The Prognostic Role of Extramural Venous Invasion in Post-Chemoradiotherapy Rectal Cancer

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

“Learning never exhausts the mind” – Leonardo Da Vinci

Dedicated to my wife, Natalie, and our daughter Yasmine who have provided the support and love that has helped me complete this thesis.

Declaration

I declare that the research and preparation of this thesis is the result of my independent work with the exception of the technical assistance acknowledged elsewhere.

This work has not already been accepted in substance for any other degree and is not currently being submitted in candidature of any other degree.

Signed……  ……..(Candidate)

Date 26th February 2015

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Abstract

Introduction

Extramural venous invasion (EMVI) is a poor prognostic factor in rectal cancer. It is detected by magnetic resonance imaging (MRI) and histopathological analysis. Neoadjuvant chemoradiotherapy (CRT) is often given to patients with locally advanced disease however the clinical outcomes of EMVI-positive tumours following such treatment remains unknown. This thesis aimed to investigate the radiological, pathological and molecular changes which occur in EMVI-positive tumours following CRT to determine whether these changes can predict prognosis.

Methods

Following a systematic review (SR) of EMVI in rectal cancer, a series of studies were conducted. Patients were identified from a prospectively maintained database of primary rectal cancers who were scheduled for CRT followed by curative surgery between 2006 and 2012. Imaging and pathology samples were reviewed and tissue samples were further processed for molecular profiling using micro-RNA analytical techniques. Data were correlated with disease-free survival (DFS), recurrence rate and patterns of relapse.

Results

SR demonstrated that EMVI is associated with locally advanced tumours, distant disease recurrence and worse overall survival. However there is a variation in technique and definitions which makes interpretation of historical studies problematic. EMVI is an important consideration in the multidisciplinary management of rectal cancer as most clinicians use it to influence treatment decisions. MRI may allow for improved detection rates of EMVI following CRT compared with routine

histopathology techniques and it is an independent prognostic factor for recurrence at 3 years. Patients with persistent EMVI following CRT have improved DFS if given adjuvant chemotherapy. Finally, EMVI-positive tumours express specific patterns of microRNA sequences.

Conclusion

EMVI is a poor prognostic factor following CRT. Patients with evidence of EMVI may be considered for intensive adjuvant chemotherapy and more frequent surveillance for distant disease. Unique molecular signatures may hold the key for future management strategies. These results have led to the development of the MARVEL Study.

Publications

Sections of this thesis have been previously presented or published as detailed below.

Peer-reviewed Papers

1. *Systematic review of prognostic importance of extramural venous invasion in rectal cancer.* M Chand, MRS Siddiqui, RI Swift, Brown G. World Journal of Gastroenterology; 22: 1721-1726.
2. *Evaluation of mesorectal lymph nodes with high-resolution MRI.* M Chand, A Wale, G Brown. Journal of Cancer and Clinical Research. 2015; 1:1021-25.
3. *Evidence for radiological and histopathological prognostic importance of detecting EMVI: recommendations for radiology and histopathopathology reporting.* M Chand, T Palmer, L Blomqvist, I Nagtegaal, NP West, G Brown. Colorectal Disease 2015; 17:468-73
4. *Total mesorectal excision optimised by pelvic MRI.* M Chand, J Nicholls, P Tekkis, G Brown, RJ Heald. Colorectal Disease. 2014; 16: 847-53.
5. *The Evolution in the Detection of Extramural Venous Invasion in Rectal Cancer - Implications for modern-day practice.* M Chand, I Swift, P Tekkis, NP West, RJ Heald, G Brown. Colorectal Cancer. 2015; 3: 481-90.
6. *EMVI-positive stage II rectal cancer has similar clinical outcomes as stage III disease following pre-operative chemoradiotherapy.* M Chand, A Bhangu, A Wotherspoon, GWH Stamp, RI Swift, P Tekkis, G Brown. Annals of Oncology. 2014; 25: 858-63.

7. *The prognostic significance of post-chemoradiotherapy high-resolution MRI and histopathology detected extramural venous invasion in rectal cancer.* M Chand, J Evans, RI Swift, P Tekkis, NP West, GWH Stamp, RJ Heald, G Brown. Annals of Surgery. 2015; 261: 473-9.

8. *Extramural venous invasion (EMVI) is a potential imaging predictive biomarker of neoadjuvant treatment in rectal cancer.* M Chand, RI Swift, P Tekkis, G Brown. Br J Cancer. 2014; 110: 19-25.

9. *Important imaging considerations in the pre-operative assessment of rectal cancer.* M Chand, G Brown. Seminars in Colon and Rectal Surgery. 2014; 25: 6-12.

10. *Adjuvant therapy decisions based on MRI assessment of extramural venous invasion and other prognostic factors: a survey of oncology and surgical practice.* M Chand, RI Swift, I Chau, P Tekkis, G Brown. Annals of RCS. 2014; 96: 543-6.

11. *Lymph status does not predict local recurrence in the TME-era.* M Chand, B Moran, R Glynne-Jones, RJ Heald, G Brown. Diseases Colon and Rectum. 2014; 57: 127-9.

12. *The importance of not overstaging mesorectal lymph nodes seen on MRI.* M Chand, RJ Heald, G Brown. Colorectal Disease. 2013; 15: 1201-1204.

13. *The role of MRI in Treatment Planning for Rectal Cancer - A Review*. RK Bott, M Chand, RI Swift, GF Nash, G Brown. Curr Cancer Ther Rev. 2013; 9:111-116.

14. *Staging and evaluation of rectal cancer and pelvic malignancy***.** M Chand, G Brown. Pelvic cancer surgery – Modern breakthroughs and future advances *2013. Eds Patel HRH, Mould T, Joseph JV, Delaney CP. Springer London*

15. *Pre-operative staging of rectal cancer.* M Chand**,** G Brown. Seminars of Colon and Rectal Surgery. 2013; 24: 114-118.

16. *How to identify tumour penetration in rectal cancer***.** Modern management of rectal cancer 2013. M Chand, G Brown. *Multidisciplinary Management of Rectal Cancer. Ed Valentini V.Springer London 2012*

17. *What are the relevant imaging factors to optimize treatment decisions in rectal cancer?* M Chand, G Brown. *Multidisciplinary Management of Rectal Cancer. Ed Valentini V. Springer 2012.*

Abstracts

ESCP Annual Conference 2014

1. Preliminary results of microRNA analysis in EMVI-positive and EMVI-negative rectal cancer

2. MRI characteristics of EMVI response to neoadjuvant chemoradiotherapy in rectal cancer

3. The effect of adjuvant chemotherapy following neoadjuvant chemoradiation on persistently positive EMVI stage II and III rectal cancer

4. The MARVEL Study Protocol

Tripartite Conference

5. Defining poor response after neoadjuvant therapy in patients with rectal cancer

6. Prognostic importance of a change in EMVI status following neoadjuvant chemoradiotherapy in rectal cancer

7. Adjuvant chemotherapy improves disease-free survival for tumours that demonstrate persistent mrEMVI following neoadjuvant chemoradiotherapy for rectal cancer

8. EMVI is associated with histopathological markers of poor prognosis locally advanced rectal cancer

BASO Annual Conference

9. mrEMVI status should be used in addition to pEMVI for treatment decision making in rectal cancer to prevent under-reporting of extramural venous invasion

10. Extramural venous invasion confers an increased risk of disease recurrence in stage II rectal cancer

11. Extramural venous invasion (EMVI) is a potential imaging predictive biomarker in rectal cancer

ESCP Annual Conference 2013

12. Regression of extramural venous invasion after neoadjuvant chemoradiotherapy improves disease-free survival in rectal cancer

13. The clinical importance of large and small-vessel EMVI in rectal cancer

14. Adjuvant chemotherapy improves survival in mrEMVI positive rectal cancer following neoadjuvant chemoradiotherapy

15. The clinical importance of a change in mrEMVI status following neoadjuvant chemoradiation

16. The clinical and prognostic importance of extramural venous invasion - a systematic review

17. Can CT be used as an alternative to MRI to make treatment decisions regarding neoadjuvant therapy?

18. Should EMVI be a consideration for adjuvant chemotherapy? A survey of surgeons and oncologists.

ACPGBI Annual Conference

19. Does the pattern of extramural venous invasion (EMVI) identified on MRI influence recurrent disease in rectal cancer?

20. Improvement of mrEMVI following pre-operative chemoradiotherapy influences disease-free survival

21. MRI-detected EMVI (mrEMVI) should be considered in addition to histopathological EMVI status with regards to treatment decisions in rectal cancer

ASCRS Annual Conference 2013

22. The effect of chemoradiotherapy on T-stage, nodal disease and CRM involvement in EMVI-positive rectal cancer

23. Comparison of sites and associated rates of recurrence in EMVI-positive rectal cancer

Acknowledgements

This work was carried out during my appointment as Honorary Clinical Research Fellow at the Royal Marsden Hospital NHS Foundation Trust and Registrar at Croydon University Hospital NHS Trust, under the supervision of Professor Gina Brown, Mr Ian Swift and Professor Paris Tekkis. I am truly grateful for the guidance and support given by all three supervisors who have inspired me to pursue a career in academic surgery.

I was responsible for gathering all data, creating and maintaining a specific database of patients with rectal cancer. All ethical approval for the experiments in this thesis was obtained myself under guidance of the above supervisors. This included recruitment of patients where necessary. I contributed and was directly involved in the reviewing of all imaging data and the development of the novel grading systems described in this thesis. Further, I was involved in the reassessment of the pathology specimens as well as all associated data collection and follow up. All data interpretation was conducted under the direct supervision of Professor Brown, Mr Swift and Professor Tekkis.

Specifically, all the MRI scans were reviewed between Professor Brown and myself individually. Where there was disagreement between the two of us, I would defer to Professor Brown's seniority and experience. This one on one tuition has also provided me with a degree of experience in interpretation of MRI rectum.

I am very grateful to Lisa Scerri (Senior Trials Coordinator in the Department of Radiology, The Royal Marsden Hospital NHS Trust) who helped me set up and maintain the patient database as well as the initial set-up and site initiations for the MARVEL Trial.

Dr Svetlana Balyasnikova and Dr Anita Wale kindly supported me in MRI interpretation and ensured that the MRI sections where accurately annotated and described where

appropriate. This is separate from the review of imaging I undertook myself with Professor Brown.

Histopathology sections were studied and reassessed by Professor Gordon Stamp and Dr Andrew Wotherspoon at The Royal Marsden Hospital NHS Trust. Specific histopathological sections were examined and scanned by Dr Nick West, Department of Pathology, Leeds University Hospital. I was involved in retrieval and transfer of all pathology materials and assisted in preparation. The staining of specific slides and laser microdissection for microRNA analysis was conducted with the help of Miss Natasha Hoare (Biomedical Sciences undergraduate student) and Mr Alex Mirnezami (Professor of Surgical Oncology), University of Southampton. I was involved in retrieval and transfer of all materials and assisted in the preparation and “cut-up” of the specimens. This was overseen by Mr Alex Mirnezami and Miss Natash Hoare.

I am truly grateful for the statistical support provided by Kjell Pennert and Karen Thomas, Consultant Statisticians, Royal Marsden Hospital NHS Trust.

I would also like to thank my co-authors in helping write the articles which have been published as a result of this work.

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Chapter 1

Overview of rectal cancer

1.1. Anatomical considerations of the rectum

The embryology and anatomy of the rectum are central to the understanding of the staging, surgery and pathology of rectal cancer. They explain the possible routes for tumour spread, both for local and metastatic disease, in addition to how surgical treatment can be applied most effectively in order to optimise patient outcomes. Furthermore, direct tumour penetration through the anatomical layers of the bowel wall into the surrounding tissue and vasculature influences prognosis and survival. Therefore, knowledge of the pelvic anatomy is essential to plan curative treatment.

1.1.1. Embryology of the hindgut and rectum

The rectum develops from the embryological hindgut as a blind-ending tube in the caudal part of the embryo. The hindgut is the distal part of the alimentary canal and includes the distal third of the transverse colon, the sigmoid colon, the rectum and the upper portion of the anal canal.

As the embryo develops the primitive alimentary canal, which is initially in broad contact with the posterior abdominal wall becomes suspended by a thin dorsal mesentery. This is the main blood supply to the primitive gut by the end of the fourth week of gestation. Gradually the gut rotates and positions itself such that it remains suspended by a central fan-shaped mesentery with three main arterial trunks supplying large sections of the bowel. These main arteries correspond to the foregut, midgut and hindgut – the inferior mesenteric artery becoming the principle vessel that supplies blood to the hindgut.

1.1.2. Anatomy of the rectum and structure of the bowel wall

The rectum forms the distal part of the lower gastrointestinal tract. It lies in the pelvis and is intimately surrounded by important neurovascular structures that control sexual function. Proximally, it is a continuation of the sigmoid colon and terminates at the anal canal. The exact beginning of the rectum remains a point of contention between anatomists and surgeons. This possibly explains the different values given for the length of the rectum that is commonly stated to be 15cm but has been quoted in the range of 13-18cm (Salerno et al., 2006).

Anatomically, the point at which the rectum begins can be delineated by the structure of the bowel wall. The colon is distinguished from the small intestine by the thickened bands of muscle which surround the bowel – the taeniae coli; the sacculations of the bowel wall between these bands – the haustra; and the fatty projections of the omentum – the omental appendices. These features are all absent in the rectum. The taeniae of the colon can be followed to the point where they spread out to form a continuous layer of longitudinal muscle forming the outer coat of the rectum. This point is sometimes called the rectosigmoid junction and lies anterior to the S3 vertebral body of the spinal column. It continues distally until it perforates a sheet of muscle comprising the floor of the pelvis – the levator ani. As the bowel exits the pelvic floor it becomes the anal canal. The condensation of muscle fibres forms a sling around the rectum creating a sharp bend of approximately 80 degrees known as the anorectal flexure. This sling of muscle provides an important mechanism for faecal continence. The portion of the rectum which lies immediately above the levator ani is known as the ampulla, and is supported by the anococcygeal ligament. This produces a dilatation of the rectum acting as a reservoir which further helps in faecal continence. Therefore,

disruption of the anatomy from surgical treatment can lead to significant functional consequences.

Immediately below the outer longitudinal coat of muscle lies a layer of circular musculature. This layer helps give the rectum its characteristic appearance when visualised anteriorly. Three internal folds of the mucosal and sub-mucosal layers which overlie the circular muscle of the rectum produce lateral flexures, transverse folds or valves of Houston. These may be referred to as the ‘rectal shelves’ and the middle shelf in the male can provide an important landmark for the pelvic surgeon where the peritoneum reflects on to the dome of the bladder.

As with most viscera contained within the abdominal and pelvic cavities, the rectum is partly covered with a thin layer of tissue called the peritoneum. This layer is in continuation with the lining of the abdomino-pelvic compartment. The peritoneal covering varies along the length of the rectum. In the upper third, it covers the anterior and lateral parts of the rectum; the middle third is only covered anteriorly and the distal third has no covering at all. The peritoneal lining reflects back on itself to become continuous with the pelvic wall. This reflection differs slightly between males and females; in males the peritoneum continues over the posterior wall of the bladder forming the floor of the recto-vesical pouch. However, in females the peritoneum passes along the posterior fornix of the vagina forming the recto-uterine pouch.

1.1.3. Vasculature and lymphatic drainage

The rectum is supplied by three arterial trunks - figure 1. The superior rectal artery is a continuation of the inferior mesenteric artery and is considered the major blood supply to the rectum. It predominantly supplies the proximal part of the rectum although there are several anastomoses which occur along its length. The two middle

rectal arteries are branches which arise from the internal iliac artery and supply the middle and distal thirds of the rectum. The inferior rectal artery comes from the internal pudendal artery and supplies the anorectal junction at its most distal part. These vessels and their tributaries produce numerous anastomoses in the submucosal layer of the rectum.

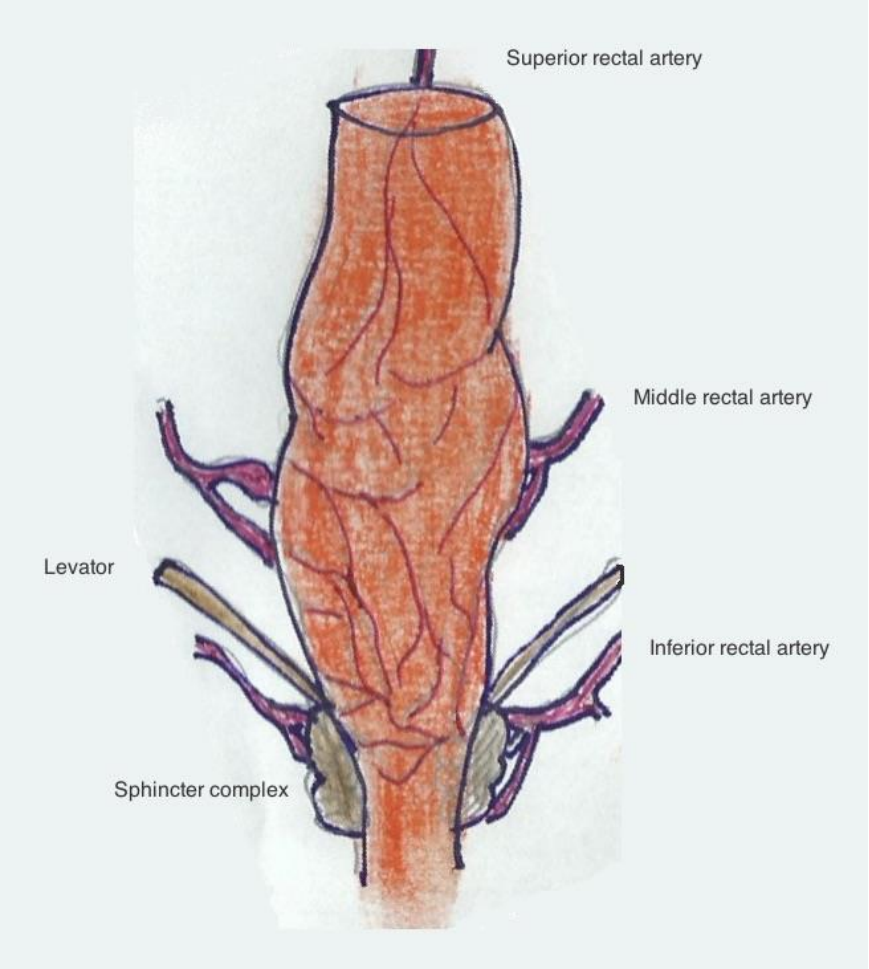


Figure 1 – Arterial anatomy of the rectum

The venous drainage of the rectum flows into the superior, middle and inferior rectal veins. These vessels drain into the inferior mesenteric, internal iliac, and internal pudendal veins, respectively. There are further anastomoses between the tributaries of these veins which flow into both the systemic and portal venous systems. The superior rectal vein drains into the portal system whilst the middle and inferior veins

drain into the systemic circulation. The importance of the venous drainage is discussed in relation to tumour spread in later sections. This is shown in figure 2.

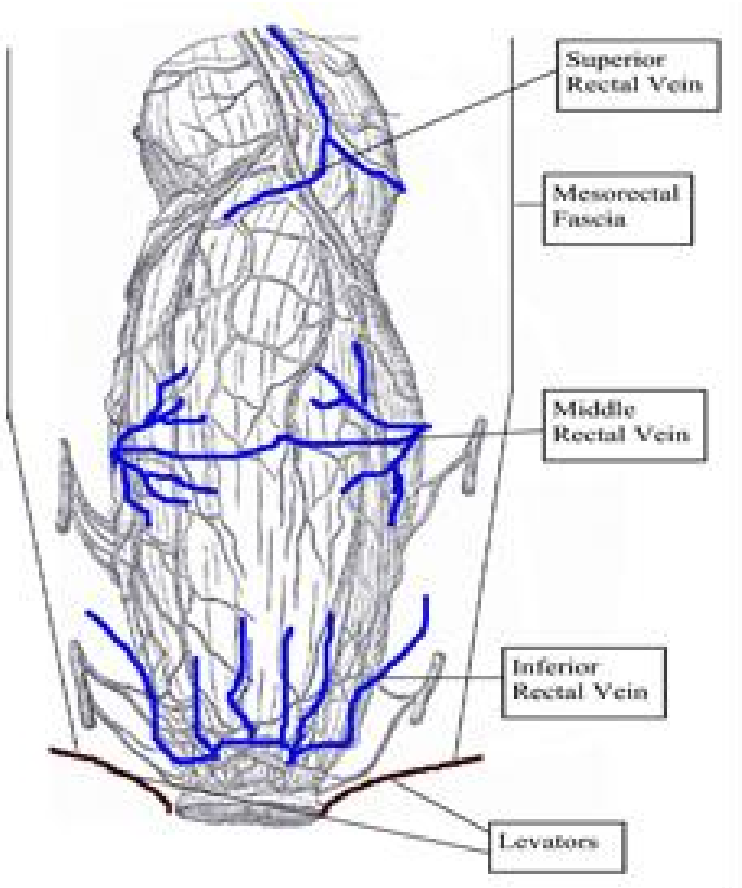


Figure 2 – Venous drainage of the rectum

The lymphatic drainage of the rectum follows the blood vessels. Therefore, in the upper third, drainage is along the superior rectal vessels which drain into the inferior mesenteric nodes, then on to the lumbar para-aortic nodes and into the thoracic duct. The middle third of the rectum drain into the middle rectal vessels then on to the internal iliac nodes before also flowing into the lumbar para-aortic nodes and thoracic

duct. The lower portion of the rectum drain along the inferior rectal and pudendal vessels before reaching the deep pelvic nodes and the para-aortic nodes. There is some drainage into the inguinal nodes.

1.1.4. The mesorectum

The rectum displays a feature not seen in the upper part of the alimentary tract - a circumferential lymphovascular envelope called the mesorectum which surrounds it along the majority of its length – figure 3. It is derived from the dorsal root mesentery and includes vascular contributions from the hindgut vessels that drain into the portal system and the iliac vessels of the systemic circulation. The outermost boundary of this fatty layer is defined by the mesorectal fascia (MRF), which seems to act as an initial oncological barrier to the spread of tumour cells.

Although the entire alimentary tract may be thought of as a muscular tube surrounded by lymphovascular mesentery and fascia, it is most pronounced and a distinct unit in the rectum. An avascular anatomical plane can be developed by careful and precise dissection in surgery of the rectum (Heald, 1988). This type of fascia is not present throughout the length of the gut and has been difficult to demonstrate in cadaveric models. This may explain the conspicuous absence of the mesorectum from the majority of anatomy textbooks. Further, the lack of such a distinct fascial plane containing the local lymphovascular vessels may be the reason why tumours of other parts of the gastro-intestinal system (oesophagus and stomach) have less favourable prognosis.

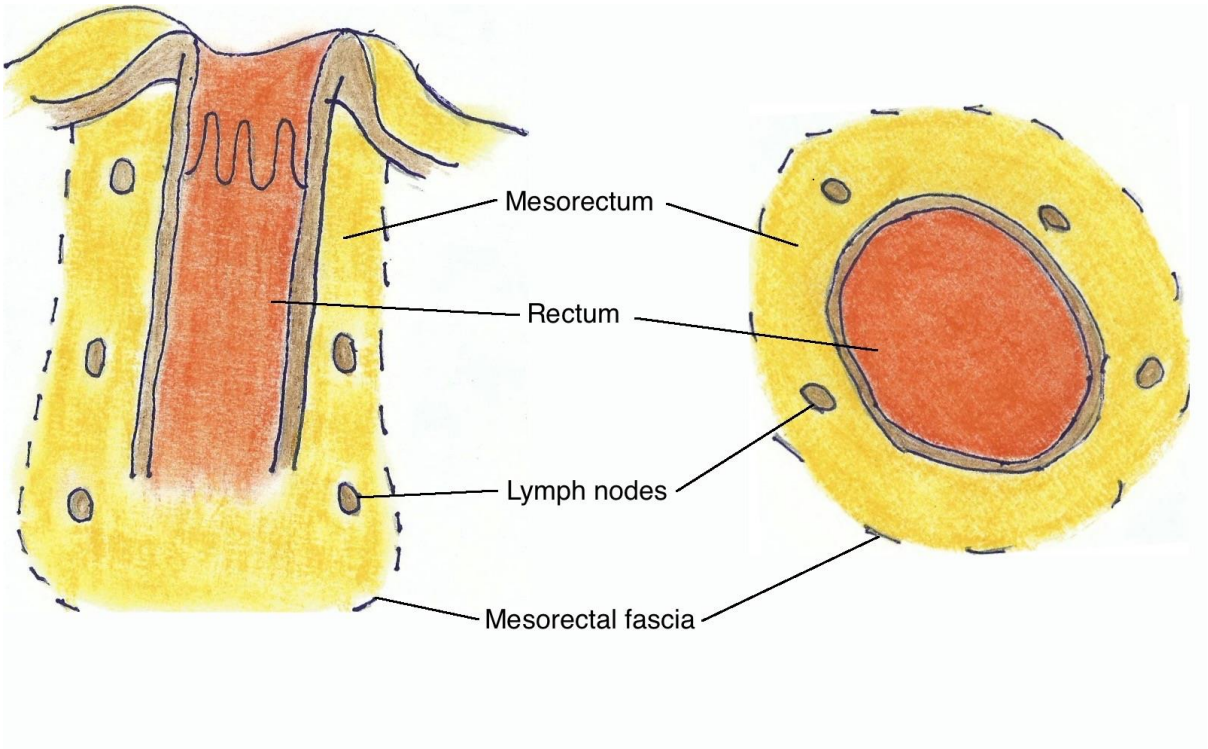


Figure 3 – Schematic diagram of the mesorectum in two planes

1.2. Surgery for rectal cancer

1.2.1. Principles of rectal cancer surgery

Miles described the first 'oncological' operation for rectal cancer in 1908 (Miles, 1908). Whilst this improved the surgical treatment of rectal cancer from an almost one hundred percent mortality, it was still associated with a huge morbidity and mortality rate compared to modern treatment. The current-day "total mesorectal excision" (TME) operation proposed by Heald, is based on the embryological origin of the rectum and pathological spread of cancer (Heald et al., 1982, Heald and Ryall, 1982). Despite oncological therapies having done much to improve survival in recent years, it is good quality oncological surgery that remains the most important determinant of prognosis in rectal cancer. The acceptance and popularisation of TME has led to much improved survival demonstrated by the low local recurrence rates of less than 5%. Conversely, poor surgery yielding a sub-standard resection specimen invariably leads to local recurrence (Quirke et al., 1986). More recent evidence from the randomised Medical Research Council of United Kingdom CR07 and National Cancer Institute of Canada – CTG CO16 (CR07) trial (Sebag-Montefiore et al., 2009) has shown the primary importance of good quality surgery and how inadequate surgery can be only *minimally* compensated for by chemoradiotherapy (Quirke et al., 2009).

As mentioned above, the fascia surrounding the mesorectum seems to act as an oncological barrier to the spread of tumour. This avascular plane can be developed by precise dissection to allow excision of the tumour with its fascial neurovascular envelope in one "monobloc" specimen. Gaining tumour clearance at the MRF that bounds the mesorectum is therefore hugely important - this boundary is referred to as the circumferential resection margin (CRM) by surgeons. The lines of excision are defined by the embryological legacy of the primitive gut.

1.3. Staging of rectal cancer

In rectal cancer, a patient's prognosis is determined by specific anatomical and tumour-related features. This information forms the basis of the various staging systems that have been developed and evolved over the years. Further, it influences treatment decisions and provides an estimate for the potential of local or distant recurrence, and ultimately long-term survival through risk-stratification models. These common prognostic factors include depth of tumour spread, the relationship of the tumour to the mesorectal fascia, the presence/absence of nodal disease, and presence/absence of tumour in the local veins – extramural venous invasion (EMVI).

1.3.2. The history of rectal cancer staging

1.3.2.1 Lockhart-Mummery staging

Lockhart-Mummery proposed the first staging system for rectal cancer in the early 1920s (Lockhart-Mummery, 1926). This classification was based on the identification of lymph nodes containing tumour and the depth of invasion. The study contained 200 patients who had undergone perineal excision. Patients were grouped in to three categories (A-C) shown in table 1. The 'five-year cure' was significantly improved in patients in stage A compared to B and C.

1.3.2.2. Dukes staging

Dukes evolved the Lockhart-Mummery system and made it more specific with regards to the spread of tumour into the bowel wall (Gordon-Watson, 1930). A study in 1932 of 215 patients classified patients into Stage A-C (Dukes, 1932). Stage A tumours were confined to the wall of the rectum compared with Stage B where tumour had

spread beyond the bowel wall into the surrounding tissues. Stage C consisted of any tumour in which there was evidence of tumour in regional lymph nodes. The more advanced the stage, the worse the prognosis. The Dukes system is still used today although modifications over the years have attempted to address the heterogeneity of tumours particularly in Stage B and C. This is shown in table 2.

1.3.2.3. Kirklin and Astler Coller Staging

Modifications of the Dukes system first gained popularity when first Kirklin (Kirklin et al., 1949), then Astler and Coller sought to address the heterogeneity within the original system.

A further modification by Astler and Coller in 1954 took into account Kirklin's modification but also included sub-staging of Dukes C tumours (Astler and Coller, 1954). The five-year survival was adversely related to increasing depth of invasion and the presence of lymph nodes. Table 3 shows the staging system and associated survival rates.

Table 1 – Lockhart-Mummery Classification

Stage	*Description*	*5-year cure rate*
A	Favourable cases where tumour had not invaded the muscle layer; no lymph node involvement	73.7%
B	Tumour spread to the muscle layer of the bowel but no appreciable lymph node involvement	44.1%
C	Large and fixed tumours with lymph node involvement	44.4%

Table 2 – Dukes original classification of rectal cancer and prognosis

Dukes Stage	*Gross description of tumour spread*	*Alive at 3 years (%)*
A	Growth limited to rectal wall	80
B	Extension of tumour to extrarectal tissues	73
C	Metastases in regional lymph nodes	7

Table 3 – Astler-Coller Staging system

Stage	*Description*	*5-year survival (%)*
A	Tumour invades submucosa	100
B1	Tumour invades muscularis Propria	66.6
B2	Tumour invades subserosa	53.9
C1	Regional lymph nodes; confined to muscularis	42.8
C2	Regional lymph nodes; beyond muscularis	22.4

1.3.2.4. TNM Staging

The original 'TNM classification' was developed by French surgeon Pierre Denoix (Denoix, 1946) and subsequently published by the Union International Contre le Cancer (UICC) ((UICC), 2010). There have been several revisions since its first proposal in the 1940s which relate to improved prognostic knowledge, resulting in further sub-divisions of the original three categories. Since 1959 these modifications have been guided by the American Joint Committee for Cancer (AJCC) where each category has been given an associated 'stage'. Each revision has been published as an edition of the AJCC Cancer Staging Manual. The current 7th edition is shown in table 4.

This system is based on three important staging sub-categories – tumour depth or penetration through the bowel wall (T); the presence and extent of nodal disease (N); and the presence of distant metastases (M). The increasing numerical value of the sub-stage indicates a worse prognosis, eg T4 is worse than T1. One or more of these combinations of TNM are associated with a stage or sub-stage.

Category	*Description*
TX	The primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ (intraepithelial or intramucosal carcinoma)
T1	Tumour invades into the submucosa
T2	Tumour invades into the muscularis propria
T3	Tumour invades through the muscularis propria into the subserosa, or into non-peritonealised pericolic or perirectal tissues
T4a	Tumour perforates visceral peritoneum
T4b	Tumour directly invades other organ or Structures
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1a	1 node
N1b	2-3 nodes
N1c	Satellites in subserosa without regional nodes
N2a	4 – 6 nodes
N2b	7 or more nodes
MX	The presence of distant metastasis cannot be assessed
M0	No distant metastasis
M1a	One organ
M1b	> one organ or peritoneum

Table 4 – 7th Edition of TNM classification of (colo)rectal cancer

The TNM system developed a way of categorising patients into a prognostic group which could standardise treatment and offer a survival estimate. However, the basis of the model is that patients can only be placed in one group – this is referred to as a 'bin model'. The first five editions of the AJCC Staging Manual changed very little, for example, the only major revision in the fifth edition compared to the first was that T2 tumours were 'downgraded' from stage II to stage I. The sixth edition highlighted some of the difficulties with this system. For example, the basis of the TNM classification is that as one progresses through the stages the prognosis, and thus survival, become worse. However current evidence would suggest that patients staged as IIb according to the sixth edition have a worse survival than patients staged IIIa [O'Connell 2004]. This led to further modification and the publishing of the current 7th edition (UICC, 2009).

In some cases, tumour may be seen as isolated deposits – tumour deposits; which are not in continuity with the primary cancer. By definition these are classified as metastases although they are confined to the mesorectal fat and thus within the local field. In 5th edition, these deposits were classified on the basis of size. Those deposits which were less than 3mm were defined as tumour deposits and those greater than 3mm as lymph nodes regardless of histological features (Sobin and Fleming, 1997). The 6th edition defined these deposits based on contour and categorised them as venous deposits if irregular in outline, and lymph nodes if smooth (Sobin, 2002). There remains confusion with regards to venous deposits with regards to their definition and implication. Pathologists have previously classified venous invasion based on the integrity of the vessel wall. Those which have an intact wall have been described as 'filling' or 'floating' type and where there is disruption of the wall it is called 'occlusive'

or ‘infiltrating’ (Sternberg et al., 2006, Ouchi et al., 1996, Talbot et al., 1981, Jass et al., 1986).

Whilst the TNM provides a universal language for the staging of tumours, there are difficulties associated with maintaining its accuracy with regards to prognosis, particularly as newer prognostic factors are identified. This also means that any evolution in the histopathological definitions must also be reflected in the staging system.

An important distinction which must be made is that of differentiation within staging, i.e. clinical, radiological or pathological. Further, the original TNM stage does not account for the use of pre-operative or neoadjuvant treatment and has thus led to the use of prefixes so to alert clinicians as to which type of stage is being offered. For example, the prefix ‘p’ denotes the pathological stage whereas ‘y’ denotes the use of neo-adjuvant treatment. More recent modifications have included prefixes such as ‘mr’ to include magnetic resonance imaging (MRI) based staging. Prefixes may be combined, for example a patient who has had neo-adjuvant treatment that is being subsequently staged by MRI would have the prefix ‘ymr’. Equally, the same patient’s pathological staging would be ‘yp’. However, it is important to appreciate that these newer modifications and their use remain personal to individual units and have not been formally introduced into the nomenclature.

1.4. Assessment of rectal cancer

1.4.1. Imaging assessment

1.4.1.1. Overview of imaging in staging of rectal cancer

In recent years, imaging has become a central part of the staging of rectal cancer. This involves assessing the local and distant spread of tumour. Choosing which imaging modality can optimally stage the extent of disease depends on the ability of the technique to identify the key prognostic features. These factors include a combination of traditional staging information such as tumour depth, nodal disease and metastatic spread – TNM classification; as well as more novel morphological characteristics such as extramural venous invasion (EMVI) and proximity of the tumour to the CRM.

The two common modalities used for the local staging of disease (spread of tumour within the pelvis) are MRI and endo-anal ultrasound (EAUS). Computed tomography (CT) is the 'gold standard' investigation for staging distant disease. This is metastatic disease outside the pelvis such as liver or lung deposits. In some circumstances where there is uncertainty regarding the presence of metastatic disease, additional complex imaging in the form of positron emission tomography (PET) may be used to delineate lesions in more detail or identify minute areas of disease. Nevertheless, whichever imaging modalities are decided on, it is important to obtain as much information on tumour behaviour, spread and morphology in order to offer patients the most beneficial treatment and the best clinical outcomes.

1.4.1.2. MRI

MRI has revolutionised the pre-operative management of patients. The image resolution has improved in recent years due to advances in sophisticated multiple element coil arrays. Modern MRI machines use multiple surface coils which are

combined in an array and connected to amplifiers with multiple receiver channels – the result of this is a single image produced by information from each coil. This configuration has superseded endorectal coils as they produce high-resolution images with much wider coverage of the local anatomy.

Its accuracy depends, however, on user expertise and correct technique during the procedure. For example, correct field alignment through the long axis of the rectum is an important determinant of accuracy. This can be seen in figure 4. Incorrect positioning and technique will lead to over- or under-staging of tumour depth and consequently influence treatment decisions – figure 5. Ideally, one should use T2-weighted images for tumour detail. It may be necessary to use thin coronal sections for low tumours in addition to the sagittal images for planning and axial images through the field. MRI is able to provide detail on the structure of the rectal wall as well as regional information about the remainder of the pelvis and perineum. Other technical information can be seen on MRI such as the presacral fascia, sphincter complex and peritoneal reflection – figure 6.

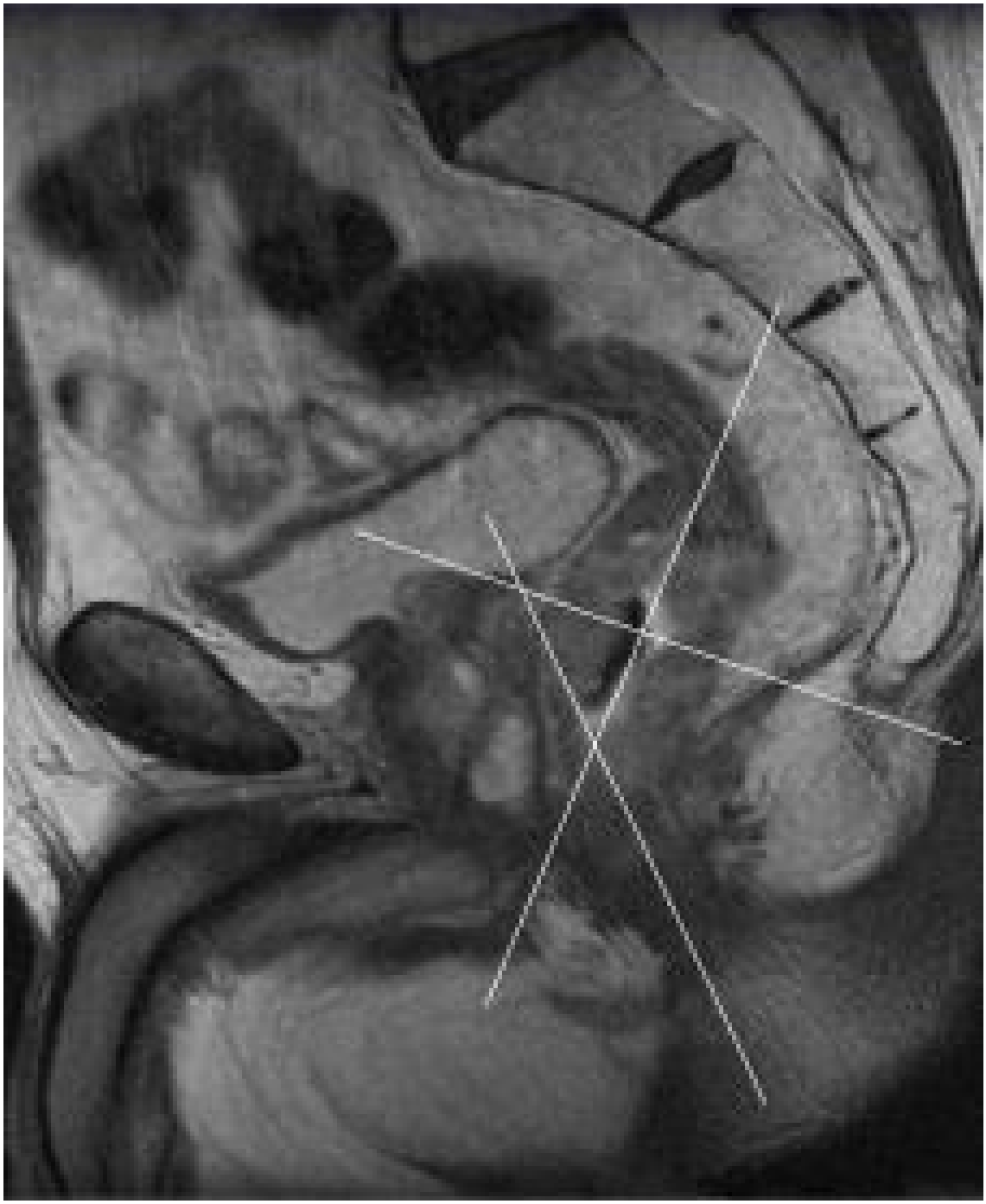


Figure 4 – Imaging planes to stage tumours of the rectum accurately. The white lines represent the axis through which the images are taken. This is of key importance to stage tumours accurately.

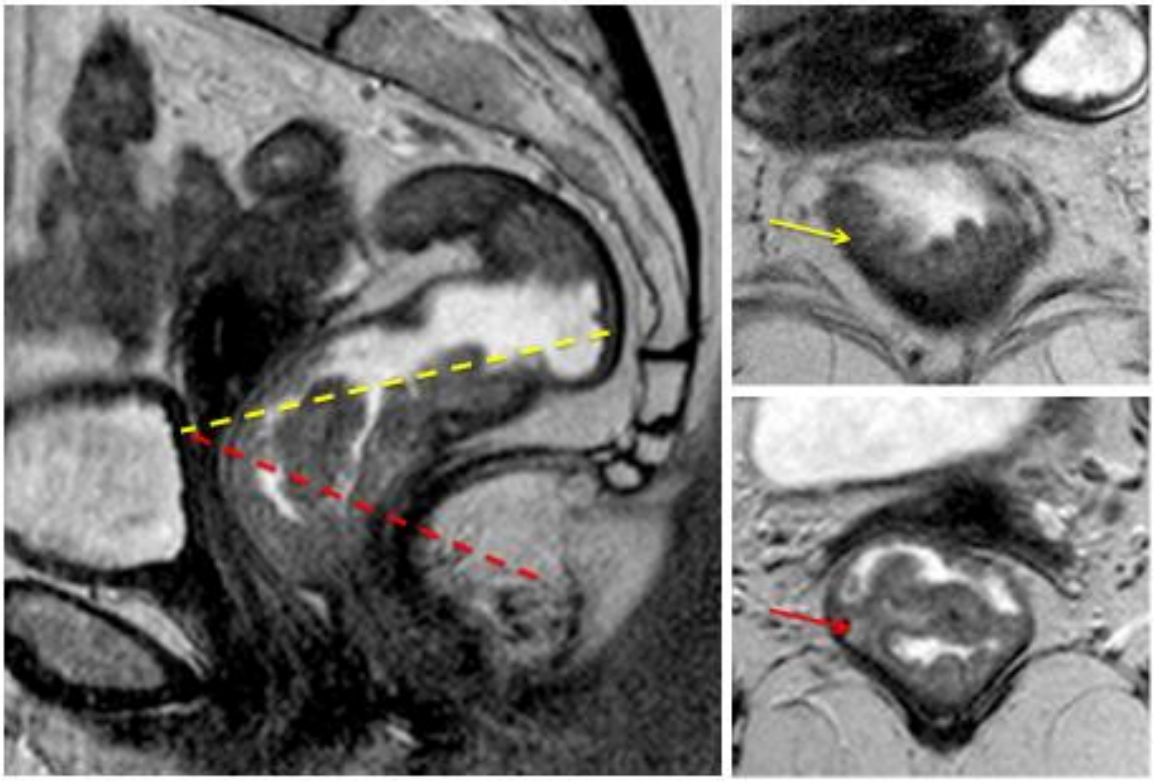


Figure 5 – Incorrect plane alignment for MRI of the rectum resulting in under-staging and over-staging of tumour. Sagittal image showing low lying rectal tumour. Yellow dashed line corresponds to the alignment of the right upper image on which tumour seems to be infiltrating through the muscularis (over-staging). Right lower image was produced using the correct alignment (red dashed line on sagittal image), perpendicular to the tumour infiltrating border. On the latter one submucosal layer is preserved thus being staged as T1 (submucosal - sm2).

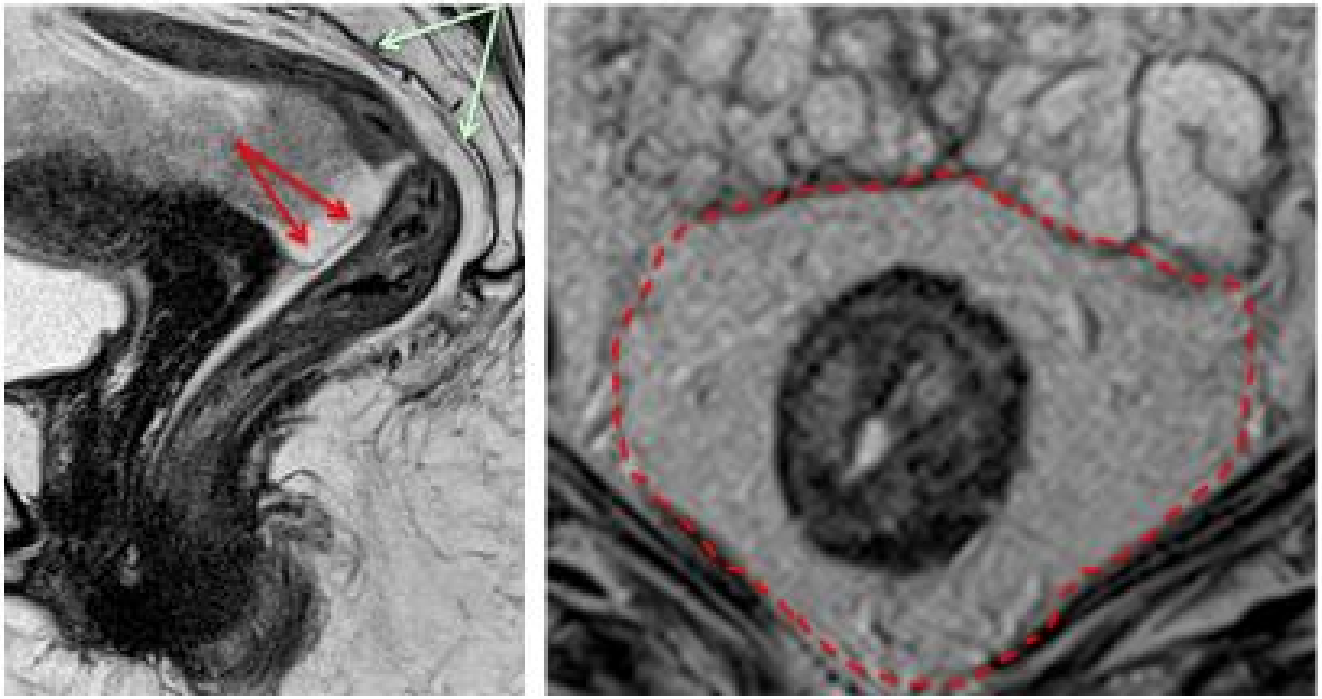


Figure 6 – Sagittal image (left) – red lines delineate peritoneal reflection, light green lines show presacral fascia. Axial image (right) - red dashed line delineate mesorectal fascia

1.4.1.3. MRI and detection of tumour depth of spread

The pattern of spread in rectal cancer is circumferential and therefore progressive disease traverses outwards through the layers of the bowel wall. The micro-structure of the bowel wall can be identified on MRI as distinct layers. Spread of tumour through the bowel into the surrounding mesorectum and beyond is associated with worsening prognosis. Furthermore, the depth of penetration of a tumour through the bowel wall also determines operative strategy and determines how 'radical' one must be in order to completely excise the tumour with adequate 'clearance'. For example, an 'early tumour' which has minimally spread into the bowel wall may be treated by local excision of the lesion without necessitating a more radical resection involving the rectum and mesorectum.

