*	**Mean survival (months)**	**95% CI**
T1-2	**< 3 mm**	29	*(22%)*	27	*(93%)*	34 mo	32 – 37 mo
	3 – 12 mm	31	*(24%)*	27	*(87%)*	33 mo	31 – 36 mo
	> 12 mm	13	*(10%)*	7	*(54%)*	24 mo	16 – 31 mo
T3-4	**< 3 mm**	3	*(2%)*	2	*(67%)*	33 mo	33 – 33 mo
	3 – 12 mm	18	*(14%)*	14	*(78%)*	31 mo	25 – 36 mo
	> 12 mm	37	*(28%)*	16	*(43%)*	22 mo	17 – 26 mo
TOTAL		131	*(100%)*	93	*(71%)*	29 mo	27 – 31 mo

Kaplan-Meier curves were generated for each of the 6 subcategories combining the binary categorisation of T staging and the subcategories of depth of extramural spread, Figure 7-9.



mrT1-2 & DEMS <3mm							
No. at risk	29	28	28	27	26	24	21
No. of events		1	1	2	2	2	2
mrT1-2 & DEMS ≥3mm-≤12mm							
No. at risk	31	30	29	28	26	23	20
No. of events		1	2	2	4	4	4
mrT1-2 & DEMS >12mm							
No. at risk	13	10	8	7	7	7	6
No. of events		3	5	6	6	6	6
mrT3-4 & DEMS <3mm							
No. at risk	3	3	3	3	3	1	0
No. of events		0	0	0	0	0	1
mrT3-4 & DEMS ≥3mm-≤12mm							
No. at risk	18	16	15	15	15	14	12
No. of events		2	3	3	3	4	4
mrT3-4 & DEMS >12mm							
No. at risk	37	27	22	20	19	15	13
No. of events		10	15	17	18	20	21

Figure 7-9: 3 year progression-free survival according to T stage and depth of extramural spread subcategorisation.

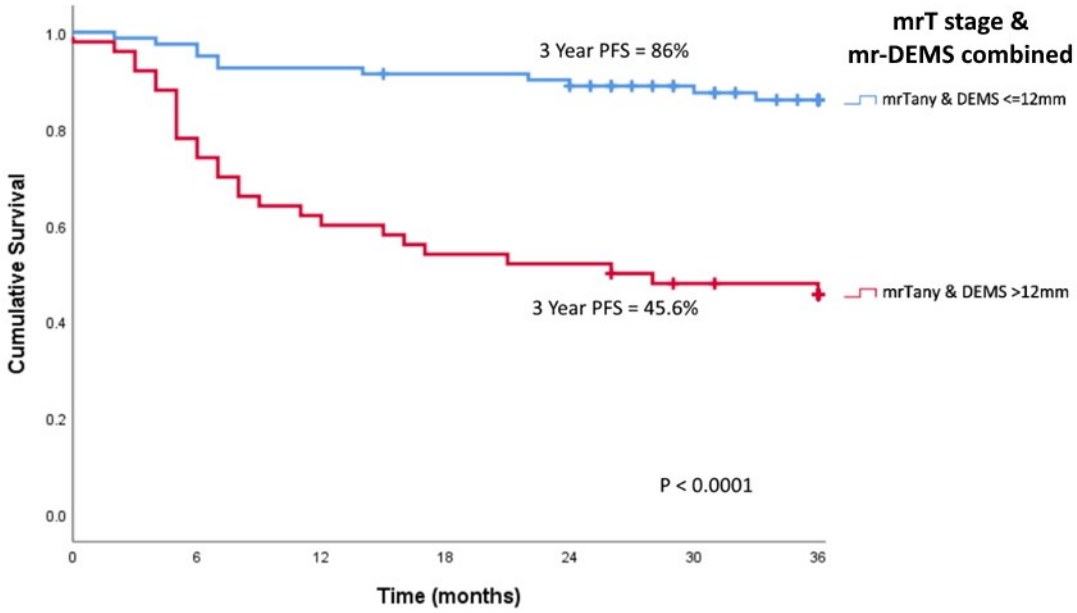
Combined, Table 7-4 and Figure 7-9 show there was worsened survival for patients with T1-2 and T3-4 cancers with depth of extramural spread > 12mm with statistically significant poorer mean survival when compared to T1-2 and T3-4 tumours with depth of extramural

spread <12mm. This also corresponded to lower mean survival for patients with depth of extramural spread >12mm, irrespective of T stage.

A binary categorisation of these groups was therefore performed with patients with depth of extramural spread <12mm (n = 81) and patients with depth of extramural spread >12mm (n = 50), irrespective of T stage, Table 7-5, Figure 7-10.

Table 7-5: Binary subcategorisation of patients according to depth of extramural spread, irrespective of T stage, with a number alive without progressive disease at 3 years and mean survival.

mrT stage	**Depth of extramural spread**	**No. patients in each subcategory**		**Alive without progressive disease at 3 years**		**Mean survival (months)**	**95% CI**
		N	**%**	**N**	**%**		
T any	**≤12 mm**	81	*(62%)*	70	*(86%)*	33 mo	31 – 35 mo
T any	**> 12mm**	50	*(38%)*	23	*(46%)*	22 mo	18 – 26 mo
TOTAL		*131*		*93*		*29 mo*	*27 – 31 mo*



mrTany & DEMS <=12mm							
No. at risk	81	77	75	73	70	62	55
No. of events		4	6	7	9	10	11
mrTany & DEMS >12mm							
No. at risk	50	37	30	27	26	22	20
No. of events		13	20	23	24	26	27

Figure 7-10: 3 year progression-free survival according to according to depth of extramural spread, irrespective of T stage

Binary categorisation of patients between those with depth of extramural spread ≤12 mm and > 12mm was performed. 3 year progression-free survival of patients with ≤12mm depth of extramural spread, irrespective of T stage was 86% (standard error 0.039) versus 3 year progression-free survival of patients with >12mm depth of extramural spread which was 45.6% (standard error 0.071), P <0.0001, Figure 7-10.

Mean survival for patients with mrDEMS >12mm was 22 months (95% CI 18 – 26 months), whereas mean survival for mrDEMS ≤12mm was 33 months (95% CI 31-35 months).

Univariate and multivariate analysis

Univariate and then multivariate Cox Regression analysis was performed to determine which of the mr-derived factors predicted for 3 year PFS and 3 year OS; namely mrT stage, mrN stage and mrDEMS. The results are tabulated in Table 7-6.

On multivariate cox regression analysis only mrDEMS was a significant predictor for 3 year PFS and 3 year OS.

Table 7-6: Univariate and multivariate cox regression analysis.

	Univariate Cox Regression				Multivariate Cox Regression			
	3 year PFS		3 year OS		3 year PFS		3 year OS	
Factor	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
mrT stage								
mrT1	1		Coefficients did not converge so a Cox Regression model could not be performed.					
mrT2	1.587	0.205-12.293						
mrT3	3.499	0.452-27.104						
mrT4	6.973	0.920-52.843						
mrT stage – Binary Categorisation								
mrT1-2	1		1		1		1	
mrT3-4	3.24	1.634-6.426	4.237	1.539-11.661	1.487	0.681-3.249	1.734	0.571-5.271
		P=0.001		**P=0.005**				
mrN stage								
mrN0	1		1					
mrN1	1.133	0.452-2.840	1.068	0.322-3.548				
mrN2	1.857	0.705-4.886	0.986	0.209-4.645				
mrN3	3.386	1.543-7.434	2.427	0.842-6.996				
		P=0.002						
mrN stage – Binary Categorisation								
mrN0	1		1		1		1	
mrN1-3	1.928	0.986-3.769	1.456	0.595-3.561	1.580	0.788-3.166	1.110	0.443-2.783
mr-depth of extramural spread (mrDEMS) – Binary Categorisation								
≤12mm	1		1		1		1	
>12mm	5.234	2.590-10.576	10.654	3.121-36.374	4.256	1.954-9.269	8.273	2.222-30.799
		P<0.0001		**P<0.0001**		**P<0.0001**		**P=0.002**

Only the statistically significant P values are given.

Discussion

I undertook a hypothesis-generating, retrospective study which aimed to validate mr-derived T staging and depth of extramural spread against 3 year PFS for patients with anal SCC.

I showed that mrT3 and mrT4 tumours had poorer 3 year PFS than mrT1 and mrT2 tumours (P <0.0001). In addition mrT3 and mrT4 tumours had increased risk of disease progression at 3 years (RR 2.73, P = 0.0009) and death within 3 years (RR 3.78, P = 0.0062). This aligns with the findings from previous papers which used clinical T staging, for example the UK ACT II study showed 80% 3 year PFS for cT1-2 tumours versus 65% for cT3-4 tumours(*James, Glynne-Jones et al. 2013*). mrT staging has not previously been tested against 3 year PFS in patients with anal cancer. 3 year PFS for mrT3-4 staging was slightly worse than shown in the ACT II study at 54.2% which may be secondary to the improved accuracy of MRI staging for the infiltration of adjacent organs, especially in smaller tumours which may be more difficult to identify clinically. This will need to be explored in a larger validation study.

ROC curve analysis determined appropriate cut-offs for the mr-derived depth of extramural spread. I showed that mr-derived depth of extramural spread is a significant and independent risk factor for 3 year PFS, and has been shown, in this series, to supercede mrT staging based on length of tumour. On multivariate Cox Regression analysis only mr-derived depth of extramural spread >12mm remained significant for the prediction of 3 year PFS and 3 year OS (P<0.0001 and P=0.002 respectively). In rectal cancer the mr-derived depth of extramural spread is a validated imaging biomarker as extramural spread >5mm is

associated with poor prognosis(*Taylor, Quirke et al. 2011c*). To date no literature has been identified which has assessed the impact of mrDEMS in anal cancer, prior to the introduction of MRI staging for anal cancer it was not possible to measure depth of extramural spread. With the widespread adoption of MRI staging there is now the basis to identify an important imaging biomarker for prognosis in anal cancer.

mrN staging predicted for 3 year PFS on the Kaplan-Meier survival analysis (P = 0.05) but not on the Cox Regression analysis. cN staging has previously been shown to also be predictive of outcomes in anal cancer in some studies, and is included as part of AJCC staging(*National Cancer Institute 2015*) but there is some disagreement In the literature related to clinical staging with other studies showing no difference in survival between node positive and node negative disease(*James, Glynne-Jones et al. 2013*). It is not surprising that the mr prediction of nodal disease in anal SCC did not predict for survival as evidence from rectal cancer shows that even though morphologic changes in the MRI appearance of nodes with malignancy can be identified*(Brown, Richards et al. 2003)* interobserver agreement is only adequate. Therefore the mr-assessment of nodal status may not be predictive of outcome due to a true absence of impact on survival from nodal staging or poor accuracy of MRI staging for nodal involvement. Furthermore with IMRT modelling of radiotherapy doses the nodal groups are adequately treated irrespective of the nodal status. In addition further evidence from rectal cancer shows that the pathways of spread in metastatic disease may not be tumour, node, metastasis in the majority of patients. Direct spread maybe a stronger surrogate for metastatic disease and that to date nodal disease has been utilised as a surrogate inappropriately.

Whilst anal SCC is more common in females*(Cancer Research UK 2019a)*, the outcomes for men are worse in my study with a 1.6 fold increased risk of death (RR 1.63, P = 0.0545). This agrees with previous publications which have also shown male gender to be a poor prognostic factor for survival*(Bartelink, Roelofsen et al. 1997)*, *(Ajani, Winter et al. 2008)*, *(Gunderson, Winter et al. 2012)*, *(Glynne-Jones, Sebag-Montefiore et al. 2013)*, *(American Joint Committee on Cancer (AJCC) 2017)*. The cause of this is unknown but may relate to the later clinical presentation of men for medical care. Although, if this was the reason, poorer progression free survival would also be expected in men, which was not demonstrated by this data.

The baseline characteristics of patients who were planned for surgical salvage of local disease was similar to those patients with locally recurrent or progressive disease who were not planned for surgical salvage. This suggests that patients who will be suitable for surgical salvage cannot be identified at baseline and a further biomarker should be explored for these patients. The number of patients undergoing surgical salvage is small consistent with the literature. A larger patient cohort would therefore be required to explored the potential role of mr-derived DEMS or another biomarker for the potential identification of patients suitable for surgical salvage.

This study has some limitations. The retrospective nature means there is some missing data and I have been unable to investigate the role of some prognostic factors on 3 year PFS, for example grade of differentiation, which has previously been shown to be a prognostic factor*(American Joint Committee on Cancer (AJCC) 2017)*.

I did not compare the interobserver agreement of readers for the assessment of mrT, mrN and mr-DEMS. Data from rectal cancer has shown good agreement between readers for these assessments*(Brown, Radcliffe et al. 2003)*. Interobserver agreement in SCC measurements can be tested as part of a future study to validate the use of mrDEMS of >12mm as a prognostic factor for SCC.

The UK PLATO study looks to modulate the treatment of anal SCC according to the primary staging. This is the first study to require the use of mr T and N staging, though it does not prescribe for the MR technique required. In addition the radiological determinants of response within this study are a mrTRG scale have not been validated prior to its use as an endpoint measure in this study*(Kochhar, Mullan et al. 2017)*. Unlike mr-TRG, mrDEMS has passed the first step of validation to becoming a clinical translatable imaging biomarker for outcomes in anal SCC.

Conclusion and post-doctoral work

This study has achieved its aims and has shown:

1. mr-derived T staging corresponds to 3 year progression-free survival
2. On multivariate cox regression analysis only mrDEMS was a significant predictor for 3 year PFS and 3 year OS, irrespective of T stage. This suggests that the depth of extramural spread is the most important factor when determining the risk of relapse for patients with anal SCC

Therefore >12mm depth of extramural spread is proposed as a novel imaging biomarker for the prognostication of anal SCC. As per the numerous guidelines for the validation of imaging biomarkers this proposed cut-off now needs to be tested in a new population of patients as the next step.

I am now working with my co-authors to identify a suitable collaborator with an independent dataset for this validation work.

CHAPTER 8 - MRI TUMOUR REGRESSION GRADING FOR PREDICTING DISEASE RELAPSE ACCORDING TO RESPONSE TO CHEMORADIOTHERAPY

This chapter is based on the manuscript **A Wale**, J Bhoday, S Yu, D Tait, G Brown. "Can magnetic resonance tumour regression grade (mrTRG) predict the timing and patterns of distant metastases in patients with locally advanced rectal cancer post chemoradiotherapy?". *Submitted to the International Journal of International Journal of Radiation Oncology • Biology • Physics on 21st June 2019.*

Introduction

Poor mrTRG response to preoperative therapy for locally advanced rectal cancer results in poorer disease-free and overall survival*(Patel, Taylor et al. 2011)*, *(Sclafani, Brown et al. 2017)*. The sites of recurrence and timing of metastatic and recurrent disease following treatment with preoperative therapy is currently unknown. In this study I aimed to determine the timing and pattern of metastatic or recurrent disease following preoperative therapy for locally advanced rectal cancer and determine whether this can be predicted by the mrTRG response to preoperative therapy.

The hypothesis was MRI tumour regression grading following chemoradiotherapy in rectal cancer can be used to risk stratify patients for disease relapse in terms of the timing and site of metastatic disease.

Methods

Patient search

Patients who underwent preoperative chemoradiotherapy/radiotherapy with post treatment MRI assessment of tumour regression grade (mrTRG) between August 2001 and October 2018 were included in this study. This study complied with our institution's service evaluation protocols and therefore did not require formal institutional review board approval. Patients already included in a trial had consented to the use of imaging for research purposes, while for other patients outside of a trial the requirement for informed consent was waived.

Inclusion and exclusion criteria

Patients who met the following inclusion criteria were eligible for the study:

1. Aged over 18 years
2. Adenocarcinoma of the rectum or sigmoid colon
3. Primary tumour treated with preoperative long course chemoradiotherapy or radiotherapy with radical intent
4. Baseline and post-treatment high-resolution rectal MRI available for review and mTRG

The first post preoperative therapy restaging MRI was included for each eligible patient. Patients who did not meet the inclusion criteria or those with metastases at diagnosis, additional malignancies, suboptimal quality imaging or mrTRG assessment of recurrent disease not the primary tumour were excluded (Figure 8-1).

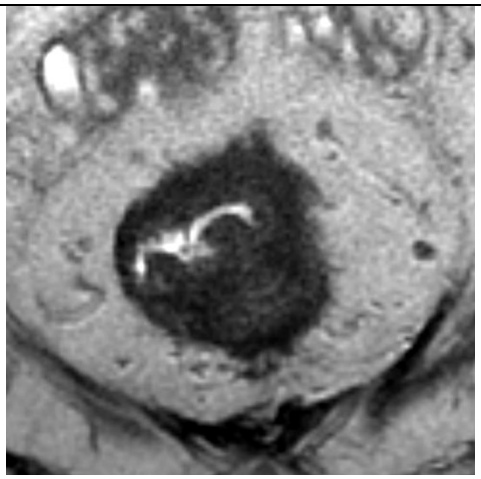
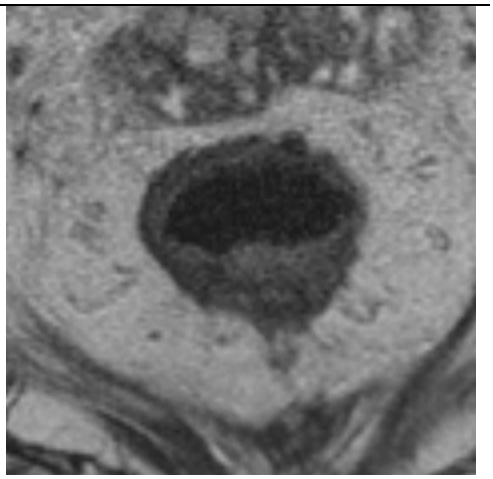
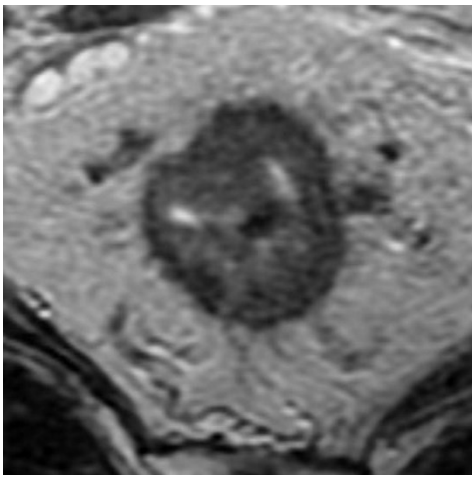
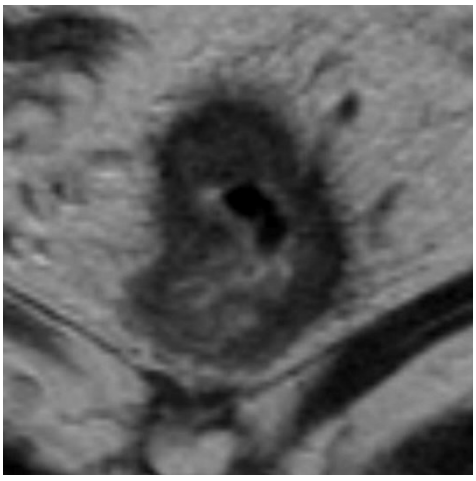
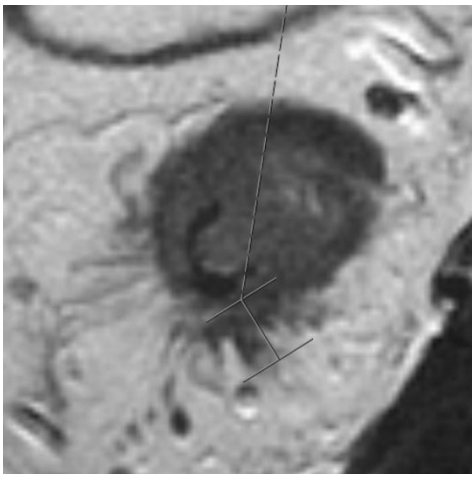
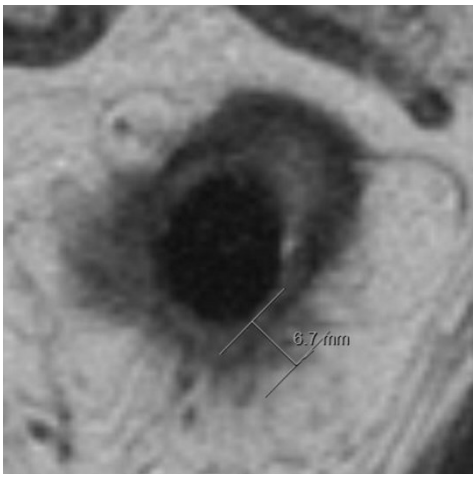
Patients received preoperative therapy in our institution if they had any of the known validated poor prognostic features on their baseline MRI:

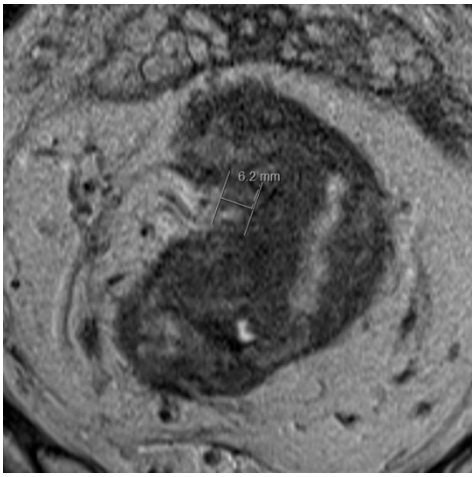
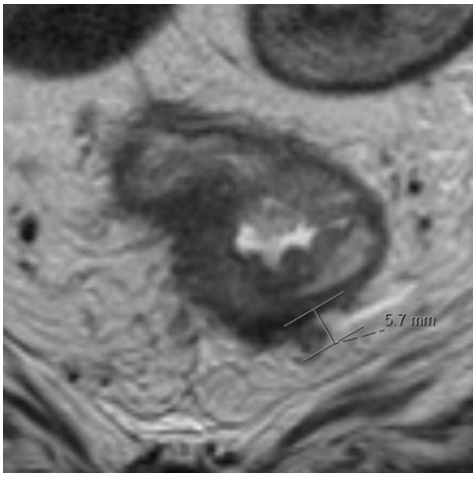
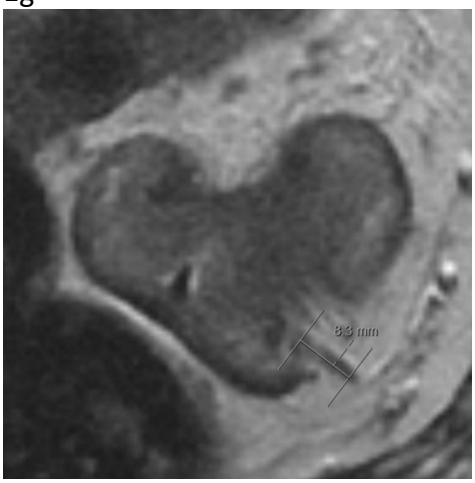
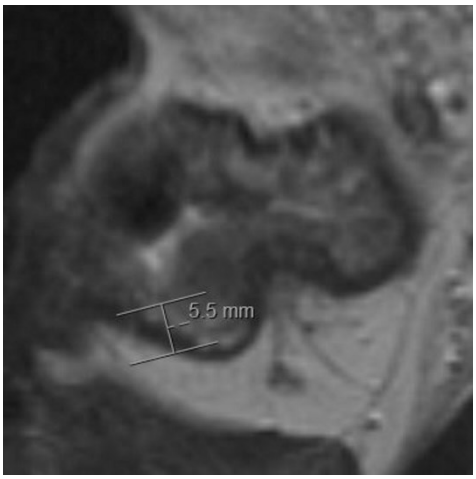
- extramural spread >5mm
- presence of extramural venous invasion and/or vascular deposits (N1c) or 4 or more tumour bearing nodules(N2)
- involvement of the circumferential resection margin or intersphincteric plane for low rectal cancer <6cm from the anal verge.