Essentially, the risk of recurrence increases with tumour depth. However, there is a distinct cut-off in terms of prognosis relating to the depth of penetration. Tumours which only minimally extend into the mesorectum, and those which are confined to the bowel wall, are considered to be 'good-prognosis' or 'low-risk' cancers. This is discussed in detail below. In comparison, when tumours spread deep into the mesorectum the risk of disease recurrence is increased.

MRI can readily identify the layers of mucosa and muscle through distinct signal characteristics. T2-weighted images are particularly useful for this. The mucosal layer is seen as a very fine line of low signal intensity overlying the much thicker and higher signal of the submucosa. Outside this the muscularis propria can be seen as a duel-layer representing the inner circular and the outer longitudinal muscle layers. The latter has a typically irregular appearance due to vessels traversing the rectal wall. At this point, the muscle layer is not a uniformly regular radiological feature and this must be appreciated to be able to identify venous invasion with accuracy. The perirectal fat is

identified as a high signal with signal void areas surrounding the relatively low signal intensity of the muscularis. This is all enveloped by the fine layer of low signal intensity representing the mesorectal fascia.

The results of initial studies which investigated the accuracy of MRI were limited by the technology relating to the coils (Hadfield et al., 1997). Furthermore, protocols which used greater than 3mm slices resulted in poor resolution and the over- or under-staging of T2 and T3 tumours (Schnall et al., 1994). Although this continues to be a problem in some units, the clinical importance of distinguishing these tumours may not be as prognostically important as first thought, i.e. negligible difference in survival. It has been shown in the results of MERCURY study that tumours which penetrate more than 5mm into the mesorectum are associated with worse survival (Patel et al., 2012, Patel et al., 2011a, Taylor et al., 2011). Tumours which penetrate into the mesorectum minimally (less than 5mm) are considered 'good prognosis' tumours. Therefore, in terms of depth of penetration, a T2 tumour or early T3 tumour (less than 5mm penetration into the mesorectum) have similar survival. Both tumours at this level of tumour penetration would not be amenable to local resection.

The depth of extramural spread into the mesorectum can be demonstrated on thin-slice high resolution MRI. In one study of 28 patients undergoing staging MRI using a four-element surface coil showed good correlation with post-operative histology (Brown et al., 1999). 21 patients out of 24 (88%) who had tumours which extended 5mm or more into the mesorectum were correctly identified on MRI. A further study of 98 patients demonstrated a kappa agreement of 0.67 between MRI and pathology (Brown et al., 2003a). Others studies in the USA and Japan have confirmed correlation between MRI and pathology to be above 80% (Akasu et al., 2005).

In the MERCURY study which included 679 patients across Europe, there was agreement between MRI and histopathology in 295 cases out of 311 undergoing primary surgery for rectal cancer (2006). In the analysis, the accuracy was further quantified by demonstrating the mean difference between MRI and histopathology -0.046mm (SD 3.85mm, 95% CI -0.487 to 0.395mm). This study formed the basis of the modern-day acceptance of the accuracy of MRI to predict tumour spread to within 1mm.

Cawthorn, Merkel and Willett were the first to report on this heterogeneity within T3 tumours. Cawthorn reported 5-year survival to be 55% for those tumours with less than 4mm penetration into the mesorectum compared to 25% when more than 4mm (Cawthorn et al., 1990). Merkel studied patient's survival characteristics with T3 tumours and used a cut-off of 5mm. Those patients with extramural spread of more than 5mm had 5-year survival rate of 54% compared with 85% for those patients whose tumours had extramural spread of less than 5mm (Merkel et al., 2001). These results were independent of lymph node involvement. These early studies highlight the importance of accurate measurement of tumour penetration into the mesorectum and those tumours with a worse prognosis, namely T3c and T3d. Therefore, the distinction between T2 and T3 tumours with less than 5mm mesorectal spread – T3a and T3b; becomes irrelevant as these patients will have minimal benefit from CRT. This has led to the sub-staging of T3 tumours which has been adopted by the UICC TNM classification since 1993. This is shown in table 5. MRI can distinguish between sub-stage of T3 tumours. However, the importance is in recognising those tumours which penetrate more deeply into the mesorectum. Figure 8 shows T3 tumours of differing sub-stage on MRI.

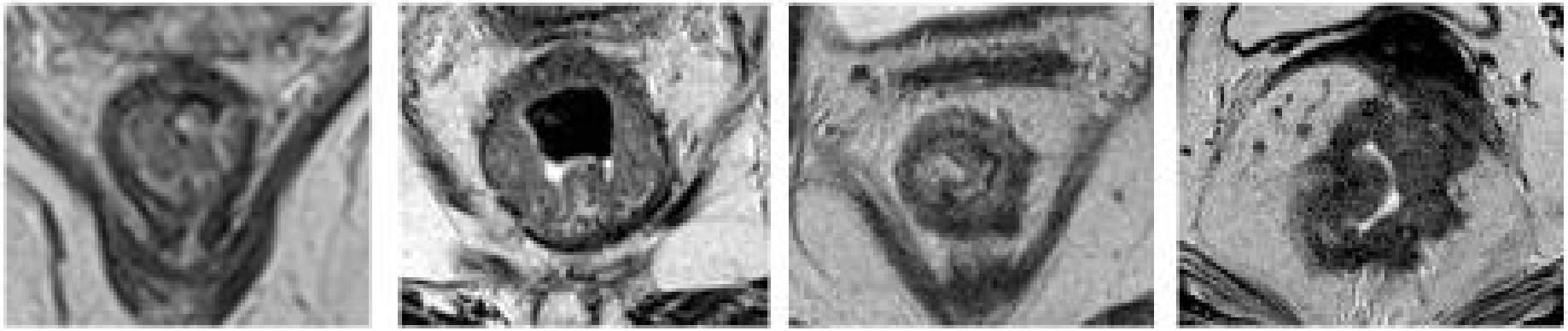
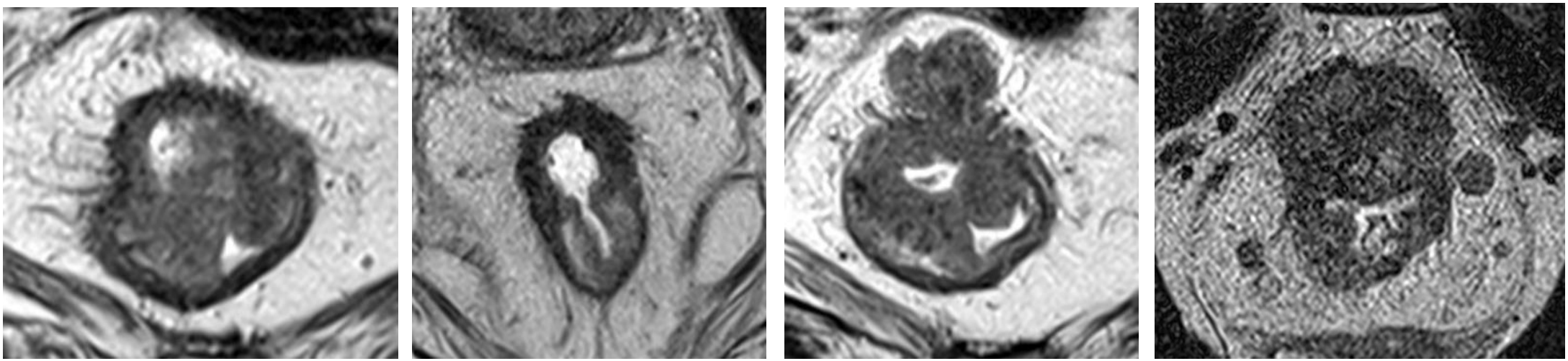


Figure 7 - Series of MRI sections showing increasing T-stage in rectal cancer. From left to right: T1 semi annular tumour infiltrating submucosal layer at 5-10 o'clock; T2 semi annular tumour infiltrating rectal wall at 9-3 o'clock within the muscularis propria; T3 tumour extends through the muscularis at 1-5 o'clock; T4 tumour invades the mesorectal fascia and posterior vaginal wall at 1-2 o'clock

Table 5 – T3 sub-classification based on penetration into the mesorectum. T3a – T3d show increasing tumour spread.

Sub-stage for T3 tumours	*Penetration into mesorectum*
A	<1mm
B	1-5mm
C	5-15mm
D	>15mm

Figure 8 – MRI sections showing T3 sub classification. From left to right: T3a; T3b; T3c; T3d.



1.4.1.4. MRI detection of the Circumferential Resection Margin (CRM)

MRI is optimal modality for identifying the CRM and mesorectum. In addition to being able to detect the depth of tumour spread into the mesorectum it can also identify the tumour edge with similar detail. Pathologists recognise a clear margin for tumour excision to be 1mm. If tumour is seen within 1mm of the CRM it is said to be a 'positive margin' or 'R1 resection' – a clear margin is referred to as 'R0' and a macroscopically involved margin as 'R2'. In the MERCURY Study, a total of 349 patients underwent pre-operative MRI assessment followed by TME surgery. All were predicted to have clear margins on MRI. 327 (94%) patients were subsequently found to have clear margins on histopathology (2006). This gave a specificity of 92%. Previously, a measured distance of 5mm on MRI had been shown to strongly correlate with a negative CRM on histology. This meant that patients were being offered CRT when tumours were within 5mm of the mesorectal fascia. However, this resulted in substantial overt-treatment of patients with safe margins. As described in later chapters, this has now changed and MRI can predict a safe margin at the 1mm level.

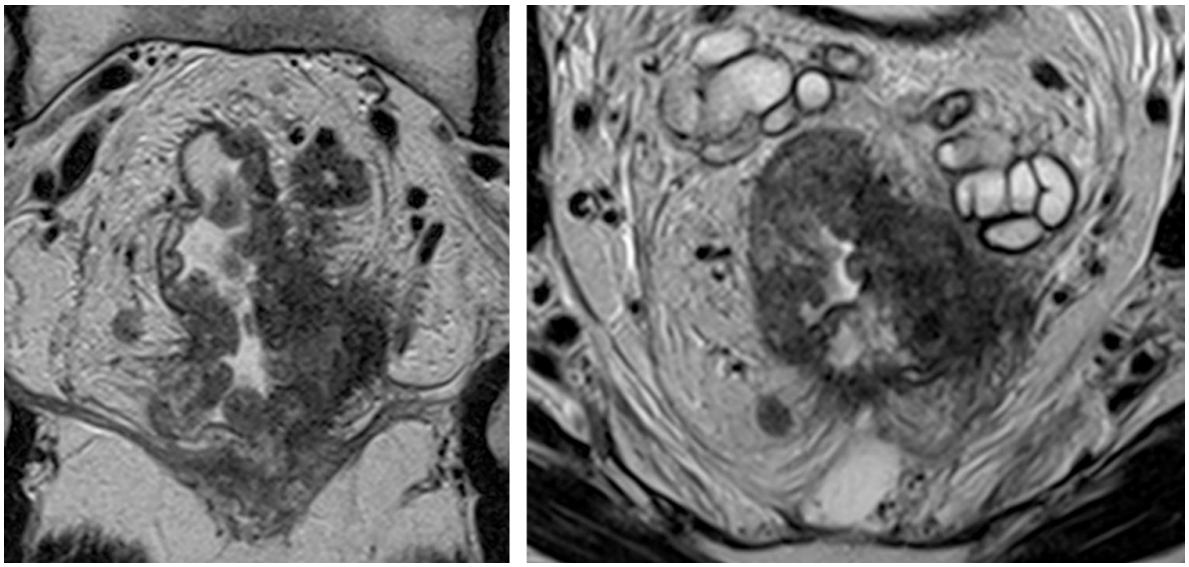


Figure 9 – MRI of CRM positive rectal cancer. Infiltration of the mesorectal fascia (at 3-4 o'clock) and tumour spread into the left pelvic lateral compartment.

1.4.1.5. MRI detection of mesorectal lymph nodes

The pre-operative detection of lymph nodes has been problematic however the evidence would suggest that MRI remains the most accurate. Pre-operative staging and subsequent treatment decisions are commonly made using MRI despite some reservations. In addition to detecting lymph nodes, the major challenge has been determining whether a node is malignant or not. Over the years, there has been a predilection for using size criteria to determine the nature of mesorectal nodes; that is, the larger the node, the more likely it will be malignant. There has been no robust trial evidence behind this or pathological correlation. A study which matched nodes from in-vivo and specimen MRIs with pathology specimens showed that there was no useful size cut-off for predicting nodal status (Brown et al., 2003c). Further, a histological survey of over 12,000 lymph nodes in rectal cancer showed considerable size overlap between normal or reactive nodes and those containing metastases (Dworak, 1991). A perceived limitation of MRI is the lack of accuracy and ability to detect nodes smaller than 3mm. Yet this may not be as clinically relevant as first appears as only 2% of nodes this size are malignant (Brown et al., 2003c).

More important than size is nodal border and the tumour signal within the node. Tumour infiltration into lymph nodes leads to characteristics radiological features which can be readily identified on MRI (Brown et al., 2003c). Tumour leads to capsular disruption causing the nodal border to become irregular as opposed to the more rounded border of benign nodes. This is shown in figure 10.

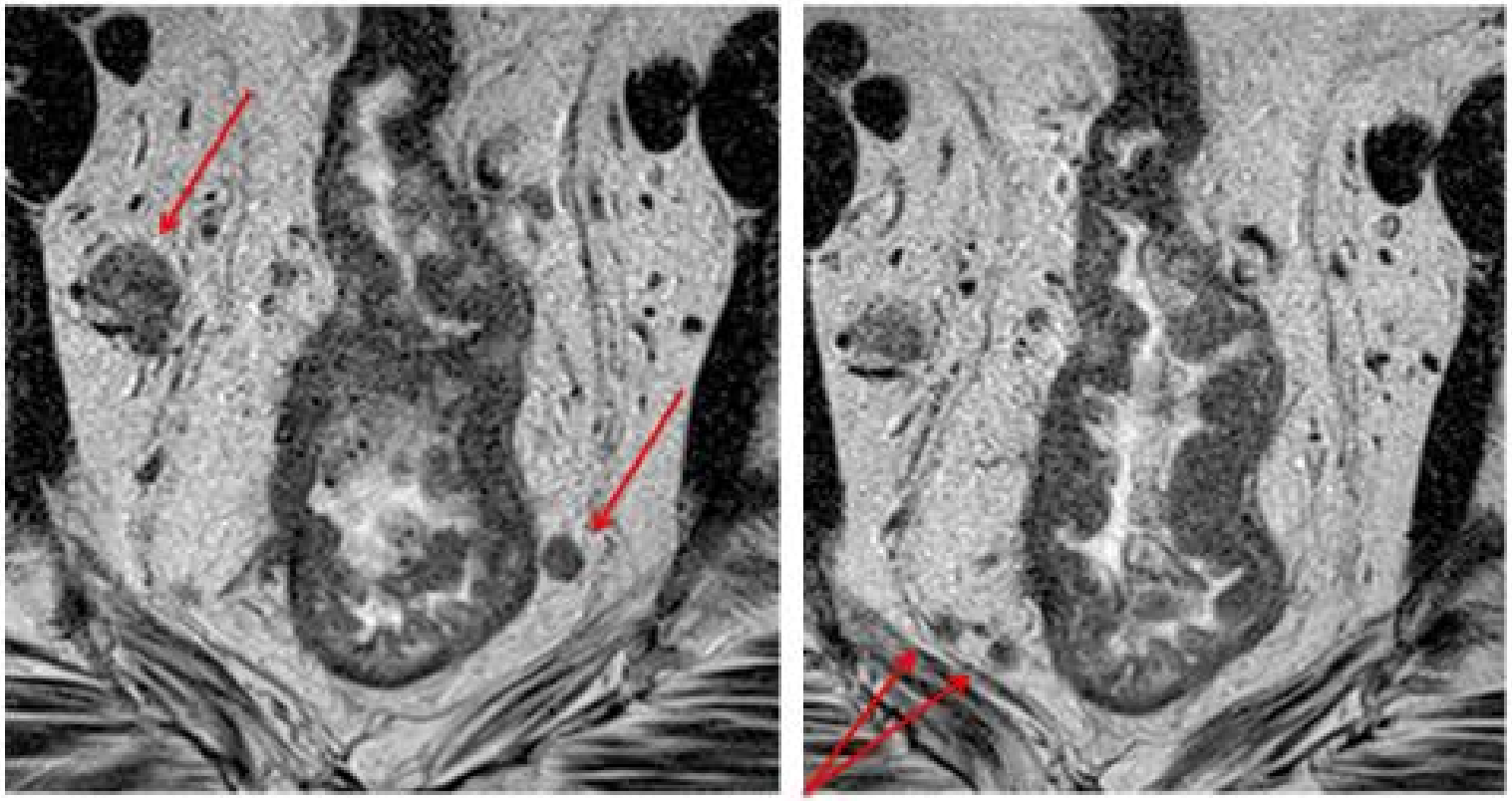


Figure 10 - Red lines show malignant mesorectal and right internal iliac nodes, which have irregular borders and heterogeneous signal.

A very small number of lymph nodes with a smooth bordered contour (<6%) has been shown to be malignant whilst those demonstrating irregular outline are malignant in over 90% of cases. Mixed signal intensity occurs due to the heterogeneity of the tumour and necrosis within the node. When using the signal characteristics and border outline together, the sensitivity is much improved. Using features of nodal border, contour and differing signal characteristics the sensitivity and specificity increases to 85% and 97% (Brown et al., 2003c).

Koh et al used ultra-small particles of iron oxide (USPIO) to attempt to detect small (>1mm) tumour foci in mesorectal nodes (Koh et al., 2004, Koh et al., 2010). In their study, mesorectal nodes were identified on T2-weighted high resolution MRI following administration of USPIO as they produced a characteristic radiological appearance. These nodes were correlated with histopathology for confirmation of malignant infiltration. The use of USPIO is not widespread and is still limited to experimental work.

Lambregts et al investigated the accuracy of gadofosveset-enhanced MRI in nodal staging and restaging following pre-operative treatment (Lambregts et al., 2011a). They found that the use of gadofosveset increased accuracy and correlation compared to standard MRI protocols as well as being reproducible.

These results suggest that even with MRI, N-staging is not sufficiently accurate for clinical use however imaging can help guide treatment decisions.

1.4.1.6. MRI detection of EMVI

To identify EMVI on MRI accurately, it is necessary to visualise the entire vasculature around the rectum, which can only be done effectively by tracing the vessels through a series of images along their length. This can help distinguish venous disease from

nodal deposits, a common mistake made even amongst experienced radiologists. Veins around the rectum are recognised on T2-weighted images as serpiginous or tortuous linear structures.

Differentiating between larger and smaller vessels can be difficult and requires a combination of signal characteristics and morphology. The larger, named vessels such as the superior and middle rectal veins appear with anatomical consistency. Ideal assessment of MRI-detected EMVI (mrEMVI) must include the following: pattern of tumour margin (extension into small veins may produce a nodular border); location of tumour relative to major vessels; vessel calibre (tumour causes vessel expansion and increase in tumour signal in the lumen); and vessel border. Smaller venules can be seen perforating the normal outer rectal wall and produce a low to intermediate signal intensity in tubular structures on T2-weighted images. The importance of EMVI and its detection is discussed in further detail in section 1.7.

1.4.1.8. Computed tomography

Currently, the optimal imaging modality for assessing metastatic spread is CT of the chest, abdomen and pelvis. This technique is accurate in identifying lesions at the common sites of metastases and is widely available in most hospitals. Combination of CT with functional imaging techniques such as PET may be more useful if there are ambiguous areas on CT. Alternatively, MRI may be used to image specific organs such as the liver in more detail if the results of CT are equivocal. Figure 11 shows liver metastases on CT.

1.4.1.5. PET

Positron emission tomography (PET) has gained increasing popularity in the last few years. Although it has had limited uses for more than a decade, the combination of PET with CT has shown to be a particularly useful tool. The principle behind PET is

the use of radioisotopes with a half-life of around 100 minutes that through tracers quantify pathological biochemical processes which are thought to precede physical changes in the anatomy. The most common tracer is fluorine-18-labelled deoxyglucose (FDG). This radioisotope acts as an analogue to glucose and identifies cells which have an increased glucose metabolism such as cancer cells.

PET/CT has been shown to have improved accuracy over CT and PET as individual examinations (Cohade et al., 2003). Yet there remain several questions about the use of PET/CT and where it fits into the repertoire of imaging modalities in the staging of rectal cancer. In the context of pre-operative staging, there is little evidence to support its routine use (Brush et al., 2011). No direct comparisons have been made in terms of accuracy of pre-operative local staging with MRI in large numbers and it is difficult to evaluate the sensitivity and specificity of this technique. However a recent study has shown that MRI and PET/CT may be used in combination for high risk patients (EMVI positive; extramural spread of >5mm; T4 disease or an involved CRM) as part of a more intensive staging process (Avallone et al., 2012). In this study, patients were stratified by MRI into whether they were at high or low risk of developing synchronous metastatic disease. Almost 21% of patients in the high risk group were confirmed to have metastatic disease on PET/CT compared to 4% in the low risk group. These patients may benefit from a further PET/CT (or liver MRI) in the initial staging process.

Recent interest has been using PET/CT to assess treatment response to pre-operative treatment with CRT. Several small studies have been published within the last few years and have indicated positive results in terms of a predictive tool for tumour regression although one of the largest studies of this type has found no benefit in serial scans to assess response and prognosis (Martoni et al., 2011, Kalff et al., 2006). The general methodology of these studies has been to perform PET/CT before and varying

times after neo-adjuvant CRT and measured the response in terms of FDG uptake. Further work is necessary before this can be confidently used as a routine modality to assess tumour response. It may be too early to draw comparisons with an established technique like MRI, however the rationale behind using PET/CT as an imaging modality, which can not only demonstrate anatomical change but also functional change, is certainly complimentary at a minimum. Figure 12 shows a positive PET/CT scan confirming the presence of recurrent disease.

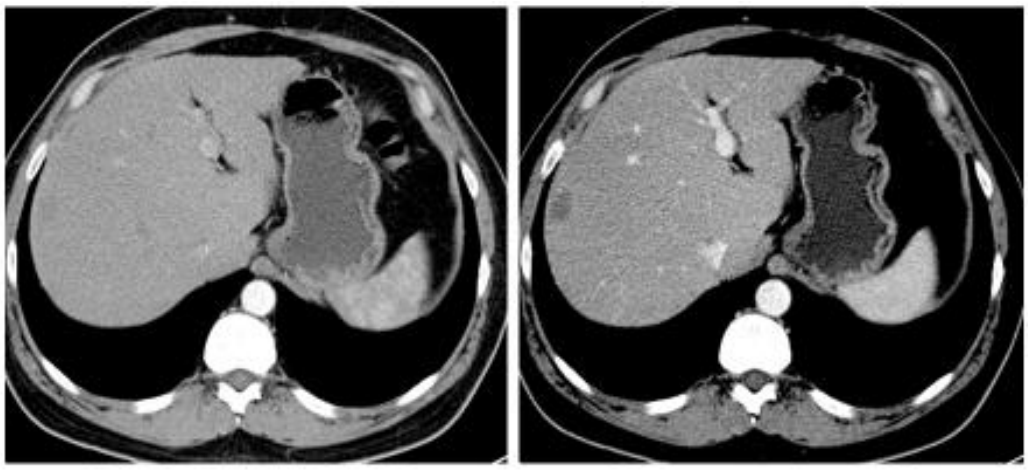


Figure 11 – This shows a staging CT of abdomen with a liver metastasis. CT arterial and venous phases – a solitary metastasis is identified in the S7/S8

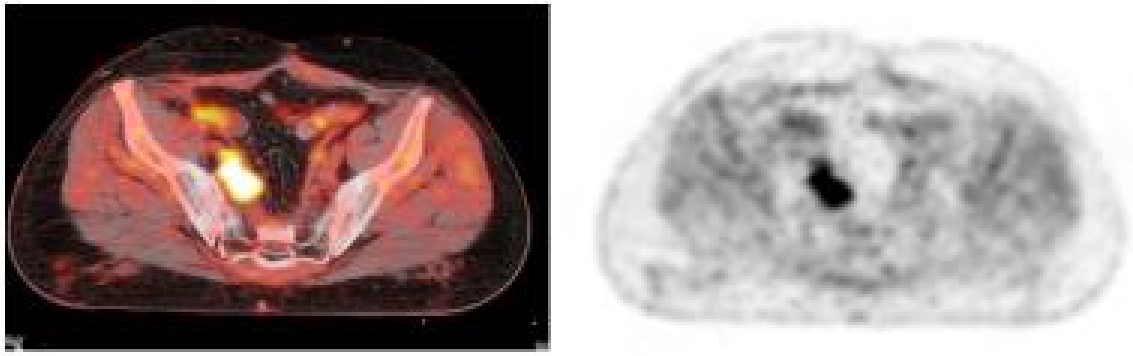


Figure 12 – PET/CT confirming the recurrent disease from the common iliac lymph nodes on the right

1.4.2. Histopathological staging of rectal cancer

1.4.2.1. Overview of pathological assessment

Optimal pathological assessment of the resection specimen following surgery is essential for a number of reasons. First, the quality of reporting directly affects the risk-stratification for patients by providing a final staging of the tumour. Secondly, there is direct influence on the treatment decisions regarding adjuvant therapy. Thirdly, it provides a reference for imaging modalities; comparison can be made between pre-operative MRI and histopathology.

Until relatively recently, the quality of pathology reporting in rectal cancer had been highly variable (Bull et al., 1997, Beattie et al., 2003) . This had been due to a combination of suboptimal preparation and dissection technique and a lack of standardisation regarding datasets. The use of proforma-based reporting has helped to encourage histopathologists to actively comment on specific factors thus leading to greater standardisation (Branston et al., 2002, Rigby et al., 1999). This has further been formalised in the UK with the Royal College of Pathologists' minimum dataset for reporting (colo)rectal cancer (Williams, 2007b).

The use of a universal staging system has helped in providing some consistency and rectal cancer should be reported by the TNM (UICC) and Dukes staging systems (see section 1.2). The two most important components of pathological reporting must relate to the important prognostic factors which will influence treatment decisions, in particular adjuvant chemotherapy; and the quality of surgery.

Quality of surgery is known to affect the risk of local recurrence - the goal of oncological surgery of the rectum is to excise the tumour with the surrounding intact mesorectum. If the mesorectum, or worse still, the tumour is breached there is an association with

local recurrence (Quirke et al., 1986, Nagtegaal et al., 2002). The prognostic factors of relevance are discussed later in more detail.

1.4.2.2. Technical considerations

Photographs of the resection specimen should ideally be taken of the anterior and posterior surfaces. Opening up the bowel to a point just above the tumour means that the tumour surface can be evaluated in detail. Assessment of the relevant prognostic factors should be made following adequate fixation in formalin. Thin sections of 3-5mm is mandatory so not to miss crucial information.



Figure 13 shows the macroscopic examination of the resected rectal cancer specimen. This image demonstrates the shiny surface of the mesorectum.

The specimen should be graded according to the quality of the surgical resection. Ideally, the mesorectum and the levator canal should be graded separately which

means that for an anterior resection operation there will be one grading compared to two for an abdomino-perineal resection (APR). There are additional features which must be identified in the APR specimen including subtle variations in the CRM. An additional assessment is made of the levator and sphincter around the anal canal and below the mesorectum. As the specimen is taken as a cylinder there should be no defects into the sphincter muscles or levators. The specimen is graded as 'levator', 'sphincteric' or 'mucosal' plane.

Mesorectal grading is either complete, moderate or incomplete depending on the quality of the specimen and correlates with the mesorectal fascial plane, intramesorectal plane and muscularis plane, respectively. Table 6 shows the differences between the grades as given by the CR07 Trial (Sebag-Montefiore et al., 2009). A 'complete' specimen has an intact mesorectum with only minor defects in the surface less than 5mm in depth. The specimen must display no coning. A 'moderate' specimen has moderate bulk to the mesorectum and with some degree of coning. The muscularis must not be exposed. An 'incomplete' specimen displays muscularis with deep defects to the mesorectum. The quality of the specimen reflects the plane of surgery encountered during the procedure.

Plane of surgery	Description
mesorectal fascial plane	Smooth mesorectum with no defect more than superficial or 5mm in depth
intramesorectal plane	Moderate bulk to mesorectum but irregularity of the mesorectal surface. No muscularis propria visible
muscularis propria plane	Substantial loss of mesorectal tissue; deep cuts on to muscularis and irregular CRM

Table 6 – CR07 definitions of specimen quality

An incomplete excision is one where there is tumour at the margin of the specimen. In rectal cancer, this is rarely at the longitudinal margin occurring in less than 2% (Guillou et al., 2005). It is more common to find tumour at the CRM, which is strongly associated with local recurrence and reduced overall survival (Wibe et al., 2002). The most accurate studies have shown that tumour within 1mm of the resection margin is a poor prognostic factor (Quirke and Morris, 2007).

1.4.2.3. Post neoadjuvant treatment assessment

As most patients present with locally advanced tumours which require pre-operative or neo-adjuvant therapy prior to surgery, pathology must be equally accurate in the identification of prognostic factors following such treatment. The same factors must consistently be reported but in addition there can be assessment of the effect of any prior treatment on tumour regression (Goldberg et al., 1994). The most common forms of neoadjuvant treatment include short or long-course and whether radiotherapy is combined with chemotherapy. These produce changes associated with fibrosis, down-staging and tumour regression depending on which regime is adopted (Wheeler et al., 1999, Shia et al., 2004).

The prefix ‘yp’ is added to the traditional TNM stage to denote that this relates to the stage after neoadjuvant treatment assessment. The predominant feature suggesting a good response to treatment is fibrosis but this may also occur as a result of radiotherapy or cancer progression. Mandard et al, have described a tumour regression grade in oesophageal cancer following neoadjuvant therapy (Mandard et al., 1994) which has been adapted to the reporting in rectal cancer. This includes tumour type, grade, margin type, lymphovascular and perineural invasion, margin involvement and lymph node status. The scale is shown in Table 7 and relies on the degree of fibrosis seen and evidence of the presence of residual cancer cells. A pathological response is defined as TRG 1-3 and has been shown to be an independent prognostic factor for disease-free survival (Vecchio et al., 2005, Bouzourene et al., 2002). Furthermore, Martin et al in their meta-analysis of the outcome following pCR reported excellent survival (90.2% OS and 87% DFS) and low rates of disease recurrence (0.7% and 8.7% for local and distant failure, respectively) (Martin et al., 2012).

Grade	**Description**
1	Complete regression of tumour; fibrosis only
2	Fibrosis with scattered tumour cells
3	Fibrosis and tumour cells; preponderance of fibrosis
4	Fibrosis and tumour cells; preponderance of tumour cells
5	Tumour without evidence of regression or fibrosis

Table 7 – Mandard grading of pathological response following neoadjuvant treatment

There are three common systems used in rectal cancer which have been developed from the original Mandard scale. Dworak et al proposed a five-point scale based on inflammatory reaction and radiation-induced vasculopathy as well as fibrosis (Dworak et al., 1997). The Rectal Cancer Regression Grade (RCRG) offered a three-point scale based around the presence of tumour cells and the degree of fibrosis. The Royal College of Pathologists in the UK uses a similar grading system to the RCRG. All three of the grading systems described above have the potential for universal application and have been shown to have good intra-observer variability and are reproducible (Bateman et al., 2009).

Although a survival benefit has been shown in patients who demonstrate a pathological complete response (pCR) following neoadjuvant therapy, the prognostic relevance of partial tumour regression is not as clear. There have been few studies which have shown an association with improved survival although a recent meta-analysis has reported a response of 50% to be associated with improved DFS (Min et al., 2011, Lee et al., 2013). As there is a current lack of universal consensus, the methodology used in this thesis is explicitly described later.

1.5. Oncological treatment for rectal cancer

There are two established curative approaches including: i) pre-operative or neo-adjuvant treatment; and ii) postoperative or adjuvant treatment. The components of either strategy include radiotherapy (RT), chemotherapy or a combination of the two.

The principles behind offering additional treatment to surgery is to help reduce the rates of local and distant recurrence. Further, locally advanced tumours may become more accessible following pre-operative treatment meaning there is a greater likelihood of complete excision. The decision to offer oncological treatment is based on the identification and recognition of adverse tumour features on presentation (in the case of neoadjuvant therapy) and following surgery (in the case of adjuvant therapy). Patients are accordingly risk-stratified depending on the type and number of adverse features identified. Accurate pre-operative imaging and post-operative pathology are critical to appropriately risk-stratify patients.

1.5.1. Neoadjuvant chemoradiotherapy

1.5.1.1 Important Radiotherapy Trials and Evidence

Study of the optimal timing and dosage of radiotherapy dates back to the 1970s. The decision to give radiotherapy pre- or post-operatively has been a balance of the prognostic outcome and the accurate selection of patients. The important issues regarding RT include the concomitant use of chemotherapy as an induction agent and whether RT could lead to a greater number of sphincter-preserving operations being performed. The advent of high-resolution imaging and the recognition of the most important adverse features associated with rectal cancer has somewhat helped to standardising treatment and regimes somewhat.

The majority of initial studies using low-dose radiotherapy of between 5-25 Gray (Gy), did not demonstrate any survival benefit over surgery alone (Abulafi and Williams, 1994). Although Goldberg et al did show with the use of 3x5Gy over 5 days followed by surgery reduced the local recurrence rate from 16% to 9% in their randomized trial of 468 patients (Goldberg et al., 1994).As the radiation dose increased to over 25 Gy however there was a concomitant improvement in local recurrence rates. The Stockholm Rectal Cancer Group showed a decrease in the local recurrence rates from 20% to 8.5%, however there was no significant difference in five--year survival and there was an associated increase risk of post-operative wound infection (8% versus 2%) (Cedermark et al., 1995). They randomised patients to receiving pre-operative radiotherapy over 5 to 7 days (25 Grays) followed by surgery or surgery alone. Longer-term follow up from patients within the Swedish Cancer Registry (median follow-up of 13 years) showed an improvement in both local recurrence (9% versus 26%) and overall survival (38% versus 30%) (Folkesson et al., 2005).

Other European trials showed a range of reduction in local disease recurrence with pre-operative RT over surgery alone. Kapiteijn et al. as part of the Dutch Colorectal Cancer Group reported a prospective randomised trial also comparing pre-operative radiotherapy and surgery with surgery alone. They explicitly mentioned TME surgery in the title of the study thereby reflecting the importance attached to the quality of surgery issue. Patients underwent a similar regime of 25 Gy divided into five fractions over five days. At two years, there was no difference in overall survival although there was a significant difference in local recurrence rates (2.4% versus 8.2%) (Kapiteijn et al., 2001). This study further reinforced the reduction of local recurrence when good quality surgery was combined with pre-operative radiotherapy. One of the criticisms of

the Swedish trial was that the local recurrence rates (26%) in the surgery group were high.

1.5.1.2. Combination chemotherapy and radiotherapy

Post-operative radiotherapy trials did not initially show any significant improvement in survival. The MRC-CR07 trial which compared pre-operative short-course RT and post-operative RT with concomitant chemotherapy for selective patients undergoing surgery showed improvement in local control (5% versus 11%) and a marginal increase in survival (Sebag-Montefiore et al., 2009). There is now clear randomized evidence that RT with or without chemotherapy is best given pre-operatively in the neo-adjuvant setting. The benefits include improved local disease control, higher compliance and reduced toxicity (Frykholm et al., 1993, Sauer et al., 2004).

It is common to offer patients with a 'locally-advanced' tumour RT with chemotherapy. There have been three randomized trials to date which have all shown an improvement in local disease control when RT is combined with chemotherapy but there is no definitive evidence indicating an overall survival benefit. Concerns have been raised about the additional toxicity of using combination chemotherapy in addition to RT and this is most likely due to the explanation of reserving such a regime for the most advanced of tumours. Bosset et al have also shown that in addition to improvement in local control by administering chemotherapy with RT, that the rate of compliance is significantly improved when chemotherapy is given pre-operatively (82% versus 42.9%) (Bosset et al., 2004). The most common chemotherapy agent used in this setting is 5-fluorouracil (5-FU) although there is some utility for oral Capecitabine (Hofheinz et al., 2012). The rationale behind using a combination of RT

and chemotherapy is that the latter has potentiating effects on RT and thus improves the response.

Current regimes of neo-adjuvant treatment are based on risk-stratification of tumours as identified on initial imaging studies. If no adverse features indicating extensive locoregional spread are detected on MRI, surgery is the most appropriate course of action despite some studies showing improvement of local recurrence for some patients if given pre-operative short-course RT (Sebag-Montefiore et al., 2009, Dahlberg et al., 1999, Kapiteijn et al., 2001, Valentini et al., 2009). Patients who exhibit poor tumour characteristics are most likely to benefit from neo-adjuvant treatment and are commonly offered RT and sensitizing chemotherapy before surgery between 6 and 12 weeks later.

1.5.1.3. Short-course versus long-course radiotherapy

A typical short course regime would consist of 5Gy given over five days (25Gy total) compared with 1.8-2.0Gy given over 25 days to a total dose of 45-50 Gy. A major concern with using short-course regimes had been the long-term morbidity in these patients (Birgisson et al., 2007). A randomised trial was carried out comparing pre-operative short-course radiotherapy with pre-operative long-course chemoradiation (Bujko et al., 2006). The study included 312 patients followed for a median follow up of 48 months. Patients were randomised to receive either 25 Gy in five fractions over five days or 50.4 Gy in 28 fractions in addition to sensitising chemotherapy (5-Fluorouracil and Leucovorin). There were also no differences in overall survival at 4 years (67.2% versus 66.2%). There were also no differences in disease-free survival or local recurrence rates although the chemoradiation group showed an increased early radiation toxicity. Despite these studies there is still no clear evidence on which

regime is optimal. National Institute of Clinical Excellence (NICE) in the UK has issued guidelines for the use of neoadjuvant therapy (Excellence, 2014). These are based on risk-stratification of patients depending on the MRI staging.

1.5.2. Adjuvant chemotherapy

Chemotherapy is most commonly used as a therapeutic rather than sensitizing agent in the postoperative or adjuvant setting. The Quick and Simple and Reliable (QUASAR) study investigated the effect of adjuvant chemotherapy in colorectal cancer patients but included a sub-analysis of rectal cancer (29% of the cohort). 5-FU and folic acid were the agents used. Some of these patients received pre-operative radiotherapy but this was only of the order of 20%. There was a statistically significant benefit seen in the rectal cancer subgroup (Gray et al., 2007).

Several Japanese studies have reported on adjuvant chemotherapy in rectal cancer but the results may not be applicable to Western practise. There are differences in oncological regimes and surgical technique. A meta-analysis of these studies has shown an improvement in both disease-free and overall survival in patients who received adjuvant chemotherapy (Sakamoto et al., 2007). Fisher et al, also showed a significant improvement in overall survival in those patients receiving adjuvant chemotherapy (Fisher et al., 1988).

With most locally advanced rectal cancers undergoing pre-operative long-course chemoradiotherapy or short-course radiotherapy, the role of adjuvant chemotherapy is less clear. A recent systematic review by Bujko et al examined four randomized trials in which patients were given neo-adjuvant radiotherapy with or without chemotherapy, and found no significant survival benefit (Bujko et al., 2010b).

Interestingly, of all the recent large-scale trials involving neoadjuvant CRT, there is no clear overall survival benefit seen despite improvement in local control.

Despite the lack of strong evidence there is a recognition that adjuvant chemotherapy may provide a survival benefit in some patients. The National Comprehensive Cancer Network Guidelines recommended postoperative chemotherapy for all patients who had received neoadjuvant CRT regardless of stage. The European Society for Medical Oncology has suggested that patients with stage III and 'high risk' stage II should be offered adjuvant chemotherapy (Glimelius and Oliveira, 2009).

1.6. Biomarkers in rectal cancer

1.6.1. Overview of biomarkers as a measure of disease activity

A biomarker may be defined as a tumour characteristic that can be objectively measured and evaluated as an indicator of a normal biological process, or pharmacological response to a therapeutic intervention (2001). They can be prognostic by providing information about outcome or predictive by providing information about the outcome for specific treatments. Biomarkers may be used in a variety of situations and serve a number of purposes. They are not limited, as most commonly thought, to monitoring of treatment response. For example, they may be a diagnostic tool which can be used for risk-stratification (staging of disease), an estimator of prognosis, and in some cases for prediction of disease response (Tejpar, 2007). Table 8 shows the categorisation of biomarkers. The study of such biomarkers can be broadly divided into imaging biomarkers; microRNA; metabolic markers; and EMT markers.

Type	**Description**
Diagnostic	Used to identify tumour type/ sub-type
Monitor	Monitors response to treatment or relapse in disease
Prognostic – informative	Correlates with survival
Prognostic – treatment guiding	Guides treatment decision making
Predictive	Guides use of specific treatment strategy according to phenotype or genotype

Table 8 – Different types of biomarkers and their application in research

1.6.2. Imaging biomarkers

The concept of an imaging biomarker is relatively new, but one which is becoming increasingly important for many phase II/III clinical trials as a surrogate end point. Imaging biomarkers provide a means to measure tumour response to a therapeutic intervention and/or detect early disease, in a non-invasive manner. Currently the imaging techniques which seek to quantify treatment response in rectal cancer can be broadly divided into those which measure tumour size and those which measure tumour activity. Whilst the former is commonly used to assess radiological response in clinical trials because of the association of size with survival functional imaging techniques may have the greater potential to uncover the underlying biological processes which lead to cancer.

Reduction in tumour size after chemoradiotherapy has been shown to be a useful biomarker (Benjamin et al., 2007). This can be measured in one, two or three dimensions by routine imaging techniques such as CT and MRI (O'Connor et al., 2008). The two commonly used criteria, World Health Organisation (WHO) (Organisation, 1979) and Response Evaluation Criteria in Solid Tumours (RECIST) (Therasse et al., 2000) have however, contrasting characteristics, in particular in the technique used to measure tumour size.

Further limitations to using size measurements have been in deciding what degree of tumour bulk reduction constitutes a significant clinical response. An example of this is has been shown by Morgan et al who investigated the effect of a VEGF receptor inhibitor on colorectal metastases, whereby significant size reduction was not met with an equally significant overall response (<10%) (Morgan et al., 2003).

Novel MRI-based tumour regression grades (mrTRG) which stratify response on the degree of fibrosis visualised in the tumour following chemoradiotherapy have, however, recently been developed. The degree of fibrosis following CRT is graded on a scale analogous to histopathological tumour regression grade described earlier (Mandard et al., 1994).

Another example is dynamic contrast-enhanced (DCE) CT/MRI which provides a detailed assessment of tumour blood flow through acquisition of data as specific contrast material passes through the vasculature. DCE-CT has the potential to identify angiogenesis and has been shown to be able to distinguish tumour from diverticular disease and detect early liver metastases (Leggett et al., 1997, Goh et al., 2007). Although reports have identified a correlation between tumour blood flow, the development of metastases and decreased survival (Goh et al., 2009, Hayano et al., 2009), this has not been translated to widespread clinical application.

Diffusion weighted imaging (DWI) assesses the Brownian movement of water molecules within cells using diffusion-weighted gradients to T2 sequences. Quantitative analysis is possible by calculation of the apparent diffusion coefficients (ADC) which are inversely correlated with tumour cellularity. DWI has been effective in detecting small liver metastases and differentiation from inflammatory lesion (Koh et al., 2008, Taouli et al., 2008, Ichikawa et al., 2006) as well as detecting lymph node metastases (Kim et al., 2008) but has been limited to mainly experimental work. Lambregts et al have shown that there may well be some utility in DWI to detect patients with a complete response following CRT (Lambregts et al., 2011d, Lambregts et al., 2011b, Lambregts et al., 2011c). In one study, they investigated the utility of DWI compared with standard MRI in 120 patients. 25 patients were found to have a complete response. The area under the ROC curve improved significantly with DWI.

Whilst DWI is currently not universally accepted as the optimal imaging modality to determine response to treatment, there is certainly some encouragement in these early small studies.

Despite some promising beginnings and ongoing study into the use of detailed imaging biomarkers, size criteria with RECIST remains the only validated tool and one which has prognostic and clinical relevance. More recently, measuring the degree of fibrosis within the tumour complex and providing a radiological-based tumour regression grade such as mrTRG has shown good results and excellent correlation with histopathology-based tumour regression grades.

1.6.3. microRNA and response to treatment

MicroRNAs are highly conserved 18-25 nucleotide non-coding RNA molecules which post-transcriptionally regulate gene expression. microRNA genes account for between 2% and 5% of the human genome and are commonly clustered within the introns of protein coding genes.(Rodriguez et al., 2004) Bioinformatic studies estimate that each microRNA interacts with hundreds of mRNA targets and as a consequence the microRNAome may regulate more than 30% of all human genes(Garzon et al., 2006, Lewis et al., 2005). As well as regulating fundamental cellular processes including differentiation, proliferation and apoptosis, microRNAs have been implicated in the pathogenesis of numerous malignancies(Garzon et al., 2009).

Growing numbers of oncogenes and tumour-suppressor genes are found to be under microRNA control and more than 50% of microRNA genes appear to be located at fragile sites or within cancer-associated genomic regions.(Iorio and Croce, 2009, Calin et al., 2004) In rectal cancer, aberrantly expressed microRNAs derail a number of cellular signal transduction and cell survival pathways including Wnt/β-catenin

pathway, EGFR pathway, and p53 function tying microRNA biology to known mutational events in the adenoma to carcinoma sequence of malignant transformation (Slaby et al., 2009).

Accumulating evidence suggests that microRNAs may also have powerful clinical applications. microRNA expression profiles are capable of discriminating tumours of different developmental origin.(Lu et al., 2005) Furthermore, the expression of individual microRNAs may be used to predict patient survival, tumour stage, the presence of lymph node metastases and the response to therapy in rectal cancer (Slaby et al., 2009, Slaby et al., 2007, Schetter et al., 2008).

Three studies have specifically examined the utility of microRNA expression signatures in predicting chemoradiotherapy response in rectal cancer (Della Vittoria Scarpati et al., 2012, Svoboda et al., 2012, Kheirelseid et al., 2013). Della Vittoria Scarpati et al, examined microRNA expression in fresh-frozen pre-treatment tumour specimens from 38 patients with locally advanced (T3/T4 Node +ve) rectal cancer and compared microRNA profiles in patients with complete (Mandard TRG 1; n=9) and incomplete (Mandard TRG >1; n=29) pathological responses to a standardised neoadjuvant chemoradiotherapy regime consisting of Capecitabine, Oxaliplatin and 45 Gy of pelvic conformal radiotherapy. Thirteen significantly differentially expressed microRNAs were subsequently validated using high sensitivity TaqMan®qRT-PCR, of which two including miR-622 and miR-630 were found to predict chemoradiotherapy response with 100% sensitivity and specificity (Della Vittoria Scarpati et al., 2012).

A similar analysis of 20 patients undergoing combined radiotherapy and Capecitabine/5-FU chemotherapy compared ‘responders’, namely those displaying a positive response to treatment (Mandard TRG 1 and 2), with ‘non-responders’

(Mandard TRG 3-5). TaqMan Low Density Arrays identified a microRNA signature consisting of eight microRNAs capable of correctly classifying 90% (9/10) of responders and 90% (9/10) of non-responders (Svoboda et al., 2012).

A third study, which used formalin fixed rather than fresh rectal cancer specimens identified a microRNA signature consisting of just three microRNAs (miR-153, miR-16 and miR-590-5p), capable of distinguishing patients with complete and incomplete responses to therapy, however the value of this data is unclear as patient demographics, tumour characteristics, study end-points and the neo-adjuvant treatment strategy were not clearly described (Kheirelseid et al., 2013).

As profiling methodology and the definition of tumour regression vary between these 3 studies, inter-study comparisons are of limited value; however it is important to note that no overlap is observed between the microRNA signatures described. This suggests that a microRNA based ‘therapy-response’ prediction tool is some way from becoming a reality however although other studies have clearly established that microRNAs do play a role in regulating the tissue response to neoadjuvant therapy in CRC(Svoboda et al., 2008, Rossi et al., 2007, Nakajima et al., 2006). Perhaps by focusing on the contribution of microRNAs within the biological pathways that govern resistance and/or sensitivity to neo-adjuvant therapy in rectal cancer, more clinically pertinent data will emerge.

1.7. The importance of EMVI in locally advanced rectal cancer in the modern management of rectal cancer

1.7.1. Background and history of EMVI

Brown and Warren first documented the association between venous invasion and metastatic disease (Brown, 1938). They conducted a study examining the resection specimens of patients with rectal carcinoma. Patients who had evidence of venous invasion were more likely to develop visceral metastases. Although a few smaller studies confirmed this finding over the next two decades, it was not until Burns et al carried out an extensive analysis of patients with colorectal cancer that the role of venous invasion was further investigated in some detail. They carried out a retrospective analysis of 338 patients with colorectal cancer of which 198 were rectal or rectosigmoid tumours. 93 (46.9%) were found to have venous invasion on histopathological examination. Further analysis of the results revealed that 74% patients with venous disease subsequently developed metastasis. With limited follow up data, they reported 119 deaths concluding that 57% of the patients died as a results of metastatic disease. This study was one of the first to highlight the difficulty for histopathologists in accurately detecting venous invasion in the resection specimen. Earlier smaller studies had not found venous invasion to be as prevalent in rectal cancer but, Burns et al utilised specialised stains of Masson Trichrome and Weigert elastic tissue stain in addition to routine Haematoxylin and Eosin (H&E) preparation in difficult cases. The histopathological criteria that pathologists adopted included the presence of intraluminal blood following an endothelial contour; communication with an identifiable vessel; and the number of endothelial cells present (a greater number indicated more likely to be a vein than a lymphatic vessel).

In 1963, Carroll et al published the largest study on venous invasion in rectal cancer expanding on Dukes and Bussey's work from St Mark's Hospital (Carroll, 1963). At the time there was an extensive follow-up programme in place at St Mark's and a large database of patients. Dukes and colleagues had already meticulously described the techniques used to identify venous invasion in resected specimens. Carroll used this series of patients who comprised a cohort between 1928 and 1952, to carry out a comparative study between a 'general' group of patients including some with evidence of venous invasion where venous invasion was not specifically sought and a 'venous' group, where pathologists had specifically sought to identify venous invasion if present. The prevalence of venous invasion was approximately 12%, which was less than most existing series at that time. In the most detailed analysis up to that time, they found that venous invasion was also associated with other adverse features such as lymph node metastases and extent of perirectal spread (penetration into the mesorectum). Using adjusted five-year overall survival as an endpoint, they also showed that venous invasion led to a significantly decreased survival (36% versus 57.4%) in the 'general' group. Metastatic disease was preferentially to the liver and associated with invasion of the superior haemorrhoidal (rectal) vein.

It was not until the seminal paper by Talbot et al in 1980 entitled *The Clinical Significance of Invasion of Veins by Rectal Cancer* (Talbot et al., 1980*)* that such a detailed analysis was performed since that undertaken by Carroll. Again with the aid of Bussey and specimens from St Mark's Hospital, 706 specimens from patients presenting with rectal carcinoma between 1960 and 1966 were retrospectively reviewed in detail. This was the first study to detail the pattern of invasion and whether invasion was within or outside of the muscle wall and the size and thickness of the vein involved. Interestingly they found the use of elastin stains unhelpful but in cases

of ambiguity opted to discount the presence of venous invasion. In their study venous invasion was demonstrated in 51.9% with 36.1% (of the total number of cases) being classified as ‘extramural’. This was the first study to state explicitly whether venous invasion was intra- or extramural. They also found an increased risk of liver metastases in patients who had venous invasion than those without (35% versus 14%) and this was more apparent in thick-walled and extramural veins. Survival was only affected when venous invasion affected the extramural veins (33% versus 73%). There was no significant difference in survival if venous invasion of intramural veins was found.

Several subsequent studies following this found similar results and researchers now began to make a distinction between intra- and extramural venous invasion. Inconsistency in techniques and definitions made the comparison of different studies problematic, however, hence the lack of a meaningful meta-analysis or systematic review on the subject.

Table 9 shows a selection of important studies which investigated the prognostic role of venous invasion and the definitions and pathological techniques used. The most common finding was that a definition of venous invasion was rare.

Author	Year	Patients	Tumour Site	ET stain	Prev
Brown & Warren	1938	170	Rectum	Yes	61%
Grinnel	1939	159	Colorectal	Yes	41%
Dukes & Bussey	1941	689	Rectum	No	17%
Seefeld & Bargen	1943	100	Rectum	Yes	20%
Sunderland	1949	210	Colorectal	Yes	28%
Madison et al	1954	42	Rectum	Yes	43%
Swinton	1959	632	Colorectal	No	25%
Carroll	1963	1996	Rectum	No	11.8%
Khankhanian et al	1977	143	Rectum	No	19% (BVI + LVI)
Talbot et al	1980	706	Rectum	Yes	52%
Rich et al	1983	142	RS/Rectum	No	17%
Phillips et al	1984	2518	Colorectal	Not specified	38.5%
Freedman et al	1984	494	Rectum	Yes	36%
Chapuis et al	1985	709	Colorectal	No	81%
Jass et al	1986	447	Rectum	No	26% (extramural only)
Sasaki et al	1987	774	Rectum	No	21% (extramural only)
Minsky et al	1988	168	RS/Rectum	Yes	48%
Krasna et al	1988	77	Colorectal	Yes	37.6%
Wiggers et al	1988	310	Colorectal	No	25%
Shirouzo et al	1991	426	Colorectal	Yes	45%
Newland et al	1993	579	Colorectal	No	29%
Harrison et al	1994	348	Rectum	Yes	21.2%
Heys et al	1994	92	Colorectal	No	8%
Ouchi et al	1996	61	Colorectal	Yes	60%
Sternberg et al	2002	81	Colorectal	Yes	70.4%
Vass et al	2004	75	Colorectal	Yes	43%
Ptok et al	2006	1043	Rectum	No	9%

Table 9 – Historical studies investigating the prognostic effect of vascular/venous invasion/ EMVI

Venous invasion, and subsequently EMVI have traditionally been identified on the routine histopathological analysis of resection specimens. Yet there remains unacceptable variability in reporting between pathologists. More recently, the increasing accuracy of high resolution magnetic resonance imaging (MRI) has meant that many prognostic factors can now be confidently identified on imaging and this includes venous invasion.

1.7.2. Histopathology definitions

Venous invasion is recognized when tumour is seen in an endothelium-lined space, either surrounded by a rim of muscle or containing red blood cells. Histological staining with standard H&E is used as routine. Pathologists classify venous invasion into three types: (i) tumour cells are found to fill the vascular lumen, with or without recanalization of the lumen (ii) tumour cells are located in the centre of the vascular lumen, distant from the vein wall (iii) tumour cells are seen to be infiltrating or occluding the wall of the vessel, showing marked thickening and inflammatory reaction. The extent of venous invasion is often described as minimal, (1-2 involved veins), intermediate (3-4 involved veins) or massive (5 or more involved veins). Note is also made of anatomical sites of invasion and whether the veins are intramural (within the bowel wall), extramural (outside the bowel wall in the peri-colonic fat or adventitia) or both.

The use of elastic stains in addition to H&E to improve EMVI detection remains contentious. Talbot et al did not find such stains helpful in detecting venous invasion and relied on meticulous specimen handling and cut-up due to the large amount of background fibrous tissue within the rectal wall itself whereas other studies by Carroll and Freedman et al have advocated the use of specialised stains (Freedman et al., 1984, Carroll, 1963). Elastic fibres are present in the adventitia of vessels, but not lymphatics, and therefore elastin stains can be used to highlight the presence of veins

and their adjacent arteries. Sternberg et al showed elastin staining to increase the probability of identifying vessel involvement by 38.5% (Sternberg et al., 2006). Vass et al demonstrated that the use of an elastin stain was more crucial in increasing the sensitivity of detection of EMVI than having the specimen examined by a moderately experienced pathologist (Vass et al., 2004).

Recently Roxburgh et al have found significant improvement in reporting of EMVI using elastin-based stains. Indeed they demonstrated that by using a specialised stain over and above the routine staining with H&E, the detection rates improved from 19% to 58% (Roxburgh et al., 2010). This study further showed that patients found to be EMVI positive on staining had a worse prognosis compared to EMVI negative patients.

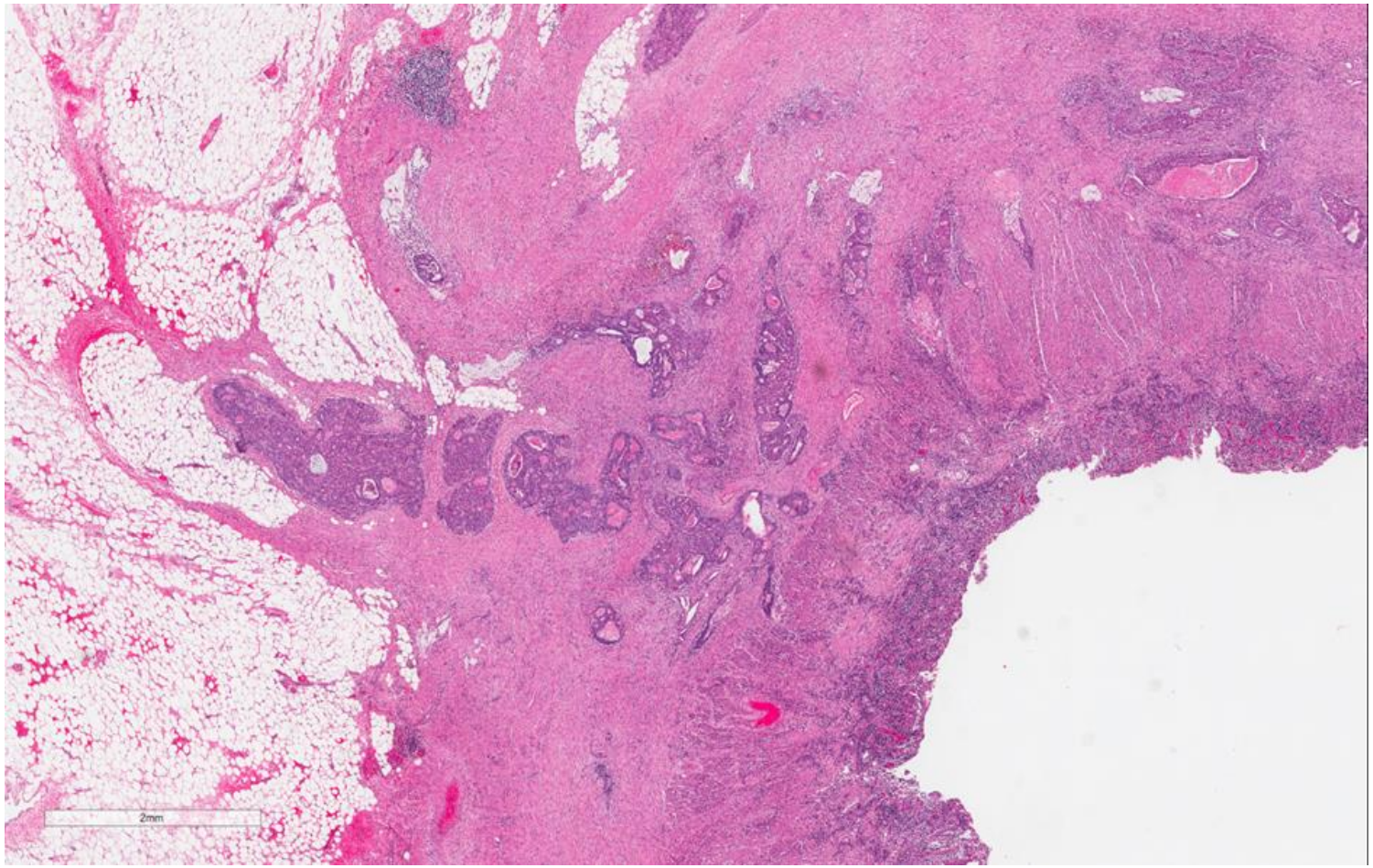


Figure 14 – Hematoxylin and eosin stain (H&E stain or HE stain) of EMVI in rectal cancer. The black arrow shows tumour extension into the vein.

Littleford et al investigated the effects of inter-observer variability on EMVI reporting (Littleford et al., 2009). Comparison of four subspecialist gastrointestinal pathologists with a specific interest in colorectal cancer found there was only “poor-to-moderate agreement.” The Medical Research Council conventional versus laparoscopic assisted surgery in patients with colorectal cancer (CLASICC) trial found a 20% difference in EMVI detection when comparing specialist GI pathologists with non-GI pathologists. Central review of the histopathology slides increased the EMVI rate from approximately 10% to 30%.

Whilst there is now active detection of EMVI and guidelines offered by the Royal College of Pathologists (Williams GT, 2007), there is still some variation on the definitions and most suitable sampling technique. And although the use of special stains undoubtedly improves the detection rate of EMVI, there is still no consensus on technique and no robust evidence that has led it to be universally accepted.

1.7.3. Radiological detection of EMVI

1.7.3.1. Incidence

In 2003, a prospective study of 98 patients with biopsy-proven rectal cancer undergoing TME surgery was carried out to determine the accuracy of MRI in identifying a number of prognostic factors including EMVI. EMVI was considered present if certain morphological features were seen on 3mm slices as defined by a serpiginous extension of tumour signal within a vascular structure (defined as a tubular structure containing signal void on T2-weighted images shown in continuity on adjacent slices) (Brown et al., 2003b). The rate of detection of EMVI on MRI (mrEMVI) was compared with histopathological assessment of resection specimens. EMVI was documented on histology if tumour was present in an extramural endothelium lined space that was either surrounded by a rim of muscle or contained blood cells. Eighteen patients had large vessel EMVI visible on H&E stain. 15 of these 18 cases found mrEMVI although more subtle involvement of tumour within smaller vessels was not demonstrated by MRI.

1.7.3.2. Radiological features

Brown et al first described in detail the MRI characteristics of EMVI. Veins around the rectum were recognised on T2-weighted images as serpiginous or tortuous linear structures. Differentiating between larger and smaller vessels was difficult and required a combination of signal characteristics and morphology. There was a signal void in the tubular structures thought to be vessels in addition to changes in contour. The larger, named vessels such as the superior and middle rectal veins appeared with anatomical consistency which helped in confident identification. Ideal assessment of mrEMVI must include the following: the pattern of tumour margin (extension into small

veins may produce a nodular border); the location of tumour relative to major vessels; the vessel calibre (tumour causes vessel expansion and increase in tumour signal in the lumen); and vessel border. Smaller venules can be seen perforating the normal outer rectal wall and produce a low to intermediate signal intensity in tubular structures on T2-weighted images. Venous invasion into these smaller venules can be recognised by their expansion and irregularity adjacent to the tumour due to contiguous tumour extension.

The prognostic significance of mrEMVI has been documented in a study by Smith et al (Smith et al., 2008a). They offered a scoring system based on previous experience which identified particular morphological and signal characteristics. This was validated against histopathology although in modern use it has been abbreviated to mrEMVI positive or negative. One hundred and forty two patients were included in the study undergoing either primary TME-surgery (n=94) or neo-adjuvant therapy followed by TME surgery (n=48). In the group in which patients underwent CRT then surgery, the incidence of mrEMVI on the initial baseline staging scans was 39.4% but fell to 24.1% following restaging. For patients with evidence of EMVI, recurrence-free survival at three years was compared between MR- and histopathlogy-detected EMVI. This was reported as 35% and 34%, respectively. Recurrence-free survival when EMVI was not present was 73.8% and 74.1% for the MRI and histopathology groups, respectively.

EMVI grade	EMVI status	Description
0	Negative	Pattern of tumour extension through muscle coat is not nodular; no adjacent vessels
1	Negative	Minimal extramural stranding; not in region of vessels
2	Negative	Stranding demonstrated in vicinity of extramural vessels; normal calibre vessels; no tumour in vessels
3	Positive	Intermediate signal intensity on vessels; only slight expansion of vessels
4	Positive	Nodular expansion of vessel with obvious tumour signal

Table 10 – Grading of EMVI as it is seen on MRI

Using similar grading criteria mrEMVI was reported in 42% of patients undergoing primary surgery for rectal cancer. 26.8% had histology proven EMVI of which 8.9% demonstrated venous invasion into vessels less than 3mm in calibre. mrEMVI with a score of more than two had 100% sensitivity and 89% specificity in identifying EMVI involving veins greater than 3mm diameter.

Sensitivity and specificity for the MRI-detection of EMVI has been reported to be between 62%-100% and 88-89% (2006, Koh et al., 2008b, Smith et al., 2008a) but this may still be an underestimate of newer, better high resolution MRI scanners. MRI-criteria for the identification of EMVI are, however, arguably more rigid. Specific

radiological features are sought which can be objectively noted to determine the presence or absence of EMVI.

1.7.4. The current limitations of EMVI being used as a factor for treatment

The lack of firm evidence has led to unacceptable variability in the treatment offered to patients who show evidence of EMVI. It is important that patients are offered an accurate estimate of the risk of disease recurrence and any survival benefit offered by oncological therapy. To attempt to resolve the actual prognostic effect of EMVI, there must be agreement on a universally accepted definition for detecting it. This will allow future studies to compare the effect of EMVI on rectal cancer in a reproducible manner. The current 'gold standard' for detection relies on histopathology but there is no consensus on methodology. As there is variation in histopathological processing techniques, the prevalence of EMVI has not been estimated accurately. Consequently, there is a risk that patients with a potentially poor prognosis are being denied optimal treatment.

MRI has been shown to be accurate in identifying EMVI on baseline imaging but there has been no evaluation of its accuracy in tumours after CRT. Many patients present with locally-advanced disease and the current practice is to offer them neoadjuvant chemoradiotherapy as described above. These patients are further staged following this treatment but the accuracy of MRI in this context has not been investigated fully. For MRI to remain an important part of decision making following CRT, it must be shown to be able to identify features such as EMVI with the same accuracy as on initial staging.

EMVI is an important consideration in stage II rectal cancers. Currently, stage II tumours are not routinely offered adjuvant chemotherapy as historical studies have

suggested that they pose little risk of disease recurrence. As more adverse features are being identified, however, it is clear that stage II cancers comprise a wide ranging group of tumours. They can be risk-stratified into groups depending on which features are present. If EMVI is shown to be a high-risk feature, adjuvant chemotherapy may improve survival and should be offered in such circumstances. Furthermore, the role of adjuvant treatment in patients who have already undergone CRT is certainly not clear. Evaluating the survival benefit in patients with persistent EMVI will aid future guidelines and form the basis of prospective randomized trials.

Tumour biology and biomarkers are being increasingly used to guide treatment. Biomarkers can be biochemical or radiological and therefore treatment response seen in adverse features like EMVI following CRT have the potential to be used as imaging biomarkers. Such imaging biomarkers like mrTRG have been investigated recently but not validated in large numbers. Using similar MRI-based TRG scores specific to individual tumour characteristics such as EMVI may further detail the survival benefit of patients who show improvement. For example, if EMVI is shown to improve in radiological terms and in survival, it may be possible to target therapies at improving this feature. Furthermore, if there is an underlying biology which explains the presence of features such as EMVI which changes in correlation with radiological characteristics following treatment and this too can be linked to survival then there may be molecular targets which can be exploited for developing new therapies. To date there is no specific profile related to recognised adverse features.

1.7.5. The future of EMVI-related trials

The recognition of EMVI as an important prognostic factor is now seen by its inclusion in data analyses for the many current trials in rectal cancer. This will allow detailed sub-analyses to be performed with respect to EMVI in the context of modern-day surgery and oncological therapies. Such analyses on the background of well-designed trials will help minimise the inconsistencies of some of the historical trials on venous invasion which meant that the results were difficult to compare. The most detailed and accurate way of determining the true predictive and prognostic value of EMVI in relation to the outcome and management of rectal cancer is to conduct a robust, well-designed clinical trial. Most of the work in this thesis has been used to design such a trial.

Chapter 2

Hypotheses

The overall aim of this thesis was to investigate the radiological and pathological changes which occur in EMVI following neoadjuvant chemoradiotherapy in rectal cancer and to relate this to survival.

2.1. Hypotheses

i. Extramural venous invasion is a poor prognostic factor in rectal cancer.

ii. EMVI produces characteristic radiological changes which can be identified and described on MRI following CRT.

iii. EMVI increases the risk of disease recurrence in stage II rectal cancer following CRT.

iv. Adjuvant chemotherapy reduces the risk of disease recurrence in patients who demonstrate persistent EMVI following CRT.

v. ymrEMVI is a poor prognostic indicator following CRT.

vi. ymrEMVI is equally accurate as ypEMVI in determining prognosis in rectal cancer following CRT.

vii. yEMVI is associated with other adverse characteristics in rectal cancer.

viii. mrEMVI can be used as an imaging biomarker to predict poor survival.

ix. Locally advanced rectal cancer with evidence of EMVI show distinct tumour profiles.

x. CRT causes a change in the rectal cancer tumour profile.

2.2. Investigations

The following investigations were undertaken to test the hypotheses in section 2.1.

i. Systematic review of the clinical relevance of EMVI in rectal cancer.

ii. A pictorial description of ymrEMVI following CRT and optimal detection strategies.

iii. The association of mrEMVI with other adverse features in rectal cancer.

iv. A survey of surgeons and oncologists in their attitudes to EMVI.

v. The clinical outcome of stage II rectal cancer with ypEMVI.

vi. The prognostic relevance of mrEMVI following CRT.

vii. The effect of adjuvant chemotherapy on persistent EMVI following CRT.

viii. mrEMVI as an imaging biomarker in rectal cancer.

ix. Differences in tumour profiles between EMVI positive and EMVI negative tumours following CRT.

x. The development of the Molecular, pathologic and MRI investigation of the prognostic and predictive importance of extramural venous invasion in rectal cancer (MARVEL) trial.

Chapter 3

Methods

3.1. Study population and design

The sub-studies within this thesis used patients' data retrieved from a prospectively maintained computer database. This is an internal database kept on a secure NHS server. It is updated in real-time and includes every 'transaction' involving the patient – consultations, tests, results, etc. This allows for accurate and detailed data collection and follow-up. From this database, a separate 'research database' was created also on the secure NHS server. These data were specific to this thesis and allowed for statistical analysis. The hospital database is continuously updated in real-time by the administrative staff within the hospital. The research database was maintained by myself and updated on a monthly basis to include any changes in follow up. This would include updated imaging, clinical follow up and treatment changes.

All original data were re-reviewed as part of the methodology. This included re-examining imaging studies, pathologic samples (where necessary) and performing further laboratory work on existing specimens as in the case of the molecular biology. The follow up data, however, used in each study was retrospective and the limitations of this are discussed in the relevant sections.

All patient data included in the analyses were obtained from the Royal Marsden Hospital (RMH) network of hospitals. Patient data were collected using the central RMH database following ethical approval. The study was classified as 'Service Evaluation' by the local ethics committee. Ethical approval was granted by the local ethics committee following the completion of a specific Service Evaluation application form. Ethical approval was granted by The Royal Marsden Hospital Research and

Development committee following submission of project protocols – Study reference SE82.

Patient inclusion criteria were dependent on the particular hypothesis being investigated however all patients were required to have primary rectal cancer with no evidence of metastatic disease on presentation (further details of the inclusion and exclusion criteria are described below in the individual study chapters). The study period encompassed January 2006 to December 2012. There was no sample size calculation for the retrospective studies as the 'timeframe' was used to include all consecutive patients. The limitations of this approach and the potential for type II statistical error is discussed in the relevant sections.

The RMH network included the following hospitals: The Royal Marsden Hospital; Croydon University Hospital (formerly Mayday Hospital); Epson and St Helier's Hospital Trust; St Georges Hospital; Basingstoke Hospital; Southampton University Hospital.

3.2. MRI technique

3.2.1. Imaging protocol

The importance of technique with particular reference to field alignment and image acquisition has been explained in section 1.5. It is equally important not only to use thin slices but also small field of view (FOV) for accurate identification. Many MRI scanners and resultant images are produced with adequate slices of 3mm but with a FOV of 22cm by 22cm and a low matrix resolution. Using correct FOV also affects the voxel size. If the voxel size is increased, resolution is lost and morphological characteristics become less obvious.

The imaging protocol employed a thin, 3-mm section turbo spin-echo T2-weighted technique with a surface pelvic phased array coil and a small field of view (160mm x 160mm, 256 x 256 matrix) with a minimum of four signal averages to ensure a 0.6 x 0.6 x 3 mm high resolution image (1mm^3 voxel size). A 1.5-T MRI scanner and four sequences were used: sagittal, coronal and at least two acquisitions in the oblique axial planes. We ensured that oblique axial scans were always perpendicular to the long axis of the rectal wall.

This high-resolution technique is recommended for optimal visualization of rectal and mesorectal anatomy (Brown et al., 2003b) and for characterization of mesorectal lymph nodes (Brown et al., 2003c). The same technique was previously used for post-treatment assessment in the MERCURY Study (2006).

Comparison of post-treatment MR images with pre-treatment MR images is conducted by ensuring that both sets of images are acquired using the same angles. Pre-treatment images are used to help locate the treated tumour, which may be difficult to visualize in patients who have had a good response to CRT. Our centre does not use

purgative bowel preparation or enemas. After initial localization imaging, large-FOV sagittal and axial images are acquired. These first two sequences allow an overview of the treated tumour, potentially involved lymph nodes, and direction of the rectal wall. This overview enables the planning of the following three high-spatial-resolution sequences that are vital for visualization of the tumour and post-treatment fibrosis. The first sequence planned is axial to the plane of the tumour and rectal wall. Thin section (maximum, 3 mm) axial T2-weighted images through the treated rectal cancer are planned using the sagittal T2-weighted images. These images are obtained perpendicular to the long axis of the rectum using a 16-cm FOV. The second sequence is oblique axial imaging for evaluation of the lymph node drainage territory. Further oblique axial imaging to ensure coverage of the draining nodes and tumour deposits, which can extend above the superior edge of tumour should be performed. The third sequence is in the coronal plane for low rectal cancers. Relying on oblique axial imaging alone can be limiting at the level of the anorectal junction. At that level, the rectal wall changes in diameter and the distance to the neighbouring tissues is smaller. The images may not show the rectal wall in its entirety and over-staging may result from partial volume averaging.

Therefore, high-resolution coronal imaging, which will show the relationship between the rectal wall and the levator muscles and between the anal sphincter complex and the intersphincteric plane, is useful for tumours in the lower one third of the rectum.

3.2.2. MRI assessment of T-staging, nodal disease and CRM involvement

MRI-assessed T staging of tumour post treatment (ymrT) was based on interpretation of local extent of persistent tumour signal intensity relative to the layers of bowel wall on T2-weighted images. T-sub-staging for both MRI (ymrT stage, after preoperative

treatment but before surgery) and pathology (ypT stage assessed after surgery) were standardized. Nodal stage post treatment was based on interpretation of lymph node border characteristics and signal intensity. A node was regarded as positive if either an irregular border or mixed signal intensity was demonstrated. Post-treatment MRI scans were evaluated for predicted circumferential resection margin status (mrCRM). A clear mrCRM was defined if the distance of tumour to the mesorectal fascia was greater than or equal to 1mm on MRI.

3.2.3. MRI assessment of height of tumour

For lower-third rectal tumours, the definition of predicted mrCRM involvement was tumour within 1mm of the levator muscle. If the tumour was present at or below the level of the puborectalis sling, the mrCRM was predicted as involved if there was invasion into the intersphincteric plane or beyond.

3.2.4. MRI assessment of EMVI

A description of the MRI characteristics of EMVI are described explicitly in the section below. Appendix A shows the reporting proforma used for data collection.

3.3. Histopathological analysis

All specimens included in the study were assessed using the TNM 7th edition staging criteria. Tumours were further classified using the T3 sub-stage as described above in Section 1 and shown in Table 5 (Hermanek et al., 1989).

Each specimen went through the exact same preparation and handling prior to a macroscopic examination leading to a grading of specimen quality including photographing (section 1.4). The surgical specimen was opened anteriorly by scissors avoiding the actual tumour. It was then pinned to a cork board under slight tension. The anterior and posterior surfaces of the mesorectum were painted with ink. The specimen was then immersed in 10% formalin for 48 hours. The position of the peritoneal reflection was noted. The fixed specimens were sliced in 3-5mm thin sections above and below the tumour with further photographs taken. If the CRM was suspected to be involved or threatened it was noted whether this was due to the primary tumour, nodal spread, dis-continuous tumour spread or EMVI. Foci of tumour in the mesorectum which measured less than 3mm were considered to be micro-satellite deposits in contrast to those larger than 3mm which were considered to be nodal deposits. The number of nodes and those containing tumour were recorded.

The distance of spread into the mesorectum and beyond the muscularis propria was recorded. In addition, the presence of EMVI, lymphatic invasion, lymphocytic infiltrate and perineural invasion are recorded. EMVI was considered to be present if the following criteria were fulfilled. The microscopic identification of EMVI relies upon of the identification of tumour cells within an endothelial or smooth muscle-lined space containing red blood cells outside the muscularis propria of the bowel wall as defined in the UK Royal College of Pathologists (RCPath) guidelines for reporting colorectal

cancer (Williams GT, 2007). Appendix B shows the reporting proforma used for data collection.

3.4. Molecular profiling and tissue microarray (TMA) analysis

Formalin-fixed paraffin embedded (FFPE) rectal tumour resection blocks were selected for further processing. Tissue specimens of four separate stage tumours were collected including EMVI-positive tumours subdivided into lymph node positive or lymph node negative and EMVI-negative subdivided into lymph node positive or lymph node negative.

3.4.1. Laser Microdissection

Paraffin embedded rectal tumours representing all four subtypes listed above were used for laser capture microdissection (LCM). LCM was used to successfully isolate epithelial cells within the rectal tumour. Tissue sections 10 micromillimetres thick were mounted on to polyethylene naphthalate (PEN) membrane glass slides and stained with Cresyl Violet. The staining procedure was as follows:

- 1 min Xylene; 30 seconds in 70% Ethanol
- 1 min in 1% Cresyl Violet acetate (in Ethanol)
- 1-2 min in 100% Ethanol.

Stained slides were allowed to dry in a fume hood and then stored for up to two weeks at room temperature with dessicant. Epithelium was microdissected using the Leica LMD microdissection platform (Leica Microsystems, UK) and collected in 50µl Digestion buffer (Recoverall Kit, Ambion) per slide. Microdissected cells were collected from 5-10 sections prior to ribonucleic acid (RNA) extraction.

LCM uses an ultraviolet (UV) laser beam to cut around selected cells, which are then lifted off the slide and collected directly into an eppendorf tube below prepared for RNA

extraction. Some samples did not contain such large amounts of RNA and therefore required more cells to be cut and collected using LCM.

3.4.2. RNA Extraction

RNA was extracted using the RecoverAll Total Nucleic Acid isolation kit (Ambion) according to the manufacturer's instructions. The nucleic acid quantity in each collected epithelium sample was determined by spectrophotometry. A minimum of 100ng of RNA was required to generate expression data on the human, rat and mouse miRCURY microRNA array allowing extra RNA for subsequent validation.

3.4.3. RNA labelling

For each sample 100ng of total RNA were labelled using a mixture of calf-intestinal alkaline phosphatase (CIP) buffer, spike-in microRNA (positive control) and CIP enzyme and was then incubated at 37°C in a polymerase chain reaction (PCR) cycler for 30 min. This was followed by a five minute incubation period at 95°C, which stops the enzymatic reaction and denatures the RNA and is then snap cooled on ice. A 'master mix' comprising labelling enzyme, dimethyl sulphoxide (DMSO), fluorescent label Hy3 and labelling buffer was added to the CIP reaction. The labelling reaction was incubated in the PCR cycler at 16°C for two hours followed by 15 min at 65°C.

3.4.3. Array Hybridization and washing

The labelling reaction was made up to 200µl with nuclease-free water and 200µl hybridisation buffer was added. Labelled RNA was denatured at 95 °C for two minutes and then placed on ice. 400 µl hybridisation mixture were pipetted into the hybridisation chamber (Corning) and the Exiqon miRCURY LNA microRNA array was gently inverted over the solution ensuring that no bubbles were trapped beneath it.

The chamber was sealed, wrapped in foil and placed in a hybridisation oven overnight at 56 °C.

After sixteen hours the chambers were dismantled and the arrays were washed in low to high stringency wash buffers (A, B and C each for two minutes) (Exiqon miRCURY array kit), followed by a few seconds in 99% ethanol. Arrays were dried briefly in a chemical hood and stored in the dark to await imaging.

3.4.4. Array scanning and data acquisition

Slides were placed face down and scanned using the GenePixPro 3.0.5 software. Scanner settings used were as follows: 532 laser wavelength to detect the Hy3 label and a Pixel size of 5 micron/pixel and 'lines to average' set at 2. A low-resolution preview scan of the whole slide allowed the positioning of a box defining the scan area. Following high resolution scanning the brightness and contrast was adjusted automatically or manually to obtain the best view. Once the image was saved as a TIFF file, the miRbase file containing the grid can was loaded and aligned with the blocks. Once all the features were aligned, the data were analysed. The results were saved and exported into an Excel spreadsheet. The mean fluorescence minus the background fluorescence could then be normalised. The panel of normalising genes on the array as well as positive and negative controls were used when determining whether the hybridisations were successful and samples were of sufficient quality to be included in the overall analysis.

3.4.5. Real-time reverse transcription-PCR

Singleplex TaqMan® microRNA assay reactions were performed for validation of profiling results. For each quantitative PCR assay, 50 ng of total RNA was converted into complementary deoxyribonucleic acid (cDNA) using a microRNA-specific reverse

transcription step and the TaqMan® MicroRNA Reverse Transcription system according to the manufacturer's instructions (Applied Biosystems, Foster City, USA). Reactions were performed in triplicate and the ABI-HT7500 qPCR instrument (Applied Biosystems, Foster City, USA) were used with the following cycling parameters:

- 95 ºC for 10 minutes
- 40 cycles of 95 ºC for 15 seconds
- 60 ºC for 60 seconds

Expression levels were normalised and calculated using cycle threshold (CT1) value technique with a reference value of 1 for a specific specimen.

3.4.6. PCR Amplification

PCR amplification was performed under recommendations by Applied Biosystems. A total volume of 20µl reaction mixture included 1.0µl TaqMan Small RNA Assay, 1.0µl of product from RT reaction, 10µl TaqMan 2X Universal Master Mix and 7.67µl Nuclease-free water and was placed into each well. The plate was sealed with an optical adhesive cover, and then centrifuged to spin down the contents and eliminate any bubbles. U6 housekeeping gene was used as an endogenous control. Each microRNA expression level was calculated using CT1 value.

3.5. Statistical analysis

Average values where calculated using mean or median where appropriate and standard deviation or interquartile range, respectively. For continuous data the mean was used and for categorical data the median value was used. Differences between groups were assessed using the Chi-squared test or Fisher's Exact test as appropriate for categorical data and student's t-test for continuous data. Survival estimates for disease-free survival (DFS) and overall survival (OS) were obtained using the Kaplan-Meier product limit method. Life tables were used to give DFS or OS at specific time-points, eg 3 years or 5 years. Patients were censored at the last point of known contact or if they died during follow-up without experiencing the outcomes of interest.

Cox's proportional hazard models were built to test the impact of confounding variables on survival (age, gender, neoadjuvant therapy, etc). These models allow the effect of predictive factors on outcome to be assessed, accounting for censored outcome, differing time of follow-up, and the interval between surgery and the adverse event of interest. Hazard ratios (HR) and 95% confidence intervals (CI) were generated. Firstly, univariate analysis was performed of individual data variables to test for significance. This was followed by multivariate analysis of all variables. In order to provide clinically and meaningful risk adjustment, several strategies were used to create multivariable models:

1. Fully adjusted model – all predictive risk factors that were judged to be clinically relevant (irrespective of statistical significance) were entered into a fixed model.

2. Parsimonious bidirectional stepwise model based on Akaike information criterion (AIC) – AIC measures the relative quality of a statistical model, trading off goodness of fit for complexity. It thus provides an accurate means for model selection,

and is not driven by significant p-values alone, which can lead to poorly fitted models. A smaller AIC indicates a better model.

3. Parsimonious forward stepwise model– a final model was built using selection based on p-values alone in order to compare to previous models. Variables significant at $p<0.1$ at univariable level were entered, and remained if $p<0.05$.

Data were analysed using SPSS 19, 20 or 21 (SPSS Inc, Chicago, Illinois) and R 3.0.0 (R Foundation for Statistical Computing).

The statistical plan for each project was reviewed and discussed with two statisticians. Approval of the statistical plan was part of the formal local ethics procedure but where there were further analyses required this was reviewed with the Royal Marsden statisticians.

Chapter 4

Systematic review on prognostic effect of EMVI in rectal cancer

4.1. Introduction

Venous invasion has been considered a significant prognostic factor in rectal cancer for well over half a century. It is well documented that tumour spread to the veins outside the muscularis propria of the bowel wall is an independent poor prognostic factor (Freedman et al., 1984, Jass et al., 1986, Talbot et al., 1981) but to what extent this confers a survival risk is still largely unknown. Improved oncological therapies and the potential for more intensive post-operative surveillance means that understanding the clinical importance of venous invasion is paramount if we are to offer patients optimal treatment.

The modern term used to describe venous invasion of tumour cells, which bears the strongest prognostic relevance in rectal cancer, specifically describes the involvement of the veins beyond the muscularis propria of the rectal wall - EMVI is defined by the presence of tumour cells within the veins beyond the outer limits of the muscularis propria and within the vicinity of the tumour. This definition is key to appreciating the limits of the historical literature and therefore how applicable it is in current day management.

The importance of EMVI as an adverse feature has been recognised by the Royal College of Pathologists (RCPath) in the UK and is therefore now included in the minimum dataset for reporting colorectal cancers (Williams, 2007b). There is a recommendation that detection rates should be at least 25%. RCPath recommends regular audit to examine the quality of reporting of features such as EMVI. The College of American Pathologists (CAP) also recognise the importance of venous invasion

although they have included it in the reporting protocol as part of ‘lymphovascular invasion’, which may have different prognostic implications (Liang et al., 2007).

This systematic review critically reviews the historical evidence for the prognostic importance of venous invasion on survival specific to rectal cancer.

4.2. Methods

4.2.1. Identification of studies

An electronic search was carried out using MEDLINE (1965-2013), EMBASE (1980-2010), CINAHL (1982-2010) and the Cochrane library databases. In addition, Google scholar and Pubmed were used to search additional articles prior to 1965. The following medical subject heading (MeSH) terms and keywords were used: 'rectal cancer'; 'venous invasion'; 'vascular invasion'; 'extramural'; and 'EMVI'. The "related articles" function was used to broaden the search and all abstracts, studies, and citations retrieved were scanned for subject relevance. The latest date of this search was 1st August 2013. All potentially relevant manuscripts were retrieved and evaluated for inclusion. Additional references from the collective libraries of the senior authors were identified. Reference lists of all relevant publications were hand-searched for additional studies missed by this search strategy, and cross referencing continued until no further relevant publications were identified. Figure 15 shows the search strategy.

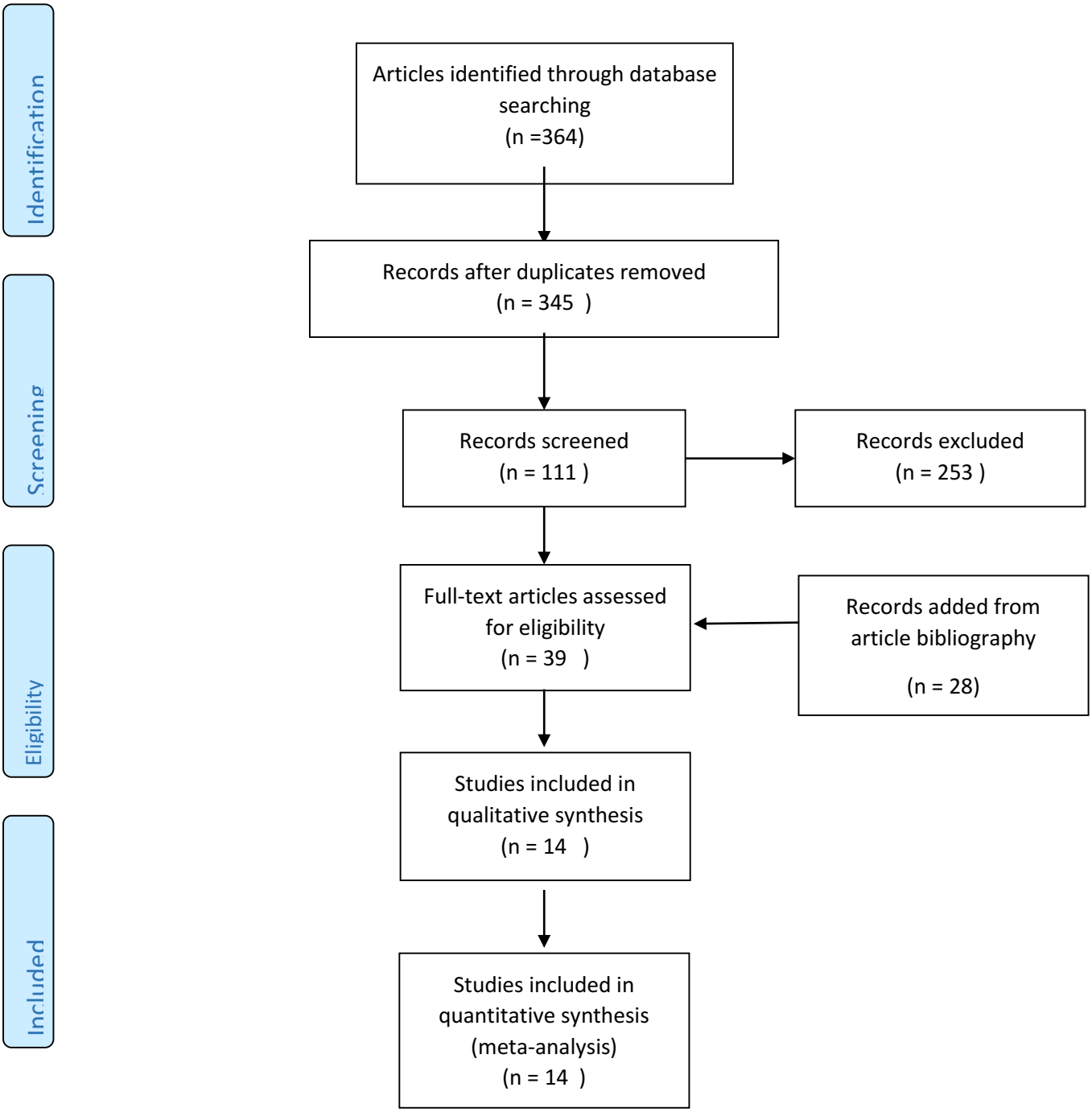


Figure 15 – PRISMA flowchart of methodology for systematic review

4.2.2. Study inclusion criteria and data extraction

Study methodology was carried out in accordance with the “Preferred Reporting for Systematic Reviews and Meta-Analyses” (PRISMA) recommendations for improving the standard of systematic reviews. Studies that met the following pre-defined criteria were included in the review process: English language publications were included, studies had to report the outcome of venous invasion or EMVI in curative rectal cancer.

Where multiple studies describing the same patient population were identified, the most recent publication was used unless additional information was imparted by earlier work. Case reports were excluded. Studies of colorectal cancer were included where there was specific analysis of rectal cancer within the study population.

Outcome measures: Studies were only included if they reported outcome information including disease recurrence and overall survival. An assessment of the quality of all eligible studies was carried out by two independent reviewers.

4.2.3. Data Synthesis

Forest plots were used to display the prevalence of EMVI positive patients and the total overall prevalence reported in the literature. We also used forest plots to illustrate the 5 year survival rate.

4.3. Results

4.3.1. Literature search and description of studies

Two reviewers performed an independent search of the literature which were cross-referenced. The outlined search strategy identified 364 publications of potential relevance. Following screening of titles and abstracts, 253 studies were excluded of which 142 articles did not report the outcome for venous invasion specifically, 32 which included patients who did not undergo surgery, 30 which described techniques only but did not report on the outcomes, 25 case reports, 24 articles described venous invasion outside the context of cancer.

This left 111 articles which were retrieved in full text. A further 28 articles were identified from the references contained in these articles, providing a total of 139 articles for evaluation. Of these 125 did not meet the detailed inclusion criteria, and were withdrawn from evaluation, leaving 14 studies published between 1935 and 2013 that were entered into the review process. For ease of description the results are presented according to prevalence, method of detection, survival; local and metastatic disease and association with other factors.

4.3.2. Study Characteristics

A total of 7262 patients in the 14 studies were included in the review. The studies were spread over seven decades (1938-2006) and six involved were retrospective. Only 6 studies commented on number of pathologists and blinding status (Harrison et al., 1994, Talbot et al., 1980, Dukes and Bussey, 1958, Seefeld and Bargen, 1943, Madison et al., 1954, Khankhanian et al., 1977). There was variation in the histological stains used, the commonest being H+E, Gieson's, elastin and Brominol.

All the included studies dealt with rectal tumours but two also incorporated rectosigmoid tumours (Seefeld and Bargen, 1943, Madison et al., 1954).

4.3.3. Prevalence

The overall prevalence of EMVI histopathologically positive patients was 26% [Random effects: Event rate 0.26 (0.18, 0.36), z=-4.3, Q=787, I2=98%]. The results for each study are graphically portrayed in figure 16.