Assessment of magnetic resonance tumour regression grade (mrTRG)

mrTRG was reported on the initial post preoperative therapy restaging MRI according to the previously reported criteria*(Patel, Blomqvist et al. 2012)*. The post-treatment MRI was compared to the baseline MRI to determine the degree of tumour signal change to fibrosis(Table 8-1).

Table 8-1: mrTRG assessment

mrTRG	Response category(25)	Baseline MRI	Post-treatment MRI
Grade 1	Complete radiological response – no evidence of treated tumour	 1a	 1b
Grade 2	Good response – dense fibrosis, minimal or no tumour signal	 1c	 1d
Grade 3	Moderate response – fibrosis predominates but tumour signal visible	 1e	 1f

Grade 4	Slight response – Less than 25% fibrosis but tumour signal predominates	 1g	 1h
Grade 5	No response – tumour shows no fibrosis	 1i	 1j

Pathological tumour regression grade (pTRG)

For patients who proceeded to surgery, the Mandard pTRG was recorded if it was available on the histopathology report or could be extrapolated. In cases where the pTRG was reported according to the Dworak pTRG scale this was extrapolated to the Mandard pTRG scale to match the mrTRG scale.

pTRG was classified according to the binary variables of good response to preoperative therapy (pTRG 1 and 2) and poor response to preoperative therapy (pTRG 3, 4 or 5).

Outcomes

Patient outcomes were obtained from the electronic patient records. The development of metastatic or recurrent disease, the date of first metastatic or recurrent disease and the site of first metastatic or recurrent disease were collected for each eligible patient, as was the date of last follow-up or death. Patients were censored at three years or their last follow-up, whichever was later. Follow-up data collection terminated on 1st March 2019.

Statistical Analysis

The primary objective was to determine the timing and sites of progressive disease for patients with a poor response to preoperative therapy for rectal or sigmoid cancer (defined as mrTRG 3, 4 or 5) compared to those with a good response to preoperative therapy for rectal or sigmoid cancer (defined as mrTRG 1 or 2). Initial studies grouped patients with a mrTRG 1, 2 and 3 as those with good response to preoperative therapy and those with an mrTRG 4 and 5 as those with a poor response*(Patel, Taylor et al. 2011)*. However a subsequent study showed those with a mrTRG 1 and 2 response had a better outcome and that the binary categorisation of patients with good (mrTRG 1-2) and poor (mrTRG 3-5) response had a better association with outcomes*(Sclafani, Brown et al. 2017)*,so this categorisation was used in this study. This study adheres to the REMARK criteria for biomarker studies.

Progressive disease was defined as the development of recurrent or metastatic disease within 36 months of the date of the baseline MRI. Patients without progressive disease or with progressive disease after 36 months were censored at 36 months or the date of last

follow-up. The number of patients with progressive disease in the mrTRG good and poor response and pTRG good and poor response groups was determined. The odds ratio and confidence limits for mrTRG resulting in metastatic and/or recurrent disease were calculated by using the Cox-Hinkley-Miettinen-Nurminen method*(Miettinen and Nurminen 1985)*. No continuous variables were assessed.

The median time to the development of progressive disease was determined for each patient and then grouped by site and response to preoperative therapy. The interquartile range was also determined.

Results

Patients

338 eligible patients were identified, 226 male (67%), mean age 62 years (range 19 to 85 years), Figure 8-1 shows a flowchart of the patients.

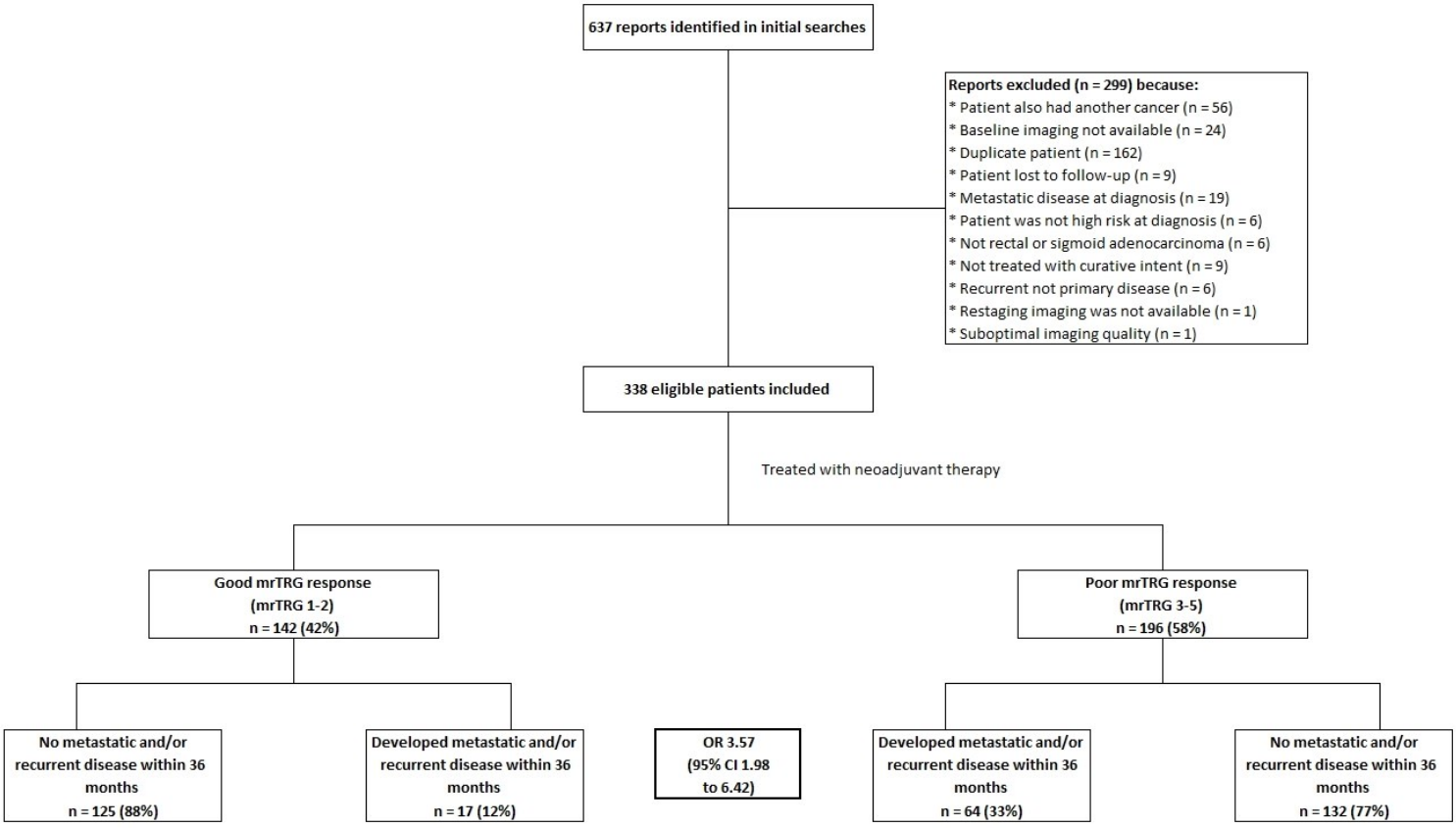


Figure 8-1: Flowchart of patients

Table 8-2 details the characteristics of the patients. As of March 2019, surviving patients had been observed for a median of 46 months (range 0 – 207 months). All patients were censored at 36 months.

Table 8-2: Table of patient demographics

Characteristic	n	*(%)*
Gender (n = 338)		
Female	112	*(33%)*
Male	226	*(67%)*
Height of tumour (n = 338)		
Low (<6 cm)	162	*(48%)*
Not low (>6 cm)	176	*(52%)*
mrT stage (at baseline) (n = 338)		
T1-3b	97	*(29%)*
T3c-4b	241	*(71%)*
mrN stage (at baseline) (n = 338)		
N0	68	*(20%)*
N1, N1c, N2	270	*(80%)*
mrEMVI (at baseline) (n = 338)		
EMVI –	91	*(27%)*
EMVI +	247	*(73%)*
mrCRM/ISP (at baseline) (n = 338)		
CRM/ISP clear	100	*(30%)*
CRM/ISP involved	238	*(70%)*
mrTRG (first MRI after CRT) (n =338)		
Good (mrTRG 1-2)	142	*(42%)*
Poor (mrTRG 3-5)	196	*(58%)*
pAJCC Stage (n = 248)		
Stage 0-2	178	*(72%)*
Stage 3	70	*(28%)*
Pathological complete response (pCR) (n = 248)		
Yes (pT0N0)	41	*(17%)*
No	207	*(84%)*
Pathological resection margin (n = 246)		
R0	226	*(92%)*
R1	20	*(8%)*
pEMVI status (n = 236)		
pEMVI –	192	*(81%)*
pEMVI +	44	*(19%)*
pTRG (n = 201)		
Good (Mandard TRG 1-2)	85	*(42%)*
Poor (Mandard TRG 3-5)	116	*(58%)*
TOTAL	**81**	***(24%)***

Development of recurrent or metastatic disease according to response to preoperative therapy

81 of the 338 patients (24%) developed metastatic or recurrent disease within 36 months; 17/142 patients (12%) with a good (mrTRG 1-2) response to preoperative therapy compared with 64/196 patients (33%) of patients with a poor (mrTRG 3-5) response to preoperative

therapy. The OR for developing metastatic or recurrent disease within 36 months in the mrTRG poor response to preoperative therapy group compared to the mrTRG good response to preoperative therapy group was 3.57 (95% CI 1.98 to 6.42).

A total of 201 patients had surgery and a pathological TRG available for review. Of these, 85/201 (42%) had a good response to preoperative therapy (pTRG 1-2) and 116/201 (58%) had a poor response to preoperative therapy. 50/201 (25%) patients with pTRG available developed recurrent or metastatic disease within 36 months; 15/85 (18%) of patients with a good pTRG response to preoperative therapy compared with 35/116 patients (30%) with a poor pTRG response to preoperative therapy. The OR for developing metastatic or recurrent disease within 36 months in the pTRG poor response to preoperative therapy group compared to the pTRG good response to preoperative therapy group was 2.02 (95% CI 1.02 to 4.00).

248 patients had surgery and pathological complete response (ypCR) data available, pathological complete response was defined as T0N0. 41/248 (17%) patients had ypCR and 66/248 (27%) developed metastatic or recurrent disease within 36 months. 5/41 (12%) patients with ypCR developed metastatic or recurrent disease within 36 months compared with 61/207 (29%) patients without ypCR developed metastatic or recurrent disease within 36 months. The OR for developing metastatic or recurrent disease within 36 months in patients without ypCR compared to those with ypCR was 3.01 (95% CI 1.13 to 8.03).

Site and timing of the development of recurrent or metastatic disease according to mrTRG response to chemoradiotherapy

The first site or sites of recurrent or metastatic disease are listed in Table 8-3. Patients with a good mrTRG had a higher rate of lung metastases (+/- other extrahepatic disease +/- pelvic recurrence) over other sites of metastases or recurrence. Patients with a poor mrTRG had higher rates of metastases at all sites, especially in the liver and lung.

Table 8-3: First site of metastatic or recurrent disease according to mrTRG response to preoperative therapy.

	mrTRG (first post-CRT MRI)			
	Good (n = 142)		**Poor** (n = 196)	
First site of metastatic or recurrent disease	**n**	**(%)**	**N**	**(%)**
Liver (+/- extrahepatic disease +/- pelvic recurrence) (n = 27)	6	(*4%*)	21	(*11%*)
Lung (+/- other extrahepatic disease +/- pelvic recurrence) (n = 31)	10	(*7%*)	21	(*11%*)
Other extrahepatic metastasis and pelvic recurrence (n = 1)	0	(*0%*)	8	(*4%*)
Pelvic recurrence (n = 15)	1	(*1%*)	14	(*7%*)
TOTAL	17	(*12%*)	64	(*33%)*

The first site listed is the predominant site of metastatic or recurrent disease and if the patient had additional sites of disease this is given in brackets, for example Liver (+/- extrahepatic disease +/- pelvic recurrence) means all of these patients had liver metastatic disease and some also had extrahepatic disease or pelvic recurrence).

The time to the development of the first site of metastatic or recurrent disease according to mrTRG is shown in Figure 8-2. For the whole cohort the median time to development of metastatic or recurrent disease was 14 months; for patients with a good (mrTRG 1-2)

response median time was 18 months and for patients with a poor (mrTRG 3-5) response median time was 13.5 months.

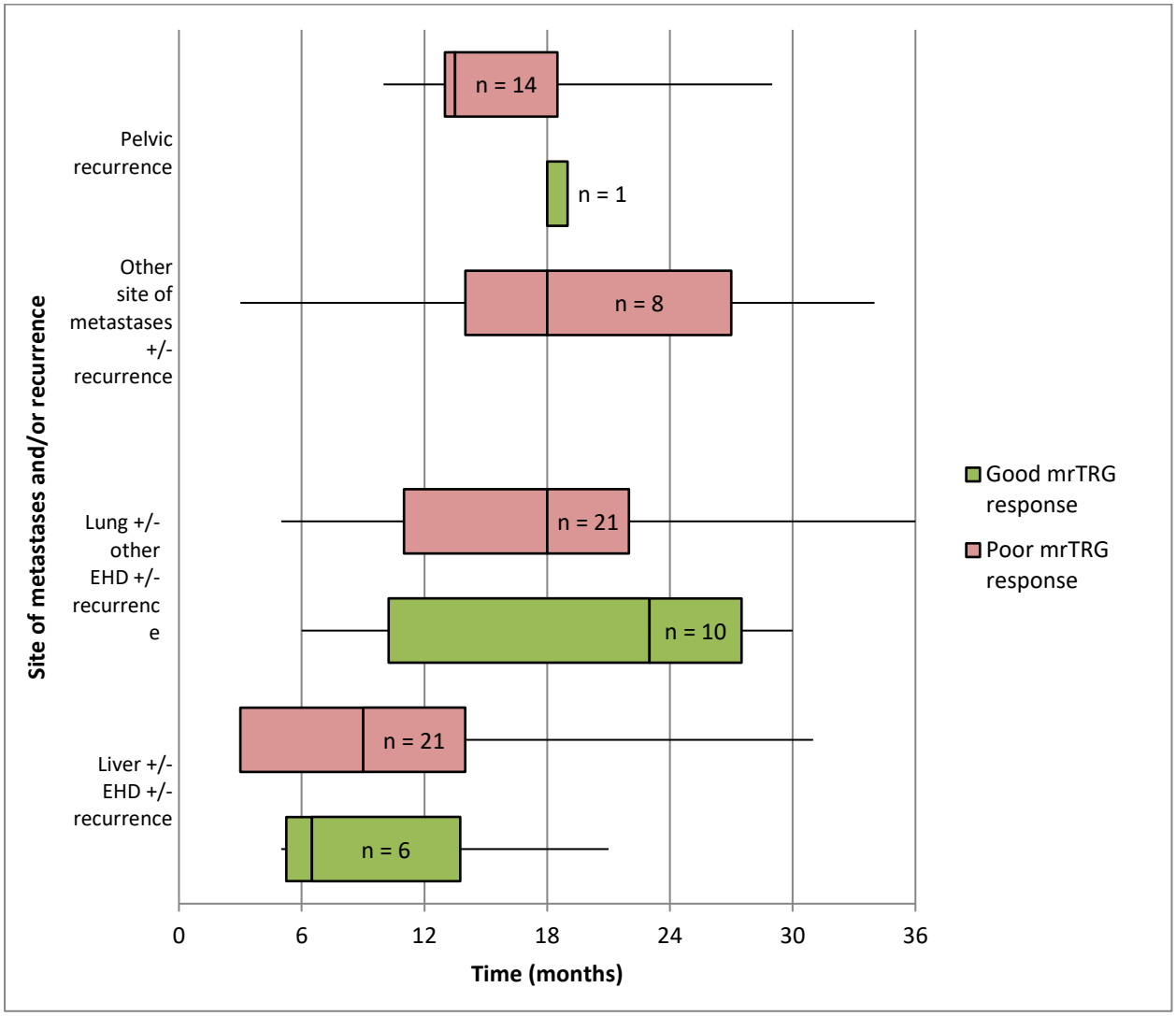


Figure 8-2: Median time to development of metastasis and/or recurrence by mrTRG response

The development of liver metastases occurred earlier than any other sites of disease; the median time to liver metastases is 6.5 months for patients with a good response to preoperative therapy and 9 months for patients with a poor response to preoperative

therapy. Median time to lung metastases was 23 months (good response) and 18 months (poor response), other sites of metastases was 18 months (poor response) and pelvic recurrence was 18 months (good response) and 13.5 months (poor response). A comparison of the site and timing of metastatic and/or recurrent disease between mrTRG and pTRG is given in Appendix 8.

Discussion

I set out to describe and compare the timing and sites of relapse for patients with rectal cancer who have a poor mrTRG response to preoperative therapy compared to those with a good mrTRG response. This study showed that the patients with a poor mrTRG response to preoperative therapy have a 3.6-fold increased risk of developing recurrent or metastatic disease with an earlier median time to the development of disease.

Overall, 81 of the 338 patients (24%) developed metastatic or recurrent disease within 36 months; 17 patients (12%) with a good (mrTRG 1-2) response to preoperative therapy and 64 patients (33%) of patients with a poor (mrTRG 3-5) response, OR 3.6 (95% CI 1.98 to 6.42). Whilst pTRG response to preoperative therapy was predictive of the development of metastatic or recurrent disease (OR 2.02 (95% CI 1.02 to 4.00)) and ypCR did predict for the development of metastatic or recurrent disease (OR 3.01 (95% CI 1.13 to 8.03)), fewer patients with a good response on ypCR were identified by both pTRG and ypCR and the OR was reduced compared to mrTRG. Compared to pTRG, mrTRG identifies more patients with a good response to preoperative therapy who have a lower frequency of metastatic or recurrent disease, allowing us to conclude that pTRG underestimates good response to preoperative therapy.

My findings concur with those of Patel *et al* and Sclafani *et al* who both showed poorer DFS for patients with poor mrTRG response to neoadjuvant therapy; P=.007 and P=0.18 respectively*(Patel, Taylor et al. 2011)*, *(Sclafani, Brown et al. 2017)*, though previous publications have not described the timing and site of recurrent and metastatic disease

according to mrTRG response to preoperative therapy. This study shows the overall median time to the development of metastatic or recurrent disease in my cohort was 14 months, but for patients with a poor mrTRG response to preoperative therapy the development of metastatic or recurrent disease was earlier, with a median time of 13.5 months. A Dutch registry study of 5671 patients found the median time to the development of metastatic disease was 18 months in patients who underwent curative treatment for the primary disease*(van Gestel, de Hingh et al. 2014)* (as in my cohort) but no authors have previously described the earlier development of metastatic or recurrent disease in patients with a poor response to preoperative therapy.

Irrespective of the response to preoperative therapy, liver (+/- extrahepatic disease +/- recurrence) was the first site of disease occurring at a median time of 6.5 to 9 months. All other sites of disease occur later irrespective of the mrTRG response to preoperative therapy. Previous publications about time to relapse have shown the liver is the most common and first site of metastatic disease*(van Gestel, de Hingh et al. 2014)*, *(van der Geest, Lam-Boer et al. 2015)*. This is hypothesised to be secondary to the portal circulation, which means the liver is the first solid organ reached by cancer cells spreading haematogeneously*(Ewing 1928)*. Similarly the Dutch registry study showed 40% of liver metastases occur within the first year, consistent with my finding that liver metastases occur earlier*(van Gestel, de Hingh et al. 2014)*.

My data showed lung metastases occur more frequently than disease at other sites in patients with a good response to treatment. This has not been demonstrated previously and it is hypothesised that it may be due to an alternative pathway of spread. Preoperative

radiotherapy obliterates the main pathway of spread which may expose other methods of tumour transport which bypass the portal circulation, such as collateral systemic circulation, and which may take longer to evolve. An equal number of lung and liver metastases occur in patients with a poor response to neoadjuvant therapy but lung metastases occur later (median time to development of lung metastases for patients with a poor mrTRG was 18 months, versus 9 months for liver metastases) consistent with previous findings*(van Gestel, de Hingh et al. 2014)*.

Previous publications have shown a poor response to preoperative therapy increases the risk of metastatic disease*(Patel, Blomqvist et al. 2012)*, *(Chand, Evans et al. 2015)*, *(Sclafani, Brown et al. 2017)*, but the link between time to relapse and mrTRG response to preoperative therapy has not previously been established. This study shows that a poor response to preoperative therapy on mrTRG results in an earlier median time to the development of metastatic or recurrent disease. A similar response was shown between pTRG and the development of metastatic or recurrent disease but the use of pTRG has inherent difficulties as described above; thus mrTRG is recommended for use in clinical practice. The findings of this study will have implications for the intensity of surveillance and counselling of patients for further therapy as it has for the presence of other poor prognostic factors*(Chand, Swift et al. 2014a)*.

There are some limitations to this study. The first is that the study was performed on a retrospective database of patients treated for locally advanced rectal cancer. There was therefore some missing data, but the number of missing datapoints was minimal and not felt to reduce the significance of the findings. Secondly, we did not test interobserver

agreement and reproducibility of mrTRG scoring in this paper. Previous studies have validated mrTRG in terms of interobserver agreement and it has been shown to be reliable*(Patel, Blomqvist et al. 2012)*, *(Siddiqui, Gormly et al. 2016)*, *(Patel, Brown et al. 2017)* with simple definitions and easy application although requiring training, specialisation and good MRI technique. These findings indicate that mrTRG can be used to give important prognostic information that will enable personalised and targeted counselling, follow-up and treatment of patients at high risk of metastatic disease based on mrTRG.

Conclusions

My results show patients with rectal cancer who have a poor mrTRG response to preoperative therapy have a 3.6-fold increase in the rate of metastatic and/or recurrent disease and an earlier median time to the development of this disease. The results also show that the liver is the earliest site of metastatic disease occurring approximately 6 months before any other sites of disease irrespective of the mrTRG response. The results have implications for patient counselling, targeted surveillance and the consideration of further therapy.

PART 3: THE APPLICATION OF IMAGING BIOMARKERS FOR THE PREDICTION OF DISEASE RELAPSE IN COLORECTAL CANCER

CHAPTER 9 - INTRODUCTION TO PART 3

Approximately 50% of patients with colorectal cancer will develop metastatic disease; liver metastases are the first and most common site of metastatic disease*(Manfredi, Lepage et al. 2006)*, *(van Gestel, de Hingh et al. 2014)*. A significant proportion of colorectal cancer deaths relate to metastatic disease in the liver*(Helling and Martin 2014)* which, if detected early, can be successfully salvaged by hepatic resection*(Khan, Wale et al. 2014)*. Unfortunately, despite the routine use of contrast-enhanced mulitdetector computed tomography (ceMDCT), the earlier diagnosis and consequent curative resection of metastatic disease still eludes us.

Imaging methods for the diagnosis of liver metastases

There are multiple imaging modalities which can each diagnose liver metastases, but they vary in their diagnostic utility and their suitability and availability for routine staging. Irrespective of the imaging modality the diagnostic utility has improved since the 1990s.

Liver ultrasound is a cheap and common modality for imaging the liver. But as with all ultrasound techniques liver ultrasound has significant inter-observer variability and limited sensitivity and specificity for the diagnosis of liver metastases. A 2005 study which evaluated the use of ultrasound for distinguishing between benign and malignant lesions found unenhanced sonography had a sensitivity of 78% and specificity of 23% for malignant lesions*(von Herbay, Vogt et al. 2004)*, however sensitivity drops to as low 20% for lesions less than 10mm*(Wernecke, Rummeny et al. 1991)*, *(Kinkel, Lu et al. 2002)*, *(Mainenti, Romano et al. 2015)*. In recent years the development of contrast-enhanced ultrasound has

increased the sensitivity to 100% and specificity to 92% for the detection of malignant lesions*(von Herbay, Vogt et al. 2004)*, but again the sensitivity drops for smaller lesions <10mm*(Westwood, Joore et al. 2013)*. In addition ultrasound is limited in its ability to provide preoperative information, including the segmental distribution of lesions, and so is not routinely used for screening for or diagnosing colorectal liver metastases*(Schima, Kulinna et al. 2005)*.

Contrast-enhanced CT of the thorax, abdomen and pelvis (CE-CT) is the routine modality used for screening and the diagnosis of metastatic disease. International guidelines, including those within the UK, recommend CE-CT at diagnosis and for the follow-up of colorectal cancer for the detection of metastatic disease*(National Institute for Health and Care Excellence (NICE) 2011a)*, *(The Royal College of Radiologists 2014)*.

Three systematic reviews and meta-analyses have been performed which examined the performance of individual imaging modalities for the diagnosis of liver metastases in patients with colorectal cancer; Bipat *et al* in 2005*(Bipat, van Leeuwen et al. 2005)* and Niekel *et al* and Floriani *et al* in 2010*(Floriani, Torri et al. 2010)*, *(Niekel, Bipat et al. 2010)*.

Niekel *et al* was the largest and most recent of these meta-analyses and reported the results of 39 prospective studies involving 3391 patients which explored the diagnostic performance of CT, MRI and PET-CT for the diagnosis of colorectal liver metastases in patients who had not previously undergone treatment*(Niekel, Bipat et al. 2010)*. CT, MRI and PET-CT performed equally well in the sensitivity for liver metastases on a per lesion basis (sensitivities of 74.4%, 80.3%, and 81.4% respectively)*(Niekel, Bipat et al. 2010)*,

findings similar to Bipat *et al(Bipat, van Leeuwen et al. 2005)*. However, on a per-patient basis the mean sensitivity of CT was 81.2%%, compared to 93.4% for MRI and 94.2% for PET-CT, and this difference in sensitivity was significant (P = 0.025)*(Niekel, Bipat et al. 2010)*. As such, whilst CT is the current standard modality for the diagnosis of liver metastases, it is limited in its sensitivity and specificity.

This is a problem significantly exacerbated for patients who are unable to have contrast enhanced CT and are left with unenhanced CT, for example due to impaired renal function. Using unenhanced CT the sensitivity for liver metastases drops to 56.1–66.7% and 52.6–56.8%, on a per-patient and per-lesion basis respectively*(Jee, Park et al. 2015)*. For lesions <1.5cm sensitivity is decreased to 28.1–34.4% (P < 0.05) and the overall size of the individual lesions is underestimated(P < 0.001)*(Jee, Park et al. 2015)*.

As shown by Niekel *et al* PET-CT has superior sensitivity for the detection of liver metastases when compared to contrast enhanced CT (P = 0.025)*(Niekel, Bipat et al. 2010)*. However, PET-CT exposes the patient to significant radiation, is lengthy and costly. A systematic review of 30 studies examining the value of PET-CT for preoperative staging found only two studies which looked at the use of PET-CT in primary colorectal cancer and concluded that there is insufficient evidence for the routine use of PET-CT for staging*(Brush, Boyd et al. 2011)*. In addition this study found limited evidence to support the use of PET-CT in the pre-operative staging of recurrent or metastatic colorectal cancer*(Brush, Boyd et al. 2011)*.

Hepatocyte specific, contrast enhanced MRI (hs-CE-MRI) and diffusion weighted MRI (DW-MRI) together are regarded as the gold standard investigation for the diagnosis of liver

metastases*(The Royal College of Radiologists 2014)*. The previous systematic reviews by Bipat *et al*, Niekel *et al* and Floriani *et al (Bipat, van Leeuwen et al. 2005)*, *(Floriani, Torri et al. 2010)*, *(Niekel, Bipat et al. 2010)* are all limited in their assessment of the diagnostic utility of MRI for the assessment of colorectal liver metastases as they did not distinguish between hs-CE-MRI and conventional gadolinium CE-MRI. Despite this both Niekel *et al* and Floriani *et al* recommended MRI as the first line modality for the assessment of colorectal liver metastases*(Floriani, Torri et al. 2010)*, *(Niekel, Bipat et al. 2010)*.

Kim *et al* performed a retrospective study of 86 patients with 179 small liver metastases (≤1.5cm) and found the combined sensitivity of hs-CE-MRI with DW-MRI was 97%, but each technique in isolation had a mean per-lesion and per-patient sensitivity of 90.7%/83.7% for hs-CE-MRI and 91.6%/83.0% for DW-MRI*(Kim, Lee et al. 2012)*. These findings agree with those of Wu *et al* who performed a meta-analysis of 11 studies (538 patients) which assessed the diagnostic performance of DW-MRI in patients with hepatic metastases, but not limited to those of colorectal origin*(Wu, Hu et al. 2013)*. This meta-analysis showed that either technique in isolation has a sensitivity of 87-90% and specificity of 90% for the diagnosis of liver metastases but that DW-MRI combined with CE-MRI had statistically significant improved sensitivity over DW-MRI alone(P = 0.03), Table 9-1*(Wu, Hu et al. 2013)*.

Table 9-1: Comparison of the diagnostic accuracy of DW-MRI) with CE-MRI from *(Wu, Hu et al. 2013)*

MRI technique	Summary sensitivity, % (95% CI)	Summary specificity, % (95% CI)	Positive likelihood ratio, % (95% CI)	Negative likelihood ratio, % (95% CI)	Diagnostic odds ratio
DW-MRI (in studies which also examined the diagnostic utility of hs-CE-MRI)	0.90 (0.82-0.94)	0.85 (0.78-0.92)	6.5 (4.2-10.0)	0.12 (0.07-0.20)	55 (34-87)
hs-CE-MRI	0.90 (0.83-0.94)	0.87 (0.78-0.93)	7.1 (3.9-12.7)	0.12 (0.07-0.20)	61 (26-139)
DW-MRI (in studies which also examined the diagnostic utility of mixed CE-MRI)	0.86 (0.78-0.92)	0.88 (0.83-0.92)	7.3 (5.2-10.2)	0.16 (0.10-0.24)	47 (31-72)
Mixed hs-CE-MRI and conventional gadolinium CE-MRI	0.85 (0.76-0.91)	0.90 (0.82-0.94)	8.2 (4.8-14.1)	0.16 (0.10-0.27)	57 (28-116)
DW-MRI combined with mixed CE-MRI	0.97 (0.90-0.99)	0.91 (0.71-0.98)	11.1 (3.0-40.7)	0.04 (0.01-0.11)	292 (96-886)

Table 9-1 also shows that the use of hs-CE-MRI has better sensitivity and specificity than studies which reported the mixed use of hs-CE-MRI and conventional gadolinium CE-MRI*(Wu, Hu et al. 2013)* further justifying the requirement to use hs-CE-MRI for the diagnosis of liver metastases.

For liver metastases of colorectal origin only the sensitivity was 92% and specificity was 87% with no significant difference between the sensitivities and specificities for liver metastases of colorectal origin and other tumour types (p = 0.38 and 0.08 respectively). There was no difference in the sensitivity or specificity of DW-MRI for liver metastases depending on the average lesion size of ≤1.5cm versus >1.5cm (p = 0.06 and 0.25 respectively)*(Wu, Hu et al. 2013)*.

These findings that hs-CE-CT and DW-MRI are the most sensitive and specific modalities for the diagnosis of colorectal liver metastases are now reflected in the Royal College of Radiologist recommendations for cross-sectional imaging in cancer management*(The Royal College of Radiologists 2014)* which recommend that MRI with hepatocyte specific contrast agents should be used for the diagnosis of liver metastases in patients with colorectal cancer.

Figure 9-1 shows the appearance of a liver metastasis on CT, DW-MRI, hs-CE-MRI and PET-CT.

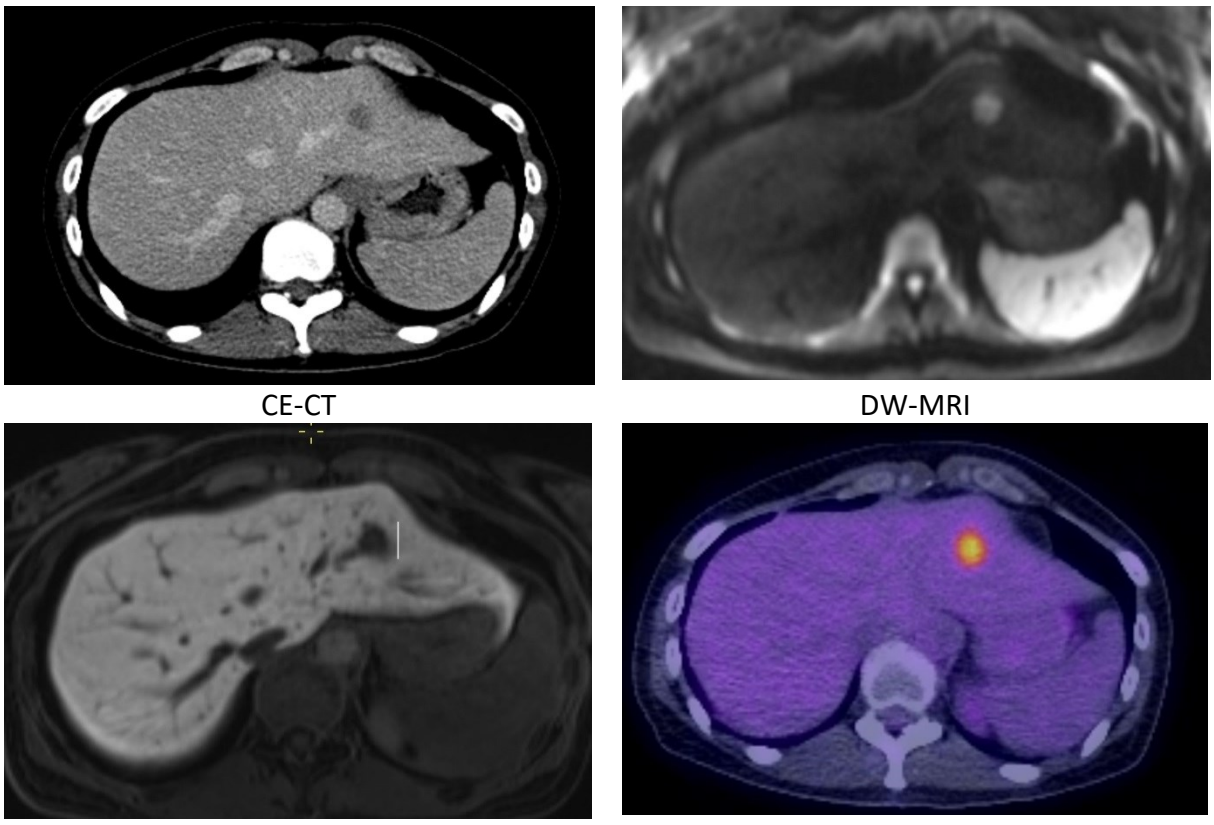


Figure 9-1: Example of a patient with a solitary liver metastasis in segment II and its appearance on CT, DW-MRI, hs-CE-MRI and PET-CT.

Screening for liver metastatic disease

The liver is the first and most common site of metastatic disease for patients with colorectal cancer*(van Gestel, de Hingh et al. 2014)* with approximately 50% of colorectal cancer patients developing liver metastases at some point.

Risk factors for the development of liver metastases

Several risk factors have been identified for the development of liver metastases, although the majority of this work has been carried out in patients who develop metachronous liver metastases following surgical resection of the primary tumour with curative intent.

Table 9-2 summarizes the findings of some of the biggest and most significant studies which have evaluated for risk factors for the development of liver metastases in colorectal cancer.

Table 9-2: Risk factors for the development of colorectal liver metastases

Paper	Cohort	N	Statistically significant risk factors for the development of metastatic disease
Studies dedicated to identifying risk factors for the development of liver metastases			
(Chuang, Su et al. 2011)	Retrospective cohort study	1099 (977 with no metastases compared to 122 with metachronous liver metastases)	On multivariate analysis: • **Preoperative serum CEA level (>5ng/ml)** – OR 1.591 (95% CI 1.065–2.377), P= 0.024 • **pT3-4** - OR 2.294 (95% CI 1.103–4.768), P= 0.026 • **pN+** - OR 2.004 (95% CI 1.324–3.031) P=0.001 • **Pathological vascular invasion** - OR 1.872 (95% CI 1.225–2.861, P = 0.004
(Augestad, Bakaki et al. 2015)	Retrospective longitudinal study	10,398	Risk factors for the development of isolated liver metastases: • **Male gender** – adjusted HR 1.45, p<0.0001 • **Primary tumour in the left side of the colon** – adjusted HR 1.63, p<0.0001 • **pN2** – adjusted HR 3.35, p<0.0001 • **pT2** – adjusted HR 2.82, p<0.0001
Non-dedicated studies which identified risk factors for the development of liver metastases			
(Chand, Bhangu et al. 2014)	Prospectively maintained database study	478	On multivariate analysis poorer DFS (including development of metastatic disease) if: • **mrEMVI positive** - adjusted HR 2.08 (95% CI 1.10-2.95), P = 0.024 • **mrEMVI positive & N+** - adjusted HR 2.74 (95% CI 1.66-4.52), P<0.001 *The poorer DFS for mrEMVI positive & N+ tumours may account for the presence of ENTDs which were reported as involved lymph nodes at the time at which this study was performed.*
(Chand, Evans et al. 2015)	Prospectively maintained database study	188	On multivariate analysis poorer DFS (including development of metastatic disease) if: • **ymrEMVI positive** – HR 1.97 (95% CI 1.01-3.90), P=0.044 • **ypEMVI positive** – HR 2.39 (95% CI 1.11-5.14), P=0.026 • **ypCRM positive** – HR 1.32 (95% CI 1.24 – 2.38), P = 0.032

Non-dedicated studies which identified risk factors for the development of liver metastases continued			
(Taylor, Quirke et al. 2014)	International multicentre interventional study (MERCURY study)	374	On multivariate analysis poorer DFS (including development of metastatic disease) if: • **MRI stage group II (TNM v 5)** – HR 2.01 (95% CI 1.09-3.61), P<0.05 • **MRI stage group III (TNM v 5)** – HR 2.42 (95% CI 1.36-4.32), P<0.05 • **mrCRM positive** – HR 1.65 (95% CI 1.01-2.69), P<0.05 • **Pathology stage II** – HR 5.26 (95% CI 2.54-11.58), P<0.001 • **Pathology stage III** – HR 9.24 (95% CI 4.46-19.13), P<0.001
(Patel, Taylor et al. 2011)	International multicentre interventional study (MERCURY study)	111	On multivariate analysis poorer DFS (including development of metastatic disease) if: • **ymrN+** - HR 2.09 (95% CI 1.06-4/15), P=0.033 • **ymrTRG 4-5** - HR 3.28 (95% CI 1.22-8.80), P=0.019
(Sclafani, Brown et al. 2017)	PAN-EX study (Pooled analysis of phase II trials EXPERT and EXPERT-C)	269	On multivariate analysis tendency to poorer 5 year RFS (including development of metastatic disease) in patients with intermediate pTRG 2 response to CRT if: • mrTRG 3-5 (poor) compared to mrTRG 1-2 (good) (mrTRG 3–5 - 5-year recurrence-free survival 6.59% versus 76.9%, P=0.18

These studies show that both pathological factors and pre-operative MRI factors have been shown to be risk factors for the development of liver metastases. However currently the presence or absence of the risk factors for the development of liver metastases are not used to risk stratify patients for surveillance or management strategies. The exception to this is in the identification of patients who could benefit from adjuvant chemotherapy to reduce their risk of disease relapse*(Chand, Rasheed et al. 2017)*.

The risk stratification of patients for surveillance according to the presence of poor prognostic risk factors could improve outcomes as it may lead to the earlier detection and treatment of colorectal liver metastases in at risk patients. Risk stratification could also avoid the potential negative impact of having to screen all patients, in terms of both financial impact and increased anxiety to patients who are low risk for the development of metastatic disease.

Requirements of a screening test for liver metastases

The World Health Organization describe cancer screening as a "distinct and more complex public health strategy"*(World Health Organization 2019)* than that of early diagnosis*(World Health Organization 2019)*. Screening tests are required to adhere to 11 key characteristics to be appropriate, as described in Table 9-3:

Table 9-3: Characteristics of a screening test*(World Health Organization 2010)*

Number	Characteristic
1	The condition should be an important health problem.
2	There should be a treatment for the condition.
3	Facilities for diagnosis and treatment should be available.
4	There should be a latent or early asymptomatic stage of the disease.
5	There should be a test for the condition.
6	The test should be acceptable to the population
7	The natural history of the disease should be adequately understood.
8	There should be an agreed policy on who to treat.
9	The total cost of finding a case should be economically balanced in relation to medical expenditure as a whole.
10	Case-finding should be a continuous process, not just a 'once and for all' project.
11	Test used should be sensitive.

CT is relatively insensitive for the detection of liver metastases, especially small volume disease. However, the further options available for the diagnosis of liver metastases, namely PET-CT and hs-CE-MRI with DW-MRI, currently do not adhere to the acceptable characteristics of a screening test. Specifically, whilst hs-CE-MRI with DW-MRI, and to a lesser extent PET-CT, are sensitive for liver metastases they may not be acceptable to patients with the requirement for contrast enhancement, long duration of the tests and in the case of PET-CT high radiation dose. Secondly PET-CT and hs-CE-CT may not be cost effective.

However, as previously shown, DW-MRI has good sensitivity for liver metastases, does not require contrast agents, has no significant additional costs and the standard sequences take approximately 10 minutes to complete. Consequently, there is increasing interest in using DW-MRI alone as a screening tool for liver metastases.

Use of DW-MRI to screen for liver metastases

Whilst DW-MRI of the liver alone has not been explored as a potential screening examination for liver metastases the potential role of whole body DW-MRI as a screening tool for metastatic disease has been explored in other cancer sites, namely non-small cell lung cancer*(Ohno, Koyama et al. 2008)*, *(Yi, Shin et al. 2008)* and whole-body MRI has been validated for the staging of myeloma*(Wale, Pawlyn et al. 2016)*.

In 2012 a multi-centre study, STREAMLINE-C, opened which aimed "to prospectively compare the diagnostic accuracy and efficiency of WB-MRI-based staging pathways with standard pathways in colorectal cancer"*(Taylor, Mallett et al. 2019)*. The study performed additional whole-body MRI in 299 patients with colorectal cancer and compared the diagnostic accuracy to standard staging investigations. The trial team were not prescriptive about the standard investigations performed. The study reported in 2019 and found 67% of patients had metastases at baseline and that pathway sensitivity was 67% (95% CI 56-78%) for whole body MRI compared to 63% (51-74%) for the standard pathway*(Taylor, Mallett et al. 2019)*. The study authors concluded that "whole-body staging pathways have similar accuracy to the standard pathways and reduce the number of tests needed, staging time, and cost"*(Taylor, Mallett et al. 2019)*.

However, considering the evidence presented previously a comparison of the accuracy of whole-body MRI against the standard clinical pathways is unlikely to result in improved clinical outcomes for patients, despite requiring a reduced number of tests, staging time and cost. In addition, whilst the un-stratified screening of patients in STREAMLINE-C showed a

cost saving it still represents a significant undertaking of time and resources for patients and hospitals with little clinical gain.

Risk-stratified screening for liver metastases

In order to ensure screening examinations adhere to the WHO guidelines it may be appropriate to screen only those patients most at risk of developing metastatic disease and only the first site of disease, i.e. the liver. This concept is supported by a study by Hunter *et al* which evaluated the incidence of synchronous liver metastases diagnosed by PET-CT and CT*(Hunter, Garant et al. 2012)*. Hunter *et al* showed that patients with rectal cancer can be risk stratified for the development of synchronous metastatic disease using the presence or absence of any validated poor prognostic features on MRI*(Hunter, Garant et al. 2012)*. The good and poor prognostic features in this study are outlined in Table 9-4.

Table 9-4: Validated MRI features used for the stratification of patients into good prognosis (low risk) and poor prognosis (high risk) by *(Hunter, Garant et al. 2012).*

Good prognosis	Poor prognosis
mrT1-T3b (depth of extramural spread ≤5mm) mrEMVI negative mrCRM/ISP clear	mrT3c-T4 (depth of extramural spread >5mm) mrEMVI positive mrCRM/ISP involved

This study then determined and compared the incidence of synchronous metastases diagnosed on PET-CT and CT combined between those patients with good prognosis and poor prognosis tumours. Hunter *et al* found that there was a 13% incidence of synchronous liver metastases for all patients*(Hunter, Garant et al. 2012)*. A comparison of the incidence of liver metastases between good and poor prognostic tumours showed patients with poor

prognostic tumours had a higher incidence of synchronous liver metastases as diagnosed by contrast enhanced CT and PET-CT at the time of the rectal MRI than patients good prognostic tumours – 20.7% vs 4.2%, OR 6.0, p<0.001*(Hunter, Garant et al. 2012)*. Hunter *et al* proposed that the identification of high risk patients at diagnosis would allow targeted further investigation of these patients. However, Hunter *et al* used PET-CT and CT which would be unsuitable as a screening test for liver metastases.