4.3.4. Survival in the presence of venous invasion

5 year overall survival rates for patients with EMVI were reported in seven studies (4, 6, 17, 18, 20-22) and the overall survival was 39.5% [Random effects: Event rate 0.395 (0.29, 0.51), z=-1.9, Q=58.06, I2=90%]. (Figure 17)

Author	Year	No. Patients	Study Design	Stain	Tumour Site	Elastin stain	No. EMVI +ve pts	Prev VI +ve	EMVI +ve 5yr survival
Brown & Warren	1938	170	Retrospective	H+E	Rectum	Yes	104	61%	No 5yr data
Dukes & Bussey (Dukes and Bussey, 1941)	1941	689	Prospective	No stain	Rectum	No	107	17%	No 5yr data
Seefeld & Bargen (Seefeld and Bargen, 1943)	1943	100	Prospective	H+E, Gieson's	Rectum	Yes	20	20%	5%
Madison et al (Madison et al., 1954)	1954	42	Prospective	Brominol, H+E, Gieson's	Rectum	Yes	19	43%	No 5yr data
Carroll (Carroll, 1963)	1963	1996	Retrospective	H+E	Rectum	No	240	11.8%	36%
Khankhanian et al (Khankhanian et al., 1977)	1977	143	Retrospective	Not stated	Rectum	No	70	19% (BVI + LVI)	Data not usable
Talbot et al (Talbot et al., 1980)	1980	706	Prospective	H+E, elastin	Rectum	Yes	366	52%	33% EMVI+ve
Rich et al (Rich et al., 1983)	1983	142	Prospective	H+E	Rectum	No	23	17%	No 5yr data
Freedman et al (Freedman et al., 1984)	1984	494	Retrospective	No comment	Rectum	Yes	89	36%	31% EMVI+ve
Jass et al (Jass et al., 1986)	1986	447	Prospective	H+E	Rectum	No	116	26% (extramural only)	41% EMVI+ve
Sasaki et al (Sasaki et al., 1987)	1987	774	Retrospective	H+E	Rectum	No	163	21% (extramural only)	No 5yr data
Minsky et al (Minsky et al., 1988)	1988	168	Retrospective	H+E, elastin	RS/Rectum	Yes	81	48%	33% EMVI+ve
Harrison et al (Harrison et al., 1994)	1994	348	Retrospective	H+E, elastin	Rectum	Yes	74	21.2%	21% EMVI+ve
Ptok et al (Ptok et al., 2007)	2006	1043	Retrospective	Not state	Rectum	No	75	9%	80.7% LVI +ve

Figure 16 – Prevalence of EMVI in studies included

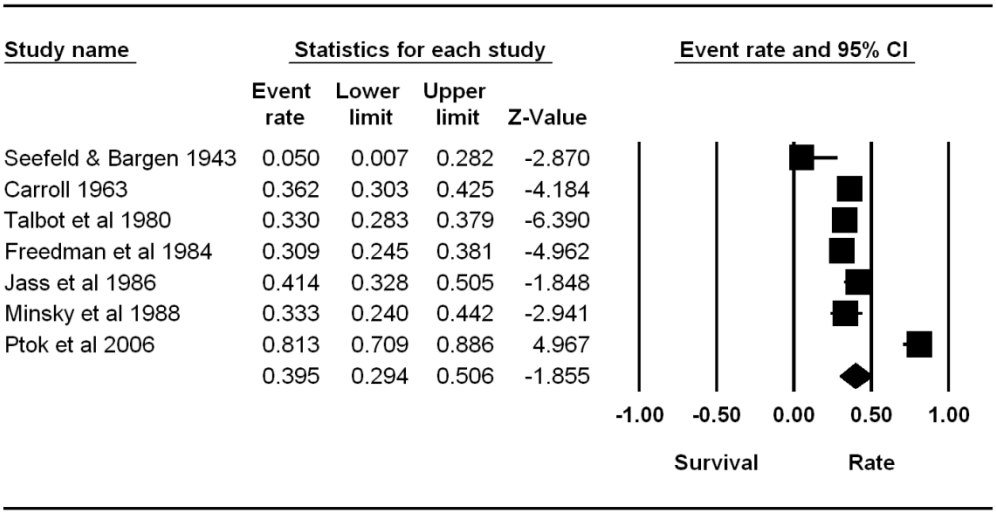


Figure 17 – Survival of patients with histopathologically confirmed EMVI

Carroll analysed the survival characteristics of 2447 patients (Carroll, 1963). Venous invasion was identified by gross dissection of the resected specimen. The corrected 5-year survival rate was compared between those patients whose resection specimen demonstrated venous invasion on histopathological analysis and patients with no evidence of venous invasion. Five year survival rates of Dukes' B tumours with venous invasion were 67.5% compared with 77.6% without and of Dukes' C with venous invasion was 23.1% compared with 32%. A further comparison using histopathological tumour grade showed a significantly lower five year survival rate in tumours of low and average histological grade when venous invasion was present (34.8% and 44.8% versus 77.3% and 60.6%).

Talbot et al examined 706 patients who were operated on with a view to cure, between 1960 and 1966 at St Marks' Hospital (Talbot et al., 1980). The tumour sections were reviewed with the help of additional stains for elastic tissue where necessary. Venous invasion was found in 51.9% of patients and of these 36.1% had extramural venous invasion. This incidence increased with Dukes stage as follows: 20%, 47%, and 64% in Dukes A, B and C. Of the 706 patients, 684 were included for survival analysis. The

5-year survival rate where venous invasion was evident was 57% compared with 73%. The survival rates according to Dukes' stage showed that when venous invasion was not present the 5-year survival rate was 86% and 46% for Dukes B and C tumours.

Freedman et al reviewed the outcomes of 769 patients with rectal cancer who underwent potentially "curative surgery" (Freedman et al., 1984). Of these only 494 were considered to have undergone "curative surgery" (the excluded cases were those where the surgeon believed there to be residual tumour) and only 21% had evidence of venous invasion. The patients with venous invasion were subdivided into submucosal, extramural or unknown with respective five year survival rates of 42%, 31% and 32%. Venous invasion was shown to be an independent factor on survival on statistical analysis.

Minsky et al found a prevalence of 48% of what was termed 'blood vessel invasion', 48% in a pathology review of 336 patients (Minsky et al., 1988). Most (71%) were classified as intramural only, and there were only 19% of patients who had extramural venous invasion. The survival analysis revealed a significant decrease when extramural venous invasion was present compared with intramural venous invasion or no evidence of blood vessel invasion at all (33% versus 72% versus 72%)They did not, however, find venous invasion to be an independent prognostic factor for 5-year survival following a proportional hazards analysis. Jass et al studied the survival characteristics of patients from St Mark's Hospital (Jass et al., 1986). All 447 patients were reported to have "extramural" venous invasion and the five year survival of patients with EMVI was 41% compared with 67% in those without.

Only one study has not demonstrated a decreased survival risk but this did not stratify between colon and rectum (Khankhanian et al., 1977). This study reviewed 143 cases

of Dukes' B colorectal cancer. Vascular invasion was found in only 18.8%. Disease-free interval and overall survival were not significantly different in these patients but interestingly, relapse was seen in 41.3% of patients without vascular invasion compared with 29.6% in those which exhibited vascular invasion implying that vascular invasion is a protective factor on survival. The details of the histopathological analysis were not given in this study.

4.3.5. Local and metastatic disease in venous invasion

Brown and Warren first showed the association between venous invasion and disease recurrence. In their study, 71% of patients with venous invasion developed recurrent disease with the vast majority of these being distant metastases. In comparison none of the 70 patients who showed no evidence of venous invasion developed metastases. Rich et al studied patterns of disease recurrence in 142 cases of rectal and rectosigmoid tumours undergoing curative surgery (Rich et al., 1983). Venous invasion was found to be an independent prognostic factor for an increased risk of local and distant disease recurrence which was independent of nodal status. In patients who had node positive disease, local recurrence was seen in 100% of patients compared with 41% when there was no venous invasion. In node negative disease this was 59% compared with 33%.

In the study of Talbot et al, patients with evidence of venous invasion had a greater incidence of liver metastases (35% compared with 14% in those patients with no evidence of venous invasion). A further comparison between the types of venous invasion showed that those patients with extramural venous invasion had liver metastases in 40% of patients compared with 23% of patients who had evidence of intramural venous invasion. The overall rate of liver metastases in all patients was 25%. When venous invasion was not seen, this was 14% and in those patients where

it was present this increased to 35%. Knudsen et al demonstrated venous invasion in 265 patients of whom 136 had metastatic disease in the liver at the time of death. This compared with liver metastases in 45 patients of the remaining 417 patients where there was no evidence of venous invasion (Knudsen et al., 1983).

A study by Horn et al reported venous invasion in 22% (28/128) patients undergoing primary “curative” surgery (Horn et al., 1991). Five year distant recurrence-free survival was 84.3% when EMVI was not present and 32.9% when present. Distant metastases occurred in 46.4% of patients with venous invasion compared with 10% without. For local recurrence this was 21.4% when venous invasion was present compared with 23% in the absence of venous invasion. The 5-year local recurrence-free survival was 75.4% and 80%, respectively but this did not reach statistical significance.

Willett et al analysed the survival of patients with node negative, T3 rectal cancer (Willett et al., 1999). None of the 117 patients included in the study were given neoadjuvant or adjuvant chemo- or radiotherapy. Survival was measured in 10-year actuarial rates for local disease control, distant disease free recurrence and recurrence free survival with rates of 76%, 68% and 62%, respectively. Multivariate analysis showed vessel involvement as well as depth of invasion and tumour grade to be independent factors for freedom from distant metastases and local recurrence.

A more recent study examined the prognostic factors which led to recurrence in 256 Dukes’ B and 74 Dukes’ C cancers after curative surgery. Univariate analysis showed that vascular invasion was significantly associated with a higher rate of disease recurrence. In the Dukes’ B group, patients with vascular invasion had 3.13 times higher risk of developing recurrence. This correlated to the same risk as Dukes’ C

patients who did not have vascular invasion. These results were also borne out in the survival analysis where Dukes' B patients with vascular invasion had a 2.61 times higher risk of death. This was also similar to patients in the Dukes' C group. Vascular invasion remained a significant risk factor on multivariate analysis.

Dresen et al analysed a subgroup of patients from the Dutch TME trial (Dresen et al., 2009). Various prognostic factors were analysed between a group of patients who developed local recurrence with a group who were disease-free at two years. Using a logistic regression model, they found EMVI associated with an increased risk of developing local recurrence (odds ratio 4.54 and 4.51 in CRM positive and negative patients). EMVI was found in 41.3% of the patients in the recurrent group compared with 13% where EMVI was not present. Similar results were reported by Takahashi et al who showed a hazard ratio of 1.90 in patients with vascular invasion (Takahashi et al., 1996).

4.3.7. Association with other prognostic factors

Recent studies have suggested an association between known prognostic factors and venous invasion implying the multifactorial nature of certain poor prognosis tumours. For example, EMVI is almost exclusively seen in T3 and T4 tumours, which gives it a positive correlation with the depth of tumour penetration of the bowel wall. But other important prognostic factors such circumferential resection margin (CRM) involvement and nodal disease, have also been shown to be associated with venous invasion.

An association with the CRM has been previously reported by Chapuis (Chapuis et al., 2006). He studied the risk factors which were associated with tumours that had a positive resection margin following surgery. The aim was to identify features which would lead to a positive resection margin. Several factors were identified on initial

analysis but using a multiple logistic regression model, nine factors proved to be significant including venous invasion (Odds Ratio 1.7). A similar study of 418 patients by Ueno et al also demonstrated that vascular invasion was a predictor of CRM involvement (Odds Ratio 2.7) (Ueno et al., 2002).

Venous invasion has also been reported to be a predictive factor for lymph node metastasis. This has been mainly studied in early rectal cancers, that is, those which are confined to the bowel wall (T1 and T2). These tumours may be treated by less radical, local excision procedures however this is dependent on the absence of nodal disease. A study of 59 patients with early rectal cancer (54 submucosal carcinoma and 5 carcinoma in-situ) reported a lymph node involvement of 9.2% (Kodaira et al., 1981). The odds of nodal disease increased 18-fold for those who had venous invasion. Venous invasion was seen in 11.8% of patients. 60% of patients with lymph node involvement had venous invasion but only 7% where there was no nodal disease. These findings were also shown by both Blumberg et al and Goldstein et al in T1 and T2 cancers (Blumberg et al., 1999, Goldstein and Hart, 1999). Goldstein studied the resection specimens of 73 abdominoperineal (APR) specimens; 11 of which had nodal disease. Amongst the associated risk factors was small vessel invasion. This association may not only be restricted to early cancers. A study by Saclarides et al of all stages of rectal cancer reported venous invasion to be strongly associated with nodal metastases (Saclarides et al., 1994).

4.4. Discussion

Historically, there has been a tendency to categorise all invasion of the vasculature including venous, arterial, lymphatic and perhaps even perineural under one pathological category. This has led to difficulty in accurately understanding which of these, if not all, are actually prognostically relevant. In recent years, there has been an increasing realisation that it is the veins that are most likely to be implicated in the development of metastatic disease and survival.

The association between venous invasion and poor prognosis has been shown in the present review to date back to the mid-20th century but the most striking finding is the huge variability in detection rates on histopathology. This ranges from 9-61% and reflects the inconsistent nature of recognition and detection. It is worth remembering that historically many pathologists were not aware of the importance of venous invasion as a prognostic factor and therefore may not have been actively seeking it. Also, the historical definitions of venous invasion did not make it clear whether detection was intramural, extramural or both. The more consistent detection rates which are found in most recent reports almost exclusively use the terminology of 'extramural venous invasion'.

Furthermore, the specialisation which exists amongst today's pathologists is another factor which would limit variability in the diagnosis of venous invasion. But the most reasonable explanation for the variation must be the lack of standardisation in detection technique and definition of morphology. This means that relying on non-standardised pathology may lead to under-reporting and thus inaccurate risk stratification of patients. Consideration should be given to the use of elastin stains to identify cases of venous invasion where there is uncertainty, in addition to reference to imaging studies such as MRI.

MRI has been shown to be accurate in identifying EMVI with reproducible results. It is also accurate in identifying EMVI following CRT using the same identification technique. CRT can destroy the morphological architecture which pathologists use to detect EMVI and thus make it difficult to identify. Such difficulty appears to be less of a problem when using MRI. A further advantage of MRI over histopathology is that pelvic imaging is able to visualise the entire rectum in-situ whereas the analysis of a small sample of the tumour is dependent on macroscopic assessment by the pathologist in the first instance, to ensure a representative area has been evaluated.

It is notable that despite being shown to be an independent adverse feature in multiple studies, it is not seen to be mandatory in many centres to offer patients oncological treatment based on its finding. This may be due to the inconsistent detection rates shown above or it may be that it is rare to find EMVI without the association with more traditional adverse features such as nodal disease or increased T-stage.

The evolution of rectal cancer management may, however, lead to a change in attitude towards EMVI if a more selective approach is taken to neoadjuvant treatment. The universal policy of irradiating all T3 tumours or any tumour that has local nodal disease may be over-treating some patients. There is accumulating evidence that not all T3 tumours behave the same and that it is depth of penetration through the mesorectum (T3 sub-stage) that is prognostic – this is discussed in detail in Chapter 1. Further, in the presence of optimal TME surgery nodal disease may not be prognostic for local recurrence. In these situations early T3 tumours, or those with N1 disease, may not benefit from neoadjuvant CRT and it may be EMVI which tips the balance.

The major limitation in the present study is the inconsistency of terminology for venous invasion. The articles which specifically refer to EMVI are more helpful as there is a

specific definition. Furthermore, many studies which have reported on venous invasion have not separated colon from rectal cancer. In addition, many of the earlier reports have not mention demographic data or tumour characteristics in much detail. The more historical studies have also been reported in an era where both surgery and oncology have evolved, which introduces an element of bias in the results.

Chapter 5

Optimal methods of detection of EMVI following CRT in rectal cancer

Aim

This chapter describes the optimal technique for imaging the rectum post-CRT and explicitly describes the process of detecting mrEMVI. There is also a description of optimal histopathological technique.

5.1. Introduction

MRI has become central to pre-operative risk-stratification of rectal cancer. The significant tumour characteristics that influence treatment decisions can be accurately and confidently identified on early MRI staging, which is now considered a mandatory investigation for rectal cancer in the UK. Each of these factors produces specific radiological characteristics which help radiologists identify their presence with reproducibility. These include tumour depth, locoregional nodal disease, involvement of the CRM and EMVI. The importance and accuracy of MRI in pre-CRT staging has been explicitly described in Chapter 1.

Locally advanced tumours which exhibit high-risk features are commonly offered CRT to reduce the risk of locoregional disease recurrence and improve survival. The prognostic relevance of the same tumour characteristics following treatment with CRT is not clear, yet some clinicians base their management plans on the initial staging regardless of the response which may have occurred. For example, if a patient demonstrates malignant mesorectal lymph nodes on initial staging (pre-treatment MRI), which are subsequently not seen on post-CRT imaging or in the resected specimen, they may still be offered adjuvant chemotherapy as ‘high-risk’ stage II

disease (based on the initial imaging). Such decisions are commonly made as part of local policy rather than national, continental or universal guidelines.

There is still no universal agreement on the best imaging modality to gauge treatment response following CRT but of the current modalities, MRI is the most accurate although may be limited by availability. The importance of MRI in its ability to measure treatment response is seen by its inclusion in many current national and international trials in rectal cancer. Furthermore, the clinical relevance in relation to survival and risk of recurrence is currently being investigated and validated prospectively against pathology for several of the traditional adverse features described in Chapter 1 – T-stage, nodal involvement and relationship of the tumour margin to the CRM. These features are given the prefix ‘y’ to denote that patients have undergone CRT.

5.2. MRI technique

The accurate assessment of mrEMVI relies on the availability of high-resolution and high-quality MRI. The technique to identify tumour characteristics optimally using MRI has been described in detail in Chapter 3. Crucially this not only requires high-resolution small 'field of view' images of the mesorectum but also higher resolution sagittal images for the appropriate planning of axial and coronal sequences. Briefly, the protocol employs a thin 3-mm section turbo spin-echo T2-weighted technique with a surface pelvic phased array coil. No bowel preparation, air insufflation or intravenous contrast agents are used. The sagittal T2 weighted images are used to plan T2-weighted small field of view axial and coronal images through the rectal cancer. These images are performed perpendicular to the long axis of the rectum and obtained using a 16-cm field of view and a 3mm section thickness resulting in a voxel size of $1.1mm^3$. Low-resolution images (voxel size greater than $1.1mm^3$), images degraded by patient motion artefact or those which are not performed perpendicular to the rectum lead to ambiguous staging and inability to determine ymrEMVI.

5.3. Pre-treatment baseline mrEMVI

EMVI displays specific radiological characteristics on MRI T2-weighted images as previously described by Brown at el and Smith et al (Smith et al., 2008b, Brown et al., 2003a). Veins around the rectum are recognised on T2-weighted images as serpiginous or tortuous linear structures. There is a signal void seen in the tubular structures thought to be vessels in addition to changes in contour. Smaller venules can be seen perforating the normal outer rectal wall and produce a low to intermediate signal intensity in tubular structures on T2-weighted images. Venous invasion into these smaller venules can be recognised by their expansion and irregularity adjacent to the tumour due to contiguous tumour extension.

These radiological features reflect the degree of venous invasion although the clinical relevance of increasing 'radiological' severity remains unknown. The prognostic importance of mrEMVI 'positive' status has, however, been documented and shown to correlate with pathology and outcomes (Smith et al., 2008a). Since this validation against histopathology, which is described in Section 1.7.3.2 by Smith et al in 2008, it has become routine practice in our institution to report on mrEMVI status. Reporting of EMVI has simultaneously become a mandatory requirement for histopathologists in the UK as part of the minimum dataset in (colo)rectal cancer (Williams, 2007a).

Briefly, ideal assessment of mrEMVI must include the following: the pattern of tumour margin (extension into small veins may produce a nodular border); the location of tumour relative to major vessels; the vessel calibre (tumour causes vessel expansion and increase in tumour signal in the lumen); and vessel border.

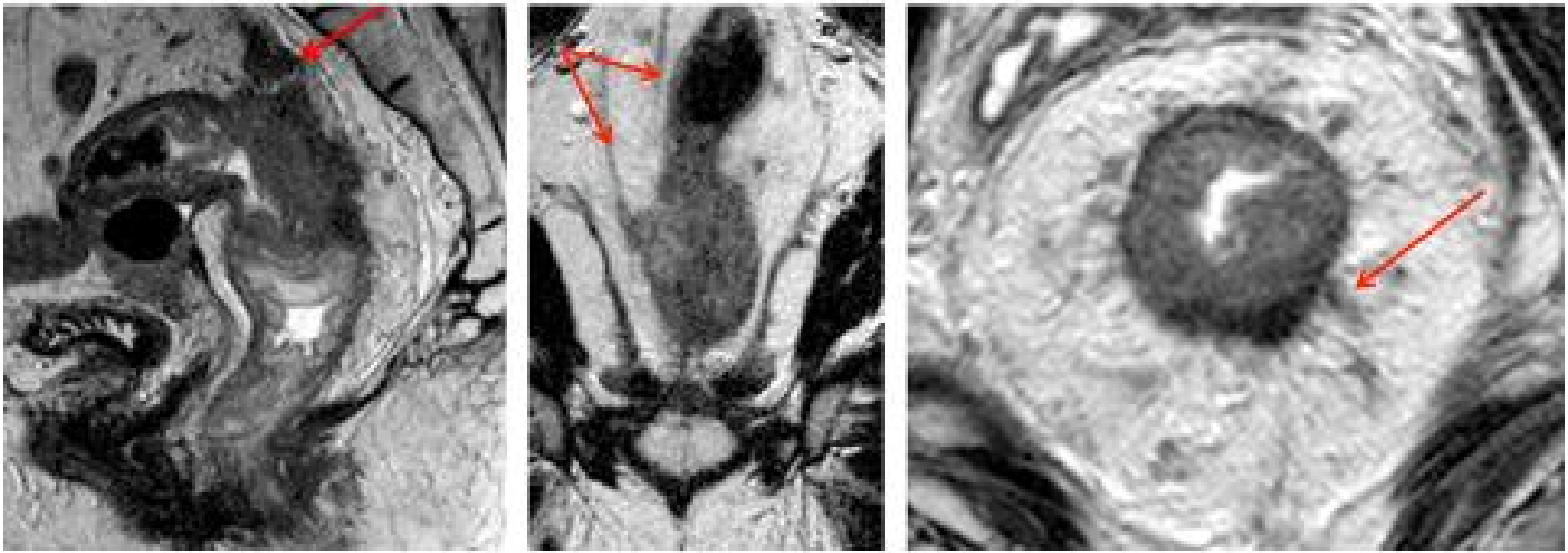


Figure 18 – Pre-treatment MRI sections showing EMVI. Large vessel invasion (superior rectal veins), medium and large venous invasion (branches of superior rectal veins and medial rectal veins), Small vein invasion – middle and lower rectal veins

5.4. Post-treatment ymrEMVI

Post-treatment EMVI can also be readily detected on MRI (ymrEMVI) using the sequences described above. CRT produces characteristic morphological change within the tumour that can be graded radiologically to reflect response. It can cause similar changes in the radiological characteristics of the extramural veins eg extension into small veins may produce a nodular border, cause vessel expansion and increase in tumour signal in the lumen. Much like the grading system proposed by Smith et al for pre-treatment mrEMVI, the MRI features of ymrEMVI can be scored according to the degree of venous invasion. We have developed a novel scale to reflect response which is similar to the tumour regression grade (TRG) used by pathologists to measure tumour response. This has been termed the mrvTRG scale and grades the response from 1-5. This scale is yet to be validated prospectively against histopathology but has been tested retrospectively and is also the subject of analysis for the prospective study described in chapter 13. The mrvTRG system is described in Table 11.

MrvTRG Score	Post-treatment mrEMVI features
1	Tumour signal replaced by vessel fibrosis
2	Less than 25% tumour; 75% fibrosis
3	25-49% tumour signal
4	50-75% tumour signal
5	Minimal fibrosis of tumour signal

Table 11 –mrvTRG grading system following CRT

The mrvTRG score reflects the degree of fibrosis seen in relation to tumour signal in the vessel. Fibrosis is seen as low signal on high-resolution T2 weighted images and the fibrosis of EMVI classically distorts the extramural vasculature. The radiologist should initially decide whether tumour signal or fibrosis is seen within the vein. Thereafter determining the exact mrvTRG depends on the relative degree of fibrosis and tumour signal within the veins. The radiological characteristics for a mrvTRG grade 2 is shown in Figure 19.

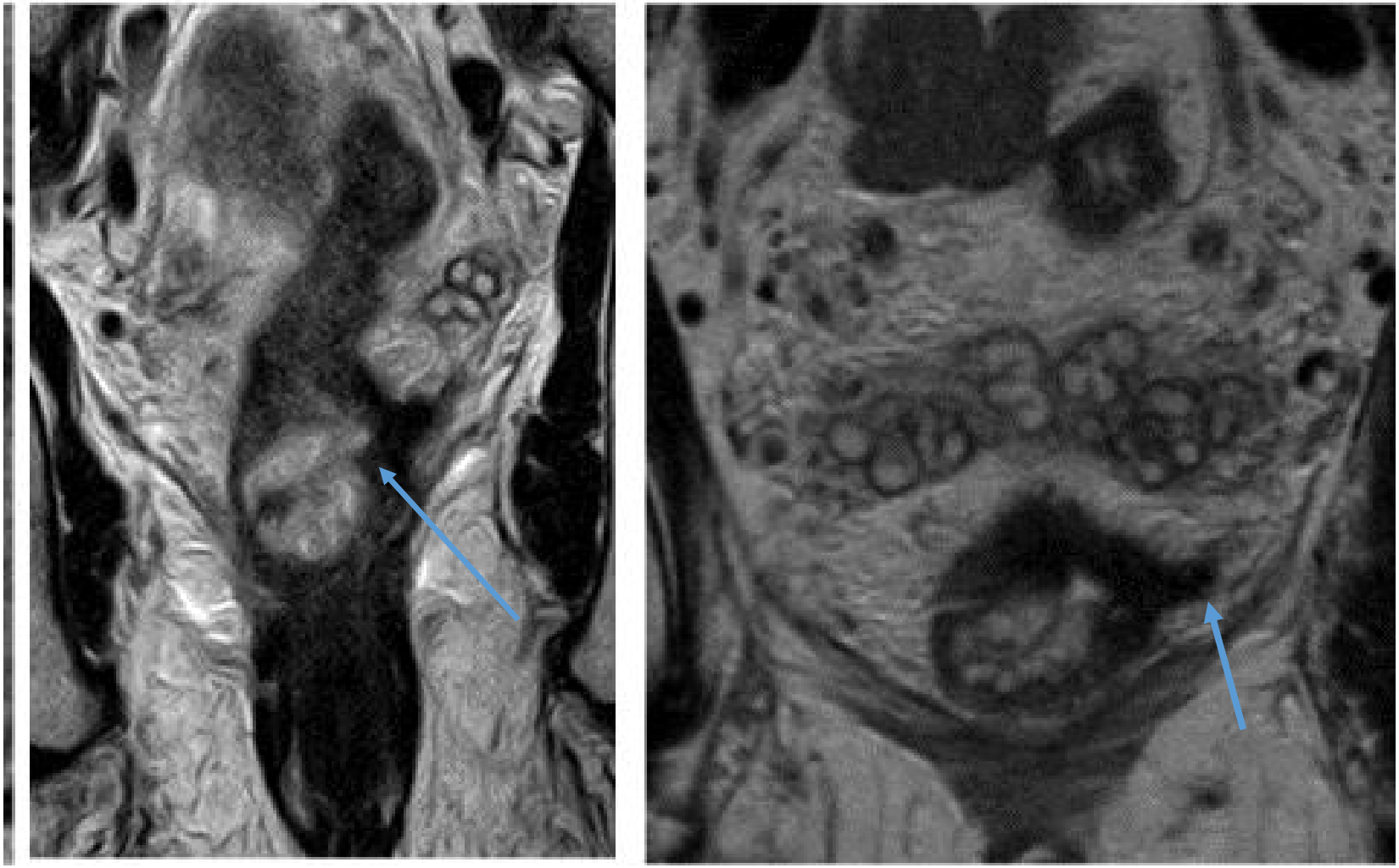


Figure 19 – mrvTRG Post CRT MRI shows fibrotic component only at the site of previously infiltrated vessels, mrvTRG2. Arrows depict the areas of fibrosis

5.5. Implications of ymrEMVI and mrvTRG

The ability to assess the treatment response after neoadjuvant CRT has exciting implications although this is yet to be prospectively validated against histopathology. If one is to continue to hold histopathology as the Gold Standard despite limitations which are discussed in further chapters, radiologists and pathologists must work together to optimise detection rates.

Using post-CRT imaging to progressively risk-stratify patients as they pass through phases of their treatment means that patients are offered optimal management strategies which are flexible. The regression grading and association with clinical outcomes means that mrEMVI has the potential to be used as an imaging biomarker in the future (this is shown and discussed in Chapter 11). If ymrEMVI can be used to predict disease progression and identify those patients are the highest risk of recurrence, it would allow clinicians to tailor surveillance and oncological treatment accordingly and ultimately improve outcomes. Ultimately, those that would most benefit from consolidation therapy would be offered such treatment without burdening lower risk patients with unnecessary morbidity.

The use of MRI in detecting a known prognostic marker of metastatic disease can also help pathologists with both techniques working hand-in-hand rather than competing. Pathological detection of EMVI following CRT (ypEMVI) is difficult as the morphological changes cause destruction in the vessel architecture and associated microanatomy which pathologists use to confirm the presence of EMVI. If there remains a singular reliance on pathology to detect EMVI then we are at risk of missing patients who would benefit from adjuvant chemotherapy. If pathologists are able to confer with their radiologist colleagues, cases where there may be suspicion of EMVI but no obvious evidence can be re-examined using MRI as a reference.

There are also adjuvant treatment implications. As the results of current trials become available, we will be able to more confidently understand the role of specific tumour characteristics on long-term survival following CRT. This will allow clinicians to offer chemotherapy to the most appropriate patients, that is, those who will benefit from such therapy without using a ‘blanket’ policy for all.

5.6. Histology-based detection

Histopathological examination is the current gold standard method by which EMVI is identified; the significance of the finding has been appreciated, at least conceptually, for over eighty years (Willis, 1930). The accurate identification of EMVI relies on meticulous examination and sampling at both the macroscopic and microscopic level; ancillary staining methods may also help to highlight the architecture of vascular structures and increase the identification rate.

5.6.1. Macroscopic examination

The macroscopic examination of rectal cancer specimens has been described in Section 3.3 in detail. Briefly, resection specimens must be appropriately opened, pinned out and fixed in formalin solution for at least 48 hours before dissection. Once adequately fixed, the specimen should be cross-sectionally sliced transversely at a thickness of three to four millimetres; these thin slices allow identification of the depth of tumour invasion and status of the circumferential resection margin.

5.6.2. Microscopic examination

The microscopic identification of EMVI relies upon of the identification of tumour cells within an endothelial or smooth muscle-lined space containing red blood cells outside the muscularis propria of the bowel wall as defined in the UK Royal College of Pathologists (RCPath) guidelines for reporting colorectal cancer (Williams GT, 2007). Due to the difficulties associated with tissue sectioning and architectural distortion by tumour or fibrosis following CRT, vascular spaces may become difficult to identify on routine haematoxylin and eosin (H & E) staining. As a result, the rate of EMVI with H & E staining has been documented to be as low as 8% (West et al., 2010), yet the

RCPath guidelines suggest that EMVI should be detected in at least 25% of cases of colorectal cancer. There is no guidance as to the expected rate in rectal cancers alone or those treated with neoadjuvant CRT. In situations where uncertainty or disagreement exists as to the nature of subserosal tumour deposits, the RCPath guidelines recommend that deeper sections through the tissue block should be performed to attempt to clarify the nature of a lesion; alternatively histochemical and immunohistochemical stains can be invaluable tools.

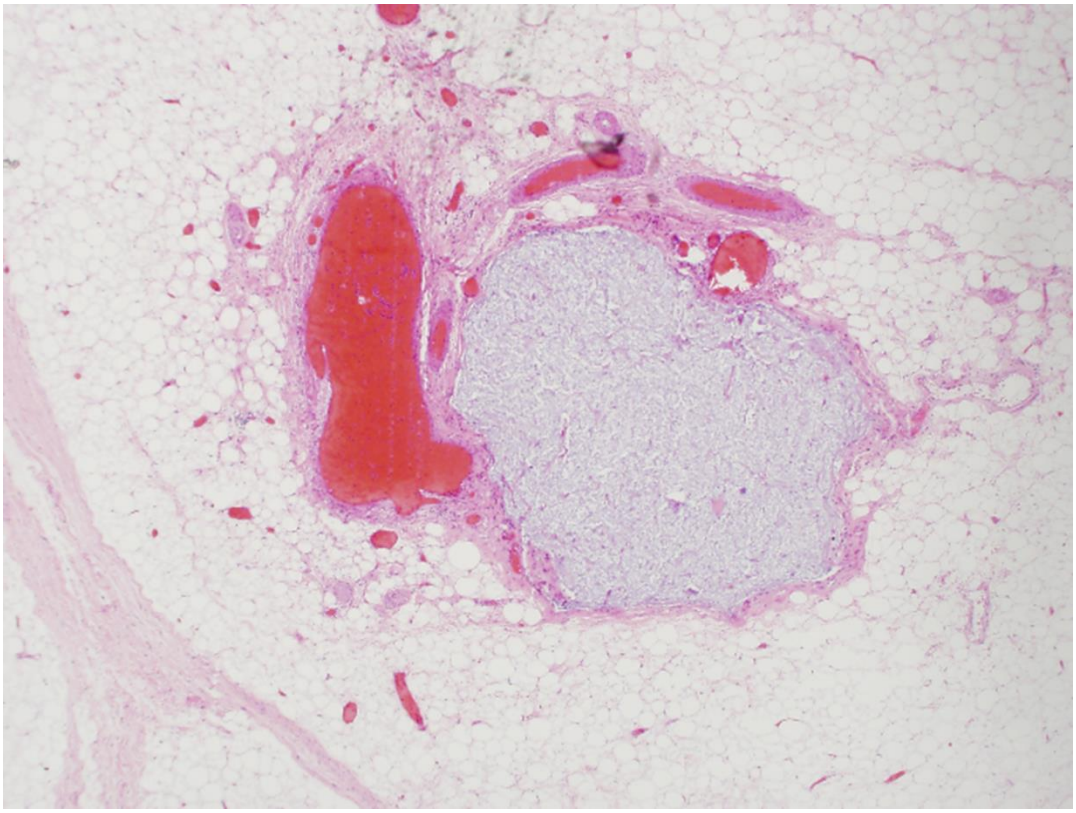


Figure 20 – EMVI in rectal cancer on H&E stain. The thick walled artery is shown next to the thinner walled vein.

EMVI was introduced into the 6th version of TNM staging when the classification of extramural tumour deposits was based on contour with irregular deposits being

classified as vascular invasion and smooth deposits lymph nodes. This classification did not make sense as vessels are generally smooth structures leading to unacceptable variation in reporting rates. For this reason, the guidance was removed from the more recent 7th addition of TNM.

5.6.3. Special Stains: histochemical staining

The elastic lamina within medium-to-large vessels provides a landmark, which can be highlighted by special stains such as orcien and elastin Van Gieson (EVG); the application of these stains has been shown to increase the detection of EMVI from 19.6 to 58% (Roxburgh et al., 2010). The major drawbacks of these methods are that they are non-specific and interpretation maybe hampered by background staining.

5.6.4. Special stains: immunohistochemistry

Immunohistochemical stains using antibodies directed towards endothelial (CD31 and CD34) or smooth muscle (smooth muscle actin) antigens within vessel walls also increase the sensitivity of histopathology-detected EMVI. The potential advantage of these methods is an increased specificity over histochemical staining, although the endothelium may become lost or obscured when a tumour embolus occupies a vessel. Kingston et al examined the rate of detection when using H & E, EVG and immunohistochemistry for CD31 and CD34 in fifty cases of colorectal cancer in which vascular invasion had previously not been identified; with the use of special stains they identified vascular invasion in 48% of cases (Kingston et al., 2007a). Although this study did not differentiate between intramural and extramural vascular invasion, it showed that EVG was the most sensitive method, however, in a minority of cases

immunohistochemical staining was still required. An example of these histochemical and immunohistochemical stains in practice is shown in Figure 21.

5.7. Limitations in detection of ymr- and ypEMVI

The perceived difficulty in interpreting post-treatment images on MRI with respect to EMVI is two-fold. Firstly, the operator must be sufficiently confident to be able to identify mrEMVI at baseline and have some indication of the severity and vessel involvement. However, following appropriate training and subsequent proforma-based reporting, it has been shown that mrEMVI can be identified with accuracy and reproducibility. Secondly, there is a perception that post-treatment MRI features can be difficult to differentiate with regards to tumour signal and fibrosis.

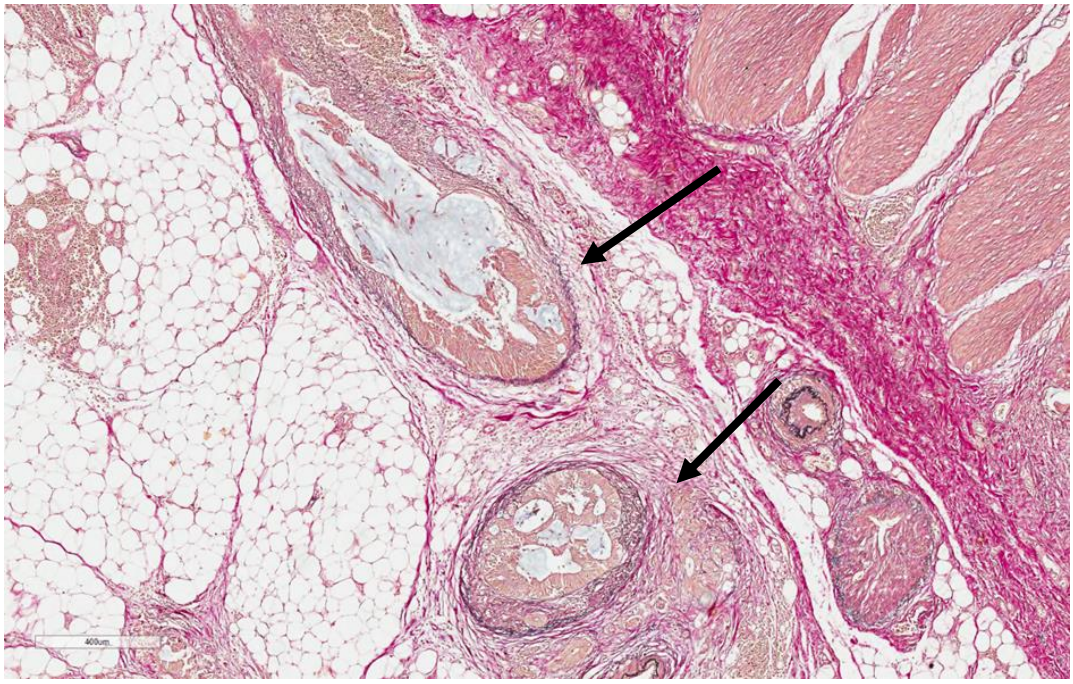
In histopathological detection, the success in identification relies on rigorous technique with appropriate sampling and sectioning. This is accompanied by the use of strict criteria to define whether pEMVI is present or absent. The use of CRT prior to surgery makes this all the more challenging but adherence to the detection criteria means that EMVI can be identified confidently, even in these situations. The use of elastin-based stains certainly have a role and there is current work being done to investigate the optimal staining technique when using these stains.

The increasing use of neoadjuvant therapy has meant that many of the traditional concepts regarding risk-stratification have been made less clear. The prognostic importance of known adverse tumour features is being re-examined in the context of pre-operative treatment. This gives us an opportunity to investigate and validate radiological factors along with pathology. EMVI has been shown to be an independent marker of poor prognosis and accurately identifiable on MRI. We have shown that

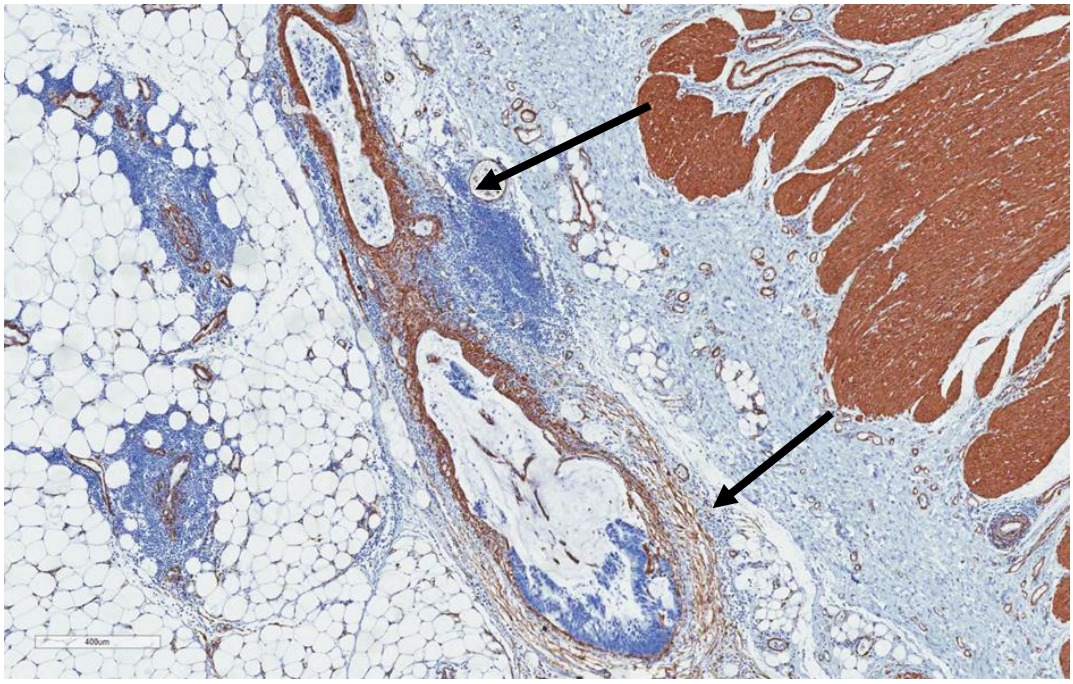
EMVI can also be seen on MRI following CRT. Further, the response to CRT can be quantified and scored against a novel regression grade and related to clinical outcomes. This MRI-based tool provides clinicians with a method of risk-stratification which can be universally applied.

Figure 21 – Special stains: elastin Van Gieson (A), and antibodies against smooth muscle actin (B), CD34 (C) and CD31 (D). The position of tumour within the vessel is highlighted by an arrow.

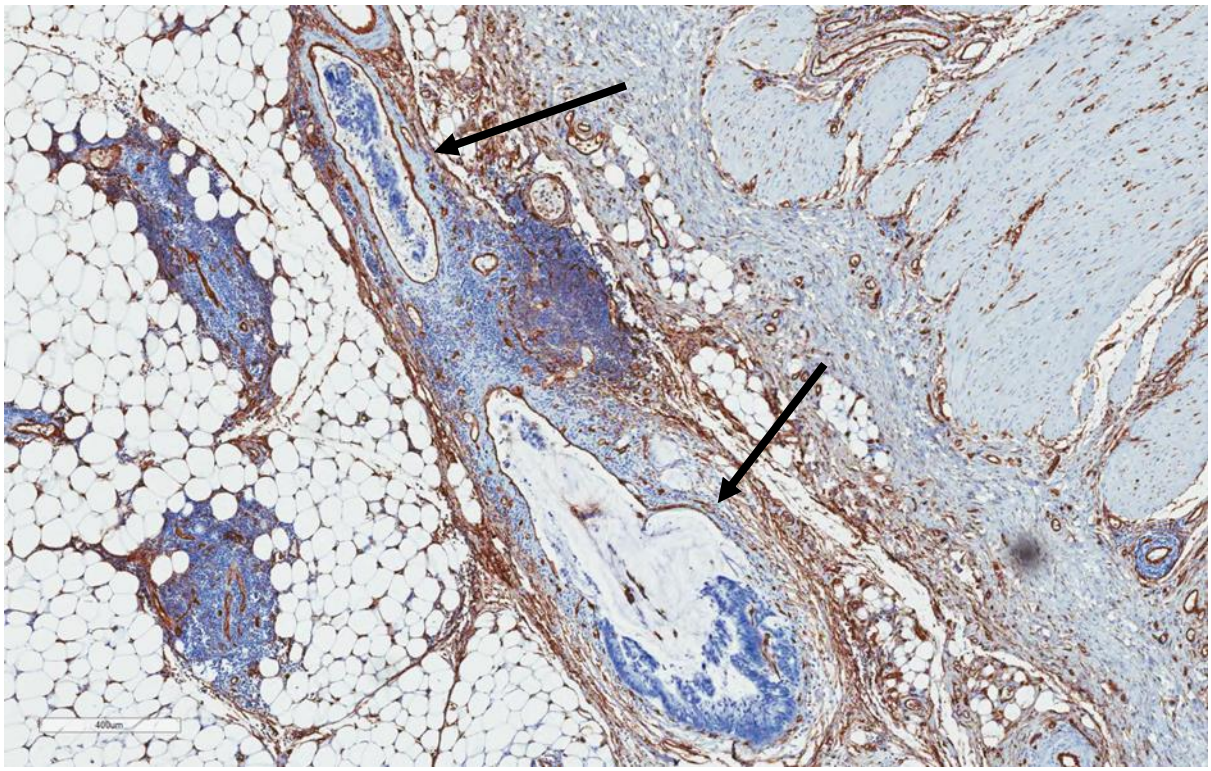
A



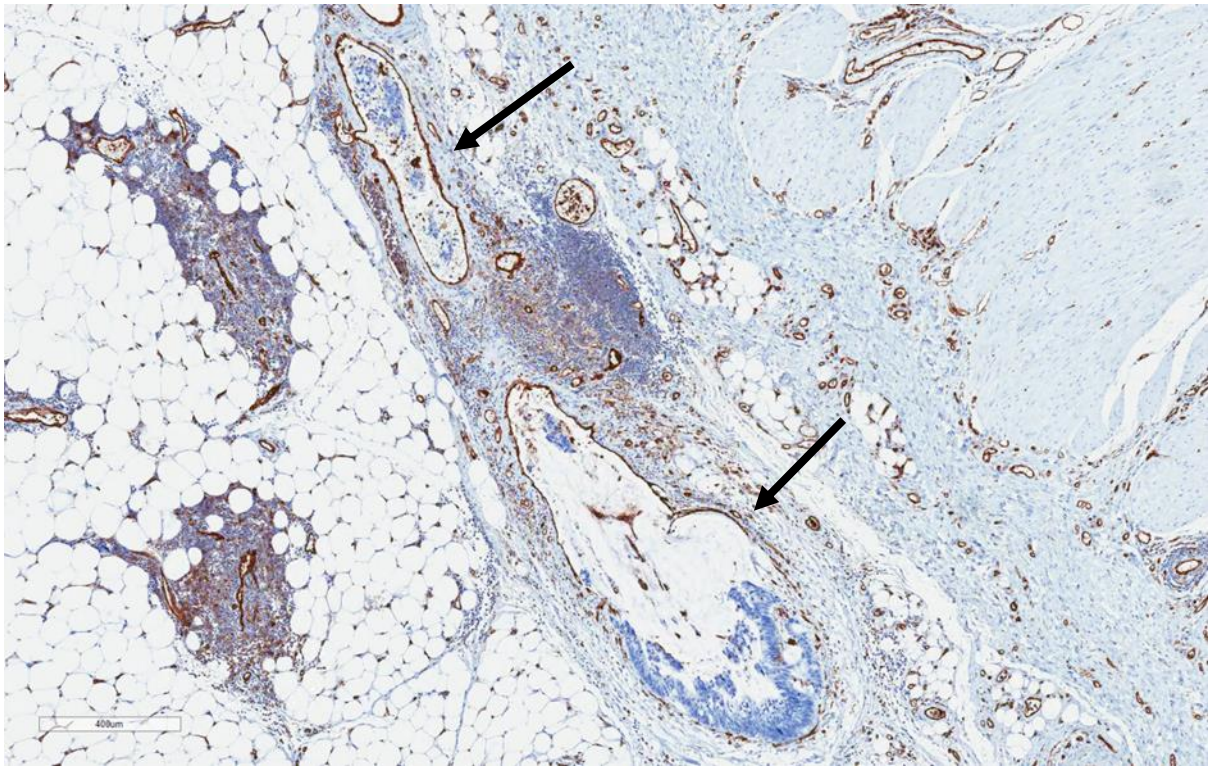
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C



D



Chapter 6

Patterns of disease recurrence in ypEMVI-positive rectal cancer

Aim

The aim of this study was to determine whether there was an association between mrEMVI and known adverse tumour characteristics such as pT stage, pN stage and pCRM status following pre-operative treatment with long-course CRT and curative surgery. Furthermore, by comparing the patterns of recurrence between these tumour sub-types, this may provide suggestions for surveillance in these high-risk patients.