I therefore hypothesized that CT alone is inadequate as the sole method of diagnosing synchronous liver metastases and that additional staging at diagnosis with liver DW-MRI will diagnose more synchronous metastases than CT alone. In addition, the validated poor prognostic factors identified as imaging biomarkers on rectal MRI could be used to risk stratify patients for screening with DW-MRI. This was initially done with a retrospective study (Chapter 10) and then I set up a prospective study (Chapter 11). The preliminary results of this prospective study are presented in Chapter 12.

CHAPTER 10 - SCREENING DIFFUSION WEIGHTED MRI OF THE LIVER RESULTS IN INCREASED DIAGNOSIS OF SYNCHRONOUS LIVER METASTASES IN HIGH RISK RECTAL CANCER: A RETROSPECTIVE COHORT STUDY

This chapter is based on the paper **A Wale**, H Harris, G Brown. "Screening diffusion weighted MRI of the liver results in increased diagnosis of synchronous liver metastases in high risk rectal cancer: a retrospective cohort study." *Manuscript submitted.*

Introduction

Liver metastases account for a significant proportion of deaths from rectal cancer*(Helling and Martin 2014)* despite routine staging with contrast enhanced mulitdetector computed tomography (ceMDCT)*(National Institute for Health and Care Excellence (NICE) 2011a)*, *(Glynne-Jones, Wyrwicz et al. 2017)*, *(National Comprehensive Cancer Network 2018)*. 13% of all patients will have synchronous liver metastases*(Hunter, Garant et al. 2012)*, but patients with MRI defined high-risk rectal cancer*(Mercury Study Group 2007)* have a 6-fold increase in the incidence of synchronous metastases diagnosed by 18-FDG PET-CT and ceMDCT*(Hunter, Garant et al. 2012)*. I investigated whether screening liver diffusion-weighted magnetic resonance imaging (DW-MRI) could be used to diagnosis synchronous liver metastases.

DW-MRI is more sensitive for liver metastases than CT or PET-CT*(Niekel, Bipat et al. 2010)*, *(Eiber, Fingerle et al. 2012)*, *(Wu, Hu et al. 2013)* but the use of DW-MRI alone to detect synchronous liver metastases has not previously been assessed . The aim of this study was to determine the prevalence of synchronous liver metastases diagnosed by DW-MRI in

patients with MRI-defined high-risk rectal cancer compared to those with MRI-defined low-risk rectal cancer. As per the results of Hunter *et al* I hypothesized that there would be a statistically significant increase in the prevalence of synchronous liver metastases in patients with MRI-defined high-risk verses low-risk rectal cancer.

Methods

Patients

Patients treated for a new diagnosis of rectal cancer in an acute general hospital in the UK between April 2011 and May 2013 were imaged with high-resolution rectal MRI and ceMDCT as per the national guidelines*(National Institute for Health and Care Excellence (NICE) 2011a)*. Patients also underwent liver DW-MRI to screen for liver metastases as per the hospital's standard imaging protocol. No matching was undertaken. Institutional review board ethical approval was granted. The requirement for informed consent was waived.

Imaging Studies

DW-MRI was performed on a 1.5T Siemens Avanto scanner with a Synergy 6 channel phased array body or spinal coil (Siemens, U.K.). T2 weighted haste axial, T2 80 and EP 2D diffusion sequences at B50, B300 and B700 with an ADC map were acquired. Patients were scanned supine during free breathing.

High-resolution rectal MRI scans were performed supine on the same MRI scanners with an empty bladder following 20mg hyoscine butylbromide (intramuscular). T2 sagittal, axial oblique and coronal oblique sequences were performed with a slice thickness of 3mm and a 200x100mm field of view were performed (voxel size 1.92mm^3).

ceMDCT scans were performed on a Phillips Brilliance 64 slice or a Phillips Ingenuity 128 slice scanner (variable kV 80 -140, smart mAs, auto collimation, reconstruction every 2.5 mm)(Phillips, N.V. USA) . 90 mls of intravenous iodinated contrast was administered at 3

ml/sec. The chest was imaged in the arterial phase and the abdomen and pelvis was imaged in the portal-venous phase.

Follow-up

Post-operative patients underwent standard follow up with ceMDCT at 9 months and 2 years and annual outpatient consultations for 5 years. Cancer-specific survival outcomes, the development of recurrence or metastases, and overall survival, date of death and date of last follow-up were collected from the patient records. Follow-up data collection commenced on 1st April 2011 and was terminated on 25th September 2018.

Evaluation

Imaging review was undertaken in 2013. ceMDCT and liver DW-MRI were reviewed by one Radiologist with > 10 years gastrointestinal (GI) subspecialist experience blinded to the rectal MRI results. Lesions were reported as liver metastases on ceMDCT only if a hypodense lesion with peripheral ring enhancement was demonstrated; otherwise the lesions were regarded as “equivocal”. Lesions were reported as suspicious for metastatic disease on DW-MRI if the lesion demonstrated restricted diffusion (high-signal) on the DWI sequence with a corresponding area of low signal on the ADC map.

Definition of events

A lesion was considered to be a true metastasis if any of the following criteria were met:

1. Biopsy or resection of the lesion confirming metastasis
2. Progression of disease (enlargement of lesion by ≥20%)

3. Response to treatment (30% reduction in maximum diameter of the lesion following treatment)

Stratification of patients

Baseline rectal MRI scans were reviewed in consensus by two specialist GI Radiologists with 7 and >10 years' experience respectively blinded to the results of the DW-MRI liver and ceMDCT. Blinding was undertaken to address potential sources of bias.

Patients were stratified into high- and low-risk groups according to findings of the rectal MRI according to the previously published reporting criteria*(Wale and Brown 2014)*(Figure 10-1). Patients were stratified as high-risk if any of the following validated poor prognostic features was present:

1. >5mm extramural spread (T3c, T3d or T4)
2. Medium or large vessel extramural venous invasion
3. Involvement of the circumferential resection margin (<1mm) or involvement of the intersphincteric plane for low rectal tumours

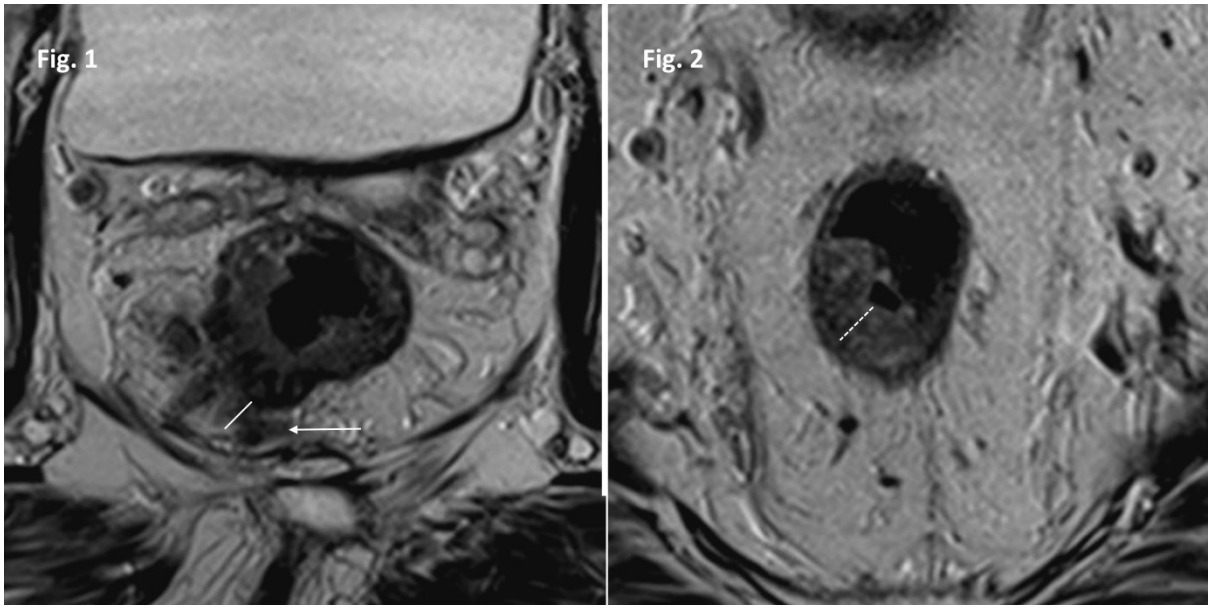


Figure 10-1: T2 weighted axial oblique small field of view images of a high-risk (1) and low-risk (2) rectal tumour.

Both tumours are semi-annular with the infiltrating border located at 7 O'Clock. (1) is a high-risk tumour with >5mm extramural spread (white line)

The incidence of lesions considered to be liver metastases was compared between the MRI-defined high-risk and low-risk groups. The effect of potential confounders and effect modifiers were not modelled as the imaging was reviewed at diagnosis before any impact of these effects and the aim of the study was to assess the effect of stratification by MRI at baseline.

Statistical Analysis

The primary outcome was to compare the prevalence of synchronous liver metastases diagnosed by DW-MRI in patients with MRI-defined high-risk versus low-risk rectal cancer. Based on previous literature*(Hunter, Garant et al. 2012)*, with an expected prevalence of 4%

in the low-risk group, showing a rate of at least 12% in the high-risk group would be considered significant with a sample size of 99 patients. Differences between groups were assessed using the Chi-squared test or one-sided Fisher's exact test as appropriate. No continuous variables were assessed.

Sensitivity and specificity of DW-MRI and ceMDCT were calculated; patients without 12 months of follow-up were excluded from the sensitivity calculation.

12 patients were lost to follow-up within 1 year and so excluded from the subgroup analysis of patients with 1-year follow-up to determine the true incidence of liver metastases compared to the incidence of liver lesions which were highly suspicious for metastases at baseline.

Survival estimates for overall survival were obtained using the Kaplan-Meier product limit method. Patients were censored at their last follow-up. Statistical analysis was performed by SPSS 25.0.0 (SPSS, Chicago, IL.) and Medcalc Software 2019. The STROBE Statement for cohort studies was completed for this study.

Results

Patients

104 patients with presumed rectal cancer were identified. 99 patients with confirmed rectal cancer (95%) had liver DW-MRI, ceMDCT and rectal MRI available for review and were included for further analysis (66 males with a mean age of 70 years (standard deviation of 6.35 years), Table 10-1.

Table 10-1: Demographics of patients included in the study. Patients with true metastatic disease at 12 months are shown for each MRI-defined high-risk feature.

			Disease status at 1 year							
	Lost to follow-up		Synchronous liver only metastatic disease		Synchronous liver & extrahepatic metastases		Synchronous extrahepatic metastatic disease only		Total with metastatic disease	
Characteristic	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)
Gender										
Female (n = 33)	5	(15)	1	(3)	1	(3)	1	(3)	3	(9)
Male (n = 66)	7	(11)	5	(8)	5	(8)	5	(8)	15	(23)
Height of tumour										
Low rectal (<6cm) (n = 25)	3	(12)	2	(8)	1	(4)	1	(4)	4	(16)
Mid rectal (6-10cm) (n = 51)	7	(14)	3	(6)	2	(4)	2	(3)	7	(14)
Upper rectal (10-15cm) (n = 23)	2	(9)	1	(4)	3	(13)	3	(13)	7	(30)
mrT stage										
T1 – T3b (n = 65)	8	(12)	2	(3)	0	(0)	0	(0)	2	(3)
T3c – T4b (n = 34)	4	(12)	4	(12)	6	(18)	6	(18)	16	(47)
mrN stage										
N0 (n = 51)	6	(12)	1	(2)	0	(0)	0	(0)	1	(2)
N1-2 (n = 12)	1	(8)	1	(8)	1	(8)	1	(8)	3	(25)
N1c (n = 36)	5	(14)	4	(3)	5	(14)	5	(14)	14	(39)
mrEMVI status										
EMVI – (n = 54)	6	(11)	1	(2)	0	(0)	0	(0)	1	(2)
EMVI + (n = 45)	6	(13)	5	(11)	6	(13)	6	(13)	17	(38)
mrCRM/ISP status										
CRM/ISP – (n = 74)	9	(12)	3	(4)	1	(1)	3	(4)	7	(9)
CRM/ISP + (n = 25)	3	(12)	3	(12)	5	(20)	3	(12)	11	(44)

Identification of liver metastases by DW-MRI and at one year

At diagnosis 10 suspicious lesions were identified by DW-MRI on a per-patient basis. 8 of these lesions were in patients with high-risk disease and 6 lesions were subsequently proven to represent metastatic disease, Table 10-2. Of the two lesions identified in patients with low risk disease one lesion was subsequently proven to represent metastatic disease.

Table 10-2: Patients with a malignant appearing lesion on either DW-MRI and ceMDCT and the results of subsequent follow-up.

Study ID	High or low risk tumour	Malignant appearing / indeterminate lesions on DW-MRI	Malignant appearing hepatic lesions on ceMDCT	Follow-up
4	Low	Yes	No	Malignant
9	Low	Yes	No	Liver lesion found to be a vascular perfusion defect on contrast enhanced MRI. Not identified on follow-up CT or MRI.
13	High	Yes	Yes	Malignant
15	High	Yes	No	Benign liver lesion – unchanged on subsequent follow-up
21	High	Yes	Yes	Malignant
54	High	Yes	Yes	Malignant
55	High	Yes	Yes	Malignant
71	High	Yes	No	Liver lesion had benign characteristics on CT but was indeterminate on DW-MRI
84	High	Yes	No	Malignant
97	High	Yes	Yes	Malignant

The OR for metastatic disease in the high-risk group compared to the low-risk group detected by DW-MRI and then confirmed as malignant on follow-up was 8.065, 95% CI 1.03 – 63.14, p = 0.018, Table 10-3.

Table 10-3: Incidence of synchronous liver metastases in high- and low-risk groups

Lesions considered liver metastases identified by:	All patients (n = 99)	High-risk group (n = 46)	Low-risk group (n = 53)	OR (95% CI)	P value
DW-MRI (3 false-positive cases)	10 (10, 5.56 to 17.6)	8 (17, 9.09 to 30.72)	2 (3.77, 1.04 to 12.75)	5.19 (1.18 to 22.79)	0.022
ceMDCT (confirmed as malignant on follow-up)	5 (5, 2.18 to 11.28)	5 (10.87, 4.73 to 23.04)	0	14.18 (0.76 to 263.81)	0.019
DW-MRI (confirmed as malignant on follow-up)	7 (7, 3.47 to 13.88)	6 (13, 6.12 to 25.67)	1 (1.89, 0.33 to 9.94)	8.065 (1.03 – 63.14)	0.018

At one year from diagnosis 12 patients were lost to follow-up (12/99, 12%). 87 patients had follow-up data available at one year, including all 10 patients who had had a suspicious lesion at diagnosis on DW-MRI. At one year 7 out of 10 patients with lesions identified at diagnosis by DW-MRI were proven to have metastases (6 in the high-risk group, 1 in the low-risk group) and a further 5 patients with high risk disease had developed liver metastases. Therefore at one year, in the whole group, there were 12 out of 87 (14%, 95% CI 8.07 to 22.58) cases of synchronous liver metastases. In the MRI-defined high-risk group there were 11 out of 40 (27.5%, 95% CI 16.11 to 42.83) confirmed liver metastases compared to 1 out of 47 (2.1%, 95% CI 3.8 to 11.11) in the low risk group. The OR for having liver metastases at one year in the high-risk group compared to the low-risk group was 17.45 (95% CI 2.14 to 142.38), and the difference in rates of liver metastases between the high-risk and low-risk groups was significant (p = 0.001)(Table 10-3, Figure 10-2).

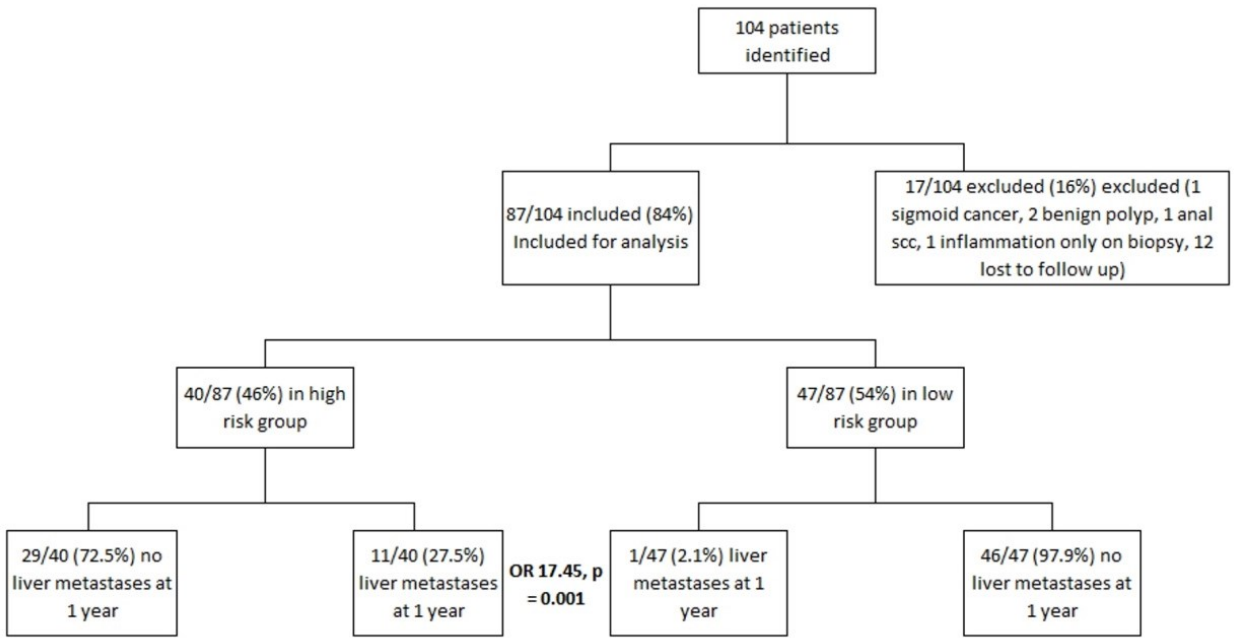


Figure 10-2: Study flow diagram demonstrating the rates of confirmed liver metastases at 1 year.

Identification of liver metastases by DW-MRI and ceMDCT

At diagnosis 5 definitely malignant lesions were identified by ceMDCT, Table 10-3. All 5 of these lesions were identified as suspicious by DW-MRI and all were subsequently proven to represent metastatic disease. There was a consistently higher proportion of patients with synchronous liver metastases in the MRI-defined high-risk group versus the low-risk group by ceMDCT (p = 0.022). The OR for confirmed metastatic disease in the high-risk group compared to the low-risk group was lower for liver metastases detected by DW-MRI (8.065, 95% CI 1.03 – 63.14) than ceMDCT (14.18, 0.76 to 263.81).

The sensitivity of DW-MRI and ceMDCT for metastatic disease was 87.5% (95% CI 47.35 to 99.67) and 71.43% (95% CI 29.04 to 96.33) respectively. The specificity of DW-MRI and ceMDCT for metastatic disease was 86.7% (95% CI 90.67 to 99.31) and 100% (95% CI 96.07 to 100) respectively.

Incidence of synchronous extrahepatic metastatic disease

12 out of 87 (14%, 95% CI 8.07 to 22.8) patients had extrahepatic metastatic disease at one year; all patients had MRI-defined high-risk disease at diagnosis. The OR for extrahepatic disease in the MRI-defined high-risk group compared to the low-risk group was 41.67 (95% CI 2.38 to 730.95) and the difference in rates of extrahepatic metastases between the high-risk group and low-risk group was significant (p = 0.01). Some patients had more than one site of extrahepatic metastatic disease. Sites were the lungs (7/12), peritoneum (2/12), pleural (1/12), spleen (1/12), nodal (3/12), bone (1/12) and not specified (3/12)(Figure 10-3).

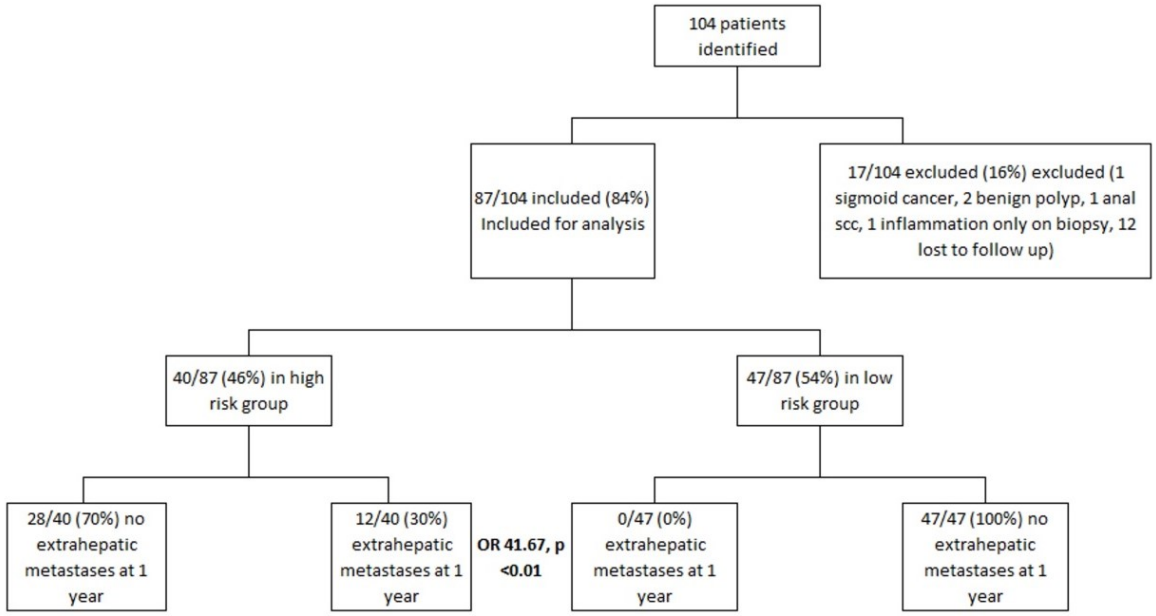
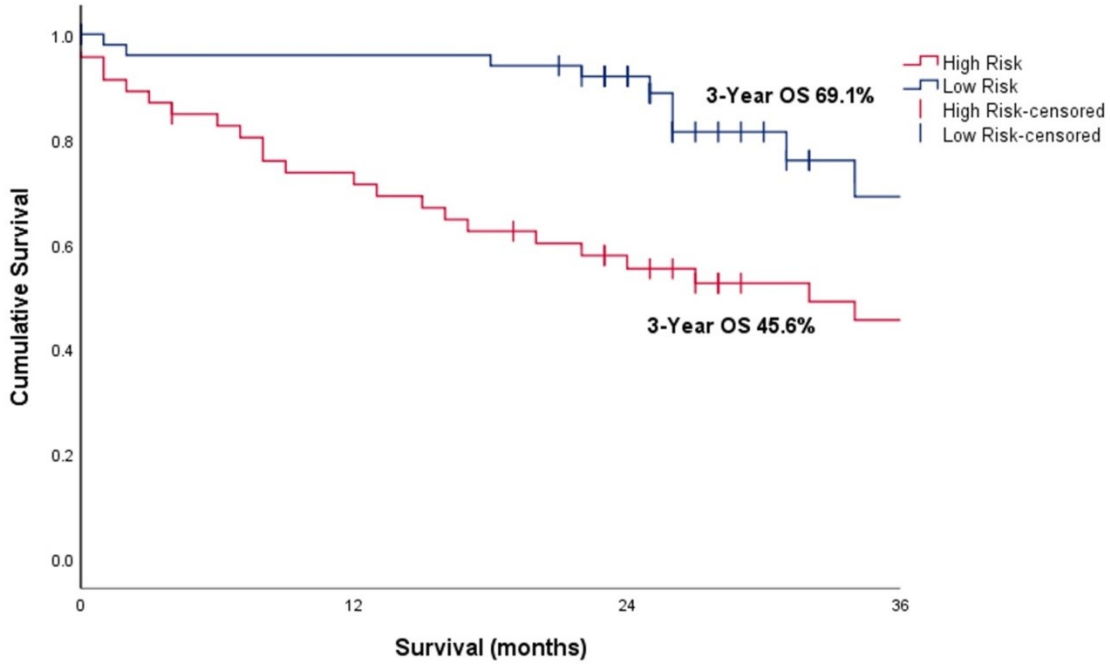


Figure 10-3: Study flow diagram demonstrating the rates of confirmed extrahepatic metastases at 1 year.

Survival Analysis

The 3-year overall survival (OS) for MRI-defined high-risk patients was significantly worse than low-risk patients – 45.6% (0.08 standard error) versus 69.1% (0.099 standard error); Mantel Cox log-rank test, p <0.05 (Figure 10-4).



Low Risk Tumours				
No. at risk	53	48	29	10
No. of events		2	4	9
High Risk Tumours				
No. at risk	46	32	22	10
No. of events		13	20	25

Figure 10-4: Comparison of 3-year overall survival between MRI-defined high-risk and low-risk patients.

Discussion

I aimed to compare the prevalence of synchronous liver metastases diagnosed by DW-MRI in patients with MRI-defined high-risk versus low-risk rectal cancer. High-resolution MRI identifies validated poor prognostic features in rectal cancer*(Merkel, Mansmann et al. 2001b)*, *(Mercury Study Group 2007)*, *(Taylor, Quirke et al. 2011a)*, *(Taylor, Quirke et al. 2014)*, *(Battersby, How et al. 2015)* and the presence or absence of poor prognostic features has been used to stratify patients into those with high-risk and low-risk primary disease*(Hunter, Garant et al. 2012)*. In this study, patients with MRI-defined high-risk disease had a statistically significant higher rate of synchronous liver metastatic disease compared to those with MRI-defined low-risk disease diagnosed by DW-MRI (OR 8.065, 95% CI 1.03 – 63.14, p = 0.018), and confirmed at 1 year (OR 17.45 (95% CI 2.14 to 142.38, p = 0.001).

In the cohort of 99 patients, DW-MRI diagnosed more confirmed synchronous liver metastases than ceMDCT; 8% (8/99) versus 5% (5/99; this did not quite reach statistical significance although the study was not powered to show this.

Patients with MRI-defined high-risk disease also had an increased rate of synchronous extrahepatic metastases (OR 41.67, 95% CI 2.38 to 730.95, p = 0.01) and significantly poorer 3-year OS than patients with MRI-defined low-risk disease; 3-year OS 45.6% versus 69.1%, p <0.05.

A previous study by Hunter *et al* demonstrated that patients with MRI-defined high-risk rectal tumours had a 6-fold increased incidence of synchronous liver metastases (OR 6.0, 95% CI 2.0-17.6, p <0.001) diagnosed by PET-CT and CT*(Hunter, Garant et al. 2012)*. This study shows patients with MRI-defined high-risk disease had a statistically significant higher rate of synchronous liver metastatic disease compared to those with MRI-defined low-risk disease diagnosed by DW-MRI and an increased number of synchronous liver metastases is diagnosed by DW-MRI over ceMDCT.

There are several reasons why this is important:

The accurate and timely pre-operative diagnosis of metastatic disease is increasingly important; patients with synchronous metastases undergo different treatment pathways which may include chemotherapy and staged resection*(Mentha, Majno et al. 2006)*, *(Aloia and Fahy 2008)*, *(Hillingso and Wille-Jorgensen 2009)*, *(Slupski, Wlodarczyk et al. 2009)*, *(Wale, Van Cutsem et al. 2018)*. Optimal investigation of all patients for metastatic disease may be desirable but is hampered by cost, time and radiation exposure as well as potential increase in patient anxiety and delays in starting treatment*(Hunter, Garant et al. 2012)*. Patients should therefore be investigated with the minimum number of high-quality investigations.

ceMDCT is the standard imaging modality for the diagnosis of liver metastases internationally, but a meta-analysis by Niekel *et al* found the sensitivity of CT, MRI and 18F-FDG PET-CT (on a per lesion basis) to be 74.4%, 80.3% and 81.4% respectively*(Niekel, Bipat et al. 2010)*. Hunter used PET-CT and CT to diagnose liver metastases but the gold standard

is contrast-enhanced MRI with DWI, especially as PET-CT is known to under-diagnose small liver metastases less than 1 cm in diameter*(Kong, Jackson et al. 2008)*. DWI-MR and contrast-enhanced MRI combined has the best sensitivity for the diagnosis of liver metastases with a sensitivity of 97%*(Eiber, Fingerle et al. 2012)* but contrast-enhanced MRI and DWI have equivalent sensitivities for the diagnosis of liver metastases, 0.9 vs 0.87 ($p>0.05$)*(Wu, Hu et al. 2013)*. The potential role of whole body DW-MRI as a screening tool for metastatic disease has been explored in other cancer sites*(Ohno, Koyama et al. 2008)*, *(Yi, Shin et al. 2008)* and staging colorectal cancer with whole-body DW-MRI is the subject of a current Phase II study*(Centre* . However, screening all patients with whole-body DW-MRI would represent a significant undertaking. My findings support those of Hunter in demonstrating that most cases of synchronous liver metastases occur in patients with MRI-defined high-risk disease, suggesting that risk-adapted additional screening with DW-MRI could be undertaken in patients with high-risk disease only. This is currently being tested prospectively in the Phase II study SERENADE, ClinicalTrials.gov Identifier: NCT02246634*(ClinicalTrials.gov* .

In this study DW-MRI identified three false-positive lesions with a resultant specificity of 86.7% (95% CI 90.67 to 99.31). This is consistent with current literature*(Niekel, Bipat et al. 2010)* as benign lesions also restrict diffusion and highlights the requirement for patients with a liver lesion which restricts diffusion to also undergo contrast-enhanced MRI with a hepatocyte-specific contrast for characterisation. DW-MRI therefore fulfils the requirement of screening tests to be highly sensitive with limited false negatives.

I found an increased incidence of extra-hepatic metastases in patients with high-risk rectal cancer which raises the possibility that these patients should undergo more targeted follow-up in the first year following diagnosis.

A potential limitation is that I only included patients with rectal cancer and not those with colon cancer. It is increasingly understood that many of the poor prognostic factors validated in rectal cancer can be readily identified on CT *(Smith, Bees et al. 2007b)* and so similar findings would be expected in patients with colon cancer. This will be tested as part of the Phase II SERENADE study, ClinicalTrials.gov Identifier: NCT02246634*(ClinicalTrials.gov)*. I didn't include nodal status in our MRI stratification of patients into high-risk and low-risk groups. Whilst nodal status is an important factor in the AJCC staging system, the assessment of nodal staging on high-resolution MRI has a relatively low sensitivity*(Kim, Beets et al. 2004)* and the other factors chosen have been validated as prognostic against patient outcomes*(Brown, Radcliffe et al. 2003)*, *(Mercury Study Group 2007)*, *(Chand, Swift et al. 2014b)*.

Finally the rectal MRIs in this study were performed with a voxel size of 1.9mm^3 rather than 1.1mm^3 as per the "high resolution" protocol validated by the MERCURY study which has been shown to be comparable to pathological staging in terms of the prediction of prognosis*(Brown, Radcliffe et al. 2003)*, *(Taylor, Quirke et al. 2014)*. The use of slightly lower resolution MRI may have reduced the proportion of high-risk patients detected in the cohort. I advocate the use of "high-resolution" imaging to be more confident in identifying low-risk patients.

Conclusions

This study showed a statistically significant difference in the prevalence of synchronous liver metastases diagnosed by DW-MRI in patients with MRI-defined high-risk versus low-risk rectal cancer. It also showed that patients with MRI-defined high-risk rectal cancer are at increased risk of extrahepatic metastases and poorer 3-year overall survival.

The results of this study therefore support the hypothesis that risk-adapted screening for liver metastases with DW-MRI could considered in patients with high-risk rectal cancer.

CHAPTER 11 - DESIGN, SET-UP AND CONDUCT OF THE SERENADE STUDY: SCREENING FOR SYNCHRONOUS METASTASES IN COLORECTAL CANCER WITH DIFFUSION-WEIGHTED MRI OF THE LIVER.

In 2013 my supervisor and I had the initial idea for the SERENADE study. The concept was to screen only those patients who had imaging-defined high-risk colorectal cancer for liver metastases with DW-MRI.

Background to the SERENADE Study

The study idea originated from previous work which had shown patients with high risk tumours had a higher incidence of synchronous liver metastases diagnosed by contrast-enhanced CT and PET-CT at diagnosis – 20.7% vs 4.2% ($p<0.001$)*(Hunter, Garant et al. 2012)*. A meta-analysis showed the sensitivity of CT, MRI and FDG PET-CT (on a per lesion basis) to be 74.4%, 80.3% and 81.4% respectively*(Niekel, Bipat et al. 2010)* but PET-CT is known to underdiagnose small liver metastases less than 1cm in diameter*(Kong, Jackson et al. 2008)*. MRI with hepatocyte-specific contrast agent and DW-MRI is regarded as the gold standard investigation for the diagnosis of liver metastases*(The Royal College of Radiologists 2014)* but hepatocyte specific contrast-enhanced MRI and DWI have equivalent sensitivities for the diagnosis of liver metastases, 0.9 vs 0.87 ($p>0.05$)*(Wu, Hu et al. 2013)* and DWI-MRI diagnosed 20% more liver metastases than CT*(Eiber, Fingerle et al. 2012)*. Therefore DW-MRI was chosen to screen for liver metastases and risk stratification was used to scan only those with high risk colorectal cancer.

Set-up of the SERENADE study

I drafted the protocol working with collaborators and statisticians and in December 2013 submitted a grant application to the Pelican Cancer Foundation for £20'000. An initial grant of £10'000 was awarded on in April 2004 which was later increased to £20'000.

I drafted the IRAS ethics application and the local institutional review board application where I defended the project resulting in institutional review board approval in March 2014. The study was added to the NIHR CRN portfolio in July 2014 and the first patient was recruited in September 2014.

To date there have been 12 amendments to the protocol, all of which I have been involved in, either through drafting protocol and CRF changes or approving these changes. I have also undertaken trial management group meetings, new site initiation meetings and answered site queries.

Patient-public involvement

Patient-public involvement (PPI) has been integral to the design and set-up of the SERENADE study, to summarise:

- PPI representatives were consulted during the set-up where they advised that patients would welcome the additional DW-MRI scan especially if it occurred at the same time as the standard restaging rectal MRI scan.
- Patients accepted the risk of a false positive result from the DW-MRI scan and were still happy to proceed.

- Recruitment has been excellent with very few eligible patients choosing not to take part. The main reason for patients declining to take part was claustrophobia which meant they did not want any more time in a MRI scanner than necessary.

Study Protocol

Hypothesis

CT alone is inadequate as the sole method of diagnosing synchronous liver metastases and additional staging at diagnosis with liver DW-MRI will diagnose more synchronous metastases than CT alone in patients with high-risk colorectal cancer.

Study design

SERENADE is a prospective phase II multicentre interventional study, performed in patients with a new diagnosis of high-risk colorectal cancer and a CT which is negative or does not confirm the presence of metastatic disease. The study aims to establish whether additional staging with liver diffusion weighted MRI diagnoses more synchronous metastases than CT alone when colorectal cancer is diagnosed and before the patient has surgery for their primary tumour, therefore patients who have CT evidence of metastatic disease are ineligible.

Patients will be identified at the multidisciplinary meeting (MDT). Prior to this they will have undergone standard staging imaging with contrast enhanced CT of the thorax, abdomen and pelvis and rectal MRI (if the patient has rectal cancer). Patients will be considered for the trial if they present with primary adenocarcinoma of the rectum or colon and have a CT scan

which is negative or does not confirm the presence of metastatic disease. They must not have had any systemic treatment for their colorectal cancer at the time of discussion in the MDT.

Patients will be given the option to enrol in the study in outpatient clinics (surgical or oncology) following the MDT meeting. Recruiting will be carried out by the local research team. Patients will be given a minimum of 24 hours to decide whether they wish to join the study and will be given the opportunity to discuss the study on the phone with a member of the research team. Following recruitment, patients will be given an appointment for a DW-MRI study of the liver. At the Royal Marsden Hospital, patients who have rectal cancer, or sigmoid cancer where MRI assessment of the primary tumour is recommended, will have a liver DW-MRI as part of their standard treatment (post-CRT) MRI. The results of the DW-MRI Liver will be discussed in the colorectal / hepatic MDT.

Patients need to complete their liver MRI scans before surgery for the primary tumour; this can be either before or after chemoradiotherapy (if the patient is to be treated with chemoradiotherapy) or before primary surgery for patients with colon cancer. This is to ensure any metastatic disease which is detected and may alter the management plan is identified before the patient undergoes primary surgery as systemic therapy is recommended for all patients with metastatic disease prior to surgery*(National Institute for Health and Care Excellence (NICE) 2011a)*.

Centres are eligible to recruit to SERENADE if they are able to undertake DW-MRI of the liver and if they are able perform MRI with liver-specific contrast agents (namely Primovist) for

the characterisation of liver lesions as per the RCR guidance*(The Royal College of Radiologists 2014)*.

Findings of the MRI will be made available to the multidisciplinary teams looking after the patients. MDTs will be free to make decisions regarding the management of patients, including the management of any identified liver metastases, according to local protocol.

Study population; inclusion and exclusion criteria

Patients with high-risk colorectal cancer (as determined by pre-operative staging (MRI/CT)) with a CT which is negative or has no confirmatory evidence of metastatic disease will be identified from the new patient lower GI MDT.

Patients with a CT which confirms metastatic disease were excluded as the purpose of the SERENADE study was to determine the added value of DW-MRI for the detection of liver metastases in patients who are presumed not to have metastatic disease.

<u>Inclusion criteria</u>

The inclusion criteria are:

1. High risk primary colorectal cancer (as determined by CT or MRI).
2. CT which is negative or no confirmatory evidence of metastatic disease.
3. Patient aged over 18 years

For the purpose of this study a high risk primary colorectal cancer is defined as a cancer which has any of the following high-risk features detected on baseline imaging (CT for colon cancer and MRI for rectal cancer)

- >5mm invasion of tumour through the muscularis propria i.e. T3c-T4
- involved circumferential margin (rectal cancer) or intersphincteric plane involved by tumour (low rectal cancer)
- Medium or large vessel extramural venous invasion (EMVI)
- N1c (extra-nodal tumour deposits)

NB: Patients with low rectal cancer (≤6cm from anal verge) whose only high-risk feature is an involved CRM/ISP are not considered to have high risk rectal cancer for the purposes for the SERENADE study.

<u>Exclusion criteria</u>

1. Patients who are unable to give consent, who withhold consent or who withdraw consent will be excluded.
2. Patient is undergoing active treatment or follow-up for another malignancy (excluding basal cell carcinoma).
3. Patient has a contraindication to CT or MRI (e.g. intraocular metal fragments, pacemaker, severe claustrophobia).
4. Patients who are pregnant or breast feeding.
5. Patients who have received systemic treatment for colorectal cancer.
6. Patients with any metastatic disease.

Study flowchart

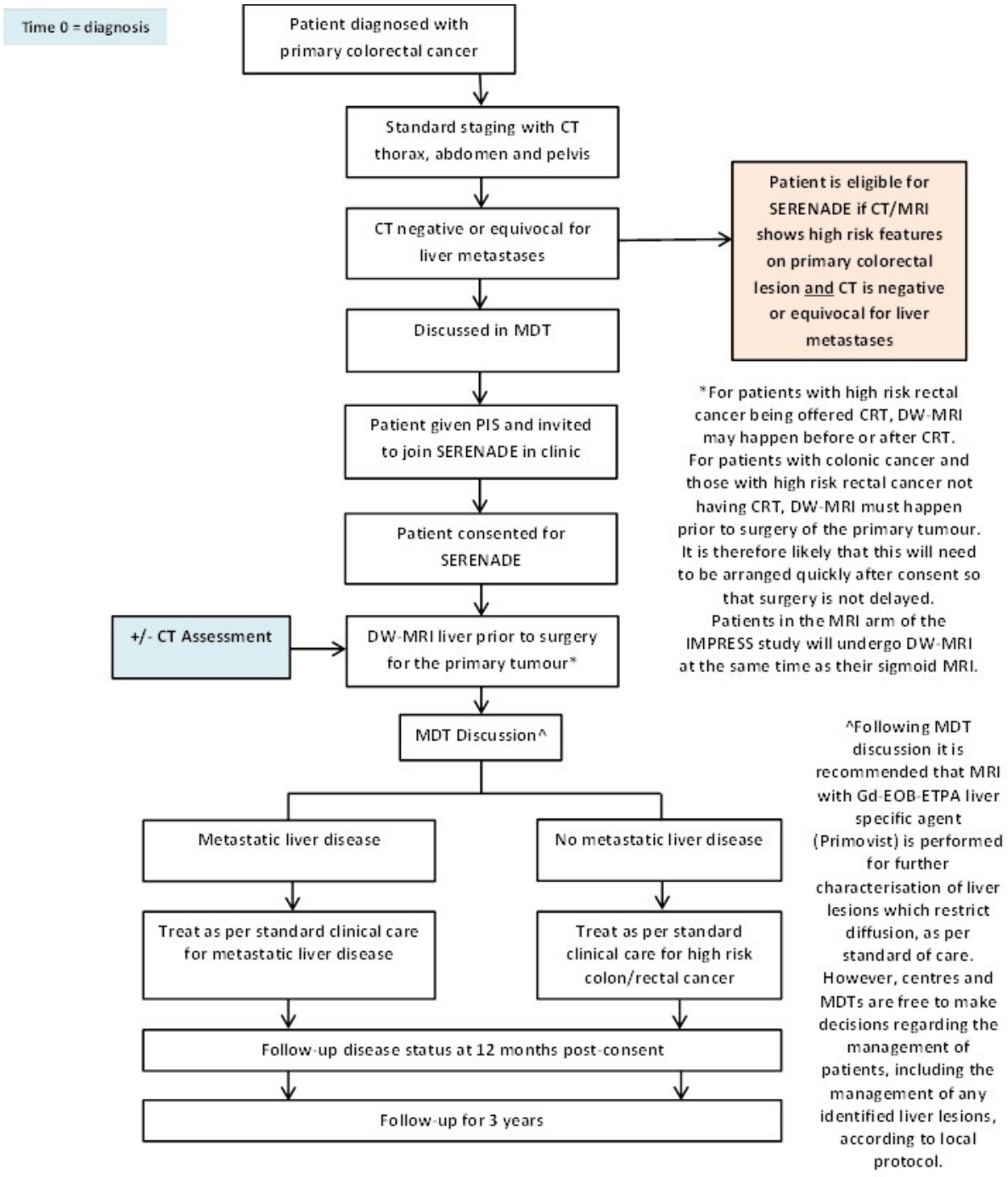


Figure 11-1: SERENADE Study Flowchart

Clinical follow-up will take place using routine out-patient consultations and any subsequent imaging as per local policy. The trial follow-up CRF will collect information at 12 months and 36 months post-CRT.

Imaging protocols

Centres are required to carry out contrast enhanced CT and high-resolution rectal MRI according to the national Royal College of Radiologist guidelines*(The Royal College of Radiologists 2014)*. The rectal MRI study described in the guidelines is based on the MERCURY Study findings which validated the use of rectal MRI*(Mercury Study Group 2006)*, *(Fowler, Beagley et al. 2007)*, *(Mercury Study Group 2007)*, *(Taylor, Swift et al. 2008)*, *(Patel, Taylor et al. 2011)*, *(Taylor, Quirke et al. 2011b)*, *(Taylor, Quirke et al. 2011c)*, *(Patel, Blomqvist et al. 2012)*, *(Taylor, Quirke et al. 2014)*. The imaging protocols are described in detail in the Chapter 3 – General Methods of this thesis.

<u>DW-MRI liver examination in the SERENADE study</u>

The individual scanner parameters for DW-MRI liver will vary between manufacturers and models therefore specific scanner parameters have not been provided. However, centres should perform 3 B values to enable accurate delineation of cystic lesions from those lesions which truly restrict diffusion.

At the Royal Marsden Hospital, we use the B values of 100, 500 and 750 but the exact B values employed are local choice. An ADC map should be generated.

T2 weighted imaging of liver +/- T1 weighted non-contrast imaging of the liver should be performed to facilitate anatomical localisation of any areas of restricted diffusion identified. Contrast-enhanced MRI imaging of the liver is not indicated as part of the SERENADE study unless area(s) of restricted diffusion are demonstrated.

Contrast-enhanced MRI of the liver

If any area(s) of restricted diffusion are demonstrated in the liver these areas require formal characterisation. The exact choice of which imaging modality is employed is at the discretion of the local MDT but an MRI liver with hepatocyte specific contrast agent (Primovist in the UK) is recommended. Some centres may choose to perform a PET-CT for the concomitant identification of extra-hepatic metastatic disease, but the MDT should be aware that the sensitivity for PET-CT for liver metastases is less than the sensitivity of MRI with hepatocyte specific contrast agent. The method of confirmation or lack of confirmation of metastatic disease will be noted as will any treatment plan made as a result of the diagnosis.

Subject Withdrawal Criteria

By consenting to the trial, patients should understand that they are consenting to follow-up, data collection and a diffusion-weighted MRI scan.

If patients choose to withdraw consent they may withdraw full consent including to any further data collection or withdrawal of partial consent in which they do not wish to have any further management within the study (only applicable if they have not yet undergone the DW-MRI scan of the liver) but is still willing to provide further data by continuing on study i.e. participating in follow-up.

Replacement of patients following withdrawal / protocol violations

Patients who withdraw full consent or those who do not meet the requirements for inclusion in the primary endpoint analysis will be replaced. The criteria for primary endpoint analysis are patients who have undergone a DW-MRI and CT prior to surgery, who have high risk features at diagnosis and at 1-year follow-up have either died of a cancer-related death or are able to complete the 1-year follow-up CRF.