6.1. Introduction

Risk-stratification of patients diagnosed with rectal cancer is undertaken using staging systems which rely primarily on three tumour characteristics – tumour penetration or spread through the bowel wall (T-stage); presence of nodal disease (N-stage); and the presence of metastatic disease (M-stage). This TNM staging system was primarily based on the histopathological findings of the resection specimen following surgery (Chapter 1.4). However in recent years, patients can be staged using similar criteria through imaging in the form of CT and MRI. The presence or absence of these features influences both neoadjuvant (if staged by imaging) and adjuvant treatment strategies (if staged by histopathology); and further estimates the risk of disease recurrence - histopathological staging following surgery is the primary determinant of adjuvant treatment decisions despite their being a lack of firm evidence regarding their prognostic value following CRT. MRI staging is more commonly used in neoadjuvant treatment decision making.

EMVI does not form part of the current staging systems but is recognised as a poor prognostic feature as described in earlier chapters. By definition, it is only found in locally advanced tumours as it is the presence of tumour cells in the veins beyond the bowel wall thus requiring tumour penetration to at least T3 stage. Therefore the majority of these tumours are often offered *neoadjuvant* treatment on the basis of increased tumour penetration regardless of the presence of other adverse features.

It is common for locally advanced tumours to exhibit more than one adverse feature but there may be a proportion of patients who will present with mrEMVI on initial staging without additional adverse features – these patients may, in fact, benefit from pre-operative treatment which is currently not the routine standard. Further and more importantly, there may be a similar proportion of patients who are also not being offered *adjuvant* treatment on the basis of pEMVI alone. This may be a relevant issue as distant progression of disease is commonly thought to be mitigated by systemic treatment with chemotherapy rather than local radiotherapy given pre-operatively.

EMVI has been shown to be associated with distant disease recurrence in Chapter 4. Tumour cells spreading through the surrounding venous channels may be part of the process and a precursor which leads to distant metastases. Presence of tumour cells in these veins as indicated by evidence of EMVI may place these patients at a higher risk of recurrence and thus be an indication for more intensive surveillance strategies or oncological treatment.

6.2. Methods

6.2.1. Patient selection

The patient selection and data collection is described in Chapter 3. This included staging characteristics, pathology staging and follow-up. Patients were grouped into 'positive' or 'negative' based on the initial mrEMVI status. Post-CRT histopathology data were compared between the groups.

6.2.2. Staging

The staging process is described in Chapter 3.

6.2.3. Primary outcome measure

The rate of recurrence was the primary endpoint and was compared between the two groups. In addition, the sites and timing of recurrence and association with ypT-stage, ypN-stage and ypCRM status were secondary measures.

6.2.4. Statistical analysis

Survival analysis was compared between the two groups. Differences between groups were assessed using the Chi-squared test or Fisher's Exact test as appropriate. Survival estimates for DFS were obtained using the Kaplan-Meier product limit method. Patients were censored at the last point of known contact or if they died during follow-up without experiencing the outcomes of interest.

6.3. Results

A total of 442 patients were included in the study. 370 patients were mrEMVI positive compared with 72 who were designated mrEMVI negative based on the initial baseline MRI scans.

6.3.1. Tumour characteristics on pathology

There was a statistically significant difference in the stage of presentation between mrEMVI positive and negative tumours. The majority of tumours which were initially mrEMVI positive were found to be ypT3 on pathology. This compared with the mrEMVI negative group where the tumours were more commonly ypT2. However there was no significant difference in those tumours which presented very late as ypT4 cancers. Less than 25% of patients in the mrEMVI positive group were given a final staging of ypT2 or less.

The final pathology nodal stage of both groups was not significantly different. The majority of patients in both groups were node-negative (stage II). This may be a reflection of success with regards the neo-adjuvant treatment. There was no difference in margin positivity between the groups or in evidence of lymphatic or perineural invasion. There were more mucinous tumours in the mrEMVI positive group and more tumour which showed evidence of lymphocytic infiltrate – $p<0.05$.

6.3.1. Timing and patterns of recurrence

The rate of recurrence was not significantly different between the two groups (25% versus 30%). However the timing of the recurrences were different. The vast majority (70%) occurred within the first two years in the mrEMVI positive group compared with less than 50% in the mrEMVI negative group. For mrEMVI negative tumours,

the majority of recurrences occurred beyond three years. In the mrEMVI positive group, recurrences were evenly spread between one, two and three years. This is shown in table 12.

	Recurrence	Year 1	Year 2	Year 3+
mrEMVI negative	18	3 (16.7%)	5 (27.8%)	10 (55.5%)
mrEMVI positive	110	35 (31.2%)	42 (38.2%)	33 (30.6%)

Table 12 – Timing of recurrence in mrEMVI positive and negative tumours

6.3.2. Site of recurrence

More than half of recurrences in the mrEMVI negative group were locoregional compared to less than1:6 in the EMVI positive group in which most were distant to the lung and liver with the latter accounting for more than 40% of disease relapse. This is shown in table 13.

Site of recurrence	**mrEMVI negative**	**mrEMVI positive**
Liver	3 (16.7%)	46 (41.8%)
Lung	5 (27.8%)	32 (29.1%)
Local	10 (55.5%)	18 (16.3%)
Other	0	14 (12.8%)

Table 13 – Patterns of recurrence in mrEMVI positive and negative tumours

Pathological characteristic		EMVI-positive (n=370)	EMVI-negative (n=72)	p-value
ypT-stage	0	33 (8.9%)	8 (11.1%)	
	1	11 (3.0%)	7 (9.7%)	
	2	53 (14.3%)	30 (41.7%)	
	3	234 (63.2%)	22 (31.4%)	
	4	22 (5.9%)	5 (6.9%)	p<0.001
ypN-stage	0	226 (61.1%)	52 (74.2%)	
	1	100 (2.7%)	13 (18.1%)	
	2	44 (11.9%)	7 (9.7%)	p<0.19
ypEMVI	Negative	280	62	
	Positive	90	10	0.07
ypCRM	Negative	348	66	
	Positive	22	6	0.62
Mucinous	No	330	39	
	Yes	40	33	<0.001
Lymphatic invasion	No	330	62	
	Yes	40	10	0.58
Perineural invasion	No	345	66	
	Yes	25	6	0.82
Lymphocytic infiltrate	No	266	42	
	Yes	104	31	0.02

Table 14 - Differences in the histopathological characteristics of mrEMVI positive and negative tumours

6.4. Discussion

The main finding of this study is that locally advanced rectal cancers that are staged as mrEMVI positive and subsequently undergo neoadjuvant CRT show earlier recurrence compared to mrEMVI negative tumours. Further, there is a preponderance to liver metastases when compared with mrEMVI negative tumours. They are also associated with a higher ypT-stage on final pathology. The difference in relapse patterns is most marked in the first year following surgery with the majority of recurrences for mrEMVI positive tumours occurring within 24 months. Consequently, 3-year DFS is significantly different between the two tumour sub-types indicating that mrEMVI positivity places patients in a high-risk category for disease recurrence.

The complex process which leads to disease recurrence is not yet understood. It is unlikely that one tumour characteristic will singularly influence metastatic spread but that the presence of specific prognostic factors will give an estimation of risk. A combination of factors and thus high-risk features may speed up the process by acting synergistically or it may be that one feature predominates over others. Regardless of this, it is not surprising to find locally advanced tumours exhibiting a number of high-risk features. That is, a tumour which has penetrated into the mesorectum is more likely to have EMVI as demonstrated in the results of this study. Interestingly, there were a number of patients who were found to be ypT2 or less in the mrEMVI positive group. By definition, EMVI should only be present in tumours which have breached the bowel wall (T3 and above). This shows that tumour regression may have taken place that has resulted in an initially T3 tumour being down-staged to T2 or less or that EMVI can exist in an irradiated T2 tumour. Either way, this is a new finding which needs further investigation in terms of prognostic relevance.

Whilst the post-operative treatment of high-risk tumours remains broadly similar for most patients, the results of this study can determine different approaches to chemotherapy in terms of timing (pre-operative versus post-operative) and duration of treatment. Furthermore, understanding the patterns of recurrence can influence surveillance timing and strategy. For example, if one particular feature has a specific target organ for metastases then it is possible to tailor surveillance accordingly to detect any disease at an early stage.

In this study, mrEMVI positive tumours have been shown to have a preponderance for liver metastases. These findings have been shown in earlier reports on EMVI which are summarised in Chapter 4. Veins are a known route of cancer spread. These provide a conduit to other organ systems and due to the anatomical venous drainage of the most of the rectum, the liver is often seen as a site of metastasis. Whilst the evidence for amending treatment strategies for EMVI is still being evaluated, there is an opportunity to tailor follow-up. The majority of recurrences occurred in the first two years which may mean that there are micro-metastases present at the time of initial treatment that just simply are not apparent on current imaging modalities. These patients have a significant risk of liver disease and it may be sensible to offer them increased surveillance with more frequent imaging of the liver. Early detection of liver metastases and subsequent prompt treatment is associated with good survival. A more standardised and consistent approach to follow-up is necessary to improve the outcome for metastases.

The most useful application of these findings is to predict prognosis before treatment and to determine treatment following CRT. There will be a proportion of patients who will have only EMVI as an adverse feature (early T3, node-negative tumours with a clear CRM). These tumours are still at risk of disease progression and should be

considered for adjuvant treatment. There appears to be variability in practise amongst clinicians with regard to adjuvant treatment and EMVI, particularly in node-negative or stage II disease (Bujko et al., 2010a). This point is addressed in subsequent chapters. It may be that patients with EMVI are only being treated with chemotherapy post-operatively because they display other high-risk features such as nodal disease or increased tumour depth. Whilst it is encouraging that these patients are being offered more intensive treatment, there still remains a proportion of patients who may be missing out on additional treatment.

The current 7th edition of the TNM staging system which is universally used in rectal cancer does not have a separate sub-stage for vascular invasion or EMVI (Table 4). The standard reporting proforma for pathologists in the UK and USA both have sections for EMVI although the USA guidelines combine vascular invasion with lymphatic invasion. Future versions of the TNM staging system may involve more tumour features such as EMVI but at present it is up to local units or national guidelines to remind clinicians of the increased risk of disease recurrence associated with EMVI.

A further interesting point from the results is that it appears that EMVI and lymph node status are independent. There is a current thinking amongst some pathologists that EMVI is a surrogate marker for lymph node status. These results do not agree with that premise. There is also the finding that mucinous tumours are more prevalent in the EMVI positive group. There is no clear explanation for this except for the fact that most EMVI positive tumours are more advanced. Mucinous tumours are aggressive and present with more advanced disease. However whether it is the more aggressive mucinous sub-type which leads to EMVI or vice versa is not known.

One major limitation of this study is the small number of patients in the mrEMVI negative group. With only 18 patients, there is the potential for a type II statistical error. Ideally, greater numbers of mrEMVI negative patients are needed to accurately describe the sites and timing of recurrences. The study is also retrospective which introduces further bias although the robust maintenance in updating the database does mitigate this bias somewhat. Follow up data is dependent on contact between the hospital and patient which means that patients may be lost to follow up in some cases.

In summary, mrEMVI positive tumours exhibit other high-risk features such as increased tumour penetration, nodal disease and CRM positive status following CRT and surgery. But there remains a proportion of patients who may have isolated mrEMVI with no other adverse features. These patients may benefit from intensive treatment as they are at risk of disease recurrence predominantly in the liver. Tailored surveillance regimes may be appropriate for the early detection of metastases.

Chapter 7

The importance of EMVI in the multidisciplinary management of rectal cancer – a survey amongst surgeons and oncologists

Aim

The aim of this study was to explore the variability between clinicians in treating patients with EMVI-positive, stage II rectal cancer. In addition, it attempted to determine to what extent the use of MRI and the per-operative identification of EMVI influences treatment decision-making.

7.1. Introduction

Treatment decisions for rectal cancer in the UK are made in the multidisciplinary team (MDT) meeting where clinicians from complementary specialities are able to discuss the optimal management strategies for individual patients. This affords clinicians the opportunity to put forward their case on treatment strategy and a decision is made through consensus. On the whole, decisions are made using the best available evidence from each discipline. Contentious issues which can lead to disagreement include the importance of specific tumour characteristics when deciding on more intensive treatment and secondly, which patients are most likely to benefit from additional treatment.

Adjuvant chemotherapy improves survival in patients with stage III rectal cancer (Fisher et al., 1988, Wolmark, 1988). Nodal disease, which distinguishes stage II from stage III disease, is known to be associated with worse survival and disease recurrence and is an independent marker of poor prognosis (Dukes and Bussey, 1958, Grinnell, 1942, Grinnell, 1950, Gilbertsen, 1960, Moossa et al., 1975). The evidence for the use of adjuvant chemotherapy in patients with stage II disease (node-negative)

is not so robust and current guidelines leave a lot of room for interpretation (Bujko et al., 2010a). In practice, the decisions patients make are often influenced by their clinicians' interpretations of the guidelines. There is currently no robust randomized trial evidence with regard to novel prognostic factors such as EMVI and the outcome of stage II rectal cancer, it is therefore not surprising that there is such variability in practise.

7.2. Methods

An electronic questionnaire was sent to a group of colorectal surgeons and oncologists. The cohort of surgeons comprised a limited membership of the Association of Coloproctology of Great Britain and Ireland (ACPGBI) who had previously given their consent to participate in such surveys. Their primary practise consisted of university and district general hospitals.

265 consultant surgeons were sent the questionnaire along with a covering letter explaining the aims of the survey and how these results may help in designing future studies. The same survey and letter were also electronically sent to a group of oncologists through the Pelican Cancer Research Foundation database - this is a national database of oncologists with a diverse practise of university and district general hospitals. A total of 99 oncologists were sent the survey. Two reminder emails were sent at 4 weeks and 12 weeks.

The survey featured nine questions including four specifically on clinical scenarios and treatment efficacy. Participants were asked to comment on the importance and detection of EMVI in their respective MDTs, their personal approach to patients with EMVI and clinical scenarios.

Responses were recorded for surgeons and oncologists as absolute numbers and percentages. These were compared using Fisher's exact test and Chi-squared where appropriate.

7.3. Results

A total of 263 surgeons were sent the electronic questionnaire of which 142 (54%) responded. This compared with 68 (69%) out of 99 oncologists who were also sent the survey. Less than 2% in each group did not answer all the questions on the survey.

7.3.1. EMVI as a treatment consideration

1.	*Is extramural venous invasion routinely commented upon in your MDT?*
2.	*Do you consider EMVI an important consideration when deciding whether your patients should have adjuvant chemotherapy?*
3.	*Should adverse features on staging MRI be considered when deciding on giving adjuvant chemotherapy?*
4.	*Would you be prepared to treat patients on MR detection of EMVI?*

Table 15 – Survey questions for EMVI

EMVI was commented on in the MDTs of all respondents either by pathology or radiology. 68.5% of surgeons would always consider EMVI when deciding treatment options whereas 30.1% would only do so in selected patients. There was a significant difference in the responses between surgeons and oncologists with surgeons being more likely to react to the presence of EMVI than oncologists - 52.9% were in favour of treating EMVI ($p<0.05$).

84.6% of surgeons and 76.5% of oncologists believed that MRI is an important consideration when deciding on treatment. When asked if mrEMVI could be used alone in treatment decision making, 35.6% of surgeons said they would and 54.8% would consider it in selected cases and 9.6% would not rely on mrEMVI alone. This

compared with the oncologists where only 13.7% prepared to treat patients on the basis of mrEMVI with a further 56.9% in selected circumstances. 29.4% would not treat patients on the basis of mrEMVI alone ($p>0.05$).

7.3.2. Clinical scenarios questions

5.	*A patient is given long-course chemoradiotherapy for malignant nodes seen on staging MRI. The final pathology shows R0 resection with a clear CRM/ node negative/ EMVI positive. Would you give adjuvant chemotherapy?*
6.	*Using the last example, what do you think the survival benefit is:*
7.	*A patient is given neo-adjuvant chemoradiotherapy for a T3c rectal cancer. There are no suspicious nodes or EMVI on staging MRI. The final pathology is T3N0M0, EMVI negative, CRM clear by 5mm. Would you give adjuvant chemotherapy?*
8.	*Using the last example, what do you think the survival benefit is?*

Table 16 – Survey questions

The majority of surgeons and oncologists were prepared to treat the patient in *Question 5* with adjuvant chemotherapy (71.2% of surgeons versus 68.6% of oncologists).

In *Question 7*, more than 88% of surgeons would not offer adjuvant chemotherapy to the patient described. 50% thought there was some benefit albeit less than 5% compared to 47.1% who thought there was no benefit whatsoever. There was a difference in the responses to the use of adjuvant chemotherapy compared to the oncologists who were more likely to offer adjuvant treatment in this case compared to the surgeons ($p<0.05$). For a patient with Stage II disease and no adverse features,

21.6% would still offer adjuvant therapy but the majority thought there was either less than 5% (54%) or no benefit at all (42%).

9.	*In the absence of an evidence base for a significant survival benefit in EMVI positive, node negative tumours would you be prepared to randomise such patients to adjuvant chemotherapy versus observation?*

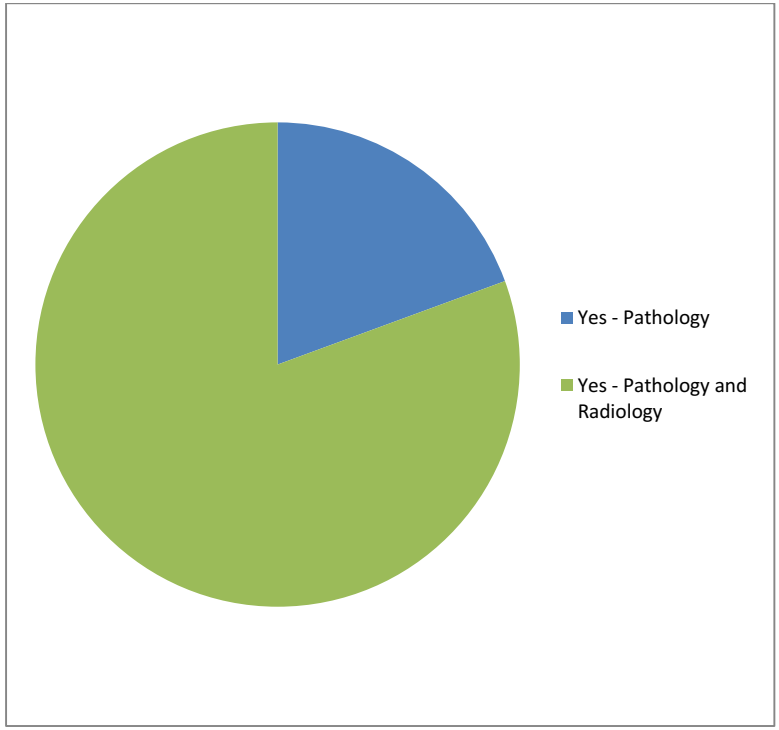
Table 17 – Survey question

77.5% of surgeons would support their patients being involved in such a trial and a further 5.6% would support this in patients over 70 years old. However, a significant percentage 16.9% would not be prepared to take part in such a trial. Interestingly, 68% of the oncologists would consider a randomized trial for Stage II patients with EMVI and only 14% would not. 18% would be prepared to involve patients over 70 years of age in the trial.

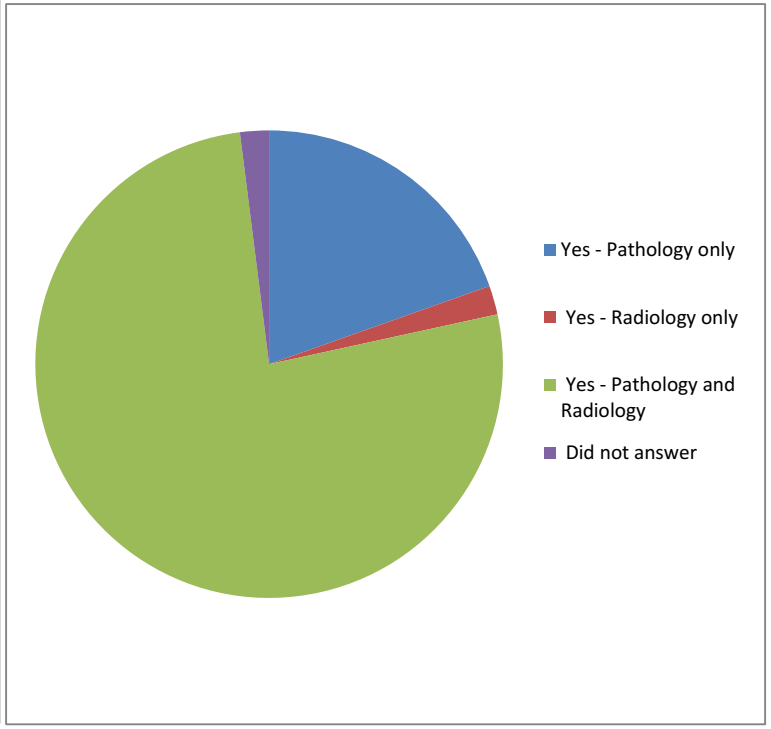
Figure 22a-h – Pie charts showing responses between surgeons and oncologists

Q1 - *Is extramural venous invasion routinely commented upon in your MDT?*

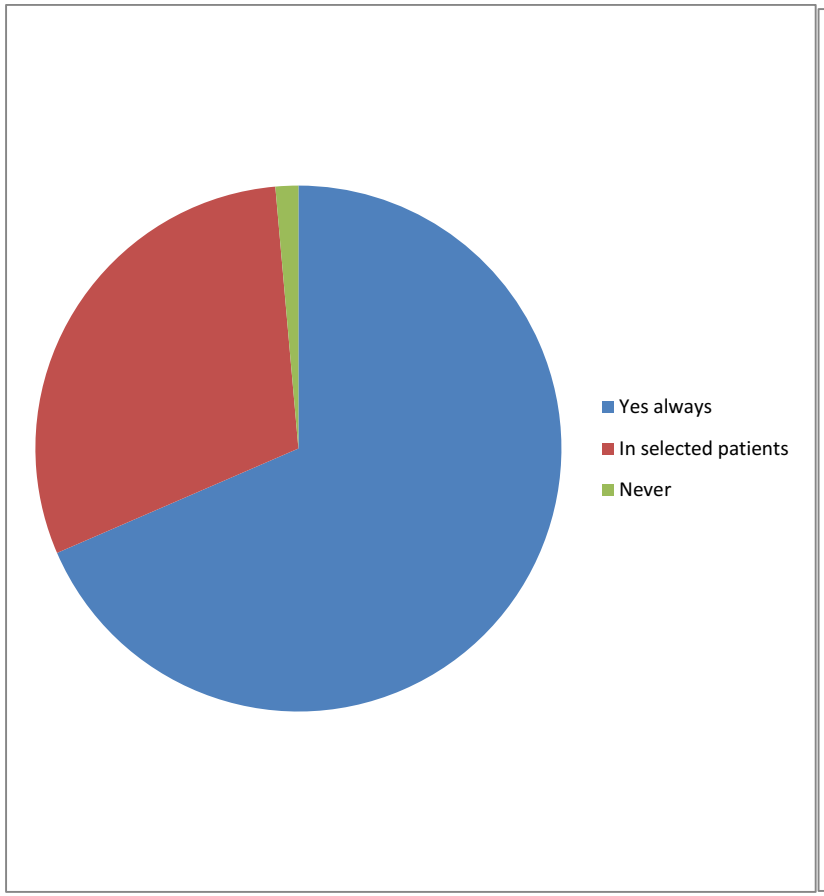
Surgeons

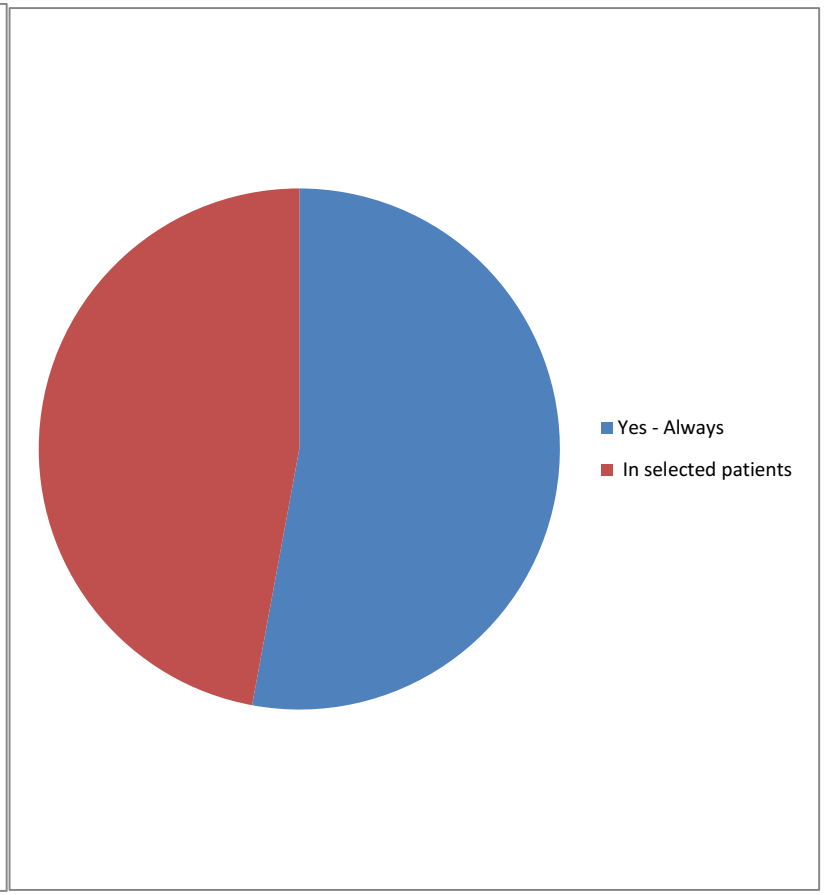


Oncologists

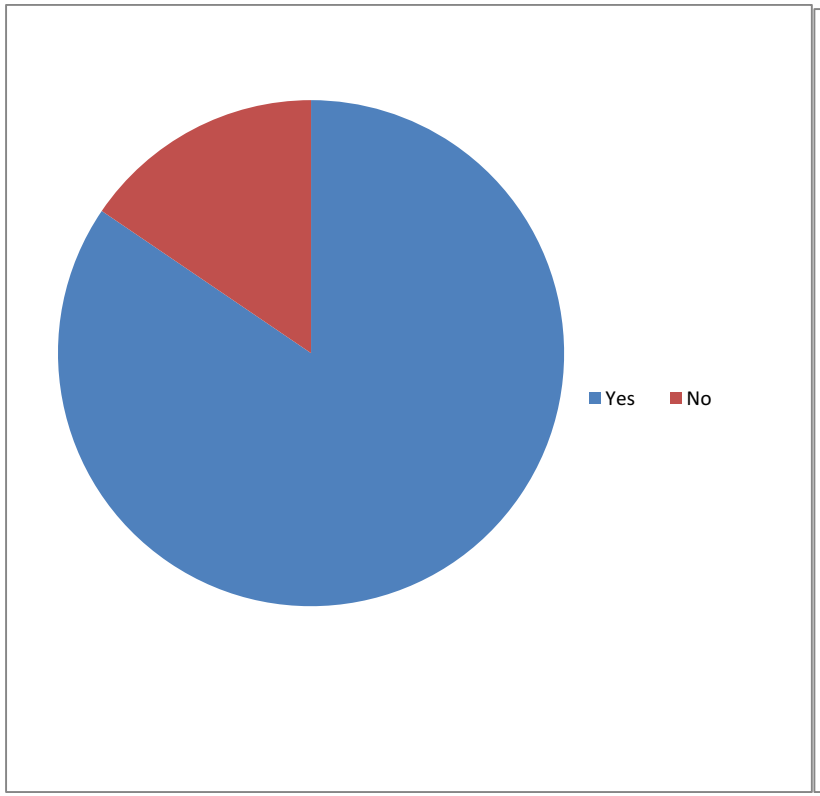


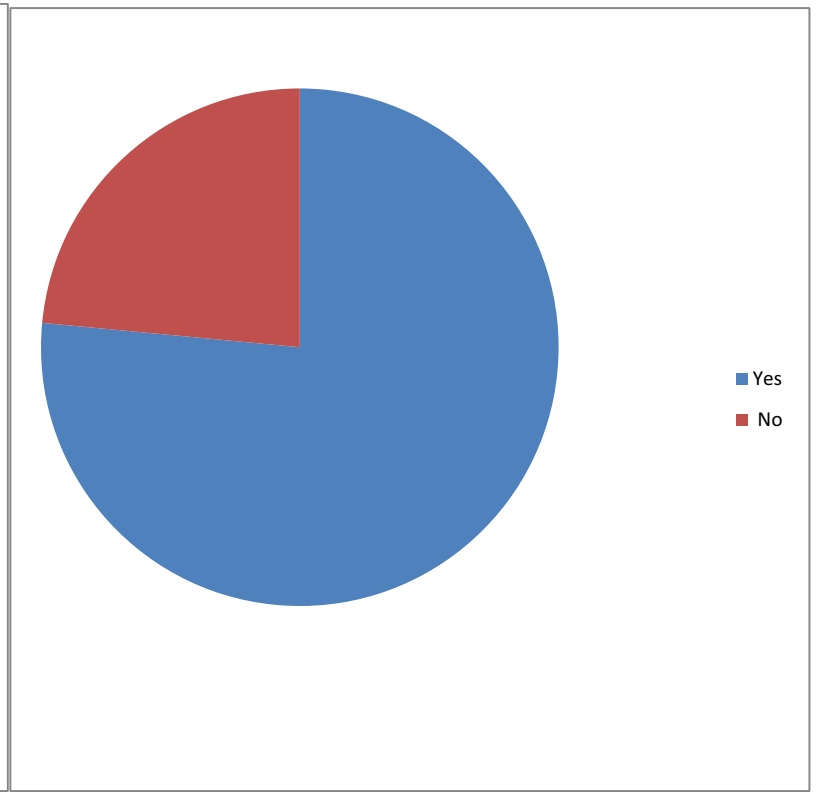
Q2 - *Do you consider EMVI an important consideration when deciding whether your patients should have adjuvant chemotherapy?*



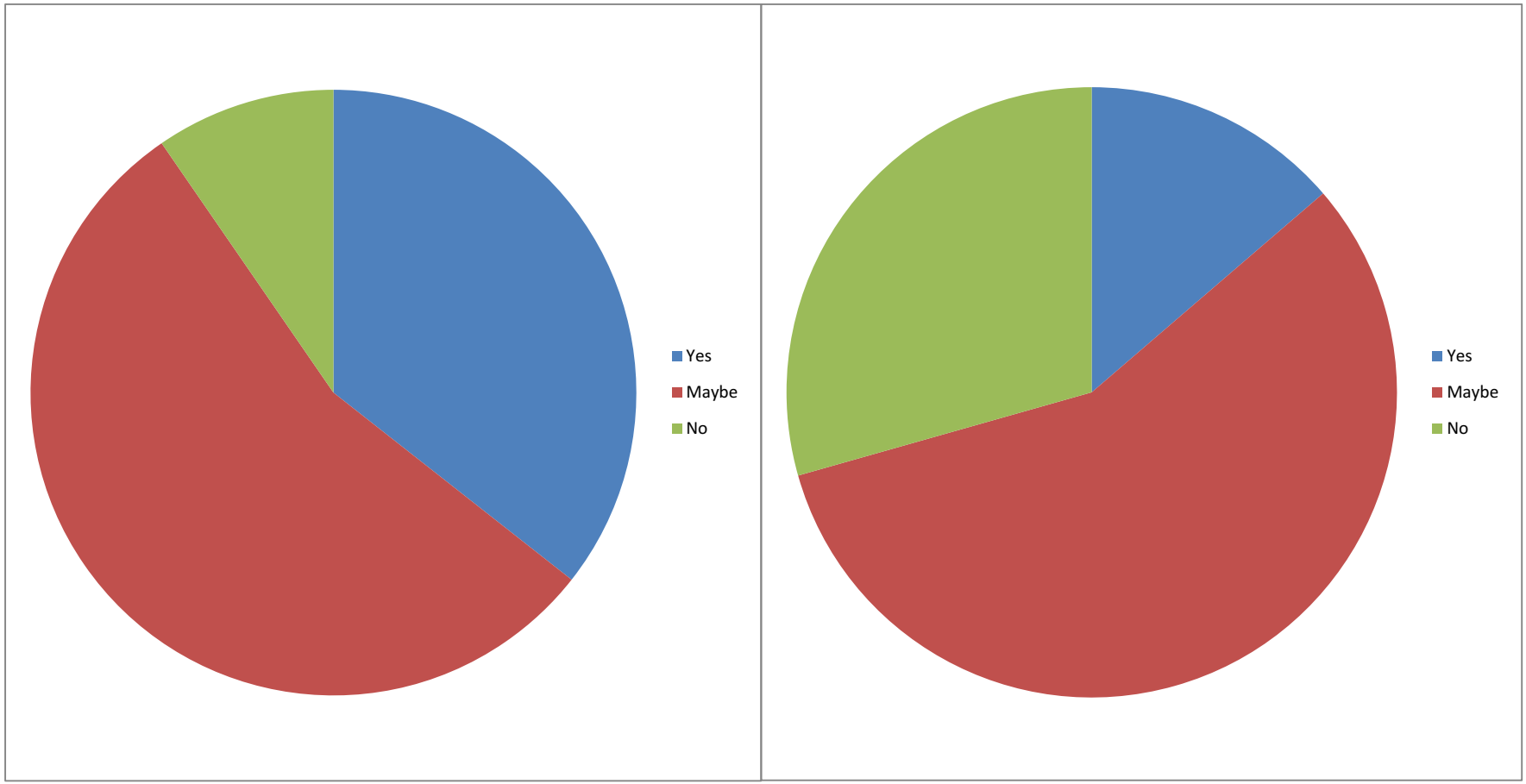


Q3 - *Should adverse features on staging MRI be considered when deciding on giving adjuvant chemotherapy?*

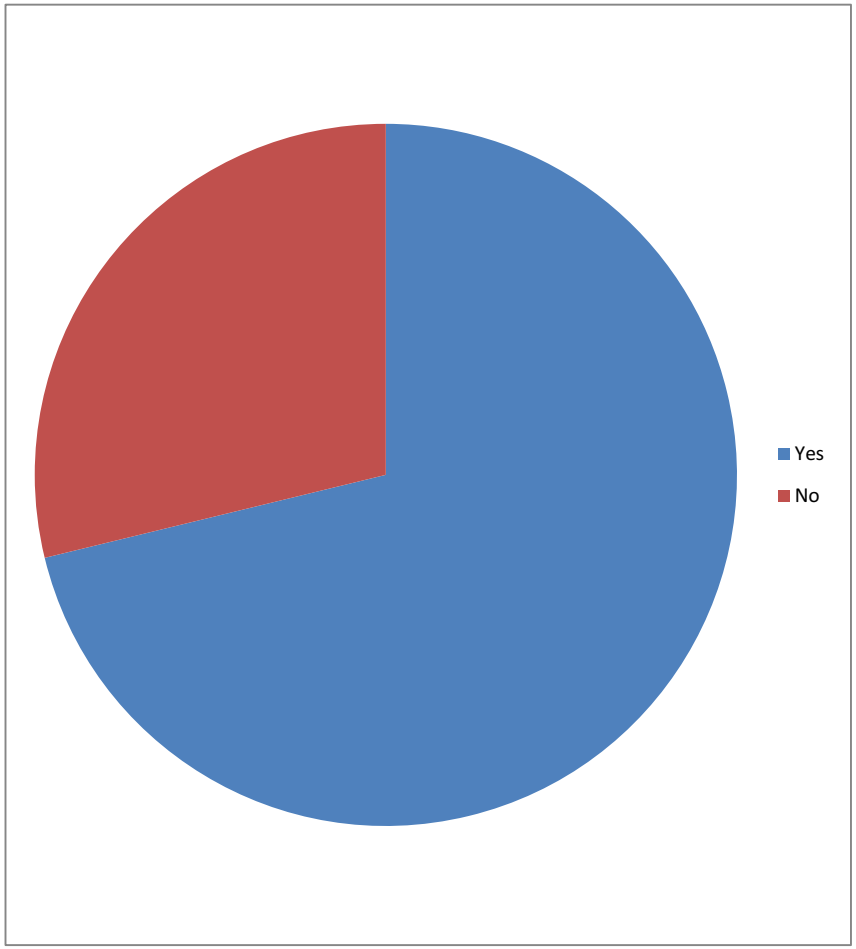


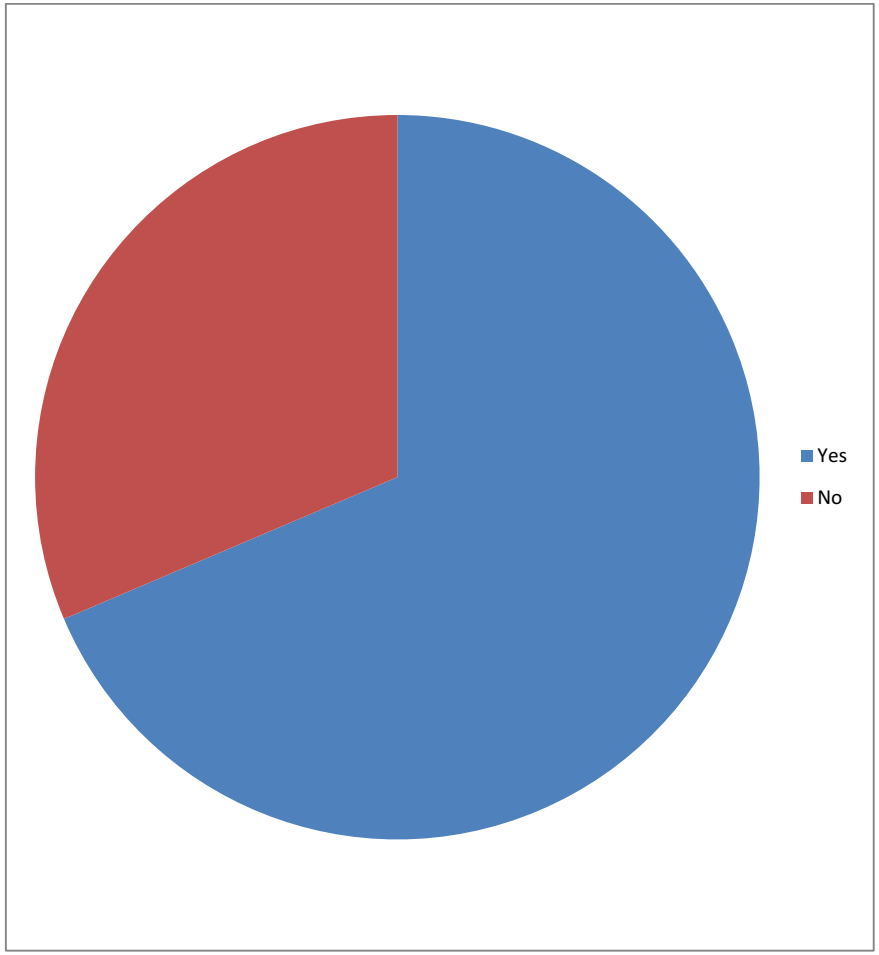


Q4 - *Would you be prepared to treat patients on MR detection of EMVI?*

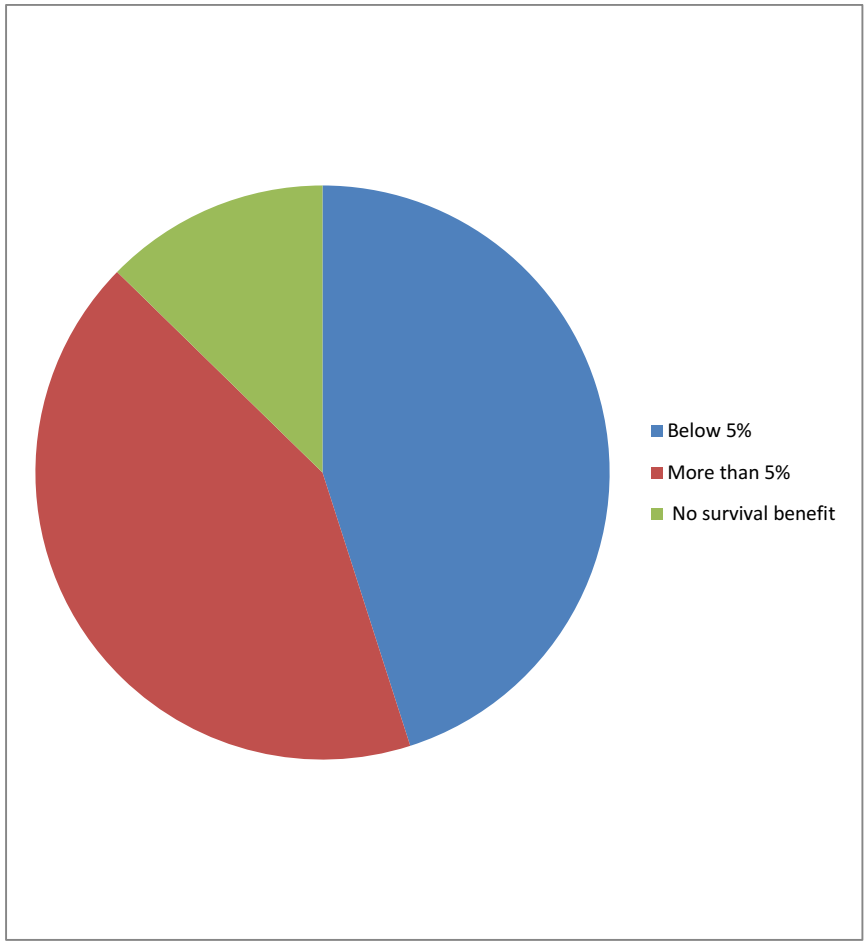


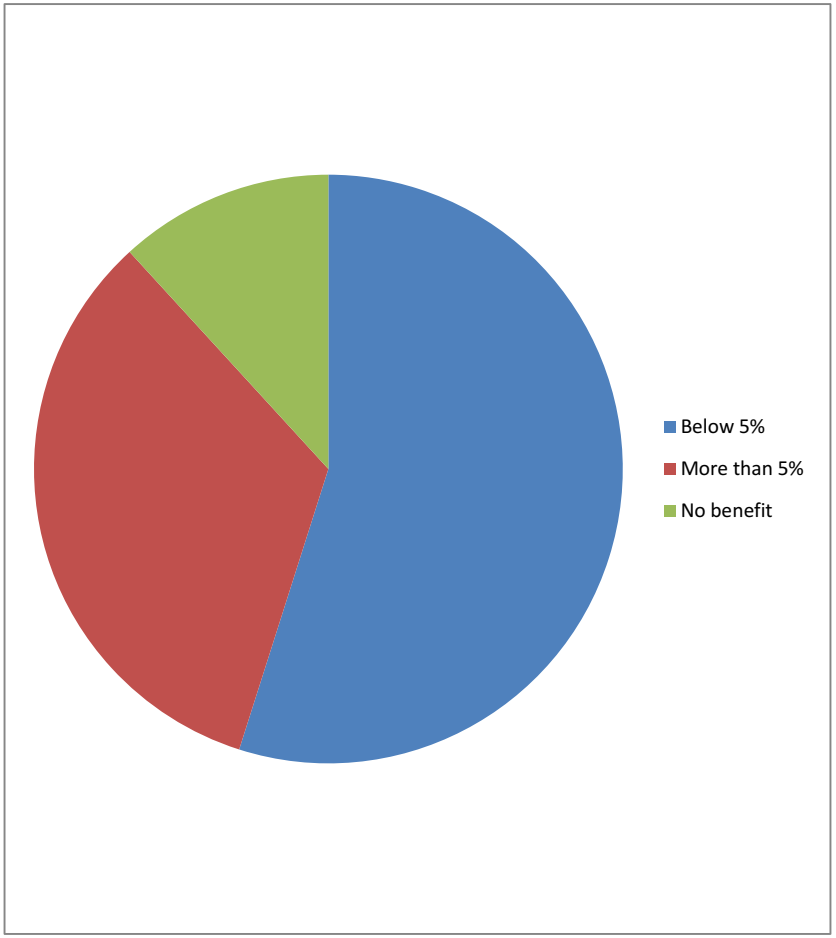
Q5 - *A patient is given long-course chemoradiotherapy for malignant nodes seen on staging MRI. The final pathology shows R0 resection with a clear CRM/ node negative/ EMVI positive. Would you give adjuvant chemotherapy?*