If a patient meets any of the following criteria for withdrawal and replacement because they are ineligible for inclusion in the primary endpoint analysis they should be replaced:

- Suboptimal imaging (protocol violation)
- Patient did not have a DW-MRI scan (protocol violation)
- Patient died of a non-cancer related cause less than 1 year after entry into the study
- Patient had a low rectal cancer (<6cm from the anal verge) with an involved CRM as their only high-risk feature (protocol violation)
- Patient underwent the DW-MRI scan after having surgery for the primary disease (protocol violation)
- Patient chose to withdraw themselves

In the above cases, patients can remain within the trial for the purposes of follow-up and data analysis, unless full consent has withdrawn, according to the treatment option to which they have been allocated.

Patients who died from a cancer-related death less than 1 year from entry into the study and patients who could not have a contrast enhanced MRI scan after a DW-MRI scan (if

applicable) should not be withdrawn and replaced as they are still eligible for primary endpoint analysis as long as they do not also meet any of the criteria for withdrawal and replacement listed above.

Endpoints

Primary Endpoint

Total percentage of patients who have synchronous liver metastases diagnosed only on DW-MRI (where all available pre-operative CT scans are negative).

Secondary endpoints

- Establish the sensitivity and specificity of DW-MRI as a screening study for synchronous liver metastases.
- To describe the cancer and survival outcomes for the study population.
- Collect the cancer specific and survival outcomes of the patients included in the study, disease free survival at 1 year and disease-free survival and overall survival at 1 and 3 years.
- To evaluate the Fong criteria of patients diagnosed (through screening) with liver metastasis in the SERENADE study and compare the distribution of patients between the different risk groups to the expected distribution in the control group (based on published data).
- To describe the treatment received by patients who are diagnosed with liver metastases.

Evaluation of outcome - definitions of liver metastases

For the sake of this study the definition of a liver metastases as diagnosed by CT is the presence of a hypodense lesion with peripheral ring enhancement on contrast enhanced CT. Any liver lesions which do not fulfil these criteria will not be regarded as being liver metastases; these lesions will be referred to as “equivocal” according to CT appearances.

The definition of liver metastases according to the appearances on DW-MRI is a lesion which demonstrates restricted diffusion (high signal) on the DWI sequence with a corresponding area of low signal on the ADC. The signal intensity of the lesion on DWI should be higher than the signal intensity on the T2 weighted sequence.

It is not only liver metastases that restrict diffusion on the DW-MRI scan, and therefore it I recommended that patients who have a liver lesion which restricts diffusion will undergo a contrast-enhanced MRI as per standard care for those with suspicious liver lesions to confirm the diagnosis of a liver metastasis. Together, the DW-MRI and contrast-enhanced MRI are highly sensitive for liver metastases. The imaging appearances of liver metastases on MRI (DW-MRI plus contrast enhanced MRI) are so specific it is highly unlikely that a patient would receive a false positive diagnosis.

Statistical Considerations

Sample Size

The primary endpoint for this study is the incidence of synchronous liver metastases additionally diagnosed by DW-MRI when all available pre-operative CT scans are negative or do not confirm the presence of metastatic disease. This is a prospective interventional study.

Considering the results of Eiber et al (2012), DW-MRI is expected to detect liver metastases in up to 15-20% of patients with a negative baseline CT*(Eiber, Fingerle et al. 2012)*.

In the study population we expect 90% of patients to have two pre-operative CT results available (baseline and post-CRT), and we expect 9% of this group (so 8.1% of all patients) to have synchronous liver metastases diagnosed on both DW-MRI and post-CRT CT, with a further 6% of this group (5.4% of all patients) to have synchronous liver metastases diagnosed on DW-MRI alone.

The remaining 10% of patients in the study will have baseline CT alone, and of these patients we expect 15% (1.5% of all patients) to have synchronous liver metastases diagnosed only on DW-MRI. Therefore, the total percentage of patients in whom we expect DW-MRI to be of additional diagnostic benefit is 6.9% (5.4% + 1.5%).

The study aims to show that the incidence of synchronous liver metastases additionally diagnosed by DW-MRI is more than 3%, assuming that the true rate is equal to 6.9%, using a

single-stage Ahern design with one sided alpha of 0.05. A total of 255 evaluable patients are required to achieve 90% power. The same total will reach 76% power should the true rate be somewhat lower at 6%.

Patients are classified as evaluable if they meet any one of the following criteria:

- Negative baseline CT, and no further CT scan planned before primary surgery, and DW-MRI with positive diagnosis confirmed as positive on follow-up.
- Negative baseline CT, and no further CT scan planned before primary surgery, and DW-MRI with negative or equivocal diagnosis.
- Negative baseline and pre-operative CT, and DW-MRI with positive diagnosis confirmed as positive on follow-up.
- Negative baseline and pre-operative CT, and DW-MRI with negative or equivocal diagnosis.

Analysis Plan

The primary endpoint of the study is the percentage of patients with synchronous liver metastasis additionally diagnosed on DW-MRI. This will be calculated as the total number of patients with a liver DW-MRI scored as positive for synchronous liver metastasis and confirmed as malignant (as per definition below), and with all available pre-operative CT scored as negative, divided by the total number of evaluable patients. An exact one-sided binomial test with alpha of 0.05 will be performed to test the null hypothesis that the true percentage of such patients is not more than 3%.

Evaluable patients have been previously described in the sample size section. Patients who were initially planned to have a post-CRT pre-operative CT scan which did not then take place due to death, withdrawal, or clinical progression are classed as unevaluable. Patients with a liver DW-MRI scored as positive for synchronous liver metastasis where this diagnosis cannot be confirmed as malignant due to early withdrawal or death from other cause are also classed as un-evaluable.

The sensitivity and specificity of DW-MRI will be determined against the gold standard definition of a malignant lesion listed below. Only patients who are followed up for 12 months will be included in this analysis

The remaining secondary endpoints are all descriptive and will be reported using summary statistics (counts and percentages, or mean, standard deviation, median, minimum and maximums). Descriptive statistics only will be used for the subgroup reporting; no formal statistical comparisons will be made. Overall survival will be measured from date of diagnosis to death using Kaplan-Meier methods, with surviving patients censored at date of last follow-up. Progression-free survival will be measured from date of diagnosis to date of progression (local or distant) or death from any cause, using Kaplan Meier methods. Patients with no recorded event will be censored at date of last follow-up. Medians and survival estimates for 1 and 3 years will be calculated with 95% confidence intervals.

A lesion will be defined as malignant if one or more of the following criteria are met:

1. Biopsy or resection of the lesion confirming metastatic disease.

2. Progression of disease whether on or off treatment defined as enlargement of lesion by 20% or more.
3. Response to neo-adjuvant therapy defined as 30% reduction in maximum diameter of the lesion following treatment.

To determine the sensitivity and specificity of DW-MRI, the number of liver metastases not detected on screening DW-MRI but detected within 1 year of diagnosis (as per the definition of synchronous liver metastases) will be measured. Sensitivity is defined as the total number of patients positive for liver metastasis on DW-MRI who also have confirmed malignant lesions within the first 12 months using the criteria above, divided by the total number of patients with confirmed malignant lesions within the first 12 months.

Specificity is defined as the total number of patients negative for liver metastasis on DW-MRI who have no confirmed malignant lesions within the first 12 months, divided by the total number of patients with no confirmed lesions within the first 12 months. Patients lost to follow-up or withdrawn before 12 months without confirmed metastases will be excluded from the sensitivity and specificity calculations. 95% exact confidence intervals will be calculated for sensitivity and specificity.

The Fong criteria of patients diagnosed (through screening) with liver metastases in the SERENADE study will be described in terms of the full 5 criteria. The distribution of patients between the different risk groups within the screened study population will be compared descriptively to the estimated distribution in a "control" population, who have not been screened, based on published data. Should the rate of metastases be much higher than the

expected rate of 15%, an exploratory univariate analysis may be carried out to assess the impact of each of the five Fong criteria on overall survival.

Quality control and quality assurance – measures to minimize and avoid bias

Imaging protocols are standardised where appropriate (see above) and the reporting criteria are predefined.

For the purposes of quality assurance on a quarterly basis all the MR imaging (rectal and liver) performed at centres outside of the Royal Marsden Hospital will be centrally reviewed. Any test results (from the Royal Marsden Hospital, or other recruiting centres) which are inadequate for technical reasons will be recorded on the CRF and the patient will be replaced in terms of the final sample size of the study. Central review of the reporting of the imaging investigations is not planned.

Protocol amendments

A summary of the major protocol amendments which have impacted upon the study design are given in Table 11-1.

Table 11-1: SERENADE major protocol amendments which have impacted upon the scientific basis of the study.

Current version	Amended version	Details of amendment	Brief rationale
V 2.1 12/11/14	V 2.0 09/07/14	• Change of study design to multicentre Addition of three new secondary objectives/endpoints: • To determine the sensitivity and specificity of DW-MRI as a screening study by determining the number of liver metastases not detected on screening DW-MRI but detected within 1 year of diagnosis (as per the definition of synchronous liver metastases). • To report on 3 year overall survival and disease free survival for the study population. • Collect the cancer specific and survival outcomes of the patients included in the study	Additional endpoints will allow for better measurement of the benefits of liver DW-MRI
V 3.0 21/04/16	V 2.1 17/11/14	Addition of one new secondary objective/endpoint: • To describe the Fong criteria of patients diagnosed (through screening) with liver metastasis in the SERENADE study and compare the distribution of patients between the different risk groups to the expected distribution in the control group (based on published data). Addition of one new aim: • To describe the Fong criteria of patients diagnosed (through screening) with liver metastasis in the SERENADE study • Extension of recruitment for an additional year to increase the number of metastases diagnosed by screening.	The Fong criteria are used for the prediction of outcomes following resection for hepatic metastases*(Fong, Fortner et al. 1999)* but to date have not been assessed as possible predictors for the development of liver metastatic disease. This secondary endpoint will allow assessment for this.

Current version	Amended version	Details of amendment	Brief rationale
V 4.0 28/08/16	V 3.0 21/04/16	• Collection of restaging CT information in new CRF where it is carried out.	• Not all patients who undergo CRT have a restaging CT prior to resection of the primary tumour but if they do and this restaging CT demonstrates liver metastases it will be clinically relevant to determine if the DW-MRI also finds metastases.
		• DW-MRI can now be carried out at any time pre-surgery for the primary colorectal tumour, instead of within 3 months of diagnosis.	• To avoid excluding patients who have a delay in CRT, for example.
		• The study will now report on one year disease free survival.	• As above
		• Further information on imaging protocols for CT and MRI/DW-MRI.	
		• Primary endpoint amended to compare DW-MRI with post-CRT CT imaging.	
		• Introduction of more stringent QA of imaging.	• Addition of central review for technical quality
		• Collection of information on the treatment of liver metastases received by trial patients.	
		• Increase of sample size from 89 to 282.	
V 5.0	V 6.1	• Remove Quality of Life data collection	• Due to poor levels of completion
V 6.1	V 6.2	• Addition of N1c as a high risk factor	• Following publication of the prognostic implications of ENTDs (reported as mrN1c) for the development of liver metastases*(Lord, Moran et al. 2018)*
		• Low rectal cancers with CRM positivity as the only high risk factor are no longer eligible for inclusion in the study	• CRM/ISP positive low rectal cancers are high risk for the development of local recurrence secondary to technical factors*(Salerno, Daniels et al. 2009)*, *(Battersby, How et al. 2015)* but CRM/ISP involvement in low rectal cancer does not appear to confer for an increased risk of metastatic disease. Therefore these patients were withdrawn and replaced.

Sites and recruitment

The SERENADE study recruited its first patient at the Royal Marsden Hospital on 18/9/2014.

To date there are 15 UK centres open for recruitment, Table 11-2.

Table 11-2: Sites open for recruitment to the SERENADE study

Site	Principle investigator	Site given green light to open	Total recruited (including subsequent withdrawals) as of 28/05/2019
Royal Marsden	Prof Gina Brown	18/09/2014*	132
Basildon & Thurrock	Miss Bryony Lovett	25/01/2019	0
Bolton	Miss Gemma Faulkner	11/12/2017	2
Broomfield Essex	Dr Peng Lee	15/02/2018	9
George Eliot	Was Dr Martin Scott-Brown, now Dr Jamal Abdulkarim	13/10/2017	3
Imperial	Was Dr Dominic Blunt, now Dr Katherine Van Ree	27/03/2018	4
Macclesfield District General	Mr Christ Smart	28/09/2017	1
Medway	Dr Iheoma Amaechi	08/12/2015	9
North Manchester	Mr Mohammud Salim Kurrimbaccus	17/07/2018*	1
Portsmouth	Was Dr Anthony Higginson, now Dr Christopher Ball	21/03/2017*	17
Queens Burton	Dr Manjusha Keni	10/02/2017	1
Royal Gwent	Dr Mark Robinson	27/10/2016	6
Royal Liverpool	Was Dr Catriona Farrell now Rebecca Wiles	17/10/2017	5
Royal Stoke	Dr Ravavarma Balasubramanium	31/07/2017	19
Salisbury	Mr Graham Branagan	26/11/2015*	49
Wythenshawe South Manchester	Miss Sarah Duff	31/01/2017*	24

*where the date the site was given the green light to open is unavailable the date the first patient was recruited is given instead.

CHAPTER 12 - PRELIMINARY RESULTS FROM THE SERENADE STUDY

The SERENADE Study is a Phase II interventional study where patients with high-risk colorectal cancer are screened for liver metastases with DW-MRI prior to having their primary surgery. The study is described in detail in the previous chapter.

The hypothesis is that diffusion-weighted MRI of the liver can be used as a screening tool for the diagnosis of synchronous liver metastases in patients with imaging-defined high-risk colorectal cancer.

Preliminary Results

Data extraction was performed on 28th May 2019 following TMG approval. 282 patients from 15 UK centres have been recruited to date, Table 12-2. 20/282 patients were withdrawn and not included in further analysis; the reasons for the withdrawals are listed in Table 12-1.

Table 12-1: Reasons patients were withdrawn from the SERENADE Study

Reason for withdrawal	No. patients
Protocol violation: Low rectal cancer with CRM/ISP involvement was the only high-risk feature	8
Protocol violation: Liver DW-MRI performed after primary surgery	2
Protocol violation: Liver DW-MRI not performed	4
Protocol violation: Eligibility criteria breached	3
Patient for palliative care only	1
Patient recruited in error	1
Suboptimal imaging	1

262 patients were included for interim analysis. The gender of recruited patients was not provided for interim analysis. Median age of patients was 65 years (range 30 – 101 years). 65% of patients had rectal cancer. Patient demographics are described in Table 12-2.

Table 12-2: Characteristics of included patients within the SERENADE study

Characteristic	n	*(%)*
Location of primary tumour (n = 262)		
Rectum	169	*(65%)*
Sigmoid	43	*(16%)*
Colon (not sigmoid)	40	*(15%)*
Unknown	10	*(4%)*
Height if rectal primary (n = 169)		
Low (<6 cm)	56	*(33%)*
Not low (>6 cm)	113	*(67%)*
T stage (at baseline on CT or MRI) (n = 262)		
T1-T3b	55	*(21%)*
T3c-T4	207	*(79%)*
mrN stage (at baseline if rectal or sigmoid primary staged by MRI (n = 183)*		
N0	17	*(9%)*
N1-2	29	*(29%)*
N1c	113	*(62%)*
EMVI (at baseline on CT or MRI) (n = 262)		
EMVI –	46	*(18%)*
EMVI +	216	*(82%)*
mrCRM/ISP (at baseline if rectal primary) (n = 169)		
CRM/ISP clear	56	*(33%)*
CRM/ISP involved	113	*(67%)*
Treatment with neoadjuvant CRT (n = 220)		
No	103	*(47%)*
Yes	117	*(53%)*

*Nodal staging has been collected for CT staging of colonic tumours but has not been made available in this preliminary dataset.

The distribution of the validated high-risk features is shown in Table 12-3. 63/262 (24%) of patients had only one high risk feature.

Table 12-3: Distribution of imaging defined high-risk features for patients included for interim analysis in the SERENADE Study.

Imaging defined high-risk feature	**n**	***(%)***
Extramural spread >5mm (T3c-T4)	207	*(79%)*
Extramural venous invasion +	216	*(82%)*
N1c disease consistent with ENTDs	118	*(45%)*
Involved circumferential resection margin / interspincteric plane	113	*(67%)*

Baseline and pre-operative CT

228/262 (87%) patients had a baseline CT data available and no patients had definite liver metastases identified on this scan as per the inclusion criteria for the study. 131/262 (50%) of patients had pre-operative CT data available; 7/131 (5%) of these studies demonstrated a liver metastasis which was not present on the baseline CT.

Screening DW-MRI study of the liver and subsequent investigations

220/262 (84%) of patients have screening DW-MRI data available. 24/220 (11%) of patients had at least one lesion which restricted diffusion on the screening DW-MRI scan of the liver. 15 patients had 1 lesion which restricted diffusion, 3 patients had 2 lesions, 1 patient had 3 lesions, 3 patients had 4 lesions and 1 patient each had 5 and 9 lesions which restricted diffusion.

14/24 (54%) of patients who had at least one lesion which restricted diffusion had liver metastasis/metastases confirmed on subsequent imaging and/or follow-up. The follow-up investigations which confirmed the presence of liver metastases are described in Table 12-4.

Table 12-4: Outcome of lesions which restrict diffusion on liver DW-MRI

Study ID	Location of primary tumour	Imaging					12 month follow-up	DW-MRI lesion confirmed as metastasis?
		Baseline CE-CT	DW-MRI	CE-MRI	Pre-operative CE-CT	PET-CT		
		Definite liver metastases?	Suspicious liver lesion?	Liver metastasis?	Definite liver metastases?	Definite liver metastases?	Liver metastases in the previous 12 months?	
POR002	COLON	NO	YES	YES				YES
POR004	RECTUM	NO	YES	YES		NO	YES	YES
RMH002	RECTUM	NO	YES		YES	NO	YES	YES
RMH010	RECTUM	NO	YES		NO		NO	NO (FP)
RMH011	SIGMOID	NO	YES	YES	NO	NO	YES	YES
RMH014	RECTUM	NO	YES		YES	NO	YES	YES
RMH025	RECTUM	NO	YES			NO	YES	YES
RMH028	RECTUM	NO	YES		YES	NO	YES	YES
RMH041	RECTUM	NO	YES	NO	NO		NO	NO (FP)
RMH043	RECTUM	NO	YES	YES	NO	NO	YES	Yes
RMH047	RECTUM	NO	YES		YES	NO	YES	Yes
RMH054	RECTUM	NO	YES	NO	NO		NO	No (FP)
RMH065	RECTUM	NO	YES		YES	NO	YES	Yes
RMH070	RECTUM	NO	YES	YES	YES	NO	YES	Yes
RMH080	RECTUM	NO	YES		YES			Yes
RMH109	RECTUM	NO	YES	NO	NO		NO	No (FP)
SDH028	COLON	NO	YES	NO			NO	No (FP)
SDH033	RECTUM	NO	YES	NO			NO	No (FP)
SDH037	SIGMOID	NO	YES	NO				No (FP)
SDH038	RECTUM	NO	YES	NO				No (FP)
SDH046	UNKNOWN	NO	YES	NO				No (FP)
SMH004	SIGMOID	NO	YES		NO	NO	YES	Unknown
SMH017	UNKNOWN	NO	YES	YES				Yes
UNM015	RECTUM	NO	YES	NO				No (FP)

Table 12-4 describes the outcomes of patients with lesions which restricted diffusion on screening DW-MRI. DW-MRI diagnosed a liver metastasis when all other pre-operative imaging was negative for liver metastases in 6/24 cases and 6/262 when considering the entire cohort (2.3%). These cases are highlighted in yellow.

17 patents had a contrast-enhanced MRI of the liver, and 16 of the 17 patients had a lesion which restricted diffusion on the DW-MRI scan of the liver. The 1 patient who had a contrast enhanced MRI without a lesion which restricted diffusion on the DW-MRI scan had a lesion which was considered benign on the DW-MRI scan. 7/17 (41%) patients who had a contrast enhanced MRI of the liver had a lesion considered to be a metastasis, including 1 patient who had a contrast enhanced MRI of the liver without a suspicious lesion on DW-MRI.

11/24 patients (46%) who had a lesion which restricted diffusion on DW-MRI underwent a PET-CT. In none of these instances were liver metastases confirmed by PET-CT despite contrast-enhanced MRI or pre-operative contrast-enhanced CT identifying liver metastases in 7/11 of these cases.

Proportion of liver metastases diagnosed by DW-MRI

The interim analysis (considering the data is incomplete) showed 13/262 (5%) of patients had a confirmed liver metastasis diagnosed by DW-MRI, with 6/262 (2.3%) patients who had a liver metastasis diagnosed by DW-MRI alone when all other standard pre-operative imaging was negative for liver metastases.

Discussion

I aimed to determine whether diffusion-weighted MRI of the liver can be used as a screening tool for the diagnosis of synchronous liver metastases in patients with imaging-defined high-risk colorectal cancer.

The interim analysis (considering the data is incomplete) of the Phase II SERENADE study has shown that 13/262 (5%) of patients had a liver metastasis diagnosed by DW-MRI. In 6 of these cases DW-MRI was the only modality which identified the liver metastasis. In the remaining 7 cases the liver metastasis was also identified on pre-operative CE-CT, however pre-operative CE-CT is not a routine procedure after the delivery of CRT nationally and so these patients may not have been identified pre-operatively in other centres. This has implications for patient staging as a 5% increase in the detection of liver metastases may warrant the adoption of DW-MRI as a screening tool for patients with high-risk colorectal cancer.

Furthermore, 11 patients who had a lesion which restricted diffusion on DW-MRI also underwent a PET-CT; in none of these patients did the PET-CT identify metastatic disease. The detail in the data is limited for this interim analysis but it may be that the lesions were <1cm where PET-CT is known to have limited sensitivity*(Niekel, Bipat et al. 2010)*.

The primary endpoint of this study is the incidence of synchronous liver metastases additionally diagnosed by DW-MRI when all available pre-operative CT scans are negative or

do not confirm the presence of metastatic disease. This requires the data to be complete and 12-month follow-up for all patients which is expected to be available at the end of 2020. The primary endpoint will therefore need to be formally assessed after this time.

Conclusion

Routine staging of patients with colorectal cancer is undertaken with CT which is relatively insensitive for liver metastases. Retrospective work for this thesis had shown that DW-MRI identified an 8-fold increase in liver metastases in patients with high-risk colorectal cancer than those with low-risk colorectal cancer. The interim analysis (considering the data is incomplete) of the Phase II SERENADE study has shown that 13/262 (5%) of patients had a liver metastasis diagnosed by DW-MRI. This has implications for patient staging as a 5% increase in the detection of liver metastases may warrant the adoption of DW-MRI as a screening tool for patients with high-risk colorectal cancer.

CHAPTER 13 - FINAL CONCLUSIONS

The overarching aim of this thesis is to investigate the use of imaging biomarkers for risk stratification for disease relapse in patients with colorectal and anal cancer, and this has been achieved in three parts.

Part 1: The development and validation of imaging biomarkers

Hypothesis: Peer-reviewed publications of prognostic studies of imaging biomarkers for liver metastases in patients with colorectal cancer do not adhere to the "REporting recommendations for tumour MARKer prognostic studies" (REMARK) guidelines.

Few biochemical or imaging biomarkers have been translated into clinical practice. For biochemical biomarkers, it was hypothesised that the incomplete reporting of biochemical biomarker studies may contribute to the lack of implementation*(McShane, Altman et al. 2005)*. This led to the REporting recommendations for tumour MARKer prognostic studies (REMARK) guidelines*(McShane, Altman et al. 2005)*, adherence to which is a requirement for publication of biochemical tumour biomarker studies*(Mallett, Timmer et al. 2010)*. Various guidelines have been published for imaging biomarker studies *(National Cancer Institute* , *(Tofts, Brix et al. 1999)*, *(Leach, Brindle et al. 2005)*, *(Hunter 2008)*, *(Woodcock and Woosley 2008)*, *(Shankar 2012)*, *(Waterton and Pylkkanen 2012)*, *(European Society of Radiology 2013)*, *(Clarke, Nordstrom et al. 2014)*, *(Boellaard, Delgado-Bolton et al. 2015)*, *(Food and Drug Administration 2015)*, *(Huang, Wang et al. 2015)*, *(Sullivan, Obuchowski et al. 2015)*, *(FDA-NIH Biomarker Working Group 2016)*, *(O'Connor, Aboagye et al. 2017)* but

there are no guidelines for reporting of these studies. It was unknown whether imaging biomarker studies also suffered from incomplete reporting.

I undertook a systematic review to determine to what extent prognostic studies of imaging biomarkers for liver metastases in patients with colorectal cancer adhered to the REMARK guidelines*(McShane, Altman et al. 2005)*. This systematic review, presented in Chapter 5, has shown deficiencies in the reporting of imaging biomarker studies are widespread in imaging biomarker research and that there is a need to apply better standards in this area. The REMARK guidelines could be made mandatory for the publication of imaging biomarker studies which may highlight the need for adherence to authors.

Part 2: Investigation of Novel Imaging Biomarkers for Disease Relapse in Anal and Colorectal Cancer.

(A) Depth of extramural spread as a prognostic imaging biomarker for anal SCC

Hypothesis: The MRI assessment of tumour length and depth of extramural spread for anal squamous cell carcinoma can be used for risk stratification for disease relapse. MRI tumour regression grading following chemoradiotherapy in rectal cancer can be used to risk stratify patients for disease relapse in terms of the timing and site of metastatic disease.

TNM staging for anal SCC is based on clinical rather than MRI staging. However, MRI staging of anal SCC is now standard practice and the clinically-derived TNM staging system has been applied to MRI staging without any validation. A number of possible prognostic biomarkers

have been proposed for anal SCC, including the size of the tumour*(Allal, Mermillod et al. 1997)*, *(Smith, Whelan et al. 2001)*, *(American Joint Committee on Cancer (AJCC) 2017)* and the depth of invasion through the bowel wall*(Smith, Whelan et al. 2001)* but to date mr-derived TN staging has not been validated for anal SCC against outcomes and the possible role of depth of extramural spread on MRI has not been investigated.

Chapter 7 described the retrospective study I undertook which aimed to validate mr-derived T staging against prognosis and investigate whether mr-derived depth of extramural spread was related to outcomes in patients with anal SCC. I showed that whilst binary categorisation of mr-derived T staging is statistically significant for the prediction of 3 year PFS, once mr-derived depth of extramural spread is added T staging is no longer predictive of outcome but a 12 mm cut-off of depth of extramural spread was predictive of outcome. On multivariate cox regression analysis only mrDEMS was a significant predictor for 3 year PFS and 3 year OS. This work has therefore proposed a novel imaging biomarker of depth of extramural spread (with a cut-off of 12mm) which now needs to be validated in another dataset. If validated depth of extramural spread could be the basis for an amendment to TNM staging and the escalation or de-escalation of treatment according to mr-derived staging.

(B) Can magnetic resonance tumour regression grade (mrTRG) predict the timing and patterns of distant metastases in patients with locally advanced rectal cancer post chemoradiotherapy?

Hypothesis: MRI tumour regression grading following chemoradiotherapy in rectal cancer can be used to risk stratify patients for disease relapse in terms of the timing and site of metastatic disease.

Poor mrTRG response to preoperative therapy for locally advanced rectal cancer results in poorer disease free and overall survival*(Patel, Taylor et al. 2011)*, *(Sclafani, Brown et al. 2017)*. However, the sites of recurrence and timing of metastatic and recurrent disease following treatment with preoperative therapy is currently unknown. Chapter 8 describes the study I undertook which aimed to determine the timing and pattern of metastatic or recurrent disease following preoperative therapy for locally advanced rectal cancer and whether this can be predicted by the imaging biomarker of mrTRG response to preoperative therapy.

I showed shows that patients with a poor mrTRG response to preoperative therapy have a 3.6-fold increase in the rate of metastatic and/or recurrent disease, with the most prevalent sites being the liver and lung, with liver occurring earlier. This has implications for personalised patient care in terms of counselling, targeted organ-based surveillance, for example liver MRI, and discussions about adjuvant therapies.

Part 3: The application of imaging biomarkers for the prediction of disease relapse in colorectal cancer.

Hypotheses:

1. **There is increased prevalence of synchronous liver metastases diagnosed by diffusion-weighted MRI in patients with MRI-defined high-risk versus low-risk rectal cancer.**
2. **Diffusion-weighted MRI of the liver can be used as a screening tool for the diagnosis of synchronous liver metastases in patients with imaging-defined high-risk colorectal cancer.**

Liver metastases account for a significant proportion of deaths from colorectal cancer*(Helling and Martin 2014)* despite routine staging with contrast enhanced mulitdetector computed tomography (ceMDCT)*(National Institute for Health and Care Excellence (NICE) 2011a)*, *(Glynne-Jones, Wyrwicz et al. 2017)*, *(National Comprehensive Cancer Network 2018)*. 13% of all patients will have synchronous liver metastases*(Hunter, Garant et al. 2012)* but patients with MRI-defined high-risk rectal cancer*(Mercury Study Group 2007)* have a 6-fold increase in the incidence of synchronous metastases diagnosed by 18-FDG PET-CT and ceMDCT*(Hunter, Garant et al. 2012)*.

I initially undertook a retrospective study to investigate whether screening liver diffusion-weighted magnetic resonance imaging (DW-MRI) could be used to diagnose synchronous liver metastases and to compare the incidence of synchronous liver metastases diagnosed by DW-MRI in high-risk and low-risk rectal cancer patients.

The results of this study, presented in Chapter 10, showed that DW-MRI diagnosed more confirmed synchronous liver metastases than ceMDCT (8% versus 5%) and that patients with high-risk rectal cancer had an 8-fold increase in the incidence of synchronous liver metastases diagnosed by DW-MRI (p <0.001) and confirmed at one year (OR 17.45, p=0.019) and poorer 3 year overall survival than patients with low-risk rectal cancer (45.6% versus 69.1%, p<0.05).

This study provided the evidence to proceed to a Phase II multi-centre interventional study, SERENADE, which I set up and ran to investigate whether DW-MRI should be used as a risk stratified screening tool for patients with high risk colorectal cancer.

Chapter 11 describes the set up and protocol of the SERENADE study and Chapter 12 describes the interim results. Whilst other studies have explored the potential role of whole-body diffusion weighted MRI as a screening tool for metastatic disease*(Taylor, Mallett et al. 2019)*, the SERENADE study is the first study to explore the potential role of risk stratified screening for liver metastases in patients with colorectal cancer.

To date 262 patients have been recruited and were included for the interim analysis. The interim analysis showed that 11% of patients had at least one lesion which restricted diffusion on the screening DW-MRI scan of the liver. 13/24 (54%) of these patients had liver metastasis/metastases confirmed on subsequent imaging and/or follow-up.

Whilst the data for this interim analysis is incomplete the 5% increase in the detection of liver metastases may warrant the adoption of DW-MRI as a screening tool for patients with high-risk colorectal cancer.

Future Work

Combining the knowledge gained from my mrTRG work which described the timing and sites of metastatic disease in patients with high-risk rectal cancer and a poor response to neoadjuvant therapy with the results from the SERENADE study which show screening DW-MRI identifies 5% more liver metastases than routine baseline staging with CT, a further study investigating intensified surveillance for patients with a poor response to neoadjuvant therapy is planned.

In addition, I am aiming to perform the validation work arising from my work which looked at the prognostic significance of depth of extramural spread for anal SCC. This work will need to be performed on another dataset and therefore I will be looking to work with collaborators outside my institution.

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CHAPTER 15 - APPENDICES

Appendix 1: Abbreviations and Definitions

Abbreviation	Definition
95% CI:	95% Confidence Interval
AJCC:	American Joint Committee on Cancer
APER:	Abdomino perineal excision of the rectum
AR:	Anterior resection
cCR:	Clinical complete response
CE-CT:	Contrast enhanced computed tomography
COMET Trial:	COncordance in MRI and Pathology Diagnosis of Extranodal Tumour Deposits Short Title: The COMET Trial
CRM:	Circumferential resection margin
CRT:	Chemoradiotherapy
CT:	Computerised tomography
DFS:	Disease-free survival
DW-MRI:	Diffusion-weighted MRI
ENTD:	Extranodal tumour deposit
EORTC:	The European Organisation for Research and Treatment of Cancer
EMVI:	Extramural vascular invasion
FSE:	Fast-spin echo
hs-CE-MRI	Hepatocyte-specific contrast enhanced MRI of the liver
IMPRESS Study:	Improving radical treatment through MRI evaluation of sigmoid cancers
IMRT:	Intensity-modulated radiation therapy
LARS:	Low anterior resection synchrome
MDT:	Multidisciplinary team
MERCURY Study:	Magnetic Resonance Imaging and Rectal Cancer European Equivalence Study
MRI:	Magnetic Resonance Imaging
mrLRP:	Magnatic resonance low rectal cancer plane
mrTRG:	Magnetic resonance tumour regression grading
KM:	Kaplan-Meier
NCI:	National Cancer Institute
OS:	Overall survival
pCR:	Pathological complete response
PET-CT:	18-FDG Positron Emission Tomography

PRESERVE Study:	PRE-therapeutic MRI assessment of Early StagE Rectal Cancer and significant Rectal Polyps to aVoid major resectional surgery: A new approach to the management of Early stage rectal cancer. The PRESERVE Study.
pTRG:	Pathological tumour regression grading
SERENADE Study:	Screening for synchronous metastases in colorectal cancer with diffusion-weighted MRI of the liver.
SCPRT:	Short-course preoperative radiotherapy
QA:	Quality assurance
RCR:	Royal College of Radiologists
TMG:	Trial Management Group
TNM:	Tumour, Node, Metastasis
TRIGGER Trial:	Using the magnetic resonance tumour regression grade (mrTRG) as a novel biomarker to stratify between good and poor responders following chemoradiotherapy in rectal cancer: a multicentre randomised control trial.
UICC:	Union for International Cancer Control
WHO:	World Health Organisation

Appendix 2: Prefixes

Prefix	Definition
TNM	Tumour, Node, Metastasis (TNM) system*(American Joint Committee on Cancer (AJCC) 2017)*
c	clinical
ct	computed tomography
mr	magnetic resonance imaging
p	pathology
y	Staging following neoadjuvant therapy

Appendix 3: Systematic Review Search Strategy

Outlined below is the search strategy for the systematic review of the reporting of prognostic imaging biomarker studies in metastatic colorectal cancer. The search was performed on 24th August 2018.

#	Database	Search term	Results
1	Medline	exp "COLORECTAL NEOPLASMS"/	182654
2	Medline	((bowel* OR colon* OR colorectal OR rectal OR rectum) ADJ3 (cancer* OR tumor* OR tumour* OR malignan* OR neoplas*)).ti,ab	173397
3	Medline	(1 OR 2)	239416
4	Medline	((liver OR hepatic) ADJ3 metastas*).ti,ab	32358
5	Medline	(3 AND 4)	13168
6	Medline	exp "TOMOGRAPHY, X-RAY COMPUTED"/	385759
7	Medline	(CT).ti,ab	291650
8	Medline	exp "MAGNETIC RESONANCE IMAGING"/	397196
9	Medline	(MRI).ti,ab	204603
10	Medline	(recist).ti,ab	3353
11	Medline	("k trans").ti,ab	712
12	Medline	(SUV).ti,ab	5156

13	Medline	(percist).ti,ab	128
14	Medline	(6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13)	899359
15	Medline	(5 AND 14)	2358
16	Medline	(predict* OR stag* OR prognos* OR evaluat* OR assess* OR indicat*).ti,ab	8394035
17	Medline	(15 AND 16)	1613
18	Medline	(review OR case OR "meta analysis").ti,ab	2983613
19	Medline	17 NOT 18	1211
20	Medline	19 [DT FROM 2005] [Languages English]	742
21	Medline	19 [DT FROM 2013] [Languages English]	363

Appendix 4: Systematic Review – Explanatory Notes for Data Extraction

Original REMARK criteria	Modified REMARK criteria for iREMARK study
Item 1: State the marker examined, the study objectives, and any pre-specified hypotheses.	
a. Description of the marker: i. Biological aspects including type of molecule or structure (e.g. protein, RNA, DNA or chromosomes) and features assessed (e.g. expression level, copy number, mutation or translocation) ii. Timing of specimen collection e.g. at diagnosis, after completion of initial therapy b. Study objectives: e.g. evaluation of the association between tumour marker value and clinical outcome, determination of whether a tumour marker contributes additional information about likely clinical outcome beyond the information provided by standard clinical or pathologic factors c. Any pre-specified hypotheses Should be formulated in terms of measures that are amenable to statistical evaluation. The distinction between pre-specified hypotheses (based on prior research or understanding of a biological mechanism, stated before the study is initiated) and new hypotheses suggested by data generated in the study need to be made.	a. Description of the imaging biomarker: i. Imaging modality ii. Use of contrast if CT iii. Sequences if MRI iv. Tracer if PET-CT v. When imaging was performed b. State study objective c. State pre-specified hypothesis if there is one

Materials and Methods
Patients
Item 2. Describe the characteristics (for example, disease stage or co-morbidities) of the study patients, including their source and inclusion and exclusion criteria.

• Source of the patients e.g. clinical trial population, healthcare system, all hospitals in a certain geographic area • Patient eligibility criteria, usually based on clinical or pathologic characteristics, should be clearly stated. As a minimum, eligibility criteria should specify the **site and stage of cancer** of the cases to be studied. Other factors including **age, treatment received, histologic type of cancer** • Exclusion criteria. If deaths occurred very early after the initiation of follow-up cases may be excluded, if done the rationale and timeframe for exclusion should be specified. • If the specimen set was assembled primarily on the basis of ready availability ("convenience" sample) this should be acknowledge • Include a flow diagram of cases • Describe how specific cases included in the study were sampled from the study population	• Source of patients • Eligibility criteria – site and stage of cancer at a minimum • Exclusion criteria
Item 3. Describe treatments received and how chosen (for example, randomized or rule-based).	
a. What treatment and the timing of that treatment	b. What treatments and timing of that treatment
Item 4. Describe type of biological material used (including control samples) and preservation and storage methods.	
c. Type of material e.g. tumour tissue, cells or DNA isolated from blood, bone marrow, urine, sputum, serum or plasma. d. Reporting according to the Biospecimen Reporting for Improved Study Quality (BRISQ) guidelines. e. Details as to whether specimen has been stored and storage conditions. Use of stabilizers f. Use of control samples e.g. biological	g. Type of imaging modality used h. Protocol for imaging study e.g. contrast use, type and timing, scanner type, sequences used and sequence parameters

Item 5. Specify the assay method used and provide (or reference) a detailed protocol, including specific reagents or kits used, quality control procedures, reproducibility assessments, quantitation methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to the study endpoint.	
i. Report assay methods completely and transparently with a level of detail that would enable another laboratory to reproduce the measurement technique. j. Report minimum amount of specimen required to perform the assay and whether there were any other assessments performed to judge the suitability of the specimen for use in the study k. Report any procedures e.g. blinded replicate samples or control reference samples that are employed to assess or promote consistency of assay results over time or between sites l. Strategies to reduce imprecision and measurement error m. Were marker assessment made blinded to clinical outcome to reduce bias.	n. Quality control procedures o. Reporting criteria for IB p. Were reporters blinded to clinical outcome?
Item 6. State the method of case selection, including whether prospective or retrospective and whether stratification or matching (for example, by stage of disease or age) was used. Specify the time period from which cases were taken, the end of the follow-up period, and the median follow-up time.	
(a) Case selection • Detailed explanation of how patients were selected for inclusion, beyond relying on prospective or retrospective e.g. where patients recruited prospectively as part of a proposed marker study or patients recruited for another purpose e.g. clinical trial or retrospective search through an existing database. • Patient selection with stratification according to clinicopathologic factors e.g. stage, based on survival experience or according to a matched design • Exactly how and when clinical,	(a) Case selection • Method of case selection • Prospective or retrospective - where patients recruited prospectively as part of a proposed marker study or patients recruited for another purpose e.g. clinical trial or retrospective search through an existing database. • Stratification or matched • How and when clinical, pathological and follow-up data

pathologic and follow-up data were collected • Marker measures extracted retrospectively from existing records, whether assays were newly performed on stored specimens or performed in real time using prospectively collected specimens (b) Time period • When study took place and over what period participants were recruited • Specific date when follow up of all participants ended plus report mean duration of follow-up • Method of calculating median follow-up e.g. reverse Kaplan Meier • How many patients were lost to follow-up or the completeness of data	(b) Time period • When study took place • When participants recruited • Date follow-up of all participants ended • Median duration of follow-up • Method of calculating median follow-up • Number patients lost to follow-up or completeness of the data
Item 7. Precisely define all clinical endpoints examined.	
• Endpoint should be precisely defined including the if patients were initially disease free, not just survival or overall survival • Indicate how cause of death was classified and source of records • Were endpoint assessments made blinded to marker measurements	• Precisely defined endpoint • How cause of death was classified if applicable • Were endpoint assessments made blinded to the marker measurements
Item 8. List all candidate variables initially examined or considered for inclusion in models.	
• Which marker measurements or clinical or pathological variables were initially considered for inclusion in models, including variables not ultimately used? • Fully define all variables and how they were measured	• Which marker measurements or clinical or pathological variables were initially considered for inclusion in models, including variables not ultimately used? • Fully define all variables and how they were measured (definitions must be full enough that the study can be reproduced without any further information, this information can be included in the appendices or in a reference if the referenced paper provides adequate detail.
Item 9. Give rationale for sample size; if the study was designed to detect a specified	

effect size, give the target power and effect size.	
• Explain the considerations that led to the sample size • What effect size will be detectable with sufficient power given the predetermined sample size	• Rationale for sample size • What effect size will be detectable with sufficient power given the predetermined sample size if applicable
Statistical Analysis Methods	
Item 10. Specify all statistical methods, including details of any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data were handled.	
• All statistical methods used in the analysis should be reported • Which decisions were pre-specified and which were made post hoc or in deviation from the original analysis plan a) Preliminary data preparation: report assessment if data quality performed prior to main statistical analysis of the data, and potential removal of data values which have been changed or removed if deemed unreliable b) Association of marker values with other variables should be described e.g. chi-square, t test c) Methods to evaluate a marker's univariable association with clinical outcome. Method of analysis e.g. logrank test or estimated effect with confidence interval in cox regression and choice of test statistic e.g. likelihood ratio test d) Multivariate analysis: - how the analysis was performed, how variables were selected e) Missing data – detailed report about the amount of missing data, why the data was missing and the number of individuals excluded because of missing data. If the missing data is assessed statistically f) Variable selection – how model selected e.g. treatments received as stratification factor. Stepwise regression or backward elimination and specific criteria used to determine inclusion or exclusion of variables from the model (e.g. P value) or best fitting model.	• All statistical methods listed • Which decisions were pre-specified and which were post hoc • Did preliminary data preparation take place? • Has possible association with other variables been tested? Association of marker values with other variables should be described e.g. chi-square, t test • Univariate methods assessed against clinical outcome including analysis methods and CI • Multivariate analysis – how analysis was performed and how variables were selected • Handling of missing data – amount of missing data, why it was missing, number excluded because of missing data and if missing data assessed statistically • Checking model assumptions if applicable

g) Checking model assumptions e.g. residual plots, time-by-covariate interactions, outliers, parametric survival	
Item 11. Clarify how marker values were handled in the analyses; if relevant, describe methods used for cutpoint determination.	
• Report how continuous variables are analysed	• Report how continuous variables are analysed

Results	
Data	
Item 12. Describe the flow of patients through the study, including the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the number of patients and the number of events.	
• Participant flow diagram	• Participant flow chart
Item 13. Report distributions of basic demographic characteristics (at least age and sex), standard (disease specific) prognostic variables, and tumor marker, including numbers of missing values.	
• Basic demographic variable and standard prognostic variables for the disease (e.g. nodal status, tumour size, presence of metastases, performance status) • Distribution of age and sex • Racial or ethic distributions • Number of patients with missing variables should be reported for each variable & number of patients for whom there is a complete dataset (all or those that affect survival) • If a subsample from a RCT or large defined cohort compare the characteristics of those with and without marker measurement to judge generalizability • Thorough description of the distribution of the marker e.g. frequency table or bar chart or mean, median, percentiles, range, standard deviation	• Distribution of patient demographics • Number of missing patients for each variable • If subset from a RCT or large defined cohort compare the characteristics of those with and without marker measurement to judge generalizability. • Distribution of the marker e.g. frequency table, bar chart, mean, median, percentiles, range, standard deviation
Item 14. Show the relation of the marker to standard prognostic variables.	
• Describe association of the marker with standard prognostic variables • New marker is most useful if it provides clinically important information beyond that given by	• Association of marker with standard prognostic variables; standard prognostic variables should be defined by the authors and identified as such.

existing prognostic variable or if it offers an advantage over other markers because its easier to measure or quantify. • Graphs can be useful • Summary description of the findings of these association assessments • Categorizing continuous variables should be avoided	
Item 15. Present univariable analyses showing the relation between the marker and outcome, with the estimated effect (for example, hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analyzed. For the effect of a tumor marker on a time-to-event outcome, a Kaplan-Meier plot is recommended.	
• First show simple association with outcome without adjustment for other characteristics with precision and uncertainty of estimates e.g. confidence intervals. P values can also be presented • If time to event a KM curve is recommended with number of patients at risk at selected time points • Show univariate regression analyses	• Univariate analysis between marker and outcome with CI an P values • KM curve if survival analysis with number of patients at selected time points
Item 16. For key multivariable analyses, report estimated effects (for example, hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model.	
• Outline model building process from the "full model" and data-dependent modelling steps to "final model" • Confidence intervals and p values • If multivariate analyses for subgroups these also need P values and CIs	• How the model was built • Multivariate analysis with CI and P values • If subgroup multivariate analysis these must also have CI and P values
Item 17. Among reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their statistical significance.	
• Evaluate whether the new marker maintains some association with clinical outcome after accounting for standard prognostic variables in a model distinguished from the other multivariate models, i.e. "standardised model", with CI and p values	• Any association between the IB and clinical outcome and the current gold standard methods of assessment, with CI and P values
Item 18. If done, report results of further investigations, such as checking assumptions, sensitivity analyses, and internal validation.	