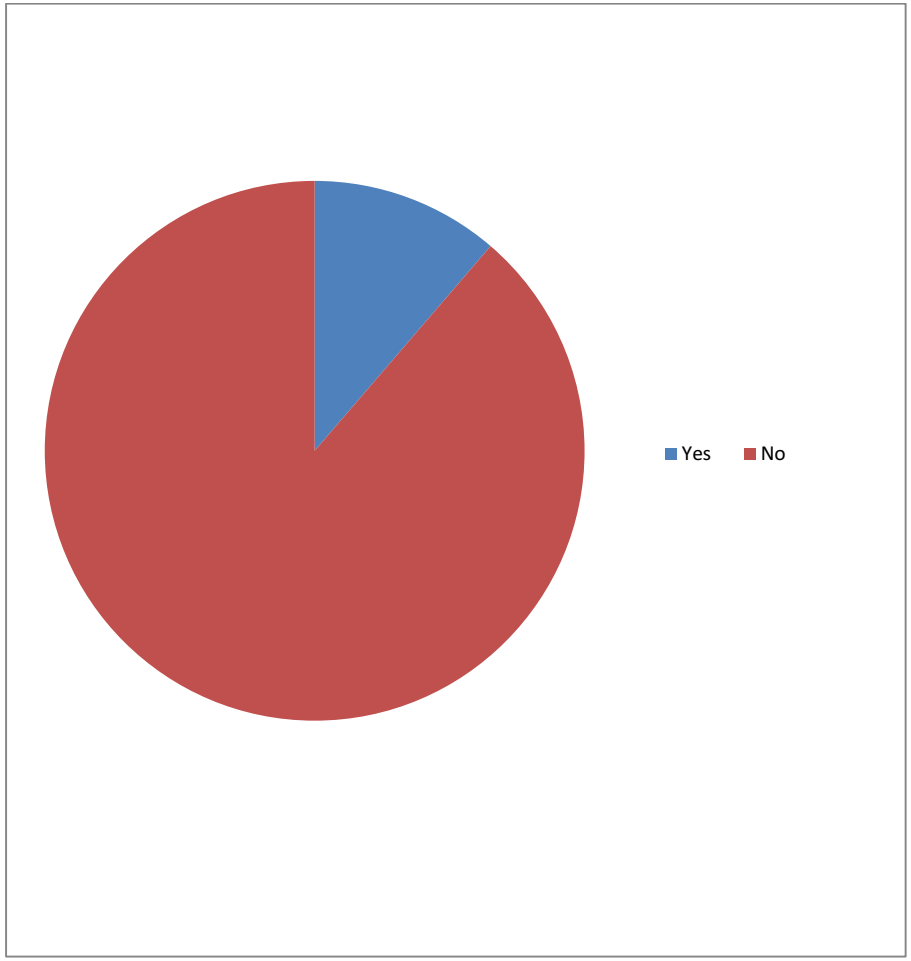


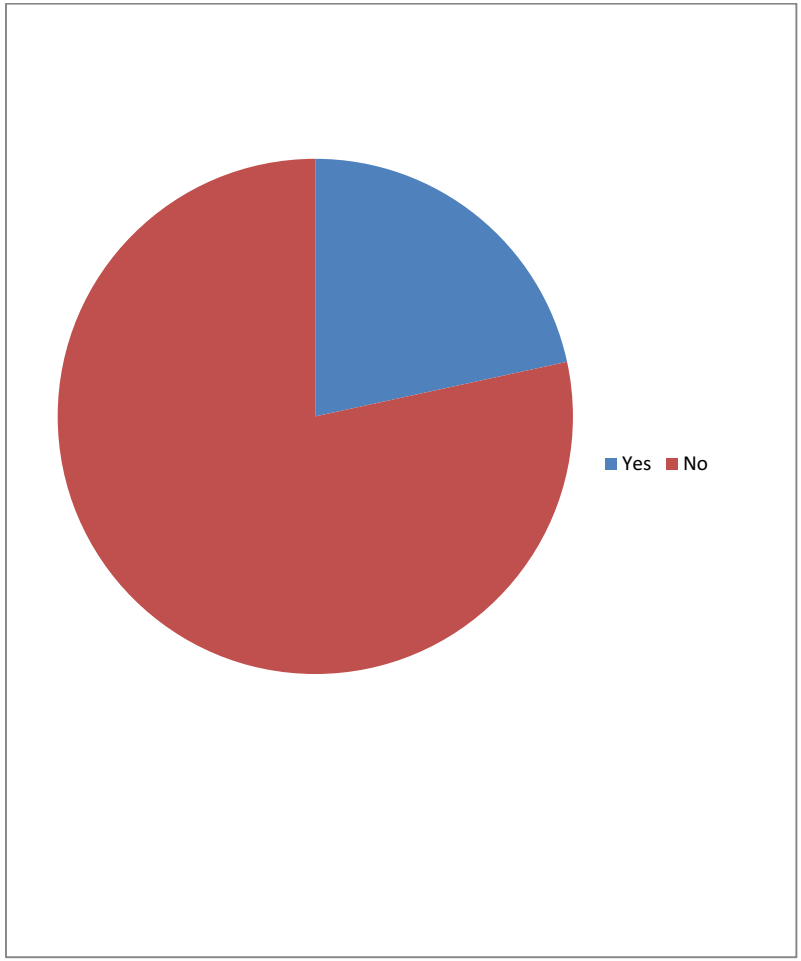
Q6 - *Using the last example, do you think the survival benefit is:*



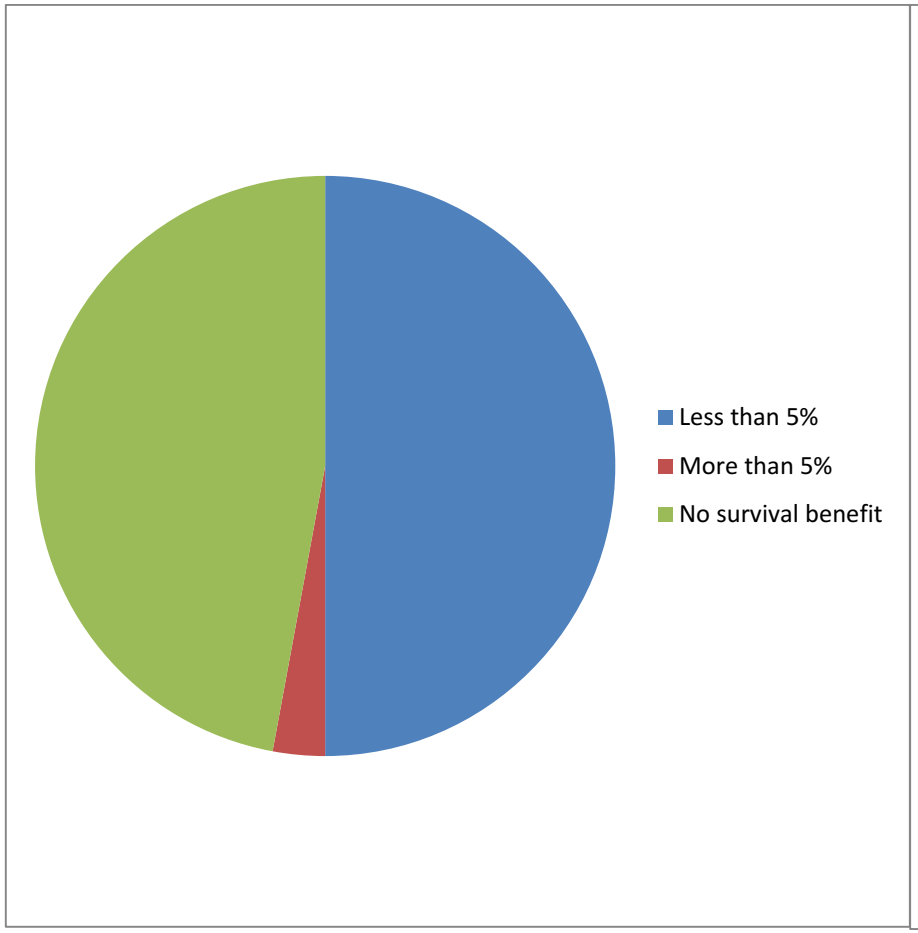


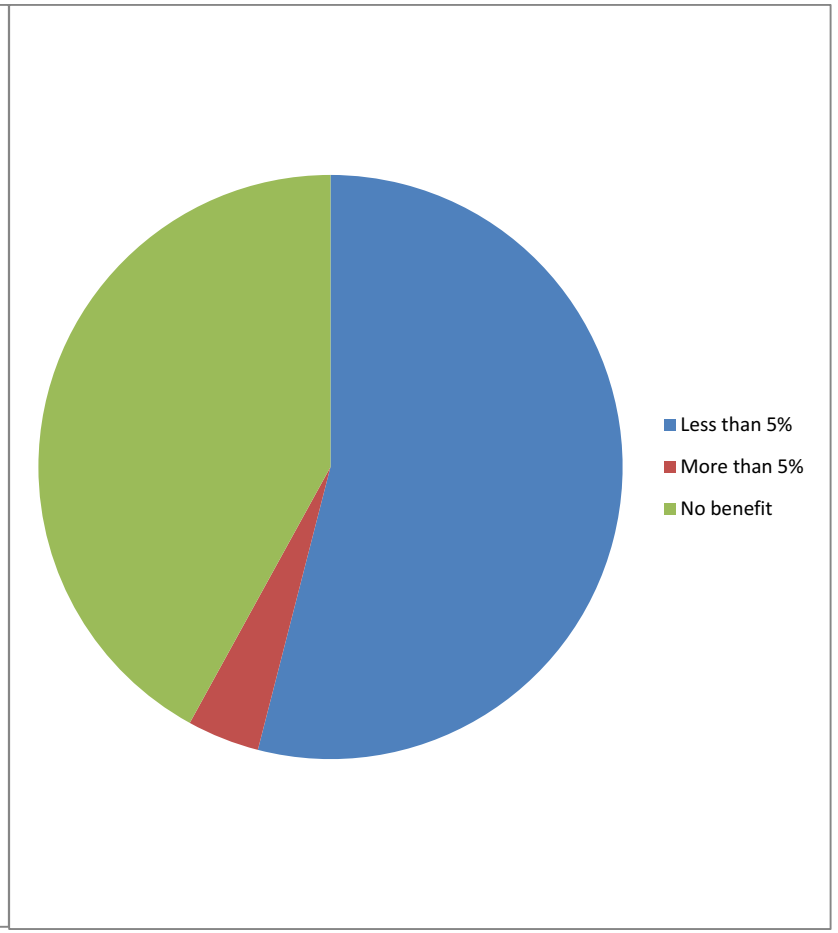
Q7 - *A patient is given neo-adjuvant chemoradiotherapy for a T3c rectal cancer. There are no suspicious nodes or EMVI on staging MRI. The final pathology is T3N0M0, EMVI negative, CRM clear by 5mm. Would you give adjuvant chemotherapy?*



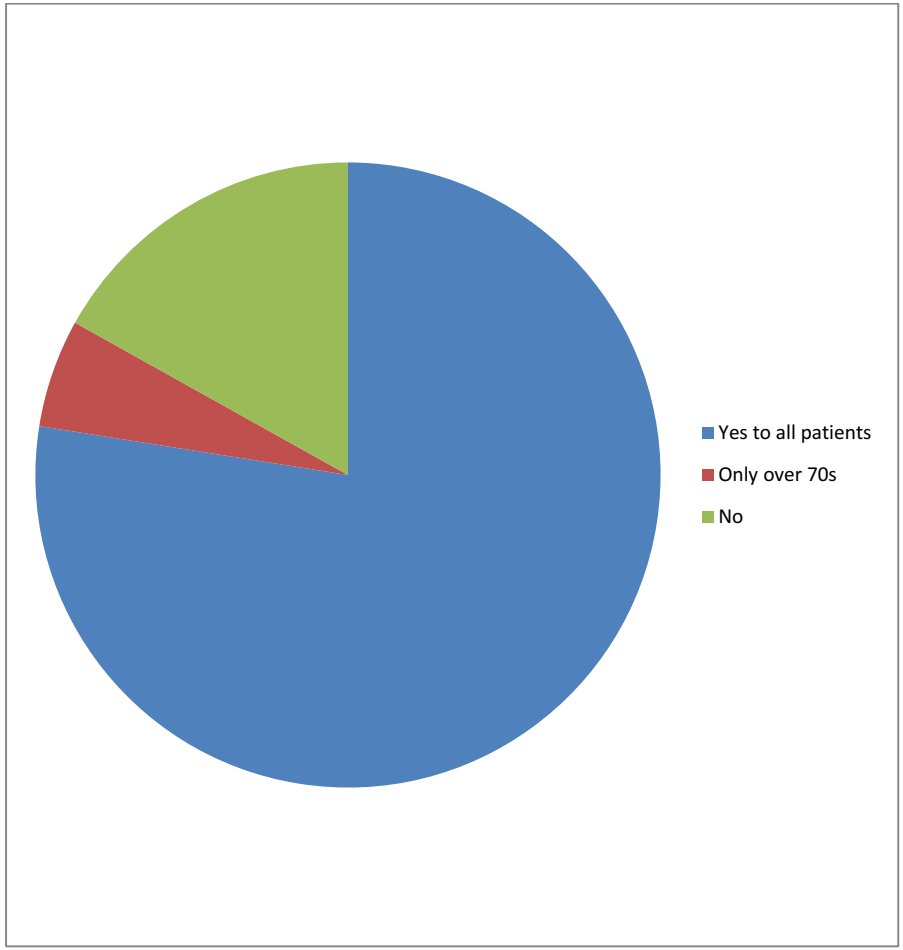


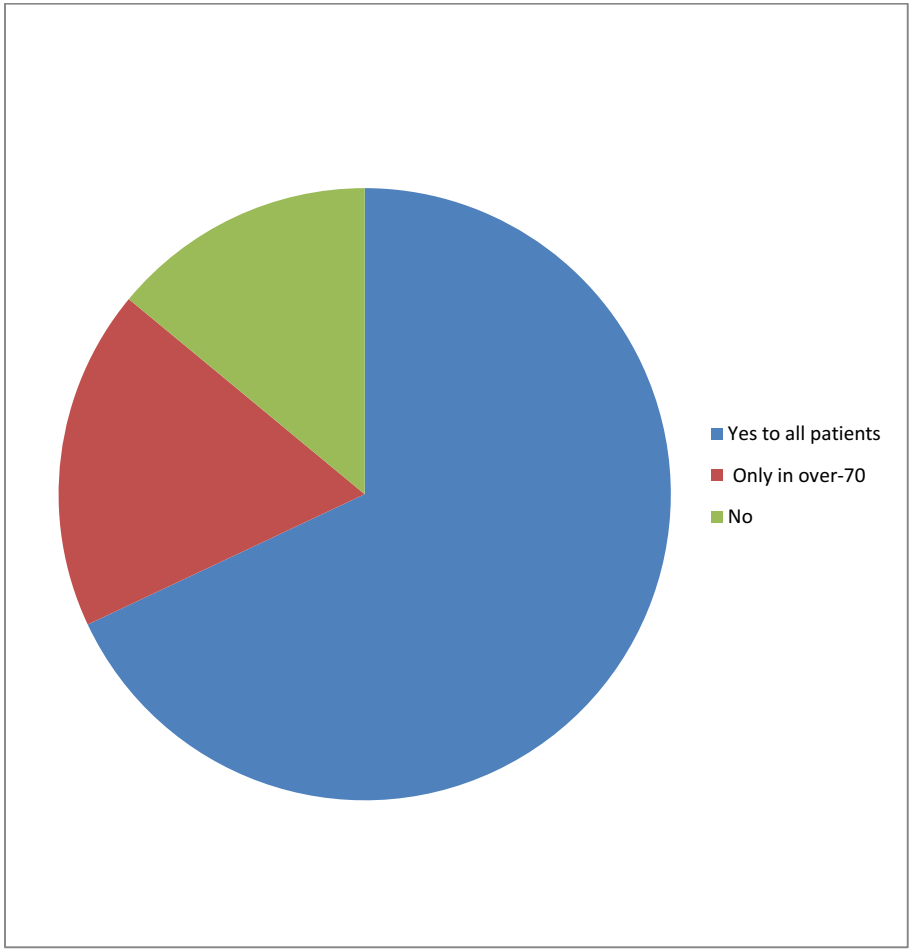
Q8 - *Using the last example, what do you think the survival benefit is?*





Q9 - *In the absence of an evidence base for a significant survival benefit in EMVI positive, node negative tumours, would you be prepared to randomise such patients to adjuvant chemotherapy versus observation?*





7.4. Discussion

The results of the survey show that EMVI is recognised by both surgeons and oncologists as a prognostic factor and discussed in almost all MDTs. Further, whether it was detected on pathology and/or radiology, it is a consideration in treatment decisions. However, oncologists seemed to be more selective in their approach to EMVI. Although this survey did not explore the relative importance of EMVI compared with other prognostic factors, it is notable that it is considered in overall management strategy. A further point highlighted is that most clinicians are now prepared to treat patients on the basis of MRI findings pre-operatively. This signals a significant shift in attitude towards risk-stratification in the last decade where MRI can now be confidently used to be more selective in which patients should be offered pre-operative treatment.

The standard of care for locally advanced high-risk rectal cancer without metastatic disease is neoadjuvant CRT (Sauer et al., 2004), this is described in detail in Chapter 1. Although there has been some recent debate surrounding the benefit of adjuvant chemotherapy in patients who have already undergone neo-adjuvant chemoradiation (Bujko et al., 2010b), it still forms the mainstay of treatment for systemic disease and the risk of metastases.

The QUASAR trial is the most frequently quoted with regard to adjuvant chemotherapy decisions for stage II tumours and the perceived survival benefit for patients is approximately 3.6% (Gray et al., 2007). Histopathological staging remains the basis for decision-making and it is important that stage II tumours which may potentially benefit from adjuvant therapy are identified through high-quality pathology reporting. The emphasis is very much on the pathologist diligently examining the specimen with appropriate sections to reveal as much information as possible. Quirke and colleagues

have extensively reported on the importance of this (Quirke and Morris, 2007, Maughan et al., 2007, Morris et al., 2007).

EMVI is arguably the most debated of these additional risk factors in deciding on oncological treatment. Yet there is little doubt that venous invasion has a prognostic influence but lack of consistent reporting in both pathology and radiology has made it difficult for clinicians to understand its exact clinical relevance. This lack of confidence in accurate detection of EMVI may be one of the reasons why it is not considered as a mandatory treatment factor.

In the present study, surgeons were more likely to rely on MRI than oncologists for decision-making (84.5% versus 76.5%) and specific to EMVI, oncologists were less likely to base treatment on mrEMVI. Despite this, the responses to the clinical scenarios of offering adjuvant chemotherapy to a patient with stage II rectal cancer with evidence of EMVI but no other adverse features and one with no adverse features whatsoever were similar (71.2% versus 68.6%). However whilst the surgeons were equally divided between the perceived survival benefit as to whether it was more or less than 5%, 54.9% of the oncologists thought there was < 5% benefit compared to 33.3% that thought it may be > 5%. The interesting point is that QUASAR states the benefit for patients in receiving adjuvant chemotherapy is 3.6%, but more than a third of surgeons and oncologists believe the benefit to be more than 5%. In the second scenario where there were no adverse features in Stage II disease, the surgeons were less likely to offer adjuvant chemotherapy (11.3%) which is consistent with the current literature compared with 21.6% of the oncologists who would offer adjuvant treatment with no obvious evidence base.

The limitations of this survey are that it did not include detailed explanations from the respondents in the rationale behind decision-making. As the results include a degree of subjectivity and not entirely based on trial evidence, it would have been interesting to understand the rationale behind some of the decision-making. It should also be noted that this questionnaire was sent to predominantly UK-based clinicians. The importance and understanding of EMVI as a prognostic factor is more apparent in the UK than other countries such as the US. Further, the central role of MRI and using it to guide treatment through risk-stratification is again more common in the UK where a more selective approach is taken in the use of pre-operative therapy. Furthermore, there is the possibility of selection bias in the present study. The database of surgeons and oncologists who were available to the survey and those who respond are more likely to do so if they are progressive in their treatment. Survey studies of all types suffer from an element of selection bias as the respondents represent a self-selected group. Lastly, the questionnaire itself was not validated in any formal manner and thus simply represents the important issues as thought by the authors of the study.

This survey reinforces the evolution in thinking with regards to adjuvant therapy in stage II disease. Factors such as EMVI should be given due consideration and the information we offer patients must be more accurate. We cannot continue to rely on historical studies which may, by no fault of design, have missed the importance of surgical technique and the emergence of novel prognostic factors which do not rely solely on pathology for identification. There appears to be an acknowledgement for a randomised controlled trial with regards to EMVI although it will be important to get the inclusion criteria and trial design correct. The effect of adjuvant chemotherapy in patients that have undergone neoadjuvant CRT and have also been down-staged must also be accounted for in any future trial design.

Chapter 8

The survival of patients with stage II rectal cancer and evidence of pEMVI following neoadjuvant chemoradiotherapy

Aim

The aim of this study was to investigate the survival of patients with stage II rectal cancer and to determine whether pEMVI status had an effect on disease recurrence.

8.1. Introduction

The complex process of treatment decision making and some of the difficult issues surrounding rectal cancer were described in the previous chapter. Staging, either pre-operatively or post-operatively (MRI versus pathology) provides a risk-stratification based on known prognostic factors, which influences the extent of any oncological treatment in addition to curative surgery. Patients with stage II disease comprise a heterogenous group with variable survival depending on the presence or absence of specific tumour features (Newland et al., 1994). Examples of these adverse features include penetration of more than 5mm into the mesorectum, peritoneal perforation, and the presence of EMVI. The influence of these prognostic factors following pre-operative therapy also remains unclear in the context of disease recurrence and the role of subsequent adjuvant chemotherapy (Bujko et al., 2010b).

Unlike patients with stage III, or lymph node positive disease, the survival benefits following the use of adjuvant chemotherapy remain unclear in patients with stage II rectal cancer. There is no specific randomized trial evidence for the benefit of adjuvant chemotherapy per stage in rectal cancer, particularly when additional adverse features are also present. Much of the guidance for the use of adjuvant chemotherapy in rectal

cancer is extrapolated from trials of colon cancer due to limited data. Furthermore, with the increasing use of neoadjuvant RT or CRT the benefits of adjuvant chemotherapy for all stages of rectal cancer is uncertain (Bujko et al., 2010b). Although there are no specific guidelines regarding the presence of EMVI, many clinicians consider EMVI when deciding on oncological treatment as seen in previous chapters. The heterogeneity in the existing trial data precludes any meaningful meta-analysis which would help resolve this issue and furthermore, prognostic factors such as EMVI have not been consistently documented in many of the historical trials.

8.2. Methods

8.2.1. Patients

Patients were identified from the prospectively maintained Royal Marsden Hospital Rectal Cancer database. Data were extracted on consecutive patients undergoing potentially curative resectional surgery for rectal cancer. Patients with synchronous tumours, undergoing local excision and those treated with palliative surgery were excluded. There was central review of all pathology and radiology by specialised GI pathologists and radiologists.

8.2.2. Staging

The staging process is described in Chapter 3.

8.2.3. Outcome measures

The primary outcome was 3-year disease-free survival from the date of surgery. The main secondary outcome was the rate of CRM positivity.

8.2.4. Definitions

Stages II disease was classified as T1-4, N0, M0 and stage III as T1-4, N1-2, M0. A positive resection margin was defined as tumour within 1mm of the circumferential resection margin. Adjuvant chemotherapy was offered to patients if they had pathologically positive nodal disease (i.e. stage III). DFS was the time from the date of surgery to the date of pelvic recurrence and/or distant disease or death due to pelvic recurrence and/or distant disease.

8.2.6. Statistical analysis

In order to assess the interaction of EMVI and nodal status, the following variables were defined which combined their positive and negative status respectively, resulting in four categories including EMVI-/N-, EMVI+/N-, EMVI-/N+, EMVI+/N+. The statistical analysis is described in Section 3.

8.3. Results

8.3.1. Demographics and treatment

A total of 478 patients were included in the study of which 168 were female (35.1%) and 310 were male (64.9%). The median age was 63.9 years (interquartile range 54.4-73.0 years). 331 (69.2%) patients had undergone neoadjuvant chemoradiotherapy with 373 (78.0%) having had sphincter-sparing surgery.

8.3.2. Histopathology staging characteristics

Only 41 (8.6%) patients had T0-2 disease compared with 437 patients (91.4%) who had either T3 or T4 tumours. 331 (69.2%) tumours were well or moderately differentiated and 147 (30.8%) were either poorly differentiated or had evidence of mucinous change or signet cells. CRM was negative in 437 (91.4%) tumours. Grouping the patients by stage, 233 (48.7%) patients had stage II disease and 245 (51.3%) had stage III.

8.3.3. Comparison of nodal and EMVI status

The prevalence of EMVI in the entire cohort was 34.9%; 57/233 stage II patients (24.5%) and 110/245 stage III patients (44.9%). Positive EMVI status was associated with neoadjuvant chemoradiotherapy, T-stage 3/4, positive CRM and nodal disease. When splitting EMVI status by presence of nodal disease (stage II or stage III), the significant differences included gender, the use of neoadjuvant chemoradiotherapy, T-stage and positive CRM. These can be seen in tables 18 and 19.

8.3.4. Survival analysis

At a median follow-up of 25 months (95% CI 1.5-36) there were 114 recurrences of which 30 (26.3%) were local recurrences. The 3 year DFS for stage II tumours was 76% and stage III tumours 62%. On univariate analysis CRM involvement was a

significant factor for reduced 3 year DFS (HR 1.92 95% CI 1.11-3.31) and was also found to be of borderline significance on multivariate analysis (HR 1.75 95% CI 1.00-3.06) – p-value 0.051. For the purposes of analysis, stage II tumours with no evidence of EMVI (EMVI-/N-) were used as reference to test the significance of EMVI and nodal status on disease recurrence. EMVI status was found to be significant on both univariate and multivariate analysis for node-negative and node-positive tumours. In the node-negative (stage II) tumours the HR was 1.95 (95% CI 1.04-3.65) on univariate and HR 2.08 (95% CI 1.10-3.95) on multivariate analysis. For node-positive (stage III) tumours HR was 2.65 (95% CI 1.63-4.30) on univariate and HR 2.74 (95% CI 1.66-4.52) on multivariate analysis. Nodal status was also found to be significant on both univariate and multivariate analysis – HR 1.71 (95% CI 1.03-2.85) and HR 1.79 (95% CI 1.04-3.07), respectively. These results are seen in table 20. The 3-year DFS EMVI and nodal status were EMVI-/N- 79% (72-86%); EMVI+/N- 59% (44-75%); EMVI-/N+ 63% (53-74%); EMVI+/N+ 50% (39-64%) (Figure 23).

Table 18 - Cohort demographics by the absence and presence of extra-mural venous invasion

		EMVI absent n=311	%	EMVI present n-167	%	Total	P-value
Age (years)	18-64	171	66.8	85	33.2	256	
	65-74	72	61.5	45	38.5	117	
	75+	68	64.8	37	35.2	105	0.612
Gender	Female	117	69.6	51	30.4	168	
	Male	194	62.6	116	37.4	310	0.122
Year of surgery	1999-2006	103	65.2	55	34.8	158	
	2007-2013	208	65.0	112	35.0	320	0.967
Neoadjuvant therapy	No	85	57.8	62	42.2	147	
	Yes	226	68.3	105	31.7	331	0.027
Sphincter preserving surgery	Yes	238	63.8	135	36.2	373	
	No	73	69.5	32	30.5	105	0.278
Pathological T-stage	T0-2	37	90.2	4	9.8	41	
	T3-4	274	62.7	163	37.3	437	<0.001
Differentiation	Well/moderate	217	65.6	114	34.4	331	
	Poor/signet/mucin	94	63.9	53	36.1	147	0.733
CRM	Negative	292	66.8	145	33.2	437	
	Positive	19	46.3	22	53.7	41	0.009
Nodal status	Negative	176	75.5	57	24.5	233	
	Positive	135	55.1	110	44.9	245	<0.001

Table 19 - Cohort demographics by the absence and presence of extra-mural venous invasion, split by lymph node status

		EMVI-/N- n=176	%	EMVI+/N- n=57	%	EMVI-/N+ n=135	%	EMVI+/N+ n=110	%	P-value
Age (years)	18-64	87	34.0	25	9.8	84	32.8	60	23.4	
	65-74	42	35.9	19	16.2	30	25.6	26	22.2	
	75+	47	44.8	13	12.4	21	20.0	24	22.9	0.091
Gender	Female	70	41.7	21	12.5	47	28.0	30	17.9	
	Male	106	34.2	36	11.6	88	28.4	80	25.8	0.033
Year of surgery	1999-2006	61	38.6	15	9.5	42	26.6	40	25.3	
	2007-2013	115	35.9	42	13.1	93	29.1	70	21.9	0.876
Neoadjuvant therapy	No	45	30.6	19	12.9	40	27.2	43	29.3	
	Yes	131	39.6	38	11.5	95	28.7	67	20.2	0.029
Sphincter preserving surgery	Yes	131	35.1	43	11.5	107	28.7	92	24.7	
	No	45	42.9	14	13.3	28	26.7	18	17.1	0.055
Pathological T-stage	T0-2	0	0.0	0	0.0	37	90.2	4	9.8	
	T3-4	176	40.3	57	13.0	98	22.4	106	24.3	<0.001
Differentiation	Well/moderate	127	38.4	41	12.4	90	27.2	73	22.1	
	Poor/signet/mucin	49	33.3	16	10.9	45	30.6	37	25.2	0.221
CRM	Negative	166	38.0	50	11.4	126	28.8	95	21.7	
	Positive	10	24.4	7	17.1	9	22.0	15	36.6	0.062

Table 20 - Univariable and fully adjusted multivariable Cox's proportional hazard models for 3 year disease free survival

		Univariable				Multivariable			
		HR	95% CI lower	95% CI upper	P value	HR	95% CI lower	95% CI upper	P value
Age (years)	18-64	Ref				Ref			
	65-74	0.97	0.62	1.50	0.873	0.92	0.58	1.44	0.701
	75+	0.83	0.51	1.35	0.452	0.91	0.55	1.51	0.716
Gender	Female	Ref				Ref			
	Male	1.45	0.96	2.19	0.076	1.37	0.90	2.08	0.145
Year of surgery	1999-2006	Ref				Ref			
	2007-2013	1.22	0.83	1.77	0.312	1.25	0.85	1.85	0.258
Neoadjuvant therapy	No	Ref				Ref			
	Yes	1.26	0.82	1.93	0.286	1.37	0.87	2.13	0.171
Sphincter preserving surgery	Yes	Ref				Ref			
	No	1.28	0.84	1.95	0.255	1.29	0.83	1.99	0.259
Pathological T-stage	T0-2	Ref				Ref			
	T3-4	1.00	0.51	1.98	0.996	1.13	0.54	2.36	0.748
Differentiation	Well/moderate	Ref				Ref			
	Poor/signet/mucin	1.09	0.74	1.60	0.677	1.09	0.73	1.63	0.661
CRM	Negative	Ref				Ref			
	Positive	1.92	1.11	3.31	0.019	1.75	1.00	3.06	0.051
EMVI/N	EMVI-/N-	Ref				Ref			
	EMVI+/N-	1.95	1.04	3.65	0.037	2.08	1.10	3.95	0.024
	EMVI-/N+	1.71	1.03	2.85	0.037	1.79	1.04	3.07	0.035
	EMVI+/N+	2.65	1.63	4.30	<0.001	2.74	1.66	4.52	<0.001

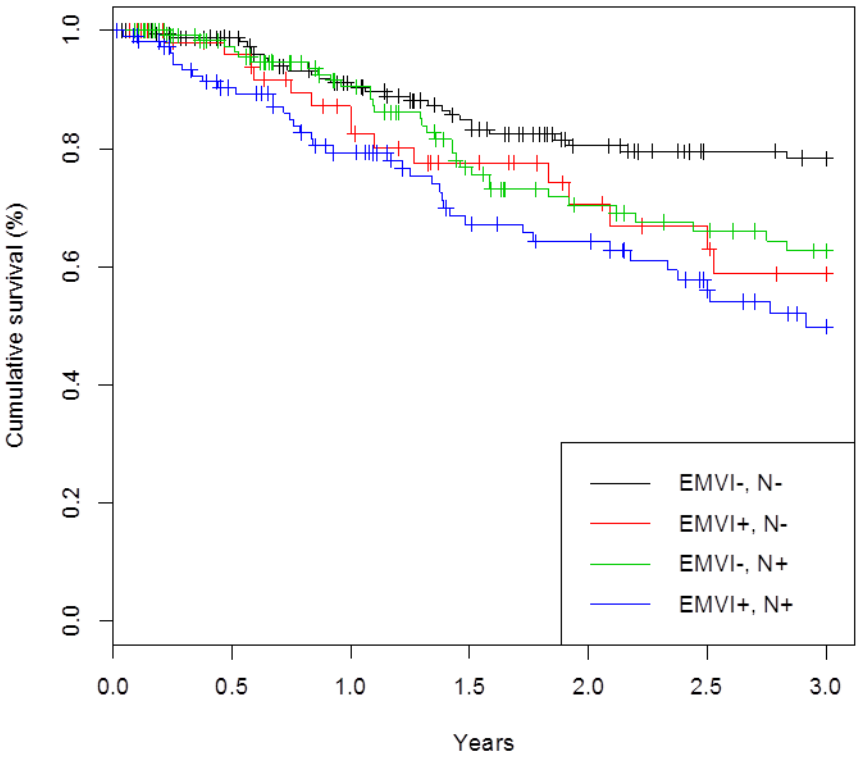


Figure 23 - Kaplan-Meier survival curve for 3-year disease free survival, stratified by EMVI and lymph node status

8.4. Discussion

The main finding of the present study is that patients with histopathological evidence of extramural venous invasion (ypEMVI) following CRT had worse 3-year disease-free survival (DFS) whether they had stage II or stage III rectal cancer. The prevalence of ypEMVI was 34.9%; stage II (24.5%) and stage III (44.9%), indicating a significant number of patients are at risk of developing metastatic disease within 3 years. The HR for ypEMVI, either alone or in combination with nodal involvement, was 2.08 and 2.74, respectively. There was an increased rate and risk of disease recurrence in ypEMVI positive patients independent of tumour stage. Notably, the 3-year DFS for patients with stage II disease and evidence of ypEMVI was similar to those who had stage III disease. It is important to note that the use of neoadjuvant treatment did not affect disease free survival which is consistent with previous reports (Sauer et al., 2004, Bosset et al., 2004). This highlights that pre-operative chemotherapy given as a sensitizer is not an adequate substitute for full-dose systemic chemotherapy.

As discussed in earlier chapters, the effect of neoadjuvant CRT leading to fibrosis and destruction of the vessel architecture can make accurate identification of EMVI even more difficult. Additional elastin stains have been recommended where there may be doubt however this practise was not undertaken in the present study. The prevalence of ypEMVI was 34.9% and specific to stage II tumours approximately 25% of cases. In our institution, EMVI is detected using guidelines offered by the Royal College of Pathologists which suggest EMVI prevalence to be a minimum of 25% (Williams, 2007a). In equivocal cases where there is suspicion of EMVI, a patient's MRI scans can perhaps be referred to as a guide prior to reviewing the tumour blocks.

The current policies for offering adjuvant chemotherapy following neoadjuvant CRT rely on histopathological identification of specific tumour characteristics known to be

associated with disease recurrence. Such policies are based on the large scale randomised trials showing a definite benefit for patients with Stage III colorectal cancer and a questionable benefit for Stage II disease (Gray et al., 2007, Bujko et al., 2010b), (James et al., 2003).

Further, the current guidance for rectal cancer is mostly derived from trials predominantly comprising colon cancers due to absence of high-quality data specific to rectal cancer trials. The observations from colon cancer trials are not necessarily translated to rectal cancer. The European Rectal Cancer Conference stated there is no evidence to support the use of adjuvant chemotherapy after preoperative chemoradiation (Valentini et al., 2009). In the QUASAR trial evaluating Stage II patients undergoing primary surgery, venous invasion was recorded in only 13% of patients – this is well below the detection rates reported currently and those recommended by the Royal College of Pathologists (Gray et al., 2007, Williams, 2007a). The trial showed that since patients in the Stage II category had DFS in excess of 80% at 3 years and chemotherapy benefit was only 3.6%, therefore adjuvant therapy should be recommended for stage II cancers with caution. Our findings and more recently published work, suggests that the true prevalence of EMVI if carefully sought using either MRI or pathology may be in the order of 25-50% (Petersen et al., 2002, Smith et al., 2008a, Roxburgh et al., 2010) and significantly greater than rates reported in the QUASAR trial. Furthermore disease free survival for EMVI positive stage II disease of 59% with a HR of 2.08 (95% CI 1.10-2.95) is substantially worse than the quoted rates and is equivalent to Stage III risk. This would suggest that the patient population evaluated in the QUASAR trial is dissimilar and therefore the proposed survival benefit of only 3.6% is unlikely to apply to patients with high-risk features such as EMVI. Further, that stage III patients have a worse outcome in terms of disease

recurrence if there is evidence of EMVI (HR 2.74 95% CI 1.66-4.52) may mean that those patients could also undergo more intensive follow-up.

The main limitation of this study is that adjuvant chemotherapy was excluded from the Cox regression analysis. It is our institution's policy to routinely offer all stage III patients combination chemotherapy with Capecitabine and Oxaliplatin. Stage II patients enter a discussion with the oncologist as to the pros and cons of further treatment based on existing published evidence. Another limitation is that this is a retrospective study. This introduces difficulty with accurate follow up and bias however the maintenance of the database described in earlier sections does well in trying to mitigate this as much as possible. Further investigation of these findings in the context of a prospective observational study would help to validate these results. Furthermore, this unit is a tertiary referral centre where the most complex and advanced cases of rectal cancer are dealt with. Thus one would expect the case mix to include more advanced disease and not necessarily represent the wider population. This may introduce further difficulty when trying to apply these findings more generally.

Currently, patients are informed that stage II disease confers a much lower risk of disease recurrence than stage III and further, that the benefit of adjuvant chemotherapy is less than 4%. This study has shown that ypEMVI status is an important independent prognostic factor for disease recurrence in both stage II and stage III disease affecting over a third of patients in these groups. The risk of disease recurrence in stage II disease is significantly increased if there is evidence of ypEMVI equivalent to node-positive (stage III) disease. Whilst the survival benefit of adjuvant chemotherapy is yet to be quantified in this context, when counselling patients following surgery for rectal cancer it is important to describe the greater than two-fold hazard ratio for developing distant or local failure

compared with negative ypEMVI status so they can make an informed decision with regards to the potential gains from systemic chemotherapy.

Chapter 9

A comparison of the prognostic effect of ypEMVI and ymrEMVI in rectal cancer

Aim

This study aimed to compare the survival between ymrEMVI and ypEMVI to determine whether they can be complimentary in prognosis based treatment decisions.

9.1. Introduction

EMVI status following pre-operative treatment is denoted ymrEMVI for MRI and ypEMVI forhistopathology. Whilst it has been accepted that pathology is the 'gold standard' for the final staging of rectal cancer – this concept was borne out of the study of patients undergoing primary surgery. The prognostic importance of EMVI following pre-operative CRT on imaging (ymrEMVI) or histopathology (ypEMVI) is not fully known. Further, the optimal technique in detecting EMVI following CRT is also not known and whether MRI and pathology can complement each other.

CRT results in specific morphological changes in the tumour complex which is most readily seen as fibrosis. This can be detected both on MRI and pathology. This change also leads, however, to architectural damage making it more difficult to identify staging characteristics, in particular EMVI. As there is no universal technique to guide pathologists when searching for EMVI in post-CRT specimens, detection may not always be optimal. The criteria for identifying EMVI on MRI is consistent whether it is sought before or after CRT.

9.2. Methods

9.2.1. Patients

The patient selection has been described in section 3. Briefly, patients were identified from the prospectively maintained Royal Marsden Hospital Rectal Cancer database. Data were extracted on consecutive patients undergoing potentially curative resectional surgery for rectal cancer with evidence of EMVI on baseline MRI. The term “EMVI” was sought from routine post-treatment radiology and pathology reports for rectal cancers.

Inclusion criteria were all adult patients that had undergone long-course chemoradiotherapy (LCRT) for locally advanced rectal cancer with no evidence of metastatic disease. Patients must have had evidence of mrEMVI on baseline staging MRI scan to be included. All patients must have undergone total mesorectal excision (TME) surgery. Exclusion criteria were patients with synchronous tumours; incomplete neo-adjuvant treatment; non-curative surgery and emergency presentation. There was central review of all pathology and radiology.

9.2.2. Pre-operative Staging

Patients underwent a minimum of two scans (before and after neo-adjuvant treatment) which were made available for review. If a patient had undergone more than one post-treatment MRI then the most recent MRI to subsequent surgery was used for the purposes of assessment. The MRI scans were reviewed independently blinded to the pathology reports.

9.2.3. MRI technique and image acquisition

This has been described in section 3.

9.2.4. Histopathology technique

The standard datasets for reporting colorectal cancers as issued by the Royal College of Pathologists (UK) were used for all examinations at our institution (Quirke and Morris, 2007). This is described in detail in section 3. All slides had been initially assessed by the local hospital before undergoing further review by our institution, blinded to the results of the pre- or post-treatment MRI.

9.2.5. Data collection

Data were collected on patient demographics, pre-operative staging (tumour depth – mrT-stage, nodal disease – mrN-stage, proximity to the circumferential resection margin (mrCRM) and mrEMVI status) and survival. Final tumour stage was documented from the pathology reports which included ypT-stage, ypN-stage, ypCRM involvement and ypEMVI status.

9.2.6. Outcome measure and statistical analysis

The primary outcome measure was three-year disease-free survival. Secondary endpoints included recurrence rates, final yT-stage, yN-stage and yCRM status.

Statistical analysis of differences between the groups was performed using Chi-squared or Fisher's exact test where appropriate. Survival analysis was undertaken between ymrEMVI and ypEMVI including rate of recurrence, time to recurrence and three year disease-free survival, which was obtained using the Kaplan-Meier product-limit and compared using the Mantel-Cox log-rank test. A p value of <0.05 was considered significant. A positive event was histology-proven recurrence (local, distant or both) or death from any cause.

Cox's proportional hazard models were built to test the impact of confounding variables on survival. This allows the effect of predictive factors on outcome to be assessed,

accounting for censored outcome, differing time of follow-up, and the interval between surgery and the adverse event of interest. Hazard ratios (HR) and 95% confidence intervals (CI) were generated.

9.3. Results

A database search revealed 188 patients who met the inclusion/exclusion criteria detailed above from a total of 580. Therefore the prevalence of EMVI at baseline MRI stage was 32.4%. Of the total number of patients, 121 were male and 67 were female. The median age was 74 +/-13 years (range 30-87 years).

9.3.1. Baseline staging

137/188 (73%) patients were staged as 'mrT-poor' (>mrT3c); 123/188 (65%) had MR nodal disease; and 81/188 (43%) were defined as potentially positive mrCRM. Therefore, the majority of the patients included in the analysis had locally advanced tumours. There were 69 low-rectal (37%); 62 mid-rectal (33%) and 57 upper-rectal (30%).

9.3.2. Post-treatment staging

At the end of treatment 5.3% of cases were pathologically CRM positive compared with 43% with threatened CRM on MRI prior to treatment. Patients were categorised into pathology and MRI detected EMVI negative or positive – ypEMVI and ymrEMVI. For each group, the final pT and pN staging was recorded from histopathological analysis of the surgical resection specimen. There were more advanced pT-stage and pN-stage cases when comparing pEMVI negative and positive patients – $p<0.05$. There was no statistical difference in CRM status between the patients with and without pEMVI but the overall prevalence of CRM involvement in the study was low. Comparison of the post-treatment staging characteristics showed similar results between mrEMVI negative and positive; there were more locally advanced pT-stage and pN-stage tumours – $p<0.05$.

9.3.3. Concordance between MRI and histopathology EMVI status

There were 99 patients (52.7%) that had evidence of persistent ymrEMVI following neoadjuvant CRT and 89 patients (47.3%) had become mrEMVI negative following treatment. Histopathology detected 36 cases (19.1%) of ypEMVI following CRT. Therefore, there may have been under-detection by histopathology or over-detection by MRI as there was a significant difference in detection rates of EMVI between the two techniques.

9.3.4. Recurrence rates

When comparing the recurrence rates of EMVI negative and positive patients whether by MRI or pathology, there was a statistically significant difference. Furthermore, the recurrence rates for ypEMVI and ymrEMVI negative patients of 23.9% and 25.8% respectively, were similar as was the case comparing ypEMVI and ymrEMVI positive groups 41.7% and 40.4%, respectively. Of the discrepant cases between MRI and histopathology, the sub-group in which patients were ymrEMVI positive but ypEMVI negative the recurrence rate was 39.4%. Patients who were mrEMVI negative on baseline staging had a recurrence rate of 22.2%. This is shown in table 22.

9.3.5. Survival analysis

The DFS for ymrEMVI positive patients was significantly worse than ymrEMVI negative patients - 42.7% (95% CI 16.8-68.6%) versus 79.2% (95% CI 70.0-88.4%); Mantel Cox log-rank test $p<0.05$. For those patients with ypEMVI there was also worsened DFS of 36.9% (95% CI 15.7-48.1%) compared with 65.9% (95% CI 56.1-75.7%) for ypEMVI negative; Mantel Cox log-rank test $p<0.05$. A comparison of MRI detected or pathology detected EMVI showed no significant difference between the groups. This was also shown to be the case comparing ymrEMVI and ypEMVI negative patients. In the sub-group of discrepant cases where patients were ymrEMVI positive but ypEMVI negative

DFS was 46.7% (95% CI 33.5-54.5%). A total of 27 patients were mrEMVI negative on baseline imaging and remained EMVI negative on MRI and histopathology after CRT. The DFS of this group of patients was 72.5% (95% CI 69.3%-77.1%), which was similar to those patients which became ymrEMVI negative from initially being mrEMVI positive ($p>0.05$). Survival curves are shown in figures 24 and 25.

Factors found to be significant on univariate analysis were tested further in multivariate regression – Table 23. Using the Cox proportional hazard regression model, mrEMVI +ve and pEMVI +ve were independently significant for predicting disease recurrence with hazard ratios of 1.97 (95% CI 1.01-3.90) and 2.39 (95% CI 1.11-5.14), respectively.