• Prognostic analysis results will have greater credibility if arguments can be made that the modelling assumptions are likely to be justifiable or that the results are not unduly sensitive to certain assumptions. • The report should mention a brief summary of the results obtained from any additional analyses that were performed or diagnostic plots that were examined for the purpose of checking assumptions or demonstrating robustness of results	• Report any further investigations to check the assumptions, sensitivity or internal validation if applicable

Discussion	
Item 19. Interpret the results in the context of the pre-specified hypotheses and other relevant studies; include a discussion of the limitations of the study.	
• Begin by briefly restating the purpose of the study and recalling any pre-specified hypotheses. • Simple summary of major findings • Clear distinction between conclusions based on pre-specified hypotheses and hypotheses suggested during the course of the data analysis • Critically evaluate the reported results including acknowledging biases or inconsistencies in the data, limitations of assay methods or design or data analysis methods • Review how prognostic value of the marker varies across the subgroups • Discussion of how the results integrate into the existing body of evidence	• State purpose of the study and any pre-specified hypotheses • Summary of main findings • Distinguish between conclusions based on pre-specified hypotheses and post-hoc hypotheses • Critically evaluate reported results considering biases, data inconsistences or analysis methods – for the purpose of this analysis a discussion of the limitations has been accepted as evidence that the author's critically evaluated their work. • How prognostic value of marker varies across the subgroups • Discuss how results relate to the existing body of evidence
Item 20: Discuss implications for future research and clinical value	
• In some cases, the results of a study will suggest that a marker has some promise for clinical value, but a firm conclusion cannot be drawn due to insufficient information. • It is helpful in the discussion of future research plans to specifically identify information that is still lacking or inadequate.	• Discuss implications for future research and clinical value, specifically identifying information that is still lacking or inadequate, for the purpose of this analysis a sentence discussing future work needed has been accepted as consideration of future research required.

Appendix 5: Systematic Review Case Report Form

Please complete one case report form per study. This form should be completed in conjunction with the REMARK scoring guidelines.

Assessor initials:

Study first author:

Study year of publication:

Study title:

Introduction

1 - Description of imaging: imaging modality:	**Yes**	**No**	
1 - Description of imaging: details of the imaging:	**Yes**	**No**	
1 - Description of imaging: when imaging was performed:	**Yes**	**No**	
1 - Study objective:	**Yes**	**No**	
1 - State pre-defined hypothesis (if applicable):	**Yes**	**No**	**N/A**
2 - Source of patients:	**Yes**	**No**	
2 - Eligibility criteria:	**Yes**	**No**	
2 - Exclusion criteria:	**Yes**	**No**	
3 - Treatment received:	**Yes**	**No**	
4 - Imaging modality:	**Yes**	**No**	
4 - Protocol for imaging:	**Yes**	**No**	

5 - Quality control procedures:	**Yes**	**No**	
5 - Reporting criteria:	**Yes**	**No**	
5 - Reporters blinded to clinical outcome:	**Yes**	**No**	
6 - Case selection: method of case selection:	**Yes**	**No**	
6 - Case selection: prospective or retrospective with details:	**Yes**	**No**	
6 - Case selection: stratification or matched?	**Yes**	**No**	
6 - Case selection: method of follow-up data collection:	**Yes**	**No**	
6 - Time period: when the study took place?	**Yes**	**No**	
6 - Time period: when the participants were recruited?	**Yes**	**No**	
6 - Time period: date the follow-up of all participants ended	**Yes**	**No**	
6 - Time period: median duration of follow-up	**Yes**	**No**	
6 - Time period: method of calculating median follow-up	**Yes**	**No**	
6 - Time period: Number patients lost to follow-up	**Yes**	**No**	
7 - Precisely defined endpoint	**Yes**	**No**	
7 - Classification of cause of death (if applicable)	**Yes**	**No**	**N/A**
7 - Were the endpoint assessments blinded?	**Yes**	**No**	
8 - Details of variables initially considered for inclusion in the model	**Yes**	**No**	
8 - Define all variables and how they were measured	**Yes**	**No**	
9 - Rationale for sample size	**Yes**	**No**	
9 - Detectable effect given sample size (if applicable)	**Yes**	**No**	**N/A**
10 - All statistical methods listed?	**Yes**	**No**	
10 - Pre-specified and post-hoc analysis decisions?	**Yes**	**No**	
10 - Preliminary data preparation	**Yes**	**No**	
10 - Possible association with other variables tested	**Yes**	**No**	

10 - Univariate methods assessed against clinical outcome	**Yes**	**No**		
10 - Multivariate analysis with details of analysis and variable selection	**Yes**	**No**		
10 - Handling of missing data	**Yes**	**No**		
10 - Checking model assumptions (if applicable):	**Yes**	**No**		
11 - How were continuous variable analysed?	**Yes**	**No**		
12 - Flow chart of patients	**Yes**	**No**		
13 - Patient demographics	**Yes**	**No**		
13 - Number of missing patients for each variable	**Yes**	**No**		
13 - Characteristics of patients if a subset from a smaller cohort?	**Yes**	**No**		
13 - Distribution of the marker	**Yes**	**No**		
14 - Marker association with standard prognostic variables	**Yes**	**No**		
15 - Univariate analysis between marker and outcome	**Yes**	**No**		
15 - Kaplan-Meier plot with the number of patients at selected time points			**Yes**	**No**
16 - How model was built?	**Yes**	**No**		
16 - Multivariate analysis	**Yes**	**No**		
16 - If subgroup multivariate analysis (if applicable)	**Yes**	**No**	**N/A**	
17 - Association between biomarker, outcome & gold standard	**Yes**	**No**		
18 - Further Investigations to check	**Yes**	**No**		
19 - Purpose of study & pre-specified hypotheses	**Yes**	**No**		
19 - Summary of main findings	**Yes**	**No**		
19 - Distinguish between pre-specified hypothesis and post-hoc conclusions			**Yes**	**No**
19 - Critical evaluation	**Yes**	**No**		
19 - How results relate to body of evidence?	**Yes**	**No**		
20 - Implications for future research	**Yes**	**No**		

Appendix 6: Bibliography of studies included in the systematic review of the reporting of prognostic imaging biomarker studies in metastatic colorectal cancer

Each of the following studies was included in the systematic review of the reporting of prognostic imaging biomarker studies in metastatic colorectal cancer (Chapter 5).

1. Abbadi RA, Sadat U, Jah A, Praseedom RK, Jamieson NV, Cheow HK, et al. Improved long-term survival after resection of colorectal liver metastases following staging with FDG positron emission tomography. Journal of surgical oncology. 2014;110(3):313-9.

2. Cheung HMC, Karanicolas PJ, Coburn N, Seth V, Law C, Milot L. Delayed tumour enhancement on gadoxetate-enhanced MRI is associated with overall survival in patients with colorectal liver metastases. European radiology. 2018.

3. Chiu KWH, Lam K-O, An H, Cheung GTC, Lau JKS, Choy T-S, et al. Long-term outcomes and recurrence pattern of 18F-FDG PET-CT complete metabolic response in the first-line treatment of metastatic colorectal cancer: a lesion-based and patient-based analysis. BMC cancer. 2018;18(1):776.

4. Correa-Gallego C, Gavane S, Grewal R, Cercek A, Klimstra DS, Gewirtz AN, et al. Prospective evaluation of 18F-fluorodeoxyglucose positron emission tomography in patients receiving hepatic arterial and systemic chemotherapy for unresectable colorectal liver metastases. HPB : the official journal of the International Hepato Pancreato Biliary Association. 2015;17(7):644-50.

5. Dimitrova EG, Chaushev BG, Conev NV, Kashlov JK, Zlatarov AK, Petrov DP, et al. Role of the pretreatment 18F-fluorodeoxyglucose positron emission tomography maximal standardized uptake value in predicting outcomes of colon liver metastases and that value's association with Beclin-1 expression. Bioscience trends. 2017;11(2):221-8.

6. Froelich MF, Heinemann V, Sommer WH, Holch JW, Schoeppe F, Hesse N, et al. CT attenuation of liver metastases before targeted therapy is a prognostic factor of overall survival in colorectal cancer patients. Results from the randomised, open-label FIRE-3/AIO KRK0306 trial. European radiology. 2018.

7. Heijmen L, ter Voert EEGW, Oyen WJG, Punt CJA, van Spronsen DJ, Heerschap A, et al. Multimodality imaging to predict response to systemic treatment in patients with advanced colorectal cancer. PloS one. 2015;10(4):e0120823.

8. Jones C, Badger SA, Stevenson M, Diamond T, McKie LD, Taylor MA, et al. PET-CT as a predictor of outcome in resectable colorectal liver metastases. European journal of gastroenterology & hepatology. 2014;26(4):466-72.

9. Kim C, Kim SY, Kim M-J, Yoon YS, Kim CW, Lee JH, et al. Clinical impact of preoperative liver MRI in the evaluation of synchronous liver metastasis of colon cancer. European radiology. 2018.

10. Kim Y-E, Joo B, Park M-S, Shin SJ, Ahn JB, Kim M-J. Dynamic Contrast-Enhanced Magnetic Resonance Imaging as a Surrogate Biomarker for Bevacizumab in Colorectal Cancer Liver Metastasis: A Single-Arm, Exploratory Trial. Cancer research and treatment : official journal of Korean Cancer Association. 2016;48(4):1210-21.

11. Lastoria S, Piccirillo MC, Caracò C, Nasti G, Aloj L, Arrichiello C, et al. Early PET/CT scan is more effective than RECIST in predicting outcome of patients with liver metastases

from colorectal cancer treated with preoperative chemotherapy plus bevacizumab. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2013;54(12):2062-9.

12. Lau LF, Murone C, Williams DS, Standish R, Lee ST, Christophi C, et al. Metabolic response evaluation for colorectal liver metastases and correlation to pathologic response and tumour markers. ANZ journal of surgery. 2018;88(3):E108.

13. Lau LF, Williams DS, Lee ST, Scott AM, Christophi C, Muralidharan V. Metabolic response to preoperative chemotherapy predicts prognosis for patients undergoing surgical resection of colorectal cancer metastatic to the liver. Annals of surgical oncology. 2014;21(7):2420-8.

14. Lee HS, Kim HO, Hong YS, Kim TW, Kim JC, Yu CS, et al. Prognostic value of metabolic parameters in patients with synchronous colorectal cancer liver metastasis following curative-intent colorectal and hepatic surgery. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2014;55(4):582-9.

15. Lee SJ, Zea R, Kim DH, Lubner MG, Deming DA, Pickhardt PJ. CT texture features of liver parenchyma for predicting development of metastatic disease and overall survival in patients with colorectal cancer. European radiology. 2018;28(4):1520-8.

16. Lim E, Wiggans MG, Shahtahmassebi G, Aroori S, Bowles MJ, Briggs CD, et al. Rebound growth of hepatic colorectal metastases after neo-adjuvant chemotherapy: effect on survival after resection. HPB : the official journal of the International Hepato Pancreato Biliary Association. 2016;18(7):586-92.

17. Mazard T, Boonsirikamchai P, Overman MJ, Asran MA, Choi H, Herron D, et al. Comparison of early radiological predictors of outcome in patients with colorectal cancer with unresectable hepatic metastases treated with bevacizumab. Gut. 2018;67(6):1095-102.

18. Mertens J, De Bruyne S, Van Damme N, Smeets P, Ceelen W, Troisi R, et al. Standardized added metabolic activity (SAM) IN ^{18}F-FDG PET assessment of treatment response in colorectal liver metastases. European journal of nuclear medicine and molecular imaging. 2013;40(8):1214-22.

19. Nishioka Y, Shindoh J, Yoshioka R, Gonoi W, Abe H, Okura N, et al. Radiological Morphology of Colorectal Liver Metastases after Preoperative Chemotherapy Predicts Tumor Viability and Postoperative Outcomes. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 2015;19(9):1653-61.

20. Rosenthal MH, Kim KW, Fuchs CS, Meyerhardt JA, Ramaiya NH. CT predictors of overall survival at initial diagnosis in patients with stage IV colorectal cancer. Abdominal imaging. 2015;40(5):1170-6.

21. Sasaki Y, Osada S, Mori R, Imai H, Tanaka Y, Matsuhashi N, et al. Determining timing of hepatectomy for colorectal cancer with distant metastasis according to imaging-based tumor shrinkage ratio. International journal of medical sciences. 2013;10(9):1231-41.

22. Seo N, Park M-S, Han K, Lee KH, Park SH, Choi GH, et al. Magnetic Resonance Imaging for Colorectal Cancer Metastasis to the Liver: Comparative Effectiveness Research for the Choice of Contrast Agents. Cancer research and treatment : official journal of Korean Cancer Association. 2018;50(1):60-70.

23. Shim J-R, Lee SD, Han S-S, Lee SJ, Lee DE, Kim S-K, et al. Prognostic significance of 18F-FDG PET/CT in patients with colorectal cancer liver metastases after hepatectomy. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2018;44(5):670-6.

24. Simpson AL, Doussot A, Creasy JM, Adams LB, Allen PJ, DeMatteo RP, et al. Computed Tomography Image Texture: A Noninvasive Prognostic Marker of Hepatic

Recurrence After Hepatectomy for Metastatic Colorectal Cancer. Annals of surgical oncology. 2017;24(9):2482-90.

25. Suzuki K, Muto Y, Ichida K, Fukui T, Takayama Y, Kakizawa N, et al. Morphological response contributes to patient selection for rescue liver resection in chemotherapy patients with initially un-resectable colorectal liver metastasis. Oncology letters. 2017;14(2):1491-9.

26. Tam HH, Collins DJ, Brown G, Chau I, Cunningham D, Leach MO, et al. The role of pre-treatment diffusion-weighted MRI in predicting long-term outcome of colorectal liver metastasis. The British journal of radiology. 2013;86(1030):20130281.

27. Tam HH, Cook GJ, Chau I, Drake B, Zerizer I, Du Y, et al. The role of routine clinical pretreatment 18F-FDG PET/CT in predicting outcome of colorectal liver metastasis. Clinical nuclear medicine. 2015;40(5):e259.

28. Tampellini M, Gned D, Baratelli C, Brizzi MP, Ottone A, Alabiso I, et al. Changes in hepatic perfusion assessed by dynamic contrast enhanced MRI, associated with morphologic evaluation, in patients with liver metastases from colorectal cancer treated with first-line chemotherapy. La Radiologia medica. 2016;121(12):950-7.

29. van Kessel CS, Samim M, Koopman M, van den Bosch MAAJ, Borel Rinkes IHM, Punt CJA, et al. Radiological heterogeneity in response to chemotherapy is associated with poor survival in patients with colorectal liver metastases. European journal of cancer (Oxford, England : 1990). 2013;49(11):2486-93.

30. Yoshita H, Hosokawa A, Ueda A, Ando T, Kajiura S, Kato H, et al. Predictive value of optimal morphologic response to first-line chemotherapy in patients with colorectal liver metastases. Digestion. 2014;89(1):43-8.

Appendix 7: Completeness of reporting of the individual elements of the REMARK criteria.

REMARK item be assessed	Yes (No. of Publications)	% Yes (No. of Publications)
Introduction		
1 - Description of imaging: imaging modality	30	*100*
1 - Description of imaging: details of the imaging	28	*93*
1 - Description of imaging: when imaging was performed	27	*90*
1 - Study objective	29	*97*
1 - State pre-defined hypothesis (if applicable)	1	*3*
Methods		
2 - Source of patients	27	*90*
2 - Eligibility criteria	29	*97*
2 - Exclusion criteria	18	*60*
3 - Treatment received	27	*90*
4 - Imaging modality	30	*100*
4 - Protocol for imaging	24	*80*
5 - Quality control procedures	1	*3*
5 - Reporting criteria	30	*100*
5 - Reporters blinded to clinical outcome	8	*27*
6 - Case selection: method of case selection	28	*93*
6 - Case selection: prospective or retrospective with details	21	*70*
6 - Case selection: stratification or matched?	2	*7*
6 - Case selection: method of follow-up data collection	13	*43*
6 - Time period: when the study took place?	5	*17*
6 - Time period: when the participants were recruited?	28	*93*
6 - Time period: date the follow-up of all participants ended	5	*17*
6 - Time period: median duration of follow-up	21	*70*
6 - Time period: method of calculating median follow-up	0	*0*
6 - Time period: Number patients lost to follow-up	12	*40*
7 - Precisely defined endpoint	22	*73*
7 - Classification of cause of death (if applicable)	0	*0*
7 - Were the endpoint assessments blinded?	8	*27*
8 - Details of variables initially considered for inclusion in the model	25	*83*
8 - Define all variables and how they were measured	29	*97*
9 - Rationale for sample size	1	*3*
9 - Detectable effect given sample size (if applicable)	2	*7*
10 - All statistical methods listed?	30	*100*
10 - Pre-specified and post-hox analysis decisions?	3	*10*
10 - Preliminary data preparation	4	*13*

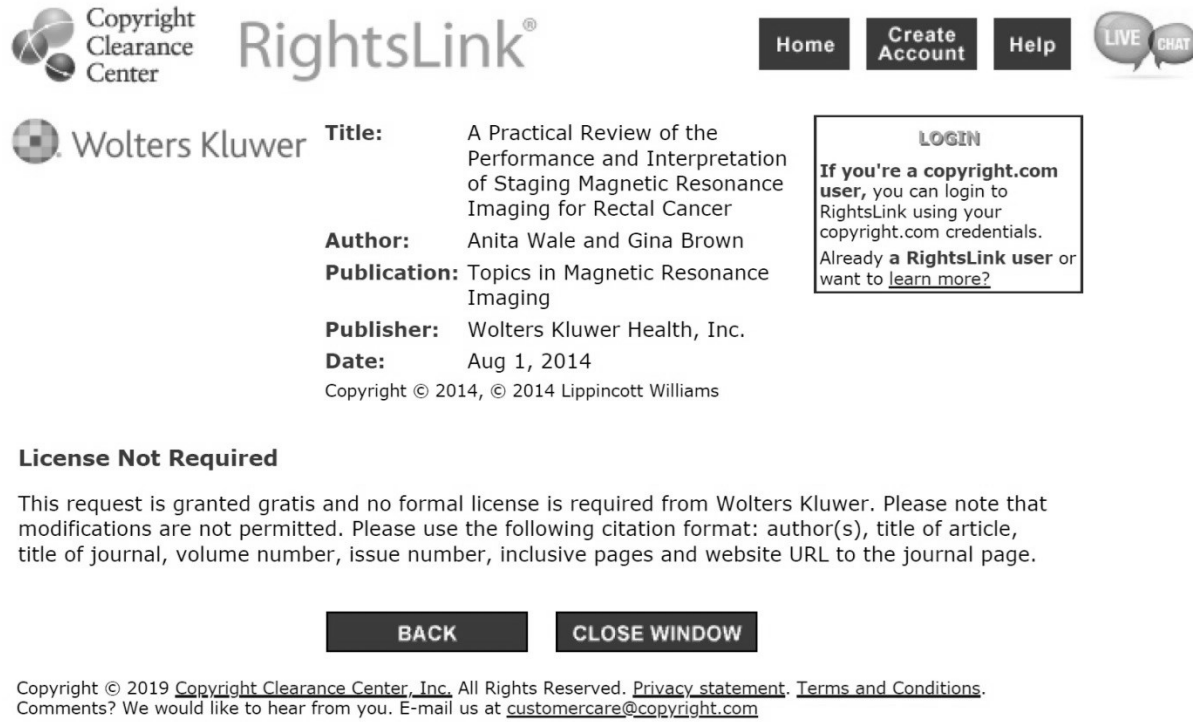
10 - Possible association with other variables tested	19	*63*
10 - Univariate methods assessed against clinical outcome	17	*57*
10 - Multivariate analysis with details of analysis and variable selection	13	*43*
10 - Handling of missing data	5	*17*
10 - Checking model assumptions if applicable	2	*7*
11 - How were continuous variable analysed?	15	*50*
Results		
12 - Flow chart of patients	5	*17*
13 - Patient demographics	30	*100*
13 - Number of missing patients for each variable	22	*73*
13 - Characteristics of patients if a subset from a smaller cohort?	3	*10*
13 - Distribution of the marker	23	*77*
14 - Marker association with standard prognostic variables	19	*63*
15 - Univariate analysis between marker and outcome	20	*67*
15 - Kaplan-Meier plot with the number of patients at selected time points	8	*27*
16 - How model was built?	15	*50*
16 - Multivariate analysis (with CI and P values)	16	*53*
16 - If subgroup multivariate analysis	0	*0*
17 - Association between biomarker, outcome & gold standard	7	*23*
18 - Further Investigations to check	0	*0*
Discussion		
19 - Purpose of study & pre-specified hypotheses	9	*30*
19 - Summary of main findings	28	*93*
19 - Distinguish between pre-specified hypothesis and post-hoc conclusions	1	*3*
19 - Critical evaluation	26	*87*
19 - How results relate to body of evidence?	29	*97*
20 - Implications for future research	20	*67*

Appendix 8: A comparison of the site and timing of metastatic and/or recurrent disease between mrTRG and pTRG

Table 15-1: Site and timing of metastatic and/or recurrent disease according to good and poor mrTRG and pTRG response to preoperative therapy.

	mrTRG (first post-CRT MRI)				**pTRG (post-operative specimen)**			
	Good (n = 142)		**Poor** (n = 196)		**Good** (n = 85)		**Poor** (n = 166)	
First site of metastatic or recurrent disease	**n**	**Median time**	**n**	**Median time**	**n**	**Median time**	**n**	**Median time**
Liver (+/- extrahepatic disease +/- pelvic recurrence)	6	6.5 mo	21	9 mo	2	11mo	13	9 mo
Lung (+/- other extrahepatic disease +/- pelvic recurrence)	10	23 mo	21	18 mo	7	18 mo	10	17 mo
Other extrahepatic metastasis and pelvic recurrence	0	N/A	8	18 mo	2	33.5 mo	5	16 mo
Pelvic recurrence	1	18 mo	14	13.5 mo	4	17 mo	7	13 mo
TOTAL	17	18 mo	64	13.5 mo	15	18 mo	35	13 mo

Appendix 9: Permission Documents



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Wolters Kluwer

Title: A Practical Review of the Performance and Interpretation of Staging Magnetic Resonance Imaging for Rectal Cancer

Author: Anita Wale and Gina Brown

Publication: Topics in Magnetic Resonance Imaging

Publisher: Wolters Kluwer Health, Inc.

Date: Aug 1, 2014

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