Staging characteristic		Post CRT Sub-groups			
		ypEMVI negative n=142	**ypEMVI positive n=46**	**ymrEMVI negative n=89**	**ymrEMVI positive n=99**
ypT-stage	PCR (T0N0)	12	0	11	3
	T1	7	1	6	1
	T2	31	2	20	13
	T3	83	36	48	72
	T4	7	9	4	10
ypN-stage	0	101	15	70	48
	1	37	20	17	39
	2	4	11	2	12
ypCRM status	Negative	136	42	87	91
	Positive	6	4	2	8
ypEMVI status	Negative	142	0	79	63
	Positive	0	46	10	36

Table 21 – Final staging characteristics following CRT and surgery

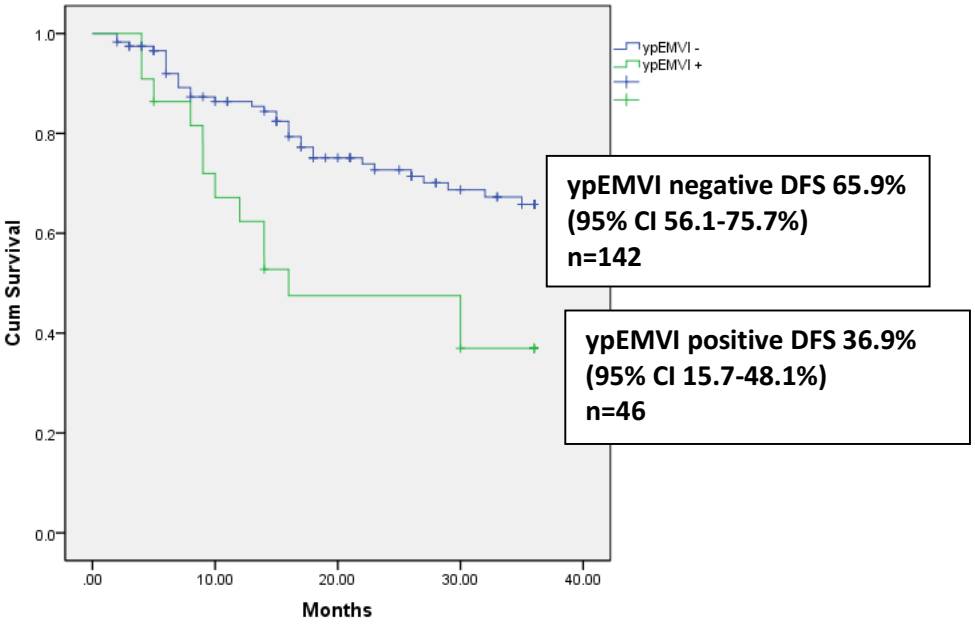
Group	Recurrences			p-value
	Local n=20	**Distant n=104**	**Total n=124**	
ypEMVI -	3	31	34/142 (23.9%)	
ypEMVI +	6	14	20/46 (43.5%)	<0.05
ymrEMVI -	3	21	23/89 (25.8%)	
ymrEMVI +	5	35	40/99 (40.4%)	<0.05
mrEMVI -	3	3	6/27 (22.2%)	

Table 22 – Recurrence rates of EMVI negative and positive tumours following CRT

Variables		Group	Patient numbers	Univariate analysis			Multivariate analysis		
				HR	95% CI	p-value	HR	95% CI	p-value
Patient characteristics	**Sex**	Female	67	Ref	0.625-1.912	0.756	Ref	0.53-1.68	0.832
		Male	121	1.093			0.93		
	Height	Upper/mid	119	Ref	0.815-2.298	0.235	Ref	0.80-2.68	0.223
		Low	69	1.369			1.46		
Baseline MR staging	**mrT stage**	Good	51	Ref	0.638-2.206	0.588	Ref	0.51-2.43	0.782
		Poor	137	1.187			1.12		
	mrN stage	Negative	65	Ref	0.691-2.071	0.523	Ref	0.90-3.28	0.199
		Positive	123	1.196			1.72		
	mrEMVI	Negative	0	Ref	0.527-1.544	0.706	Ref	0.42-1.89	0.078
		Positive	188	0.902			0.89		
	mrCRM	Negative	107	Ref	0.497-1.441	0.539	Ref	0.44-1.62	0.617
		Positive	81	0.846			0.85		
Post-CRT preoperative MR staging	**ymrT stage**	Good	116	Ref	0.723-2.052	0.459	Ref	0.54-1.89	0.984
		Poor	72	1.218			1.01		
	ymrN stage	Negative	104	Ref	0.701-1.982	0.534	Ref	0.21-0.91	0.206
		Positive	84	1.179			0.431		
	ymrEMVI	Negative	89	Ref	1.237-4.323	0.004	Ref	1.01-3.90	0.044
		Positive	99	1.987			1.97		
	ymrCRM	Clear	148	Ref	0.674-2.354	0.469	Ref	0.50-2.67	0.729
		Involved/threatened	40	1.26			1.16		
Final pathology staging	**ypT**	Good	54	Ref	0.695-1.279	0.534	Ref	0.11-8.62	0.994
		Poor	134	1.125			0.99		
	ypN	Negative	118	Ref	1.724-4.878	<0.001	Ref	0.91-12.82	0.069
		Positive	70	2.912			3.41		
	ypEMVI	Negative	142	Ref	2.088-6.281	<0.001	Ref	1.11-5.14	0.026
		Positive	46	3.889			2.39		
	ypCRM	Negative	178	Ref	1.421-7.907	0.006	Ref	1.24-2.38	0.032
		Positive	10	3.352			1.32		

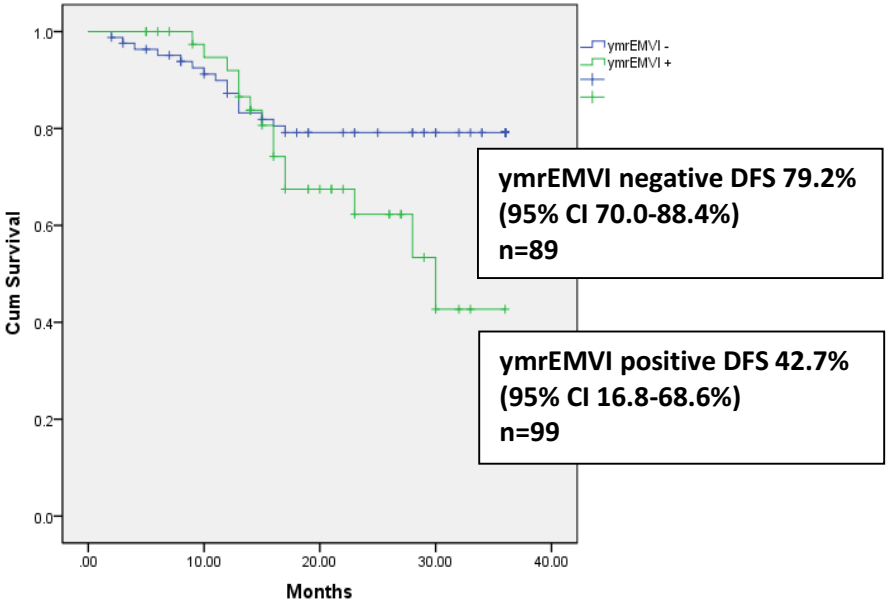
Table 23 – Univariate and multivariate analysis (Cox proportional Hazards for disease free survival) by clinical, preoperative MRI and postoperative histopathology characteristics

Figure 24 – Comparison of 3-year Disease-free Survival between ypEMVI -ve and ypEMVI +ve



	Recurrences	
ypEMVI -	34/142	23.9%
ypEMVI +	20/46	43.5%

Figure 25 – Comparison of survival outcome of 3-year DFS between ymrEMVI -ve and ymrEMVI +ve patients



	Recurrences	
ymrEMVI -	23/89	25.8%
ymrEMVI +	40/99	40.4%

9.4. Discussion

This is the first study to examine the independent clinical relevance of post CRT yEMVI status. The main finding of the present study was that disease-free survival was significantly reduced for patients who had either MRI or histopathological evidence of EMVI following pre-operative CRT, and that this was independent of yT and yN stage. DFS for ymrEMVI-positive patients was significantly worse than for ymrEMVI-negative patients. Equally, patients with ypEMVI had a DFS of 36.9% compared with 65.9% for ypEMVI-negative. The difference in survival between patients whohave converted from EMVI positive to negative on MRI or histopathology, versus those who showed persistent EMVI implies that this is of predictive as well as of prognostic relevance.

Pre-treatment EMVI status has been previously shown to be a prognostic factor (Smith et al., 2008a). In that study mrEMVI status was not shown to be an independent variable, only the initial T-stage was significant. The present study differs as we have only evaluated locally advanced tumours undergoing preoperative CRT. These were almost exclusively T3 and above hence EMVI status was independent of T stage in our series. The overall prevalence of EMVI in the present study at baseline was similar but following CRT, ymrEMVI was shown to be an independent variable for disease recurrence.

The importance of possible post-CRT prognostic factors and their role in disease recurrence is still not well understood and there is still no randomized trial evidence confirming the benefit of adjuvant chemotherapy after preoperative therapy (Bujko et al., 2010b). We have shown that disease relapse rates for persistent mrEMVI at three years are 40.4% with a HR of 1.97 compared with mrEMVI negative patients in whom relapse rates are 25.8%. This information may be of relevance when counselling

patients of the ongoing risks and potential gains of adjuvant chemotherapy after CRT in the absence of randomised trials that have specifically addressed this question. The present study suggests that patients could be risk-stratified before and after CRT based on their mrEMVI status and advised accordingly.

Examining the results of ymrEMVI and ypEMVI detection reveals a striking difference in detection. Of the initial 188 mrEMVI positive patients, 99 remained EMVI positive on MRI with only 36 (36.4%) being detected on histopathology. Significantly the prognosis for mrEMVI positive tumours that were pathologically ypEMVI negative was as poor and significantly worse than patients with no ymrEMVI or ypEMVI involvement. Furthermore both the survival and recurrence rates of patients with no evidence of mrEMVI on baseline staging prior to CRT were similar to those patients who had become EMVI negative following CRT. This finding would suggest that MRI is a better modality for detection of EMVI in patients who have undergone CRT than “routine” histopathology. Perhaps if these discrepant cases were re-examined with specialised stains specifically looking for EMVI, histopathology detection rates may be increased and similar to that of MRI.

CRT leads to fibrosis which can distort the normal micro-architecture which guides pathologists to identify venous invasion leading to a known false-negative rate during histopathology analysis (Liang et al., 2007, Ouchi et al., 1996). Whereas a post-CRT MRI clearly shows massive tumour extension into the extramural veins which can be followed anatomically along the line of the vessel, the cross sections taken for examination do not permit the pathologist to follow the vessels in the coronal or sagittal plane explaining difficulty pathologists have in detecting EMVI. CRT often destroys the features that must be present for histopathological detection yet the vascular anatomy

remains obvious on MRI. This is the most likely explanation of low detection rates by histopathology in the present study.

There may be some gain in histopathological examination by taking additional sections at deeper levels using specialised stains if necessary in cases where mrEMVI is suspected but not confirmed on pathology. Recent studies have reported the improved detection of EMVI when using an elastin stain (Kingston et al., 2007b, Inoue et al., 1992, Vass et al., 2004, Roxburgh et al., 2010). Furthermore, the actual processing and sectioning technique influences detection rates (Howlett et al., 2009). The Royal College of Pathologists has recommended that a minimum of four blocks from the tumour specimen should be evaluated to identify certain prognostic factors including EMVI. One study examined the potential to "miss" EMVI when too few blocks were examined and found that 41.3% of EMVI-positive tumours would be missed if only two blocks were examined. This compares with only 4% of cases being missed If five blocks are examined (Talbot et al., 1981). It should be borne in mind that pathologists only section a relatively small sample of the specimen (5x5 micron sections) whereas MRI has the ability to visualise the entire rectum with tumour in-situ.

There are several limitations of this study. Firstly, it is not universal practice for rectal cancers to be re-staged with MRI following CRT and thus there is a reluctance to rely on consequent MRI findings to guide treatment strategies. Secondly, the MRI scans were reviewed by a specialist GI radiologist and it may be argued that general radiologists may not have the same accuracy for detection of mrEMVI or ymrEMVI without specialised training. Assessment for MRI staging factors after treatment has, however, been shown to be reliable if a high resolution protocol and consistency in the use of reporting criteria are followed (2006). A further limitation is that this is a retrospective study but, the MRI scans were prospectively reviewed through a blinded

process and the data used was analysed from a prospectively kept computer database.

In summary, the pre and post CRT evaluation of EMVI is of prognostic and predictive relevance. The finding of persistent EMVI is more likely to be detected using high resolution MRI than conventional pathology and this has independent prognostic significance for disease free survival. Where MRI is used to restage patients after chemoradiotherapy, ymrEMVI information may be used to counsel patients regarding the ongoing risk of metastatic disease and potential effectiveness of adjuvant chemotherapy.

Chapter 10

The survival benefit of adjuvant chemotherapy of patients with persistent mrEMVI following neoadjuvant chemoradiotherapy for rectal cancer

Aim

The aim of this study was to determine if a survival advantage from adjuvant chemotherapy is observed in patients with persistent mrEMVI following CRT.

10.1. Introduction

The role of adjuvant chemotherapy in rectal cancer remains contentious as discussed in previous chapters. The evidence-base is simply lacking when compared to colon cancer. Furthermore, the survival benefit of adjuvant chemotherapy in patients who have already undergone CRT is even less clear. Briefly, although CRT leads to decreased local recurrence, no large-scale studies have shown an improvement in overall survival. Therefore, there is still be a role for adjuvant chemotherapy to continue to improve long-term survival but this may involve a change in strategy from clinicians in terms of timing and duration.

EMVI can be considered a 'high-risk' feature in rectal cancer and stage II tumours which demonstrate EMVI have been shown to have similar survival as stage III tumours following CRT (Chapter 8). Perhaps it may be prudent to consider less traditional tumour characteristics such as EMVI to guide treatment in the context of pre-operative chemoradiotherapy. That is, for patients who have undergone surgery after CRT, using the MRI scans as evidence of mrEMVI may be a novel method of deciding on whether to offer adjuvant chemotherapy.

10.2 Methods

10.2.1. Patients

Patients were identified from the Royal Marsden Hospital Rectal Cancer. Data were extracted on consecutive patients undergoing curative treatment for locally advanced rectal cancer. Treatment included long-course neoadjuvant CRT followed by surgery.

Adjuvant chemotherapy was given to selected patients based on the presence or absence of specific adverse or 'high-risk' features (nodal disease, increasing tumour penetration into the mesorectum, threat of the circumferential resection margin and in selected cases for EMVI). Patients with synchronous tumours, undergoing local excision and those treated with palliative surgery were excluded. There was central review of all pathology and radiology by specialised GI pathologists and radiologists, respectively.

10.2.2. Staging

The staging of patients is described in Chapter 3.

10.2.3. Outcome measures

The primary outcome was 3-year disease-free survival from the date of surgery. The main secondary outcome was recurrence rate.

10.2.4. Definitions

Performance status was defined according to the Eastern Cooperative Oncology Group (ECOG) classification and shown in table 24.

Classification	Description
0	Asymptomatic – fully able
1	Symptomatic but fully ambulatory; restricted in strenuous physical activity
2	Symptomatic; <50% time in bed
3	Symptomatic; >50% time in bed
4	Bedbound
5	Death

Table 24 – (ECOG) Classification of performance status

10.2.6. Statistical analysis

Analysis has been described in section 3. Briefly, differences between groups were assessed using the Chi-squared test or Fisher's Exact test as appropriate (if there were fewer than five patients in any one group, Fisher's test was used instead of Chi-squared). Survival estimates for DFS were obtained using the Kaplan-Meier product limit method. Patients were censored at the last point of known contact or if they died during follow-up without experiencing the outcomes of interest.

Cox's proportional hazard models were built to test the impact of confounding variables on survival (age, gender, performance status, pathological T-stage and N stage, CRM involvement). Hazard ratios (HR) and 95% confidence intervals (CI) were generated.

10.3 Results

10.3.1. Demographics and treatment

A total of 226 of 631 (35.8%) had evidence of persistent mrEMVI following CRT including 72 female and 154 male patients. The median age was 63.5 (IQR: 54.8- 72). 158 patients had undergone adjuvant chemotherapy with 68 being observed only following surgery. This is shown in table 25.

		Adjuvant chemotherapy N 158	Observation N 68	Total	P-value
Performance status	0-1	145	61	206	
	2-3	13	7	20	0.14
Gender	Female	44	28	72	
	Male	114	40	154	0.09
Pathological T-stage	T0-2	32	18	50	
	T3-4	126	50	176	0.34
Nodal status	Negative	109	48	157	
	Positive	49	20	69	0.95
CRM	Negative	149	63	212	
	Positive	9	5	14	0.76

Table 25 - Cohort characteristics split by the adjuvant chemotherapy

10.3.2. Histopathology staging characteristics

10.3.3. Comparison of adjuvant chemotherapy and observation groups

Patients were grouped into whether or not they had received adjuvant chemotherapy (adjuvant versus observation). Both groups were matched in terms of age, performance status and final pathological staging characteristics (T and N stage, and CRM status). The majority of patients in both groups were of performance status 0 or 1; and had locally advanced disease in terms of T-stage.

10.3.4. Survival analysis

At a median follow-up of 26 months (2-84) there were 68 recurrences of which 12 (17.6%) were local. The 3 year DFS rates for patients who received adjuvant chemotherapy was 74.6% and observation only 53.7% (Figure 26). (Mantel Cox Log Rank Test – p=0.02). On univariate analysis CRM involvement was a significant factor for reduced 3 year DFS (HR 2.564 95% CI 1.274-5.162) and but not on multivariate analysis. Pathological nodal status and performance status of 3 were found to be of borderline significance on univariate analysis (HR 1.587, 95% CI 0.983-2.562, p-value-0.06; HR 3.298, 95% CI 1.009-10.780, p-value-0.05) but not on multivariate analysis.

Months

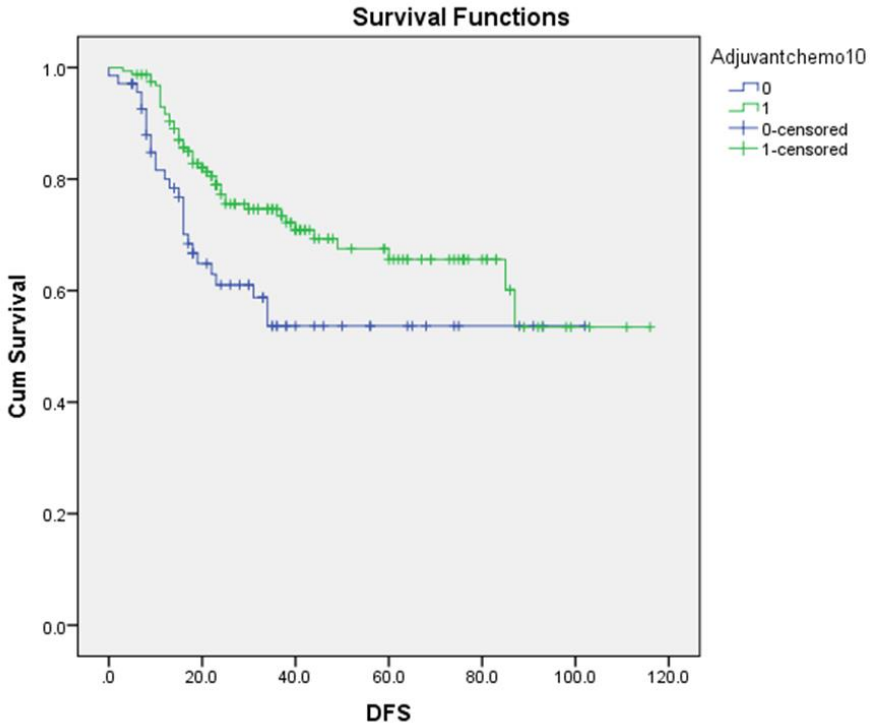


Figure 26 – Kaplan Meier curve comparing DFS between adjuvant chemotherapy group with observation-only group

For the purposes of analysis, patients who received adjuvant chemotherapy were used as reference to test the significance of chemotherapy on disease recurrence. Adjuvant chemotherapy had a survival benefit on both univariate and multivariate analysis (HR 0.569, 95% CI 0.352-0.919, p-value-0.021; HR 0.484, 95% CI 0.290-0.809, p-value =0.006). These results are seen in table 26.

		Univariable				Multivariable			
		HR	95% CI lower	95% CI upper	P value	HR	95% CI lower	95% CI upper	P value
Gender	Female	Ref				Ref			
	Male	.857	.531	1.384	0.528	.949	0.563	1.598	0.843
Pathological T-stage	T0	Ref				Ref			
	T1	1.258	0.141	11.266	0.837	1.633	.166	16.068	.674
	T2	0.607	0.152	2.427	0.480	.822	.183	3.683	.798
	T3	2.103	0.761	5.810	0.152	2.571	.771	8.577	.125
	T4	1.729	0.488	6.131	0.396	1.270	.276	5.847	.759
Pathological N-stage	Negative	Ref				Ref			
	Positive	1.587	.983	2.562	0.059	1.570	.921	2.677	.098
CRM	Negative	Ref				Ref			
	Positive	2.564	1.274	5.162	0.008	3.753	1.547	9.107	0.03
Performance status	0	Ref				Ref			
	1	.836	.509	1.373	.478	.860	.507	1.457	.574
	2	1.050	.439	2.511	.913	1.029	.421	2.512	.950
	3	3.298	1.009	10.780	.048	2.136	.609	7.497	.236
Adjuvant chemotherapy	No	Ref				Ref			
	Yes	0.569	.352	.919	0.021	.484	.290	.809	.006

Table 26 - Univariable and fully adjusted multivariable Cox's proportional hazard models for 3 year disease free survival

10.4 Discussion

The main finding of the present study is that patients who receive adjuvant chemotherapy following CRT and curative surgery have a decreased risk of developing disease recurrence over three years. This was independent of age and performance status in addition to nodal disease. The hazard ratio for disease recurrence for patients receiving chemotherapy was 0.48. They also had a significantly improved disease-free survival at 3 years compared to patients undergoing clinical follow-up alone (74.6% versus 53.7%). Although the majority of patients in the study had adjuvant chemotherapy, approximately 30% did not implying there is a significant number of patients who may benefit from additional treatment. It is notable that nodal disease was not an independent factor for disease recurrence in matched patients who have previously undergone neo-adjuvant treatment and oncologically successful surgery.

There remains a lack of consensus on the use of adjuvant chemotherapy following CRT. The current European and North American guidelines recommend that all patients with stage III and 'high-risk' stage II rectal cancers are offered adjuvant chemotherapy although this is not underpinned by a robust evidence base. However, the survival benefit of further treatment, with up to six months of 5-FU-based chemotherapy in patients who may have already had a significant response from pre-operative treatment, is unknown and further confuses the issue. In fact paradoxically, there is some suggestion that patients who have had minimal response from neoadjuvant treatment indeed show no survival benefit following adjuvant chemotherapy (Janjan et al., 2001, Das et al., 2006, Collette et al., 2007). This has led to a variability in practise amongst clinicians described in Chapter 6.

Age is most commonly cited as a reason for not recommending or offering adjuvant treatment (Schrag et al., 2001, Potosky et al., 2002, Ayanian et al., 2003) however there is good evidence to show comparable outcomes for elderly patients who are given such treatment in both colon and rectal cancer (Sargent et al., 2001, Popescu et al., 1999). With an increasing elderly population and a drive towards improving outcomes for this group of patients, we will no doubt see a rise in the number of elderly patients being offered adjuvant treatment in the future. Another interesting point is that co-morbidity or performance status, which are often used as reasons why eligible patients did not receive adjuvant chemotherapy (Abraham et al., 2006). However it is important to appreciate that patients have already been through both neo-adjuvant treatment and major surgery which means that unless it is the initial treatment that has led to a decrease in performance status, this factor becomes redundant.

The design of the present study and the subsequent analysis attempted to address some of these points which have previously explained the variability in practise with regards to adjuvant chemotherapy. EMVI can be considered to be a 'high-risk' factor and patients should be offered adjuvant treatment to reduce the risk of disease recurrence. The patients in the study were matched in terms of age, performance status and final staging which means the results of the multivariate analysis show a true independence for disease recurrence.

The difficulties with relying solely on histopathology to detect EMVI following CRT has been described in earlier sections. If mrEMVI can be reliably identified in a reproducible manner then it seems sensible to use if for decision making. Using radiological characteristics to guide and inform treatment decision-making is universally done so with regards to neoadjuvant treatment. Extending this to adjuvant treatment seems a natural progression particularly if there is difficulty in interpreting

routine pathological analysis following CRT and there is a potential for patients who may benefit from further treatment to be missed. In addition to this, the results would be known to clinicians before surgery thus helping plan for future treatment and inform patients at an earlier stage.

Limitations of the study include a lack of information regarding the decision-making process following surgery. Knowledge of whether treatment was not recommended or whether not offered would have given further understanding to any variability in practise. Also, there was no information regarding severity and extent of any operative complications. This is known to be a factor in delaying or withholding adjuvant treatment (Cheung et al., 2009) and may have played a role in this study. Understandably, if a patient has endured a challenging journey through the first phases of their treatment they are less likely to consider further therapy particularly if there is a risk of morbidity. Further limitations include that this is a retrospective study and which can impact on the accuracy of the follow-up data. The database at the Royal Marsden from which the data is extracted is comprehensive across all hospital specialties and is prospectively maintained but nevertheless there is still the potential for inaccurate follow-up data. This is not a randomized study which also means that the results are subject to bias. There was no sample size calculation so the results can suffer from a type 2 error. The numbers are small and it would be interesting to develop a randomized study using expected differences in three year DFS.

Chapter 11

Extramural venous invasion (EMVI) is a potential imaging predictive biomarker of neoadjuvant treatment in rectal cancer

Aim

This study aimed to demonstrate that improvement in the degree of MRI-detected EMVI following CRT results in improved survival. Further, by relating the radiological improvement in EMVI to better outcomes in terms of disease recurrence and time to recurrence this would suggest that mrEMVI could be used as a predictive imaging biomarker.

11.1. Introduction

The challenges associated with solely relying on histopathology techniques to detect EMVI have been discussed in some detail in the preceding chapters. The pertinent issues include direct difficulties in interpreting the resection specimens, particularly following CRT and if only simple stains are used. Other issues include the delay in diagnosis – histopathological analysis is performed following surgery. And further, the results of the histopathological analysis do not provide a stratified measure of the severity of EMVI. Some of the advantages of using MRI to detect EMVI have also been presented in preceding chapters. These include timing of detection, grading the severity and being able to demonstrate vascular anatomy in vivo and thus tumour invasion can be readily identified.

EMVI may play a role in pre-operative risk-stratification and further, influence decisions with regard to adjuvant chemotherapy or more intensive neoadjuvant treatment. EMVI responds to CRT by causing vessel fibrosis which can be detected on MRI. These

radiological changes may result in improved survival. The prognostic effect of MRI-detected EMVI (mrEMVI) has previously been examined (Smith et al., 2008a) and described in detail in earlier sections. The potential to grade regression of EMVI following neoadjuvant treatment means that it could be used as an imaging biomarker to measure effectiveness of such treatment.

11.2. Methods

The patient selection has been described in Section 3. Briefly, a retrospective analysis of prospectively collected data was conducted examining the staging and post-treatment MRI scans of patients who had presented with primary rectal cancer and MRI evidence of EMVI. The MRI scans were re-reviewed to capture the additional data on changes in mrEMVI.

The study fulfilled the relevant Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK) criteria (McShane et al., 2005).

11.2.1. The REMARK Criteria for reporting biomarkers

The REMARK criteria were born out of a need to develop a standardised reporting tool for potential biomarkers. Several hundred reports have emerged investigating novel biomarkers to predict disease activity in cancer but the overwhelming majority have failed to translate into meaningful clinical application.

Much of the reason behind this poor yield has been the lack of reporting criteria which has meant that many studies have not been replicated to reach the same conclusions.

The REMARK guidelines are analogous to similar guidelines used for the reporting of randomized controlled trials or observational trails such as Consolidated Standard for Reporting Trials (CONSORT); and Standards for Reporting Diagnostic Accuracy Studies (STARD). The criteria include reporting of specifics of hypotheses, study design, methodology, results presentation and statistical analysis amongst others. The full REMARK guidelines are shown in Appendix C.

11.2.1. Chemoradiotherapy and post-operative chemotherapy regime

The CRT regime is described in Chapter 4. The post-operative chemotherapy is also described in Chapter 4. The policy of our institution is to routinely give patients with stage III disease adjuvant chemotherapy in addition to patients who demonstrate high-risk features on baseline imaging and therefore require pre-operative therapy. For patients with stage II disease following pre-operative treatment, a discussion is had between patient and oncologist regarding the benefits of further adjuvant treatment.

11.2.3. Radiological mr-staging

The local policy of our institution is for all patients with rectal cancer to routinely undergo local staging with MRI and for those patients who have CRT to have further MRI scans to assess treatment response. Each patient underwent a minimum of two MRI scans, before and after CRT, which were available for review. Where patients had undergone more than one post-CRT MRI scan, the most recent scan immediately prior to surgery was reviewed for the purposes of analysis.

mrT-stage was categorised into either “good” or “poor” based on the extent of tumour spread into the mesorectum. “T-good” included all tumours staged as T3b or better whereas T3c or worse were recorded “T-poor”. mrN stage was categorised as either positive or negative, and mrCRM was considered positive if the tumour edge was within 1mm of the circumferential resection margin. These characteristics were recorded pre-treatment and post-treatment (mr- and ymr-stage).

ymrEMVI status was recorded as either positive or negative (positive status was a score of 3 or 4 on the MRI-EMVI grading system described in earlier sections). Further MRI assessment for the extent and site of EMVI was recorded in addition to the degree of regression. This was categorised as large-vessel mrEMVI if tumour invasion was

identified into superior, middle or inferior rectal veins; or small-vessel disease if mrEMVI was seen into non-anatomical vessels. mrvTRG was used to categorise the degree of EMVI regression. The criteria for the mrvTRG score has been presented in earlier sections. The treatment response was measured by the degree of fibrosis present in the extramural vasculature.

Fibrosis in contradistinction to tumour is characterised on MRI as dense low (dark/black) signal intensity rather than the nodular intermediate (relatively brighter/grey) signal intensity of tumour. This is best seen on post-CRT T2-weighted MRI, the areas of fibrosis have very low signal intensity, whereas areas of residual tumour have intermediate signal-intensity. The signal intensity of fibrosis is similar to that of the muscularis propria, and signal intensity of residual tumour is similar to that of baseline tumour.

11.2.4. Histopathology staging

Final histopathology staging (yp-stage) included T-stage; N-stage; EMVI status and CRM involvement.

11.2.5. Statistical analysis

The response of EMVI to CRT was measured using the mrvTRG scale described above. For the purposes of analysis, patients were categorised into two groups based on whether there had been more or less than 50% fibrosis seen within the lumen of the affected vessels – mrvTRG1-3 (good mr venous responder) and mrvTRG4-5 (poor mr venous responder). Survival included recurrence rates; site of recurrences; and 3 year disease-free survival (DFS).

Differences in staging characteristics between groups was analysed using Fisher's exact test and Chi-squared, where appropriate. Survival curves for DFS were

calculated using the Kaplan Meier product limit method; differences between survival curves for mrvTRG levels were tested for significance using the Mantel-Cox log rank test. An event was radiological or pathological detection of recurrent/metastatic disease or death from any cause. Time to event was recorded from the start of treatment date. Multivariate analysis was performed using Cox regression for time to recurrence. A p-value of <0.05 was considered significant. Hazard ratios were recorded with 95% confidence intervals.

11.3. Results

A total of 88 patients who were mrEMVI positive met the inclusion criteria and had MRI images available for review. 62 were included in the final analysis. Reasons for exclusion were metastatic disease on presentation, incomplete neoadjuvant treatment, and EMVI not evident on MRI review. 21 patients were female and 41 male. The median age was 68 (range 28-87).

11.3.1. Pre-treatment mr-staging

60 patients (97%) were staged as mrT-poor and 44 (71%) patients also had MR defined nodal disease at the time of diagnosis. mrEMVI was present in all 62 patients; of these, 50 (81%) had evidence of large-vessel mrEMVI. The height of the lower edge of the tumour from the anal verge was measured on MRI for each patient - 21 low rectal (0-5cm); 15 mid-rectal (6-10cm); 26 upper rectal (>10cm). The CRM was considered threatened on MRI if the tumour margin spread to within 1mm – same as histopathology criteria. 31 patients (50%) had a positive mrCRM.

11.3.2. Post-treatment ymr-staging

11.3.2.1. ymr-EMVI

35 patients (56%) had more than 50% fibrosis of EMVI following CRT (“good venous responders”). This included 12 patients where there was complete fibrosis of EMVI (mrvTRG1) which is considered to be a change in EMVI status from positive to negative. 27 patients (44%) demonstrated less than 50% fibrosis of EMVI (“poor venous responders”).

In the good venous responder, 20 of 35 patients showed evidence of large vessel EMVI; 2 patients had small-vessel and disease and 12 patients showed no evidence

of EMVI. In the poor venous responders, 26 of 27 patients had large-vessel EMVI and only 1 patient with small-vessel disease.

11.3.2.2. Other staging characteristics

Following CRT, 13 of 35 (37%) patients were now staged as T-poor; 11 patients had nodal disease (31%); and 5 patients (14%) showed that the CRM was still threatened or involved in the good venous responders. Of the 27 poor venous responders, 26 were still T-poor (96%); 18 (67%) had nodal disease; and 17 (63%) had an involved CRM. The pre- and post-CRT staging characteristics are shown in table 27.

Staging characteristic		Baseline staging n=62	Post- CRT		p-value
			MrvTRG 1-3 n=35	MrvTRG 4-5 n=27	
mrT-stage	Good (mrT1-T3b)	60	22	1	<0.05
	Poor (>mrT3b)	2	13	26	
mrN-stage	0	18	24	9	<0.05
	1/ 2	44	11	18	
EMVI vessel type	No evidence	0	12	0	>0.05 (small and large vessel)
	Small vessel	12	3	1	
	Large vessel	50	20	26	
mrCRM status	Negative	31	30	10	<0.05
	Positive	31	5	17	

Table 27 – Pre and Post-treatment MRI staging characteristics (ymr-stage)

11.3.3. Histopathology staging

The majority of the good venous responders were staged as ypT3 (17/35). One patient had a pathological complete response with no residual tumour found; 12 patients were staged as ypT2 and 5 patients were ypT4. For nodal staging – 11 patients were node-negative and 24 patients had N1/N2 disease. The CRM was clear of tumour in all patients.

In the poor venous responders group, one patient had a pathological complete response and there was one patient staged as ypT2. 22 patients had ypT3 stage and 3 patients ypT4. 14 patients had node-negative disease whilst 13 patients had N1/N2 disease. 2 patients had a positive CRM. These are shown in table 28.

Staging characteristic		**No of patients**		**p-value**
		MrvTRG 1-3	**MrvTRG 4-5**	
ypT-stage	pCR	1	1	<0.05
	T2	12	1	
	T3	17	22	
	T4	5	3	
ypN-stage	0	24	13	0.12
	1/ 2	11	14	
ypCRM status	Negative	35	25	N/A
	Positive	0	2	

Table 28 – Post-treatment histopathological staging (yp-stage)

11.3.4. Survival analysis

Only 3 patients (9%) developed recurrence from the good venous responders – 2 hepatic; 1 local recurrence. 3-year disease-free survival (DFS) was 87.8%. In comparison 12 patients (44%) developed disease recurrence from the poor venous responders, with the majority being hepatic metastases – 7 hepatic; 3 pulmonary; 2 local. 3-year DFS was 45.8%. A Mantel-Cox log-rank test comparing survival showed a statistically significant difference – p=0.013. The survival curves of the two groups are shown in figure 27.

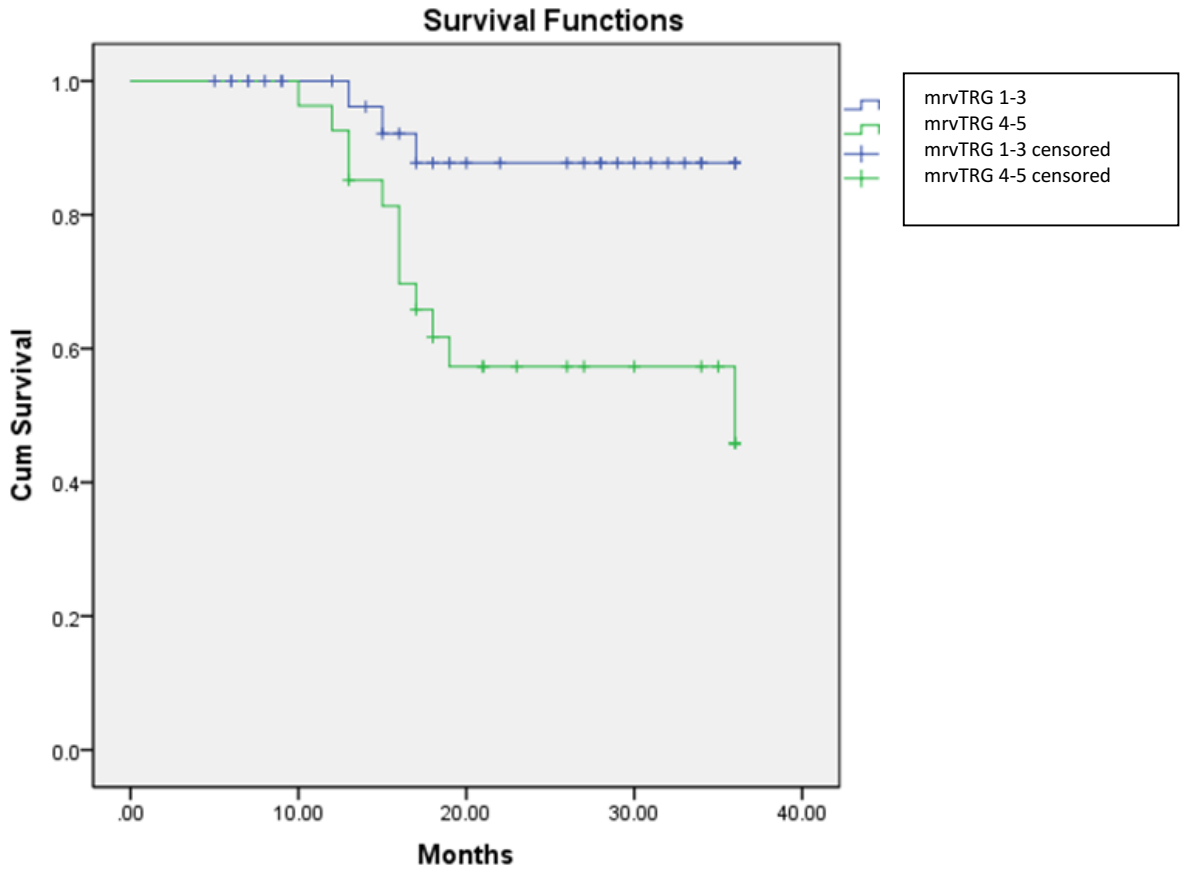


Figure 27 – Kaplan Meier curves for disease-free survival (DFS) for patients mrvTRG 1-3 and mrvTRG 4-5

On multivariate analysis of specific patient and tumour characteristics using Cox regression, only regression of EMVI by more than 50% (mrvTRG1-3) was shown to be significant for improved disease-free survival and recurrence – p = 0.013. The hazard ratio of a patient developing recurrence following CRT and surgery if there was less than 50% fibrosis of EMVI was 5.748 (95% CI 1.442 – 22.905). This is shown in table 29.

Variable	Hazard ratio	95% CI	p-value
Sex			
Female	1.000		
Male	1.479	0.527 – 4.149	0.458
Height from anal verge			
>5cm	1.000		
<5cm	0.634	0.248 – 1.620	0.341
EMVI regression			
mrTRG 1-3	1.000		
mrTRG 4-5	5.748	1.442 – 22.905	0.013
ypT stage			
T3b or better	1.000		
T3c or worse	0.265	0.067 – 1.052	0.059
ypN stage			
N0	1.000		
N1/2	2.255	0.891 – 5.710	0.086
ypCRM status			
negative	1.000		
positive	1.197	0.136 – 10.496	0.871

Table 29 – Multivariate analysis of prognostic factors for disease-free survival following neoadjuvant chemoradiation and TME surgery

11.4. Discussion

This study has shown that good regression of mrEMVI with more than 50% fibrosis is associated with significant improvement in disease-free survival, independent of final pathological staging (T-stage, nodal status and CRM status). This finding was seen in 56% of patients initially presenting with mrEMVI. 3-year disease-free survival (DFS) was only 45.8% when there was less than 50% fibrosis (poor venous responders) compared with 87.8% in good venous responders. In addition, the recurrence rates were 9% for good mrEMVI versus 44% for poor mrEMVI responders. This is the first study to stratify the tumour regression grade of EMVI following neoadjuvant treatment using REMARK criteria for investigating imaging biomarkers. These results would suggest that mrEMVI could be used as a predictive imaging biomarker and that it is worthwhile targeting patients with mrEMVI for further oncological treatment such as neo-adjuvant chemotherapy rather than just chemo-sensitisation to elicit further down-staging of mrEMVI, since those patients showing a significant degree of fibrosis of mrEMVI have improved survival.

Using detailed MRI assessment enables more effective risk assessment and therefore therapeutic stratification early in the patient treatment pathway. The most important recognised factors that influence neoadjuvant treatment are depth of tumour penetration, and in particular the extent of spread into the mesorectum and beyond (T3-sub-stage and T4), and proximity of the tumour edge to the circumferential resection margin (CRM) – these have been described in earlier sections.

The response to neoadjuvant treatment can be measured histopathologically by the use of tumour regression grades (Mandard et al., 1994, Suarez et al., 2008). The regression grading enables an assessment of response in terms of cytological

changes and stromal changes including fibrosis which have been show to correlate well against outcomes. There has been recent investigation in the use of MRI-based regression grades (mrTRG). Understanding how a tumour will respond to treatment using mrTRG scores pre-operatively, increases the chance that surgery will result in a successful oncological outcome and further allows for intensive oncological treatment if patients have not shown an adequate response. However there have been no specific investigation of response to mrEMVI as a predictive tool.

The consistent depiction on MRI of the larger veins such as the superior and inferior rectal veins, aids identifying the type and size of vessel involved. MRI is therefore more accurate in identifying large-vessel EMVI than smaller-vessel disease (Smith et al., 2008a). We observed that the majority of patients had large-vessel disease initially and where there was less than 50% fibrosis of EMVI large-vessel disease predominated. This confirms the importance, first shown by Talbot, of large vessel disease over smaller vessel (Talbot et al., 1981). Whilst small-vessel EMVI may be more difficult to identify both radiologically and histopathologically, it may in fact be of little clinical consequence.

Following CRT, 38 patients showed more than 50% fibrosis (mrvTRG score of 1-3). Interestingly, of these 38 patients, 12 patients showed complete regression of mrEMVI and would be reported as EMVI-negative on MRI - no evidence of EMVI. Only one patient who had become mrEMVI negative developed a recurrence – hepatic metastases. There was also improvement in all other prognostic factors as one would expect. As the study cohort all underwent CRT, it is, by definition, a high-risk group of patients. This can be seen by the baseline staging characteristics which show that almost all patients were staged as T-poor. In our institution, not all T3 patients are universally irradiated and nodal disease alone is not an indication for neo-adjuvant

therapy. In the mrvTRG 1-3 group, the majority of patients were staged T3 but the CRM was negative in all patients. Although there were a few patients who were radiologically staged to have CRM involvement following CRT on MRI, this information would have been known to the surgeon allowing them to make appropriate surgical decisions to ensure a clear margin. In the poor venous responders, T3 tumours predominated and the CRM was involved in 3 patients out of 28. More than half of patients still had nodal disease although this was not statistically relevant difference between the groups. Further, on multivariate analysis, nodal disease was not a significant factor and only mrvTRG grade was significant.

These results suggest that EMVI regression is a prognostic indicator for disease recurrence in addition to being predictive as well. Patients who show little fibrosis of EMVI following CRT are more likely to develop metastatic disease. The hazard ratio of 5.748 is significant and suggests that if patients show minimal regression of EMVI following standard CRT that more intensive pre-operative chemotherapy to further treat EMVI may improve disease recurrence. These patients are at risk of developing metastases and should be considered for aggressive therapy pre- or post-operatively. This also raises the issue of more frequent follow-up as currently there is no universal consensus on rectal cancer surveillance after curative surgery. Whilst patients who are enrolled in trials have a defined follow-up strategy, the timing of imaging, serum CEA and colonoscopy varies from centre to centre. Patients who are identified to be high-risk of metastatic disease should be considered for more frequent follow-up, and in particular more frequent liver imaging.

One of the perceived limitations of this study is the use of a specialist gastrointestinal radiologist to stage the MRI scans. It is not always possible for patients' imaging studies to be reported by specialist radiologists and sub-specialisation has not been

accepted in the UK as yet. It has been previously shown in the Mercury study that technique and assessment of MRI can be standardised, particularly when a specific proforma-based system is adopted (Taylor et al., 2010, Taylor et al., 2008). Further, this is retrospective analysis which introduces bias. However the data collected was done so from routinely undertaken proforma reports for histopathology and the MRI scans were re-reviewed by the authors. The numbers in the study are also small as this is a pilot study. This introduces the possibility of bias and statistical error (type II). The development of the MARVEL trial (described in later chapters) will help prospectively validate these results.

In summary, neoadjuvant therapy is known to improve survival and downstage disease in terms of depth of tumour spread, regional lymph node and CRM involvement. There have been no studies of the effect of chemoradiation on EMVI in terms of morphological change or effect on survival. This is the first study to quantify the radiological changes which occur in EMVI following CRT and how they relate to survival. Patients in whom there has been more than 50% fibrosis and regression of EMVI show significant improvement in disease-free survival. In patients where there has been less than 50% regression, consideration should be made for additional neoadjuvant therapy. The regression and fibrotic changes in EMVI following CRT are identifiable on MRI and by showing that mrEMVI treatment response correlates with improvement in disease recurrence, we suggest that it has a role as a predictive imaging biomarker.

Chapter 12

Differences in molecular profile between ypEMVI negative and ypEMVI positive rectal cancer and the changes following neoadjuvant chemoradiotherapy

Aim

This study primarily focuses on a sub-type of advanced rectal tumours that exhibit EMVI and aims to characterise gene expression differences in malignant cells from these tumours compared to EMVI negative rectal tumours. Identification of genes involved in the development pathway, in tumours expressing EMVI positive status, can allow the identification of potential therapies which could hope to prevent these genes (specifically microRNAs) from being over or unexpressed preventing vascular invasion.

12.1. Introduction

Numerous efforts using molecular profiling have attempted to categorize cancers such that patients with an increased risk of recurrence and metastasis can be identified. The behaviour of malignant cells lining rectum depends upon both genetic mutations and gene expression changes, which it may be possible to identify. This knowledge may mean that we can predict which cancers will respond to various drugs and treatments such as irradiation and spare some patients unnecessary morbidity.

Pre-operative assessment and tumour staging is critical for recognising more aggressive and advanced colorectal tumours and can improve prognostic outcome, for example by using molecular methods. Inaccuracy of staging in some cases has led to some patients being staged with a low stage tumour when it is in fact a higher stage

tumour and therefore should get treatment intensification. This highlights the importance of accurate pre-operative staging in clinical management especially when identifying patients who would benefit from CRT. Molecular profiling will help ensure future accurate staging, most likely in conjunction with high resolution imaging techniques such as MRI.

Responses of tumours to neoadjuvant CRT can be quite varied between patients and for some patients the little benefit of the neoadjuvant treatment regime is eclipsed by the significant adverse side-effects of the therapy (Birgisson et al., 2007). However for some patients, CRT prior to surgery can significantly reduce tumour size thus improving the chance of complete tumour removal during surgery. Therefore establishing a robust identification criteria for those who would benefit from CRT is also advantageous.

Previous efforts using molecular markers alongside gene expression profiling have hoped to define patients with an increased risk of disseminated disease. With a large variation in the reported prevalence of EMVI in surgical resection specimens it indicates there are problems when assessing EMVI status. The preceding chapters have attempted to address this however there may be more novel ways in which EMVI could be detected. Identification of genetic markers and further understanding of the molecular mechanisms which lead to EMVI may aid in developing newer treatment strategies. The identification of new diagnostic and prognostic biomarkers for EMVI is needed to help characterise the tumour and complement treatment selection strategies.

Deregulation of gene expression is a hallmark of cancer. With the technology available today, molecular and genetic profiling allows us to recognize and understand the

fundamental mechanisms, which regulate the behaviour of rectal cancer. MicroRNAs (microRNAs) have recently been described as “managers of gene expression” (Mirnezami et al., 2009) regulating biological processes including proliferation, cellular differentiation, apoptosis and stem cell self-renewal. MicroRNAs are short non-coding regulatory molecules which base-pair with the 3’ -UTR region of protein coding mRNAs resulting either in degradation of the mRNA or inhibition of translation. Primary or pri-microRNAs are first transcribed in the nucleus by polymerase II, as long hairpin structures. These structures are processed by the ribonuclease ‘Drosha’ to form shorter double stranded pre-microRNAs. These pre-microRNAs are subsequently exported out of the nucleus where they are further trimmed by the endonuclease Dicer to produce short mature microRNAs. These 22-25 base pair duplexes are integrated into the RNA-induced silencing protein complex (RISC) (Bartel, 2004). Mature microRNAs escort the RISC to supress gene expression by binding to their target mRNA.

So far more than 1400 microRNAs have been identified and are estimated to regulate up to 30% of the human genome. With microRNAs having hundreds of transcripts as direct or indirect targets and more than one microRNA having the ability to assemble on a single transcript, the possible regulatory potential by microRNAs is vast. Such deregulation of genes particularly those involved in tumour suppression, cell cycling, apoptosis and invasion can lead to cancer formation and progression. In recent years, significant advancement in microRNA research studies using high-throughput analysis of pathological samples has expanded our knowledge of a variety of cancers including colorectal cancer and breast cancer (Sorlie et al., 2006). Technology such as expression profiling using microarrays would allow the identification of any significant variations in microRNA gene expression between different sub-types of cancer such

as EMVI positive rectal cancer and EMVI negative tumours revealing new potential biomarkers and therapeutic targets.

12.2. Methods

12.2.1. Clinical Sample Selection

The patient selection and tissue sampling is described in Section 3. Briefly, tissue samples used in this study were obtained from a licenced and approved tissue bank at Southampton General Hospital and the Royal Marsden Hospital. Formalin-fixed paraffin embedded (FFPE) rectal tumour resection blocks were selected from patients with locally advanced adenocarcinoma of the rectum and showing no evidence of distant metastases. Patients initially underwent a pre-operative MRI scan, prior to long-course chemoradiotherapy (LCRT) and then surgery. Patients were also subjected to a pre-operative MRI scan as part of their treatment plan. Both scans were assessed using a scoring proforma to resolve basic characteristics and the post-treatment MRI scan was compared to the histopathology analysis of the surgical specimen.

Tissue specimens of four subtypes were collected; EMVI positive tumours subdivided into lymph node positive or lymph node negative, and EMVI negative subdivided into lymph node positive or lymph node negative. The clinical and pathological information for patients within the criteria was recorded into a database. Written informed consent forms were obtained from each patient

Samples were further analysed following laser micro-dissection and RNA extracted for analysis. These techniques are explicitly described in Section 3.

Results 12.3

12.3.1. Demographics and tumour characteristics

A total of 30 samples were selected for analysis. 12 EMVI-positive tumours were compared with 18 EMVI-negative. These samples were collected on the basis of MRI-detected EMVI on post-CRT MRI scans (ymrEMVI status). The patient characteristics and tumour characteristics are shown in table 30.

Sex	Male	18
	Female	12
Age	Median	69.5
Height from anal verge	Low	8
	Middle	9
	Upper	13/01/1900
T-stage	Good	7
	Poor	23
N-stage	Negative	11
	Positive	19
CRM status	Negative	18
	Positive	12

Table 30 – Patient demographics and MRI-based tumour characteristics

12.3.2. Survival

The recurrence rate of the EMVI-positive group was 7/12 (58.3%) versus 5/18 (27.8%). 3-year DFS was 25.8% in the EMVI-positive group compared with 72.9% in the EMVI-negative group ($p<0.05$).

12.3.3. MicroRNA analysis

Comparison of the EMVI positive and EMVI negative sample demonstrated a difference in three microRNA sequences - hsa-miR-1277-5p; hsa-miR-4764-3p; hsa-miR-873-3p. The difference in expression was calculated as a 'fold difference' which corresponded to under- or over-expression of the sequence. This is shown in table 31.

microRNA sequence	**Fold Difference**	
	EMVI positive	**EMVI negative**
hsa-miR-1277-5p	0.49	1.00
hsa-miR-4764-3p	2.33	1.00
hsa-miR-873-3p	0.49	1.00

Table 31 – Difference in microRNA expression between EMVI positive and negative tumours.

Comparison was also made between tumours which were node-negative or node-negative (stage II versus stage III) tumours with evidence of EMVI. A further four sequences were identified that demonstrated differential expression. This is shown in table 32.

microRNA sequence	**Node negative**	**Node positive**
hsa-miR-1914-5p	1	0.48
hsa-miR-4666b	1	3.74
hsa-miR-4482-5p	1	3.37
hsa-miR-634	1	0.43

Table 32 – Difference in microRNA expression between stage II and stage III tumours

12.4. Discussion

The results of this study show that individual rectal cancer sub-types have distinct molecular profiles. Differences in expression were also demonstrated in three specific microRNA sequences between ypEMVI negative and ypEMVI positive tumours. Node-positive and node-negative tumours were shown to have different expressions in four different and specific microRNA sequences. This study is the first to show that ypEMVI positive tumours have inherent biological profiles when compared to EMVI negative tumours, which adds to the differences in clinical outcomes that are already known. These differences can be identified in a unique gene signature through microRNA sequencing.

Traditional techniques to detect tumour characteristics are being challenged in an attempt to discover increasingly accurate methods of risk-stratifying and ultimately treating patients with cancer. Following the lead of breast cancer where much excellent work has identified novel therapeutic targets, rectal cancer is now being investigated through molecular profiling techniques. The premise of detecting tumour characteristics through routine histopathological methods remains the 'Gold Standard' however we have shown in earlier chapters how high resolution imaging techniques such as MRI can complement this.

High-throughput analysis of gene signatures is a more novel technique which has great potential. It allows clinicians to stratify patients on differences identified on a molecular level and thus more accurately than gross pathology methods. By linking these profiles to clinical outcome data, we are able to develop highly accurate surveillance and treatment strategies.

The clinical outcome data between node-negative and node-positive tumours is not disputed. The patients in this study whose tumours' were further analysed had all undergone pre-operative CRT. The difference in microRNA expression between tumours with distinct nodal status following CRT is an important finding. This implies that despite CRT the tumours still have inherent biological differences which may result in specific clinical outcome patterns.

Differences were also noted between the EMVI sub-types. Earlier chapters have demonstrated different clinical outcome data between these sub-types. The results of this study add to the clinical observations which show that EMVI is an important factor post-CRT. The use of the radiological and clinical data strengthens the case for discovering therapeutic targets based on EMVI status. The difference in expression between EMVI positive and negative tumours is a basis for therapy. If we can target the specific sequences which are unique to EMVI positivity we have the opportunity to improve survival.

The next step in this process is to validate these results both internally and externally. Further, determining where the microRNA sequences act in known tumorigenesis pathways is important in developing future therapies. However there are certain limitations in this particular study. Firstly, the laser micro-dissection did not specifically identify areas of tumour where there was known EMVI. The sample were taken from tumour specimen but not correlated directly to extramural veins. This introduces the idea that specific areas of the tumour may have unique profiles and that the area containing extramural veins laden with cancer cells has a different profile than an area of tumour without veins. This is an important investigation for the future. For example, do the molecular profiles of liver metastases mimic those of EMVI positive tumours in general, or are they specifically related to the profile of tumour specimen within the

extramural veins? Secondly, this study only investigated the molecular profiles of patients following CRT. It would be interesting to know whether the profiles had changed following CRT by comparing samples before and after. This may help explain the mechanisms and target of CRT in individual patients.

In summary, the results of this study are interesting and demonstrate that tumours with distinct morphological and radiological characteristics also have distinct molecular profiles. However, the clinical relevance and application of these results are a long way off. There needs to be further validation and investigation of EMVI sub-type. For example, verification with quantitative PCR followed by external validation. Further, there a number of new avenues which must be explored to confirm these results with greater accuracy and detail to ensure that the most appropriate therapeutic targets are manipulated.

Chapter 13

Development of the 'MARVEL' Study – The Molecular and Radiological Evaluation of EMVI in Rectal Cancer

Aim

This prospective study will identify important differences between key rectal cancer tumour subtypes, and in turn will fuel the formulation of new hypotheses. Identification of reliable pathological markers of EMVI pathways (from both the primary tumour sample, but more importantly from the pre-operative biopsies) has real potential for taking us a step closer to more personalized management of rectal cancer by establishing prognostic biomarkers reflective of disease type, but also through the underlying biology that may be highlighted (with its promise of therapeutic translation).

13.1. Introduction

The preceding chapters have discussed in detail and presented results to explain that optimal treatment of rectal cancer relies on accurate pre-operative risk stratification and subsequent multidisciplinary management. Treatment decisions take place in the context of the MDT and involve clinicians from different specialities. The identification of adverse tumour characteristics underpins this strategy. EMVI is an example of this and has been shown in some detail to be a poor prognostic factor in rectal cancer that confers an increased risk of disease recurrence (Chapters 1 and 5). Traditionally, EMVI has been identified on histopathological analysis of surgical resection specimens alone. And as pathology has been historically considered to be the 'gold standard' for staging, diagnosis is not always possible on biopsy if one were to enquire

about the pre-operative EMVI status. Therefore there is over-reliance of post-operative specimens.

CRT is widely accepted as beneficial to selected patients in terms of decreased risk of local recurrence. Until now, very little has been known about the long-term outcome and response to CRT of EMVI positive tumours. The results of the studies in earlier sections have helped better understand the morphological and clinical outcomes of such tumours.

New analytic techniques using principles of miniaturization are becoming available for gene and molecular profiling. The use of microarrays can be readily applied to the profiling of rectal cancer. Using these techniques, it is possible to identify differences in the genetic profiles between EMVI positive and EMVI negative tumours and whether any changes are observed following CRT. This may then be correlated with clinical behaviour over the medium and long-term with regards to local recurrence, distant metastases and overall survival.

13.2. Methods

13.2.1. Trial Design

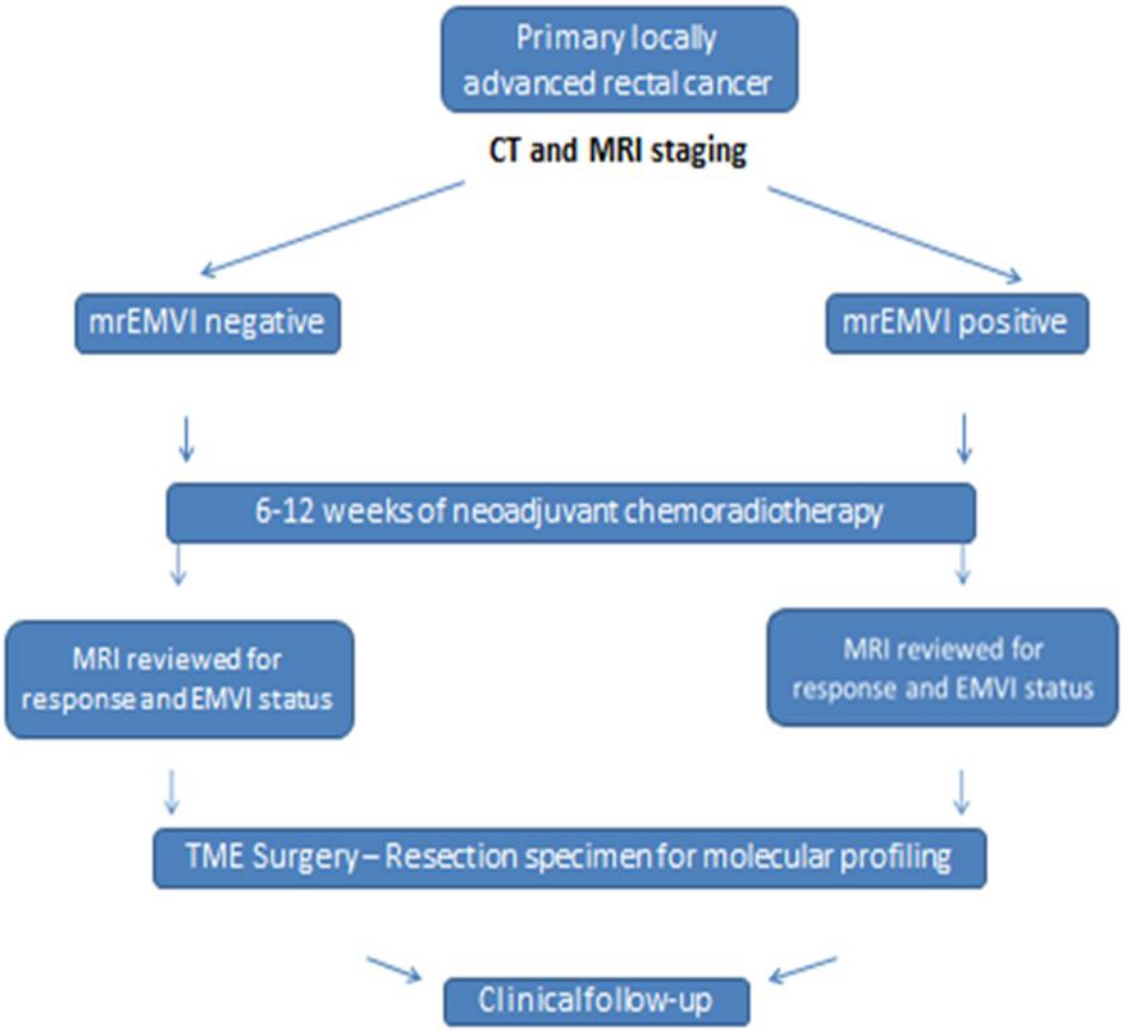


Figure 28 – Flowchart showing MARVEL trial design

This will be a prospective study comparing two groups of patients undergoing the same treatment regime for locally advanced rectal cancer. The study aims to investigate the radiological and pathological effects of pre-operative CRT on EMVI in rectal cancer. The treatment regime for locally advanced tumours will be dictated by

the hospital – the study protocol does not interfere with local policy. However, pre-operative treatment must include long-course CRT. Surgery must be considered curative and performed using total mesorectal excision (TME) principles.

The primary objective will be to document a difference in relapse free survival (RFS) at 1 and 3 years between mrEMVI positive and mrEMVI negative rectal cancer. Our definition of RFS will include patients who are alive at the end of follow-up with no evidence of recurrence. Secondary endpoints include the rate of conversion of EMVI positive to negative following CRT and the response rates of EMVI tumours in relation to other adverse features such as T stage, nodal stage, CRM involvement and TRG. The final objective is to document the differences in the underlying profiles of EMVI positive and negative adenocarcinoma, and whether there is any change in these profiles following CRT and relate this to clinical outcome. By identifying differences between the underlying profiles relating to EMVI status, we will be able to propose potential therapeutic targets.

13.2.2. Participants

Patients will be identified at the multidisciplinary meeting (MDT) held at the local hospital. They must be able to undergo CRT followed by curative surgery. Recruitment will take place on a consecutive patient basis. As part of the staging process, it must be local policy to perform local staging with MRI and staging for distant disease with CT scan of the thorax, abdomen and pelvis. Patients will be divided into those who are EMVI positive or EMVI negative depending on the baseline staging MRI scan.

Patients will be given the option to enrol in the study in the outpatient clinic (surgical or oncology) following the MDT meeting. This will occur in the local hospital and the local research team will carry out recruitment. Patients are free to consent to join the

trial on the same day they read the patient information sheet, as this trial is non-interventional. This study will not require patients to undergo any additional or further investigations or procedures outside the treatment pathway.

CRT would be given over a 3-month period followed by repeat MRI scanning to determine response and potential for surgery – the neoadjuvant treatment strategy will be in line with the current NICE proposals (Appendix XX). They will consent to allow analysis of their biopsy and surgical specimen, which may include genetic analysis. Patients in the trial will be treated as determined by the MDT responsible for their care. All staging information determined as part of this study will be made available to the MDT. The trial will have no impact on the treatment given to patients.

Following recruitment, biopsy specimens will be retrieved and “banked” for analysis. Tissue blocks and slides from local units will be requested by the Royal Marsden Hospital once recruitment has been confirmed. All imaging studies will be performed at the local hospital and reported on the clinical reporting form (CRF) by a member of the research team. Once patients have completed pre-operative CRT they must undergo further MRI scanning as part of their treatment strategy to assess local response. These MRI scans will be reported on the CRF. Providing the patients are able to proceed to surgery they will continue in the trial. Central review of MRI scans will take place at the Royal Marsden Hospital, Sutton. Routine histopathology reporting will be performed by the local hospital and reported on the pathology CRF by a member of the research team. The surgical specimens will be retrieved and “banked” following surgery for pathological analysis. Clinical follow up will take place using routine outpatient consultations and any subsequent imaging as per local policy.

13.2.3. Inclusion Criteria

- Locally advanced primary adenocarcinoma of the rectum (requiring pre-operative treatment); diagnosed on tissue biopsy
- Adult patients – over 18 years
- Able to undergo curative (TME) surgery
- Able to undergo MRI and CT with relevant contrast agent
- Able to undergo LCRT

Exclusion Criteria

- Metastatic disease at presentation
- Emergency diagnosis/treatment
- Unable to undergo staging (MRI and CT) or treatment procedures (LCRT/surgery)

13.2.4. Sample size

The primary endpoint for this study is disease relapse at 3 years. There will be interim analysis at one year. This is a non-matched prospective case-control study. We have estimated relapse at year one for EMVI positive patients to be in the order of 33% and EMVI negative to be 16%. At year 3 we estimate 75% relapse in patients with EMVI positive rectal cancer compared with 35% of EMVI negative tumours. Using previous work, we estimate that 25% of EMVI positive rectal cancer becomes EMVI negative following CRT. Recruitment will take place over a two-year period, with an additional three years of follow-up. We anticipate a maximum of 5% loss to follow up at three

years. A total of 40% of all patients are expected to be EMVI positive prior to treatment. Based on these estimates, the study would have 91% power to show a difference in relapse rate with a total of 80 patients (48 EMVI negative and 32 EMVI positive), using a two-sided alpha of 0.05.

The laboratory work is an observational hypothesis. There are no statistical calculations or power analyses that can be done at this time, as there isn't any existing data that can be used to extrapolate from. If a biomarker is found, it would need to be internally and externally validated formally (in further studies).

13.2.5. Analysis methods

All time to event endpoints will be calculated using Kaplan-Meier methods. Cox regression will be used to estimate hazard ratios and 95% confidence intervals. All tests will be two-sided with alpha 0.05 unless otherwise stated. The primary endpoint of time to relapse will be measured from the date of surgery. Those patients who are found to have irresectable disease after CRT will be withdrawn from the trial. Patients who do not have successful resection (local disease remaining after surgery) will be included with relapse at time 0. Date of relapse in all other patients will be defined as the date of first positive diagnosis of local, nodal or distant relapse. Patients will be grouped as EMVI positive or negative according to their status pre-CRT, and relapse rates compared between the groups using a log- rank test. Relapse rates at 1 and 3 years will be calculated for both groups, and overall, with 95% confidence intervals. Cox regression will be used to calculate a hazard ratio and 95% confidence interval.

In patients with EMVI positive status pre-CRT, relapse rates will be compared between patients who remain positive following CRT, and those who achieve EMVI negative

status following CRT. Pathological T and N stage (from the surgical specimen) will be compared between the two groups using a Chi-squared or Fisher's exact test.

13.4. Discussion

This is a multicentre observational trial which is currently open to recruitment. One of the main objectives of this trial is to investigate the prognostic and predictive effect of EMVI following CRT. Whilst several studies have reported on the survival for specific tumour characteristics including CRM, T-stage, nodal disease and EMVI, whether or these conclusions apply following CRT is yet to be validated in large numbers. MRI has been recently shown to be effective in assessing CRT response but this needs to be done prospectively in such a trial.

In earlier chapters we have seen that EMVI is becoming increasingly recognized as an important prognostic factor in rectal cancer. This has been shown to adversely affect survival for Stage II tumours as well as being a potential imaging biomarker. These initial studies support the need for a large-scale prospective trial and to determine whether oncological treatment strategies need to consider EMVI more directly.

The results of this trial will be available in 2017. The Approval letter for the MARVEL trial is shown in Appendix D.

Chapter 14

Conclusions

14.1. Recent and current literature

14.1.1. Optimal histopathological detection of pEMVI – the future.

Since the commencement of this thesis in 2010, there have been other investigators who have examined the importance of EMVI in similar contexts. In earlier sections, the difficulty in accurately identifying EMVI using routine histopathological methods and stains has been explained. One of the controversies in the detection of pEMVI has been whether or not to use more specialised stains, particularly those which stain for elastin. This has now been examined in greater detail in the last few years.

A large-scale retrospective study from Scotland examined more than 400 specimens of both colon and rectal cancer. They carried out a comparison of pEMVI detection rates between using an elastica stain and with normal routine H&E staining. The 3-year survival were no different between the groups however the rate of detection improved from 18% to 58% (Roxburgh et al., 2010). They also found that staining for elastica improved the prognostic value of EMVI. A direct comparison between using H&E alone versus elastic staining and H&E in 53 patients demonstrated an increase in the area under the receiver operator curve (ROC) from 0.58, P = 0.293 (H&E alone) to 0.74, P = 0.003 for venous invasion detected using the elastica method. Although they have not examined rectal cancer specifically, they have also made the argument that accurate testing for EMVI is important so not to exclude patients from vital adjuvant therapy (Roxburgh and Foulis, 2011).

The experience of pathologists in Canada was reported by Messenger. A population-based survey was sent to 361 pathologists that yielded a 64.9% response rate. The majority of respondents stated they reported EMVI in less than 10% of specimens and only 9.1% reporting detection rates above 20% (Messenger et al., 2011). Interestingly, special stains were also used if there was suspicion of EMVI but by just over 50% of pathologists.

A further and more recent study from Toronto has further examined the utility of elastin stains. The investigators sent forty slides to six specialist gastro-intestinal (GI) and six non-specialist pathologists to look for EMVI. Initially they were only stained with H&E and subsequently resent following staining for elastin – Movat stained. The overall rate of detection improved from 19.6% to 46.4% ($p>0.001$). Further results from this study showed an improved detection rate for specialist GI pathologists and better inter-observer agreement when Movat stain was used - H&E: $\kappa=0.23$ vs. Movat: $\kappa=0.41$ (Kirsch et al., 2013).

In a recent systematic review exploring the detection methods of pEMVI, the use of elastic stains were found to be beneficial with an overall detection rate of 43.6% (van Wyk et al., 2014). However rates of detection have been reported as high as 71% for all stages of disease in colorectal cancer (Roxburgh et al., 2014, Sejben et al., 2010).

There appears to be a realisation that EMVI is an important consideration for adjuvant therapy and accurate detection is crucial to be able to offer patients optimal survival. Furthermore, there is also a shared concern that the current methods of detection are resulting in underestimates for the true prevalence of EMVI and therefore there is the real possibility that we may be denying patients optimal adjuvant treatment. The routine use of specialised stains, in particular elastin-based stains, will most likely

become the standard for histopathology. There is accumulating evidence that using elastin stains improves detection rates and it may be the case in the future that the use of these stains becomes incorporated into universal guidelines for pathologists.

14.1.2. The use of MRI-based scales to measure CRT treatment response.

In earlier chapters, the use of MRI to detect and grade EMVI in response to CRT has been shown as prognostically important. Indeed, it may be possible to use mrvTRG as a predictor of disease progression and to determine which patients require increased surveillance and intensified chemotherapy regimes. Such a scale has recently been shown to predict survival following CRT by using radiological changes to the tumour as a whole.

Studies by the MERCURY Group have demonstrated the accuracy of the MRI-based TRG scale (mrTRG). A study in 2011 from the MERCURY Group examined the response to CRT of 111 patients with locally-advanced rectal cancer (Patel et al., 2011b). They compared the tumour regression grading of MRI and histopathology. Further they investigated the use of MRI to predict ‘good’ and ‘poor’ responders using specific radiological criteria. MRI assessment of TRG was an independent predictor of poor survival and recurrence. Hazard ratios (HR) of 4.40 (95% CI, 1.65 to 11.7) and 3.28 (95% CI, 1.22 to 8.80) were seen with ‘poor’ mrTRG for overall survival and 3-year DFS, respectively. And for an MRI predicted CRM involvement was independently significant for local recurrence with HR of 4.25 (95% CI, 1.45 to 12.51). This compared to ypT stage and ypCRM status as independent predictors of recurrence and survival.

14.2. Final Conclusions and future perspectives

The treatment for rectal cancer is evolving and consequently staging systems have followed to maintain accurate risk-stratification of patients. Historically, clinicians used evidence of tumour depth, nodal disease and metastatic spread to offer relatively simplified treatments, primarily involving surgical resection. However as more tumour features have been shown to be related to prognosis in addition to more detailed information becoming known about the more traditional adverse features, patients have been offered more tailored treatments.

Most patients present with what is described as “locally advanced” disease, which means that the tumour has penetrated through the bowel wall into the surrounding mesorectum. These patients require radical resection of their tumours under the surgical principles set out in ‘total mesorectal excision’ (Heald et al., 1982). Furthermore, they are likely to benefit from additional oncological therapy. Tumour characteristics associated with this include features such as nodal disease and EMVI. Offering neoadjuvant CRT to patients is the most common treatment strategy for locally advanced tumours. However the proportion of patients who are likely to benefit from such treatment is not as clear as previously thought, and features such as EMVI may be underestimated at present. For example, most centres will pre-operatively irradiate tumours whose only adverse feature is involved mesorectal nodes under the assumption that these nodes are a risk for local recurrence. But considering the anatomy of lymphatic drainage in the mesorectum does not cross the mesorectal fascia and that optimal TME surgery will involve high ligation of the inferior mesenteric artery and excision en-bloc of the tumour, rectum and mesorectum attached to the vascular pedicle, it seems illogical to offer radiotherapy for this reason alone (Chand et al., 2013b).

The rationale for this approach is based on historical data which did not exclusively examine patients' who had undergone optimal surgery in the CR07 trial for example. Indeed, in those patients who did undergo TME surgery the benefit of radiotherapy was marginal. Using neoadjuvant therapy more selectively will mean that patients who are more likely to benefit from the treatment are offered it whilst those who are less likely are not subject to the additional morbidity associated with an intensive treatment such as radiotherapy (Chand et al., 2013a).

The future of rectal cancer treatment will be based on identifying those patients who are most at risk of disease recurrence and offering them more intensive treatment whilst at the same time not burdening patients who are unlikely to benefit from additional treatment with added morbidity. This policy of selective oncological treatment must be based on accurately identifying those adverse factors which are most associated with disease recurrence that can be treated with current therapies. EMVI may be one such feature and by using more accurate techniques and different approaches to detection, it will be possible to truly understand its prognostic relevance in rectal cancer.

EMVI has become increasingly recognised as a poor prognostic factor and improving consistency of its definition along with more accurate detection has meant that its true prognostic value is being recognized. Although there is still no randomized trial evidence which definitively shows it to be a predictive factor in rectal cancer, there is growing level 2 and 3 evidence as described above. A review of the current literature shows a huge variation in detection rates over the years. The vast majority of these studies have been based on patients undergoing primary surgery with only a small proportion having adjuvant chemotherapy. The evolution in the management of rectal cancer has led to a greater number of patients being offered pre-operative CRT for a

period of 6-12 weeks. This has made these historical studies redundant in one aspect such that the prognostic effect of EMVI once it has been exposed to both chemotherapy and radiotherapy may not be the same as in those patients who have undergone primary surgery.

A major advance in the detection of EMVI has been the increasing use of MRI. It has now become integral to the staging of rectal cancer and despite the popularity of endo-anal ultrasound in the USA, MRI remains the mainstay of local staging for the majority of patients. Thus EMVI can be first detected at the time of initial staging at diagnosis compared with the historical studies described above, where the first time EMVI was detected was following surgery and analysis of the resection specimens by the pathologist. In addition, the role of MRI in the treatment pathway allows morphological changes in EMVI to be quantified and compared with the post-operative pathology. This has provided many exciting opportunities to understand the prognostic relevance of EMVI in the modern-era of rectal cancer management.

MRI is routinely used to risk-stratify patients early in the management pathway and forms the basis of treatment-decision making for neoadjuvant CRT. The last decade has seen the acceptance of high-resolution MRI to inform clinicians with regards to decision-making. The identification of the CRM is the most important of adverse features. However, to varying degrees the identification of tumour spread into the mesorectum (T3 sub-stage) and nodal disease have also been reasons for neoadjuvant therapy. The results of trials have shown that neoadjuvant therapy in combination with TME surgery have improved the rates of local recurrence significantly, but this has not translated to an improvement in overall survival (Glimelius, 2013). The devastating effects of local recurrence have been dramatically reduced but it is the systemic disease which is leading to death from rectal cancer and

it is this which needs to be targeted in the future. Developing new strategies to reduce the risk of distant disease and ultimately improve overall survival must be the goal of future studies. It is unlikely that radiotherapy will have a significant role to play in this and the mainstay of treatment will be chemotherapy either in the adjuvant, or possibly, the neoadjuvant setting. The difficulty will be convincing patients that rather than opting for an operation after a period of chemoradiotherapy, that they should continue with a further six months of chemotherapy whilst alleviating their concerns of the risk of the disease becoming more advanced. Furthermore, there is a risk of over-treating patients and there must be sufficient robust evidence behind such a strategy. Aside from the side effects associated with a prolonged period of systemic chemotherapy, patients can be wary from protracted treatment particularly if this results in lethargy. This resistance from patients to continue with systemic chemotherapy has been seen in the post-operative setting whereby patients who have already undergone intensive pre-operative treatment followed by a major operation are reluctant to undergo further systemic treatment for a limited benefit (Khrizman et al., 2013).

The survey conducted in Chapter 7 investigating the role of MRI in treatment decision-making demonstrated the acceptance by most clinicians in using high quality imaging to guide decisions. Whilst MRI has an established role in influencing neoadjuvant therapy, it is encouraging that there is an appetite for its use in adjuvant treatment. Our institution has a policy of using a combination of pathological and radiological factors to decide on adjuvant treatment. For example, a stage II tumour which has no adverse features following CRT would normally not be offered adjuvant chemotherapy in most centres across Europe. Whilst the NCNN guidelines (NCNN, 2013) suggest that all patients who have undergone neoadjuvant therapy for locally advanced disease should be offered adjuvant chemotherapy regardless of pathological staging,

there is no evidence behind this stance and further it is difficult to explain this to patients in a quantifiable manner. In our institution, the initial staging MRI scans are re-visited to look for nodal disease; and if this proves to be positive for mesorectal nodes patients are offered further chemotherapy. Again, there is no evidence behind this but highlights the variability in practise shown in the survey results.

Another aspect of this variability in practise is the perceived survival benefit of adjuvant treatments. Even within the oncology community, there is variation in the survival benefit offered by adjuvant chemotherapy which means patients are being told differing information about the same treatment. Until there is clear evidence and associated guidelines, patients will be offered potentially inaccurate information to base their decisions on – either under- or over-representing survival benefit.

Interestingly, there remains a reluctance to use MRI following CRT for decision-making and a persistent reliance on pathology. Direct comparison of like-for-like (MRI versus MRI) seems to be the obvious and intuitive thing to do when it comes to guiding decision-making. The same MRI sequences and protocols are used for initial staging and for post-CRT imaging which is ideal for comparison of treatment effect and study of prognostic factors. The effect of tumour regression was originally derived in oesophageal cancer following neoadjuvant treatment (Mandard et al., 1994). There have been recent reports describing the accuracy of MRI-based regression grades on measuring the effect of treatment response in rectal cancer (Patel et al., 2011b). In these reports, an MRI-derived TRG scale (mrTRG) has been shown to be independently predictive of disease-free survival and overall survival. An argument against using such a scale is that once the patient has had surgery and been finally staged by pathology, the mrTRG scale is redundant. Yet there are associated difficulties in relying on pathology alone.

Firstly, there is no robust evidence to suggest that adjuvant chemotherapy following surgery in patients who have undergone neoadjuvant chemoradiation show any survival benefit. However, patients in whom there has been tumour regression following variable neoadjuvant chemotherapy regimes show improved survival. This would suggest that evaluating disease response by MRI and then deciding on further neo-adjuvant treatment may be a better strategy than the use of post-operative chemotherapy. Therefore, tumour features such as EMVI and their response to CRT could be objectively measured on MRI and used to decide whether further pre-operative chemotherapy is beneficial.

Secondly, using pathology alone may result in a significant number of patients with EMVI being missed. A major challenge in the detection method for patients has been the effect of CRT on the architectural features pathologists use to identify EMVI. Accurate detection of adverse features relies on standardisation of technique and definition, both of which are currently lacking in pathology. The gradual acceptance of pro-forma based reporting actively encourages the pathologist to seek out features such as EMVI and is no doubt the future of pathology reporting. However even with more accurate techniques than currently in routine use, much of the detection of EMVI is based around the identification of specific architectural features. The fibrosis which results from CRT produces anatomical distortion thus ‘hiding’ the presence of EMVI (Messenger et al., 2011).

But before the correct techniques and definitions are applied to the specimens, it is important that they tumour is adequately sampled and that it is cut up in a manner which is optimal for EMVI identification. This has been exhaustively described by Quirke et al (Quirke and Morris, 2007). The pathologist can only look for EMVI in the specimen they have been provided - another disadvantage of using pathology alone.

All these problematic issues with identifying ypEMVI alone can be helped by the use of MRI. Certainly, a starting point would be to use MRI as a reference which would 'guide' pathological analysis. Although, MRI interpretation post CRT has been labelled difficult by some, using the same criteria for identification EMVI can be accurately sought. It is also independent of the quality of the resection specimen and sampling by the pathologist. The entire rectum can be seen in-situ therefore giving a better picture of the anatomical relationship of the tumour and surrounding vasculature.

Traditionally, the use of adjuvant chemotherapy and prognostication of patients following surgery has been based on studies involving primary surgery. Much of this evidence is still used in rectal cancer for which there is scarce data on the effect of CRT on tumour features. The effect of neoadjuvant therapy with the use of radiotherapy +/- chemotherapy in varying doses is clear on local recurrence. There is little dispute that local recurrence rates have fallen not simply because of improved surgical technique but the addition of pre-operative treatment. Yet the overall survival of these patients is unchanged. The interpretation must be that it is the systemic disease which ultimately affects survival which is not adequately treated by the use of radiation and limited chemotherapy. Therefore the role of adjuvant chemotherapy becomes very interesting. But how effective is chemotherapy in patients who have already undergone neoadjuvant CRT and can we continue to use the same pre-treatment features to guide such treatment?

If we relate the use of oncological treatment to the surgical anatomy of the rectum, it may give us some clues. The rectum as we have seen earlier is surrounded by an embryological fatty envelope. This acts as an oncological barrier which contains the lymphatic channels and nodes which can act as conduits for tumour cells. Many centres use the presence of tumour in these nodes as a reason to offer radiotherapy

prior to surgery. These nodes and their channels do not cross the mesorectal fascia and if good quality surgery under TME principles is performed, will be excised en-bloc with the tumour. The rationale for this treatment is based on studies in which TME surgery was not an inclusion criteria and thus specimen quality was variable (Chand et al., 2014). Alternatively, the veins outside the bowel wall do cross the mesorectal fascia and these too can act as conduits of disease progression. Could it be a possibility that post-CRT we are chasing the 'wrong' adverse feature and it is actually EMVI that we should be pursuing?

The importance of EMVI and MRI-based detection of adverse features is being increasingly recognised. The main problem is that there is still no robust randomized evidence that shows that either (y)pEMVI or (y)mrEMVI are predictive factors for survival. It is unlikely that without this evidence that oncologists will be willing to treat patients on the basis of anecdotal evidence alone. The design of current trials has tried to address this question somewhat – for example, MARVEL (Chapter 13) has the opportunity to assess EMVI directly. The results of the survey above show the confusion. Even within a learned community there is a great variability in the benefit of chemotherapy in EMVI-positive patients and whether mrEMVI should be used as a prognostic factor for decision-making. In these situations, it is strong level 1 evidence in the form of a prospective randomized trial or meta-analysis that can provide guidelines.

The discordance between mrEMVI and pEMVI remains problematic and may hinder the interpretation of future trials investigating the clinical relevance of EMVI. We have already described some of the reasons behind under-reporting in pathology particularly after CRT. However, the question of whether mrEMVI is a true reflection of venous invasion must also be addressed. Without direct correlation between optimal

histopathological analysis, mrEMVI will continue to suffer scepticism. One possibility is that mrEMVI may not be exactly the same as pEMVI. The radiological characteristics which define mrEMVI may be demonstrating some other phenomenon such as a combination of lymphovascular, perivascular and perineural invasion. If this is the case, perhaps looking at mrEMVI as an entirely separate prognostic factor may be more fair comparison. After all, whether mrEMVI and pEMVI are the same phenomenon or not, the results of these studies in addition to others continue to show mrEMVI as an independent marker of poor prognosis. On the other hand, if the discordance between mrEMVI and pEMVI are simply due to over-diagnosis of one versus under-diagnosis of the other, optimising definitions and techniques for both radiologists and pathologists should help to bridge this gap. Further work involving detailed correlation of tumour between MRI image slices and representative sample from the tissue blocks will aid development of consistent and accurate techniques. This work is currently being undertaken by our research group and is ongoing but is not included in this report.

The issue of adjuvant chemotherapy for patients with EMVI will need further resolution. There are two main issues; firstly, there is component of the current staging systems that accounts for EMVI which means including this in the treatment discussion whether that be in the forum of the MDT or with patients alone is an additional conversation. Secondly, the fact that most tumours with EMVI will have associated poor prognostic features means that they will most likely undergo neoadjuvant CRT. The role of adjuvant chemotherapy in this setting is unclear as already discussed. This leads on to the need for a new, randomized trial where the survival benefit in patients post CRT that show evidence of persistent EMVI can be evaluated. The design of such a trial will be challenging and at minimum will suffer from a limited number of patients. To

accurately identify the predictive and prognostic effect of EMVI post CRT, patients will need to have been given a pathological stage II and show evidence of mrEMVI on the post CRT images. There will be only 300-400 patients per year that fit such a criteria and a sample size will be in the order of 400 patients to determine a 20% difference in 3-year DFS at the 90% power level. This doesn't preclude a trial but highlights some of the initial hurdles in the design phase.

The role of biomarkers will continue to increase in the future and diversify to involve different disciplines, for example, molecular biology, imaging; biochemistry. A concern of some clinicians is that pushing more intensive treatments to poor prognosis tumours is over-treating patients with potentially little gain. Conversely, if a subset of patients with poor prognosis are 'missing out' on treatment because of under-diagnosis, we may be doing these patients a dis-service. More accurate identification of patients who are not only at significant risk of poor outcomes, but also will benefit the most from additional intensive treatment is the goal of biomarker medicine.

The utility of biomarkers is in their ability to identify high-risk populations. It is unlikely that one particular sequence of cDNA, RNA or microRNA will be able to guide treatment for all patients and that combinations of adverse biomarkers will result in more accurate risk stratification. Studies which seek to validate molecular biomarkers and in particular microRNAs, report under- or overexpression being linked with survival. These sequences can then be targeted for therapeutic exploitation. Clearly, as with any pharmaceutical product that attempts to enter the market, stringent testing will need to be overcome to ensure that these sequences not only target cancels for reduction in aggressiveness but also do not affect normal host tissue leading to undesired consequences. This is a fundamental difference compared to imaging biomarkers which are able to identify high-risk populations and monitor disease

response to therapy but not currently a target for therapeutic purposes. This highlights the interaction between biomarkers of differing types (as explained in chapter 10). Whilst one type may seek to detect and monitor disease, another may be able to treat disease.

On a more fundamental level, identifying changes in the underlying biology of tumour following treatment and linking these changes to survival allows targeting of specific areas of the tumour. This can be demonstrated in the changing of EMVI status which improves disease free survival but also has an underlying molecular change. If studies such as MARVEL are able to show these changes between the biopsies and resection specimens, we will be able to simplify further the targeting of microRNAs and gain a better understanding of the pathways involved in disease progression and metastases. The results of TMA studies above have identified specific sequences which are unique to EMVI tumours. Clearly, validation both internally and externally will be required before any consideration can be made of therapeutic translation but the results are encouraging. The use of such novel technologies is a must if we are to understand the natural biology of these diseases and will greatly enhance the large scale studies currently underway examining the clinical effects of different treatment groups and validation of prognostic markers of disease progression and survival.

There is certainly a long way to go before unique microRNA sequences can be exploited therapeutically. microRNA 21 is considered by many to have the greatest potential of the current crop of microRNAs associated with colorectal cancer (Asangani et al., 2008). However, this along with other sequences such as those identified in the above study need accurate characterisation into the tumorigenesis and progression of disease pathways.

The pattern of recurrences highlights another important consideration in the general management strategies of high-risk EMI positive patients. The majority of recurrences occurred in the liver suggesting a preponderance for this site. Whilst the underlying pathways for liver metastases are still not fully known, there must be an anatomical association through the vasculature which leads to this phenomenon. The mechanisms of metastatic spread and thus the potential targets of treatment in the future still need further investigation however in the meantime, patients who are considered at risk of liver metastases should be offered more frequent follow-up. This may include shorter periods between imaging of the liver and clinical follow-up appointments in an attempt to identify potential disease recurrence at the earliest of stages. Therefore tailoring surveillance regimes based on an individual's risk may be preferable compared with a more generalised approach. Currently, there is no universal consensus on follow-up for patients at risk of disease recurrence and strategies vary from unit to unit in terms of endoscopic, radiological and clinical follow-up. Unifying a follow-up protocol will also give patients more confidence in their general treatment knowing that wherever they are treated they will receive similar surveillance.

A further point which has possibly led to an underestimation of the significance of EMVI in influencing treatment strategies is the association with other known adverse features. A recent randomised study by Rullier et al, comparing neoadjuvant radiotherapy plus capecitabine to radiotherapy plus capecitabine plus oxaliplatin in T3-4, M0 rectal cancer showed an association between a positive CRM and vascular invasion (Rullier et al., 2013). In patients with a positive CRM, 19.6% had vascular invasion compared with 6.2% of patients who did not have any evidence of invasion. This was found to be significant on multivariate analysis.

By definition, EMVI is found exclusively in locally advanced tumours in terms of tumour penetration and nodal disease. This means that these tumours would have been offered neoadjuvant therapy in any case, and as current regimes are standardised whether such tumour have EMVI or not will not change the management. Although this is an important limitation of EMVI as a prognostic factor influencing neoadjuvant treatment, it is adjuvant treatment where there is more potential for changing treatment strategies. With the current lack of strong evidence on the prognostic implications of tumour features following CRT, there is an opportunity to help define guidelines. To do this, and plan appropriate trials to validate hypotheses of EMVI being associated with disease recurrence and that EMVI can be treated by intensive chemotherapy regimes, there must be a fundamental shift away from traditional adverse features such as nodal disease. The QUASAR trial continues as the basis of evidence for many clinicians despite only a limited number of rectal cancers in the cohort studied and that no patients had CRT. However to isolate patients following CRT with only EMVI as a recognised adverse feature will be difficult. This has been explained above but briefly, there must be a universal consensus on EMVI detection and as the number of patients will be relatively small, any investigation will require a multicentre design. Approximately, 15000 patients are diagnosed with rectal cancer per year, however using results in the above studies for determining the prevalence of ymr/pEMVI, ypT3b or less, ypN0, CRM clear tumours will equate to around 300-400 patients only. Recruitment for such a trial will be difficult and require a multicentre design.

Chapter 15

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Appendix

Appendix A

Patient Study code:

Patient MRI datasheet

Radiologist Date:

Patient Initials:

Date of Birth:

Baseline Scan:	Yes: date / /	No
Post Pre-op Treatment Scan:	Yes: date / /	No
Scan inadequate for technical reasons	Yes	No
Tumour height from anal verge (mm.):		
Tumour height from puboretalis sling (mm):		
Craniocaudal length of tumour (mm):		

MRI T-Stage	
T1 Submucosa	
T2 muscularis	
T3a : <1mm	
T3b : 1-5mm	
T3c : 5-15mm	
T3d : >15mm	
T4a Into adjacent organs / external sphincter	
T4b perforation of visceral peritoneum	

Distance of tumour edge to:
mesorectal fascia CRM <1mm ☐
intersphincteric plane < 1mm ☐

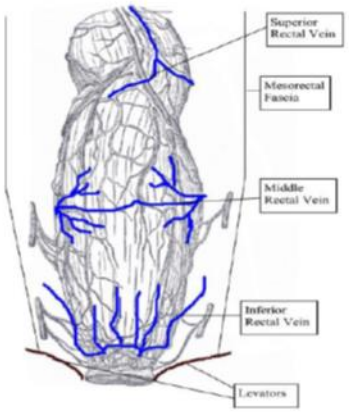
Closest CRM relates to
Tumour ☐
EMVI ☐
Lymph node ☐
Tumour deposit ☐

Extramural venous invasion: **Yes** **No**
If yes, score: (see EMVI scoring sheet supplement)

Minimal vascular spread ☐
Slight expansion of veins by tumour ☐
Clear and definite irregular expansion of a vein ☐

Is this large-vessel or small-vessel disease?
Large-vessel Small-vessel

Name the vessel:
Inferior rectal ☐
Middle rectal branch ☐
Superior rectal vein branch ☐
Non-anatomical vein ☐



Mesorectal Lymph Node Morphology: **High resolution**

No nodes/only high signal nodes ☐
< 4 nodes with either irregular border or mixed signal ☐
< 4 nodes with both irregular border and mixed signal ☐
>= 4 nodes with irregular border or mixed signal ☐

Certainty of malignancy:
Unlikely ☐ Doubtful ☐ Definite ☐

Appendix B

Addressograph

Pathology Reporting Form

Pathologist:	Date:
Study Code:	Surgeon:
Patient initials:	Operation date …/…/20...
Date of Birth:	Sex M F

Biopsy report

Type: Adenocarcinoma ☐ Mucinous ☐

Differentiation: Poor ☐ Well/mod ☐

Comments:

Histology

Type: Adenocarcinoma Yes ☐ No ☐

Differentiation:(By predominate type) Poor ☐ Well/Mod ☐

Other tumour type (Please State) ……

Maximum extramural spread of tumour

- Above sphincters (from edge of muscularis propria) …..mm
- At sphincters (from edge of internal sphincter) …..mm

Minimum distance of tumour to CRM from outer edge of tumour …..mm

Is the resection complete? (>1mm to the CRM) Yes ☐ No ☐

Metastatic Spread

No of Nodes examined …….. No. of positive nodes ……..

Apical Node positive Yes ☐ No ☐

ExtramuralVascular invasion Yes ☐ No ☐

Extra – nodal deposits seen ☐ Yes ☐ No

CRM Involved ☐ Yes ☐ No

Maximum length of margin involvement ……mm

Mode of CRM Involvement: ☐ Direct ☐ Vascular
☐ Node ☐ Tumour Satellite

Site of CRM Involvement ☐ At mesorectum ☐ Below Mesorectum (at levators/sphincters)

Tissue at CRM: ☐ Normal tissue ☐ Fibrosis
☐ Tumour

Distance from dentate line to lower border of Mesorectum ……..mm

Plane of resection

Mesorectum (all specimens)

Plane ☐ Muscularis propria ☐ Intramesorectal ☐ Mesorectal

Post neoadjuvant therapy tumour regression

☐ No regression ☐ Minimal regression ☐ Moderate regression

☐ Good regression ☐ Total regression

Other comments
……
……
……
……
……

Appendix C

Guidelines for the REporting of tumor MARKer studies (REMARK)

Introduction

State the marker examined, the study objectives, and any prespecified hypotheses

Materials and methods

Patients

Describe the characteristics (e.g., disease stage or comorbidities) of the study patients, including their source and inclusion and exclusion criteria

Describe treatments received and how chosen (e.g., randomized or rule-based)

Specimen characteristics

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

Assay methods

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 end point

Study design

State the method of case selection, including whether the study design was 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

Precisely define all clinical end points examined

List all candidate variables initially examined or considered for inclusion in models

Give rationale for sample size; if the study was designed to detect a specified effect size, give the target power and effect size

Statistical analysis methods

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

Clarify how marker values were handled in the analyses; if relevant, describe methods used for cutpoint determination

Results

Data

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 numbers of patients and the number of events

Report distributions of basic demographic characteristics (at least age and sex), standard (disease-specific) prognostic variables, and tumor marker, including numbers of missing values

Analysis and presentation

Show the relation of the marker to standard prognostic variables

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 analyzed. For the effect of a tumor marker on a time-to-event outcome, a Kaplan–Meier plot is recommended

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

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

If done, report results of further investigations, such as checking assumptions, sensitivity analyses, and internal validation

Discussion

Interpret the results in the context of the prespecified hypotheses and other relevant studies; include a discussion of limitations of the study

Discuss implications for future research and clinical value

Appendix D

NHS
Health Research Authority

National Research Ethics Service

NRES Committee London - Fulham
HRA NRES Centre Manchester
Barlow House
3rd Floor, 4 Minshull Street
Manchester
M1 3DZ

Telephone: 0161 625 7109
Facsimile: 01641 625 7299

24 October 2012

Dr Gina Brown
Consultant Radiologist
Royal Marsden Hospital
Downs Road
Sutton
SM2 5PT

Dear Dr Brown,

Study title: **Radiological And Pathological Analysis Of Extramural Venous Invasion In Rectal Cancer**

REC reference: **12/LO/1735**

Thank you for your email dated 17/10/12, responding to the Proportionate Review Sub-Committee's request for changes to the documentation for the above study.

The revised documentation has been reviewed and approved by the sub-committee.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.